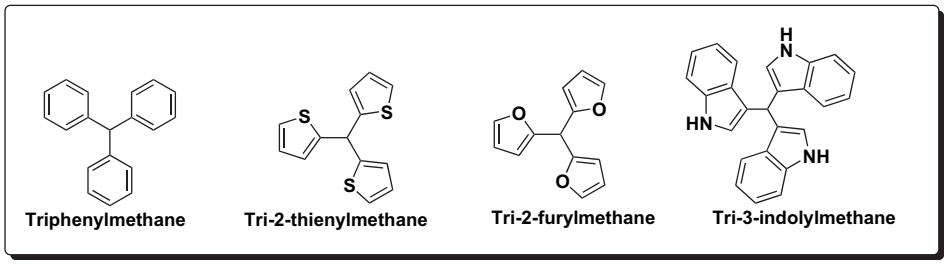
ELSEVIER

Tetrahedron

Tetrahedron Vol. 62, No. 29, 2006

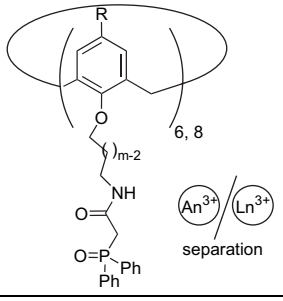
Contents

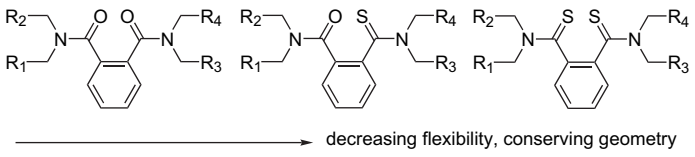
REPORT

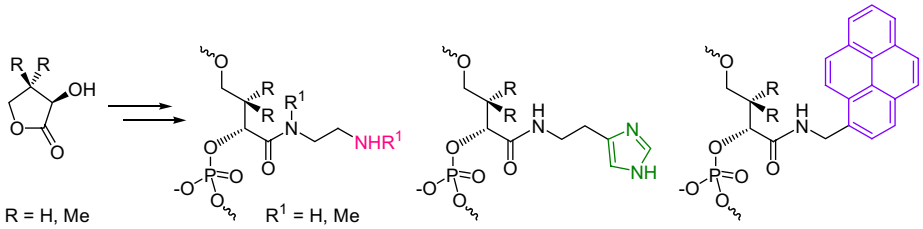


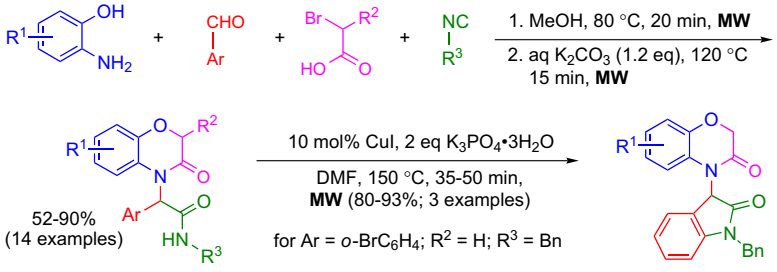
The major developments in the synthesis, reactions, and applications of triaryl- and triheteroarylmethanes are described.

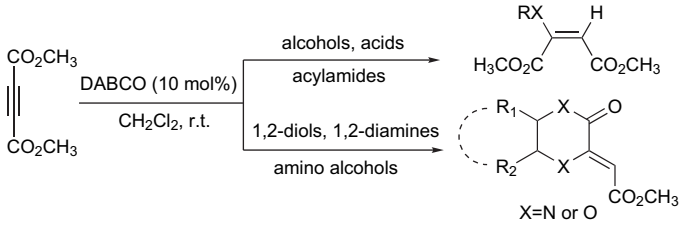
ARTICLES

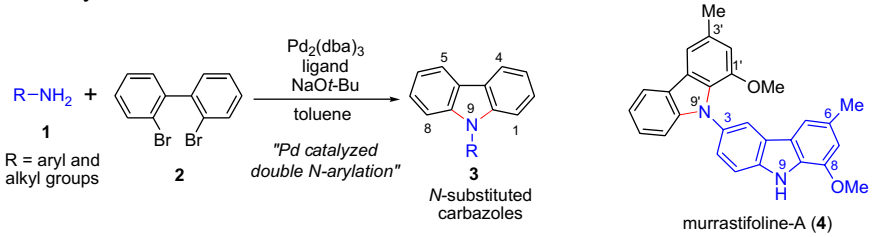




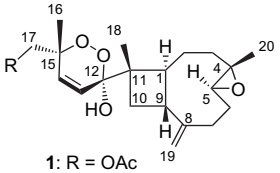


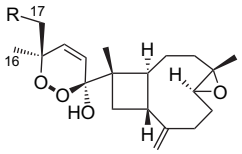


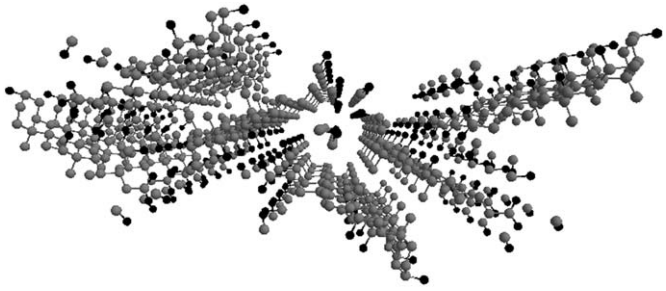


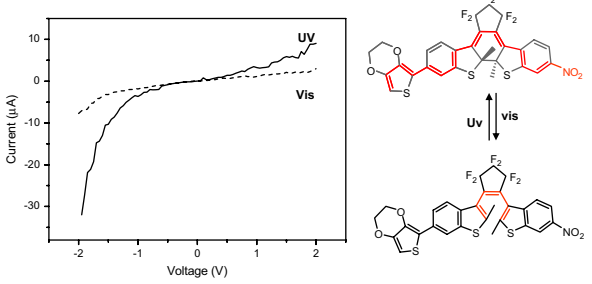


Aryl and aliphatic amines including *tert*-butylamine and a protected glucopyranosylamine were effectively transformed into the corresponding *N*-substituted carbazoles by way of the Pd-catalyzed double N-arylation. The first total synthesis of murrastifoline-A, a biscarbazole alkaloid, based on this methodology was also accomplished.

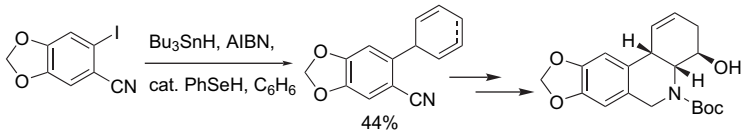


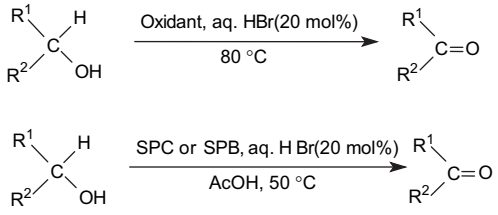


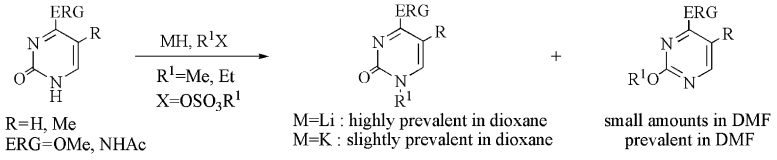


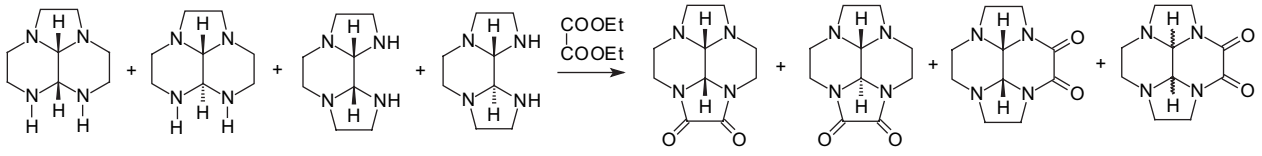


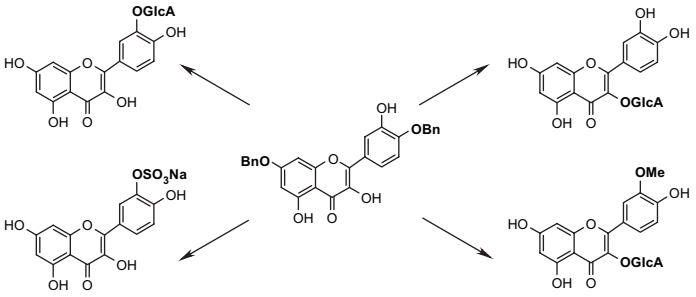
Several acnistins and withajardins showed leishmanicidal activity. The 3D-QSAR models predict the same affects for both leishmanicidal and toxicity activities.

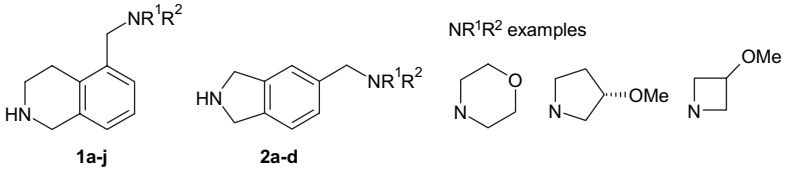




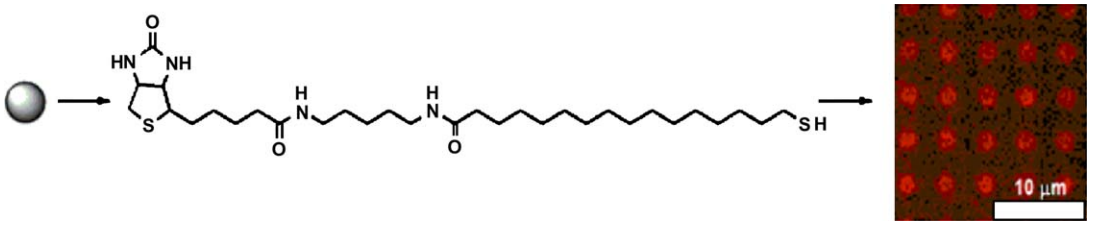


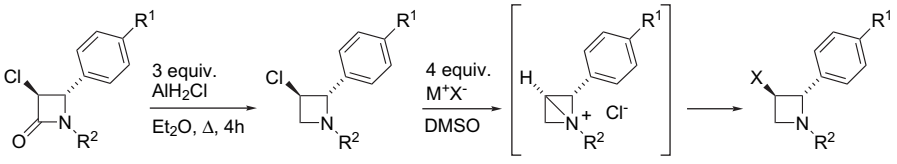


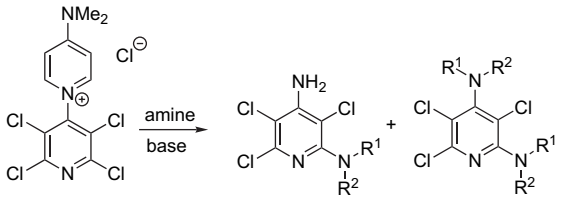


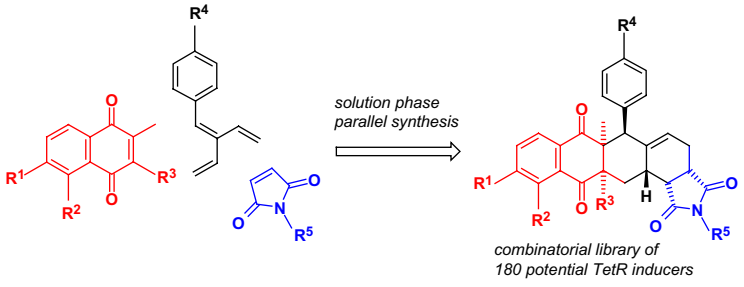


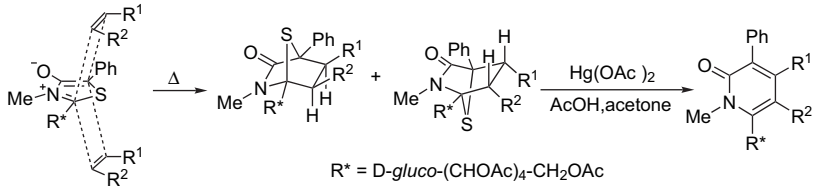
The synthesis of 10 substituted aminomethylene tetrahydro-isoquinolines **1a–j** (eight steps from 5-hydroxyisoquinoline) and four similarly substituted dihydro-isoindoles **2a–d** is described.

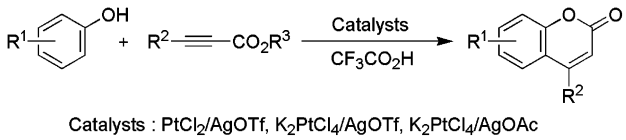


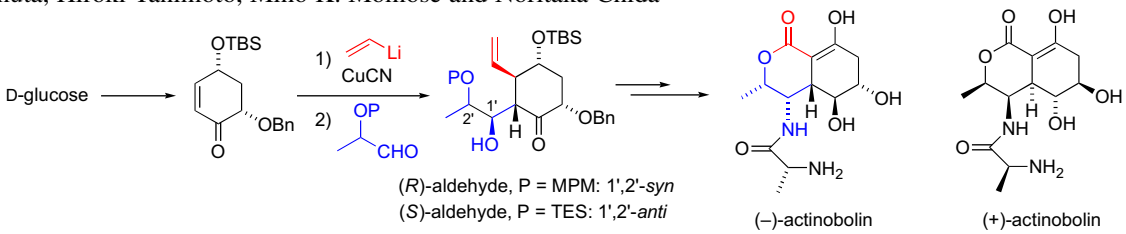




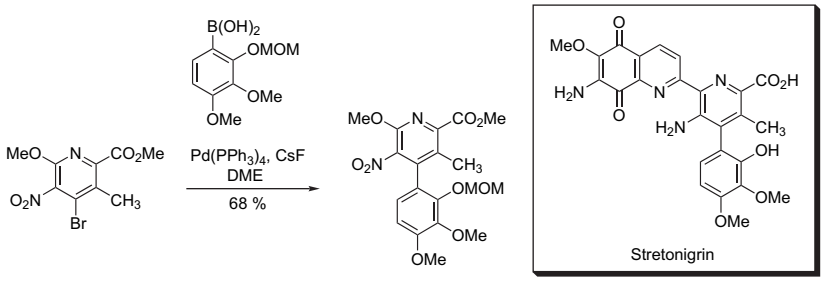


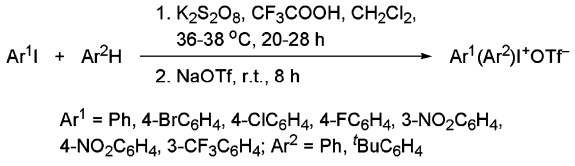


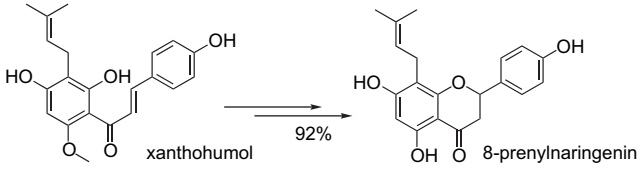


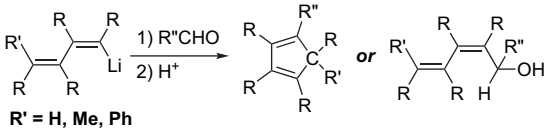


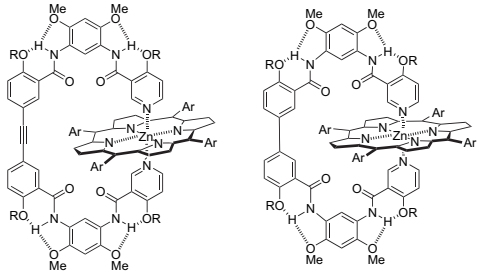
Total synthesis of (−)-actinobolin starting from D-glucose is described. Stereocontrolled three-component coupling reactions of a cyclohexenone derived from D-glucose by way of Ferrier's carbocyclization, with vinyl cuprate and 2-alkoxypropanal effectively constructed the carbon framework of actinobolin. The formal synthesis of (+)-actinobolin starting from D-glucose was also accomplished.

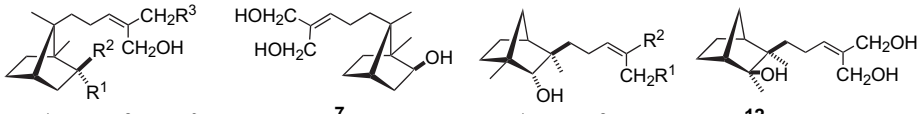


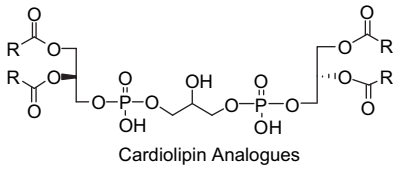


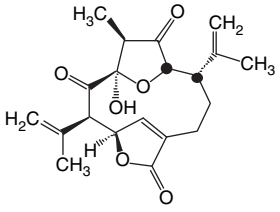


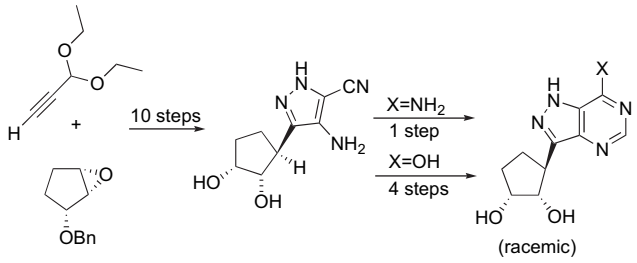


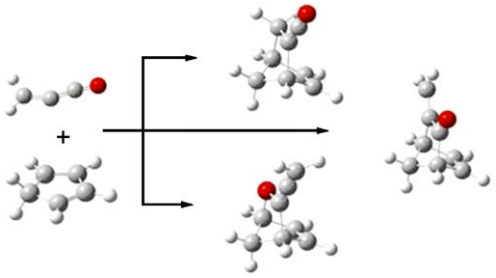


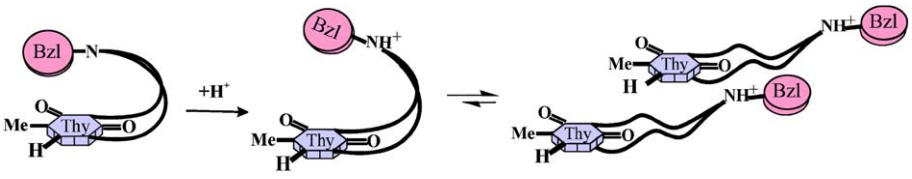


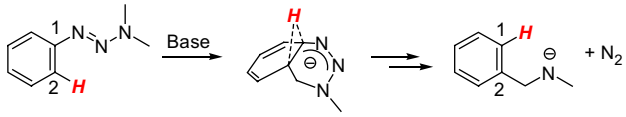




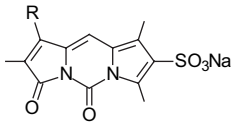






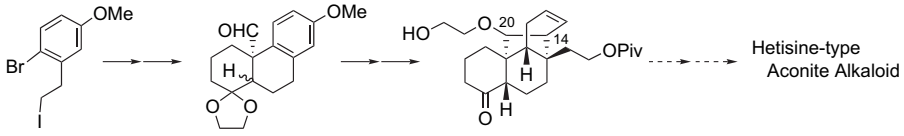


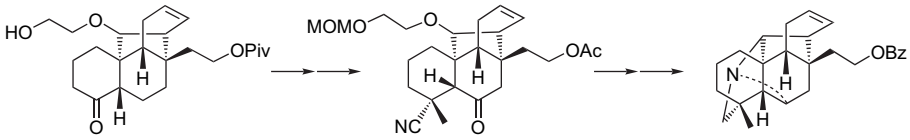
Intramolecular 1,2-Proton shift (Possible path)

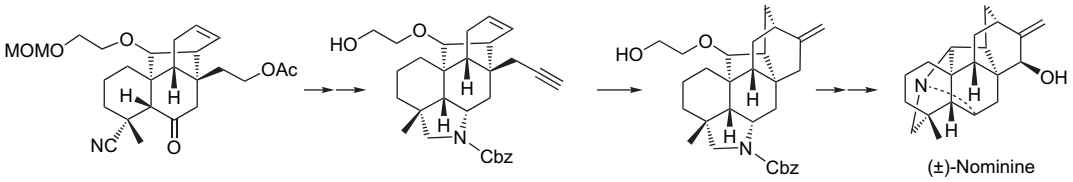


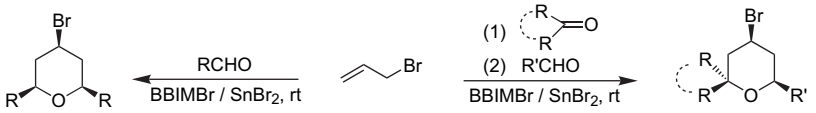
1: R = C_6F_5
2: R = *p*-$EtOC_6F_4$

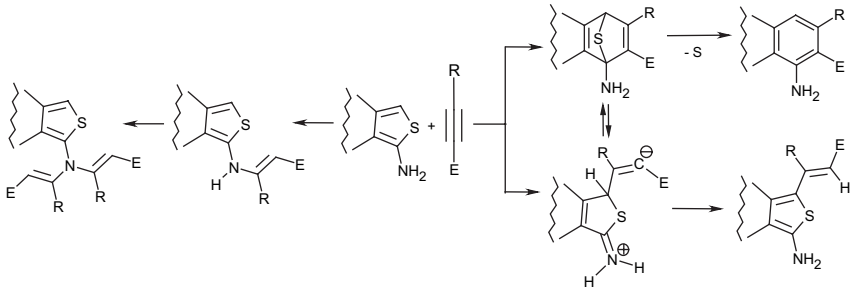
Fluorinated *N*,*N*′-carbonyl-bridged dipyrrinones, synthesized and found to be highly fluorescent (ϕ_F 0.4–0.6) are potential ^{19}F MRI and fluorescence imaging agents for probing hepatic metabolism.

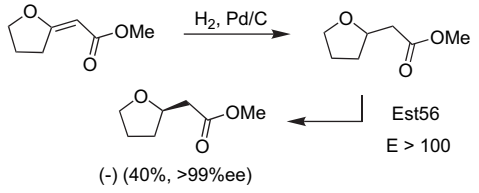












*Corresponding author
Supplementary data available via ScienceDirect

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Recent advances in the chemistry of triaryl- and triheteroarylmethanes

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Contents

1. Introduction

Triarylmethanes (TAMs) have attracted the attention of many scientists mainly because of the interesting properties associated with their derivatives. The versatility of TAM is clear from their high end applications. For instance, simple triarylmethanes have been reported to be active against intestinal helminths, filariae, trichomonads, and trypanosomes[1] and the phenol derivatives of TAM have been known to exhibit antioxidant properties, antitumor activity, and inhibitory activity toward histidine protein kinase.[2]

Keywords: Triarylmethanes; Triheteroarylmethanes.

Abbreviations: Ar, aryl; Bt, benzotriazolyl; CAN, cerium(IV) ammonium nitrate; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DME, 1,2-dimethoxyethane; DMSO, dimethylsulfoxide; ISC, inter system crossing; INDO, intermediate neglect of differential overlap; LAH, lithium aluminum hydride; LDA, lithium diisopropylamide; NBS, *N*-bromosuccinimide; NLO, non-linear optical; OTf, triflate; PTSA, *p*-toluenesulfonic acid; PPA, polyphosphoric acid; TFA, trifluoroacetic acid; TMEDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; TMS, trimethylsilyl; binap, 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl.

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doi:10.1016/j.tet.2006.04.081

The acid-labile nature of the trityl group made it a celebrated protecting group for nucleosides, carbohydrates, etc.[3] The well-known dyes generated from TAM including Crystal Violet, Malachite Green, Sunset Orange, Pararosanilin, Victoria Blue, etc. (Fig. 1) exhibit stable quinoid structures.[4] Malachite Green has long been used to control the fungal and protozoan infections in fish[5] and it shows selective phototoxicity toward tumor cells.[6]

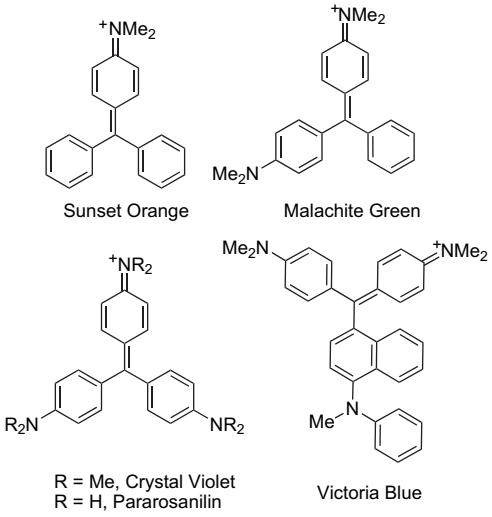


Figure 1.

TAM leucocyanides and dyes are useful in many photochemical reactions.[7] TAM derived from pyrene and terephthalaldehyde are used in polymer chemistry and they possess ferromagnetic properties. In addition, these molecules are potential synthons for the construction of complex three-dimensional network systems. Three known heterocyclic analogs of TAM are tri-2-thienylmethane, tri-2-furylmethane, and triindolylmethane (Fig. 2) and their chemistry has been investigated to some extent.

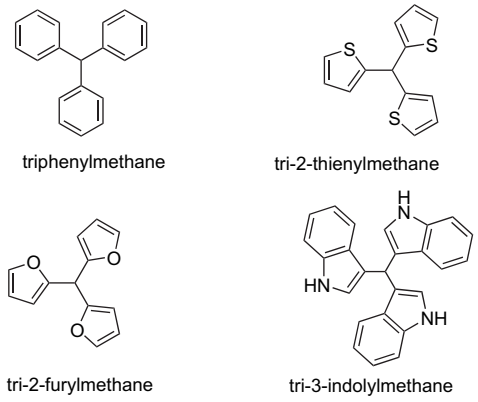


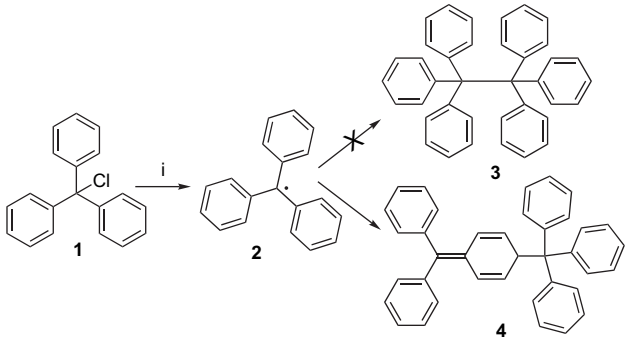
Figure 2.

The present report is aimed at highlighting the recent chemistry of triarylmethanes and at serving as a pointer to their diverse applications. The main aspects covered in the first part are the literature on triphenylmethyl radicals and cations, especially their synthesis and reactions. An account of TAM dyes and TAM leuco-derivatives is also provided in this part. Since the photochemical and photophysical aspects of TAM dyes were reviewed earlier,[8] such details have been excluded from this review. In the second part, the chemistry of symmetric triheteroarylmethanes like tri-2-thienylmethane, tri-2-furylmethane, and triindolylmethane is discussed.

2. Chemistry of triarylmethanes

2.1. TAM radicals

The experimental demonstration of the existence of the triphenylmethyl radical **2** by Gomberg is a milestone in the development of mechanistic organic chemistry.[9] The radical, prepared by the action of zinc powder on triphenylmethyl chloride **1** (Scheme 1), was shown to be stable for weeks in the dry crystalline state. The dimer of the triphenylmethyl radical was thought to be hexaphenylethane **3**, which was later disproved by the establishment of its correct quinoid structure **4** by Lankamp et al.[10–12] The trityl radical reacts readily with oxygen, yielding the corresponding peroxide and, with iodine, it forms the iodotriarylmethane. The peroxide on exposure to sulfuric acid is transformed into the triarylmethanol.[9]



Scheme 1. (i) Zn/Ag.

Later, Gomberg prepared a radical more stable than **2** by substituting –OR groups on the phenyl rings.[13]

2.2. TAM carbenium ions

Trityl halides were the first source of stable carbocations and, later, tritylcarbinols were found to be more useful for the generation of carbocations.[14] Breslow et al. have investigated carbenium ions of the type **5**, with more than one group available for interaction with the cationic center (Fig. 3).[15] Substituents X contain heteroatoms that can coordinate to the cationic center in a process called intramolecular solvation, while substituents Y can offer resonance stabilization to the carbocation. Carbenium ions required for the studies were prepared from the corresponding carbinols, which in turn, were obtained by the reaction of lithiobenzene derivatives with benzophenone, ethyl benzoate or diethyl carbonate.

NMR and ultraviolet spectroscopy, pK_R+ measurements, and quenching studies of a series of such TAM cations with potentially coordinating side chains in the *ortho*

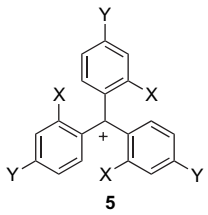
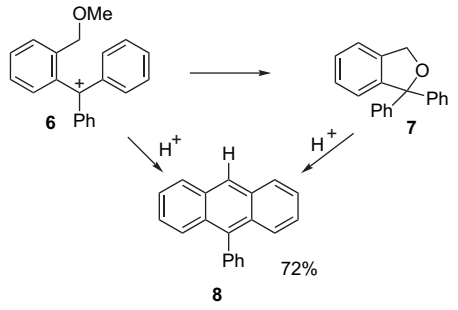


Figure 3.

position have shown two types of ions, viz., those in which side chains are completely uncoordinated with the cation and those in which cyclization occurred with the side chain. The decomposition reaction of a solution of (*o*-methoxymethyl)triphenylmethyl cation **6** results in the formation of α,α-diphenyldihydroisobenzofuran **7**. This, on further treatment with acid, gets converted into 9-phenylanthracene **8**, thus exemplifying side-chain cyclization (Scheme 2).



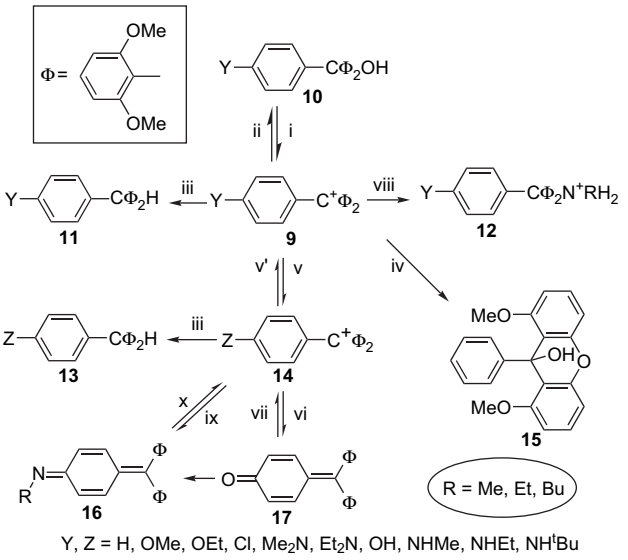
Scheme 2.

Triarylmethanols bearing *o*-methoxy groups on the phenyl ring were synthesized by Wada, and the reduction of the corresponding cation was investigated in various alcohols.[16] The high basicity of triarylmethane with additional methoxy groups at the *ortho* and *para* positions is explained by through-space interactions of a pair of 2p electrons of an *o*-methoxy oxygen with the empty 2p orbital of the resulting carbocation.

Wada et al. have also investigated the aromatic nucleophilic substitution of triarylcarbenium ions of the type **9** [(4-$YC_6H_4)C\Phi_2]^+$ where Φ=2,6-$(OMe)_2C_6H_3$; Y=OMe, NMe_2, Cl, OH.[17] Depending on the reaction conditions, the carbenium salts gave different products (**10–17**, Scheme 3). Arnett et al. have investigated the thermodynamic stability of such carbocations in sulfolane.[18]

2.3. TAM dyes

The photochemical and photophysical properties of triphenylmethane dyes in solid and liquid media were reviewed by Duxbury.[8] The photochemical reactivity of triphenylmethane dyes is both complex and interesting and has captured the attention of many scientists. These dyes have been considered as promising candidates in textile industry because of their brilliant colors and high tinctorial strength. They are found to be useful in laser spectroscopy as saturable mode absorbers for mode locking, because of their low fluorescence yield and fast non-radiative decay.



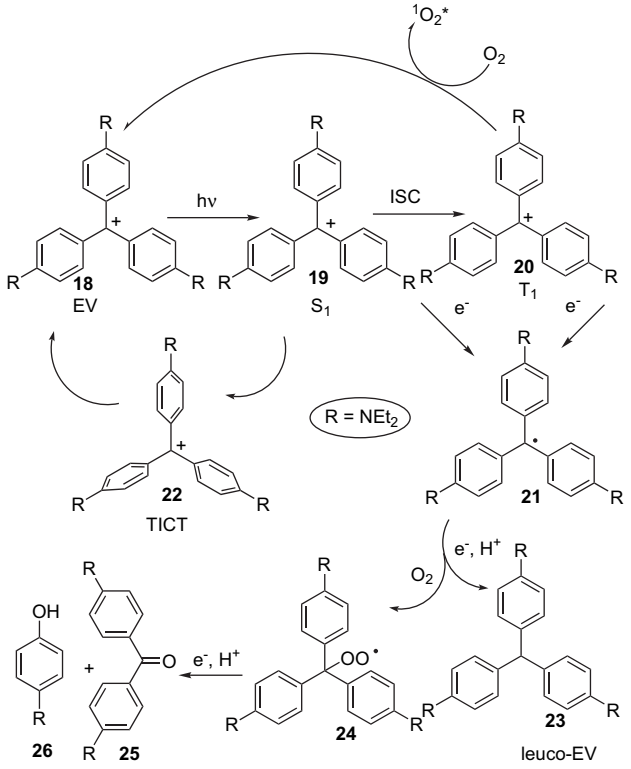
Scheme 3. (i) $+H^+$; (ii) $+OH^-$; (iii) $+RCH_2OH$, $-RCHO$; (iv) H_2O; (v) +HZ; (v′) +HY; (vi) $-H^+$; (vii) $+H^+$; (viii) $+NRH_2$; (ix) $-H^+$; (x) $+H^+$.

The TAM dyes exhibit interesting spectral features, consequent to their structure and their interaction with the physical and chemical environment (concentration of the dye, pH of the solution, pressure, temperature, variation in the counter anion etc.). It was found that such dyes show two types of absorption bands, viz. x- and y-band, where the x-band corresponds to the promotion of an electron from the nonbonding orbital to the lowest antibonding orbital, resulting in a high electron density on the central carbon atom, while the y-band arises from the excitation of an electron from the second highest occupied bonding orbital to the lowest vacant orbital.

The fluorescence lifetime of TAM dyes in a low-viscosity medium is typically in the picosecond range as a consequence of the fast relaxation process that occurs via rotational motions of the aryl rings.[19] In the context of the search for new sensitizers for photodynamic therapy, Baptista et al. have studied the effect of biological host bovine serum albumin (BSA) on Ethyl Violet (EV) fluorescence at 20 °C (pH∼7.3).[20] The BSA binding sites were very efficient in preventing the free rotor motion in the dye, as indicated by an increment in EV fluorescence as a function of BSA concentration. It is observed that, in addition to a singlet and a triplet state, this process involves a twisted intermediate charge-transfer state (TICT). The proposed mechanism involves a two-electron process that can account for the formation of the products **23** and **25** (Scheme 4).

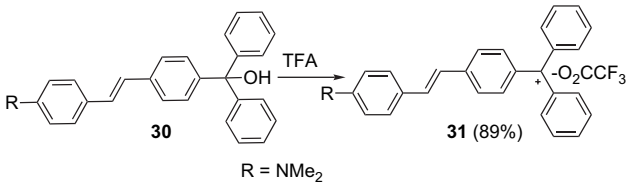
Sengupta et al. have used New Fuchsin (**27**), a cheap commercial dye for the synthesis of a tritolan dendron **29**, based on tetraphenylmethane scaffold (Scheme 5).[21] Energy transfer studies were carried out with the dendrimer **29**, in which the tolan part is the donor moiety and the anthracene core, the acceptor.

Screttas and co-workers synthesized a series of TAM dyes based on the stilbenyl group and, because these are nonlinear chromophores, such dyes exhibited second-order



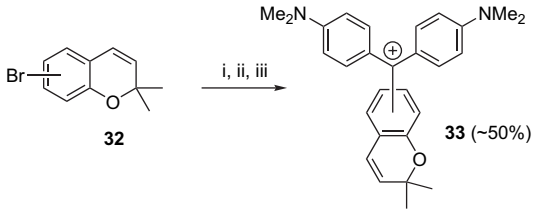
Scheme 4.

NLO properties.[22] Carbinols were obtained by the reaction of stilbenyllithium with ketones, ethyl benzoate or diethyl carbonate and the transformation into the corresponding cations was affected by dissolving the carbinols in TFA (Scheme 6).



Scheme 6.

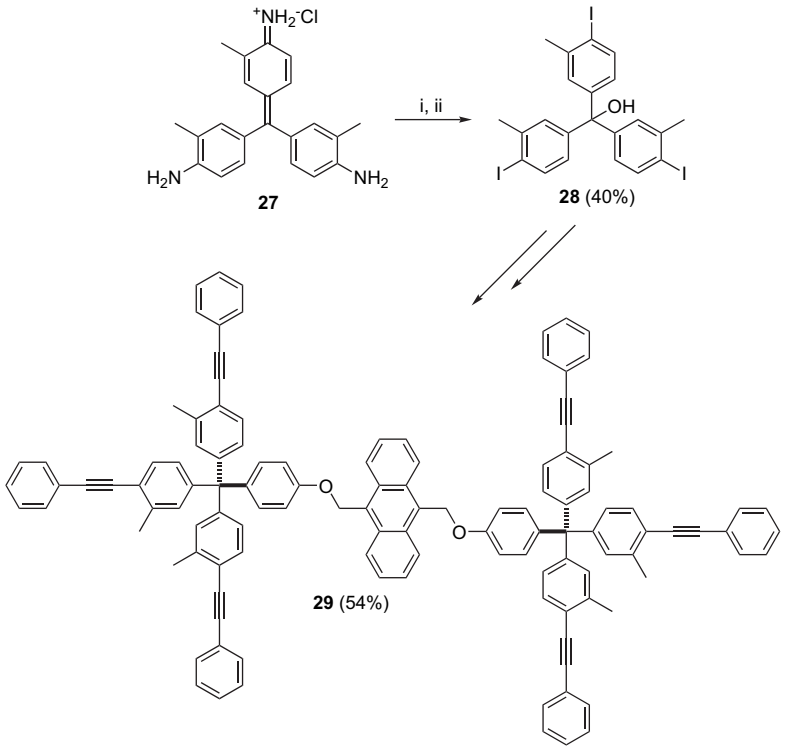
Clayton et al. have synthesized TAM dyes containing a 2*H*-1-benzopyran unit and the spectral parameters of these dyes were similar to that of the naphthyl derivative of Malachite Green.[23] Compound **33** was obtained by the reaction of lithiochromenes with 4,4′-bis(dimethylamino)benzophenone and subsequent treatment with acids (Scheme 7).



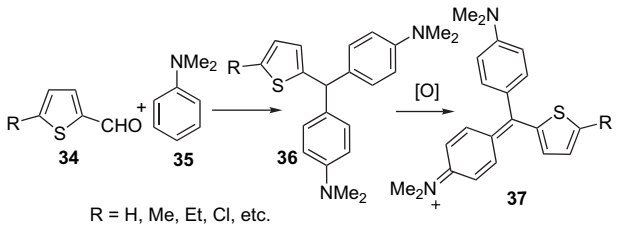
Scheme 7. (i) BuLi, TMEDA, Et_2O; (ii) $(4\text{-}NMe_2C_6H_4)_2CO$; (iii) H^+.

2.4. Heterocyclic dyes

The method for the synthesis of dyes incorporating heterocycles like thiophene involves the condensation of substituted thiophene aldehydes with dimethylaniline in the presence of anhydrous $ZnCl_2$, forming leuco-bases, which on oxidation afford the corresponding dyes (Scheme 8).[24,25]



Scheme 5. (i) Dil H_2SO_4, $NaNO_2$, 0 °C; (ii) KI, H_2O, rt.



Scheme 8.

The dyes could be isolated as crystals and the absorption spectra of such dyes also showed three principal bands, as in the case of the triphenylmethane dyes, viz. x-, y-, and x′-band. Substitution of the phenyl group of Malachite Green with thiophene shifts the y-band toward red, which may be attributed to the electron-releasing nature of the thiophene ring, compared to the phenyl ring. It was observed that, when the basicity of the nitrogen atoms increases, the absorption frequency is lowered. Dyes of the similar category containing thianaphthalene were also synthesized and investigated by Ghaisas and co-workers.[26] Thianaphthalene—Malachite Green also exhibited a similar bathochromic shift of the x-band.

2.5. Leucohydroxides and leucocyanides

By the use of suitable photocleavable compounds, the laser-induced pH jump technique can be used for the generation of a proton or hydroxide-ion pulse, which in turn, can be used for the study of chemical and biochemical processes. Triphenylmethane leuco-derivatives are well-known photochromic molecules, which dissociate into ion pairs under UV irradiation with the production of the intensely colored triphenylmethyl cation. Irie et al. have selected TAM leucohydroxide as the hydroxide-ion emitter in the laser-induced pH jump technique.[27,28] In order to overcome the insolubility in water, quaternary ammonium salts or sulfonate groups were introduced on the molecule (Fig. 4). On irradiation, the pH of the solution increased, because of the ejection of hydroxide ions from the excited TAM leucohydroxide.

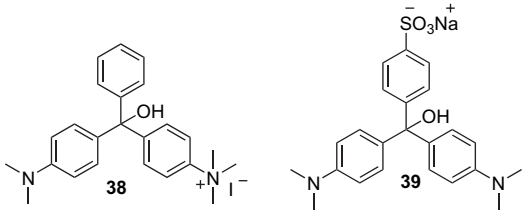
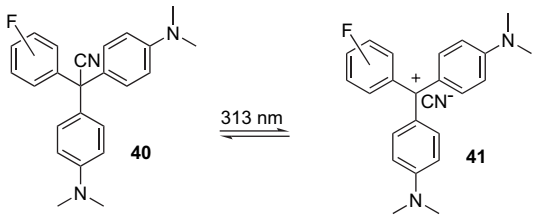


Figure 4.

Holmes showed that photoionization of the leucocyanides of Malachite Green, Crystal Violet, and Sunset Orange occurs only in a solvent with high dielectric constant.[29] Non-acid-sensitive color formers for photochemically cured, cationic polymers were developed by Malpert et al. by making use of the fact that the leuconitriles undergo heterolytic cleavage of the cyanide moiety upon irradiation with UV light, forming the colored cation (Scheme 9).[30] By suitably controlling the concentration or by changing the irradiation dose, leaving the substituents on the phenyl ring anionic, it is possible to tune the color formation.[31] The effect of ring fluorination on the absorption spectrum of Malachite Green was investigated by Hallas et al.[32]



Scheme 9.

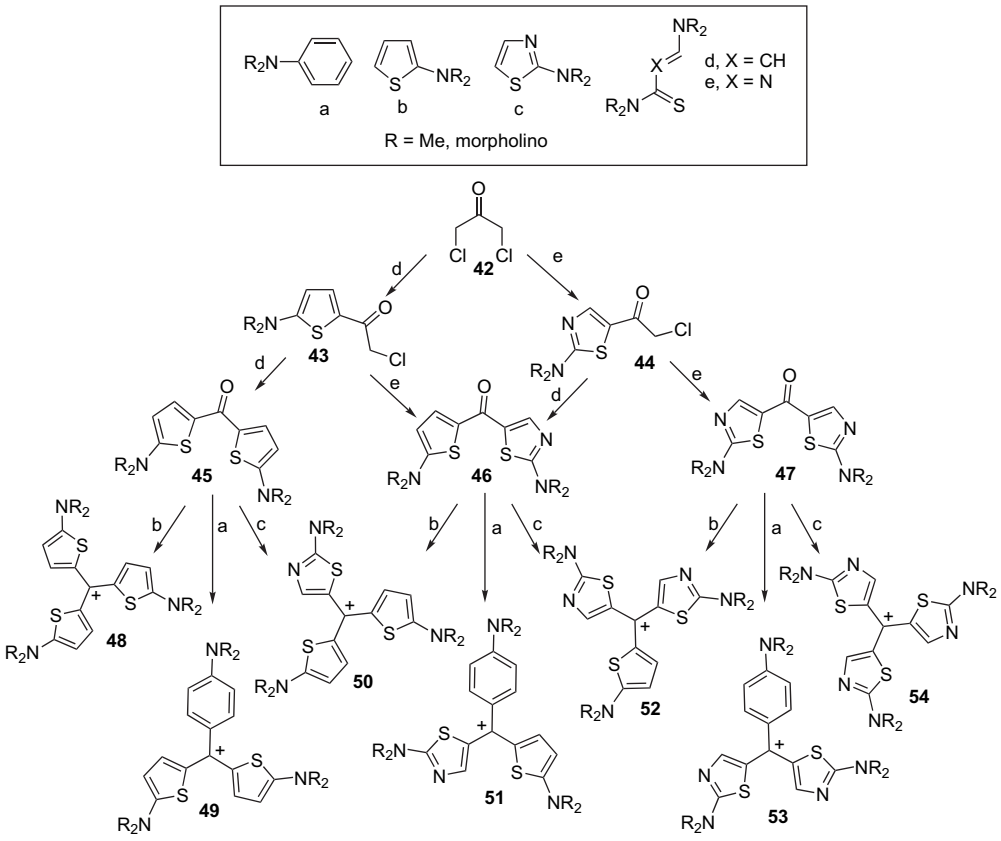
Recently, Noack et al. have shown that the heterocyclic analog of Michler's ketone could be efficiently employed for the synthesis of various heterocyclic Crystal Violet dyes (**48–54**, Scheme 10) and, according to theoretical studies, these molecules should display special NLO behavior.[33]

2.6. Triarylmethanol derivatives

Triarylmethanol derivatives were studied extensively because of their photocleavable nature, which gives an intense color change on irradiation by UV–vis light, both in polar solvents and in the solid state.[26,34,35] The clathrate-forming property of triarylmethanol derivatives with solvents like acetone, methanol, DMSO, dioxane, etc. is deemed to have application involving the concentration, separation, and purification of such solvents from industrial and environmental solutions and vapors.[36,37] Triarylsilanol derivatives also possess such inclusion properties.[38,39] Hayashi et al. have demonstrated that fluorine-substituted triarylmethanols exhibit properties different from those of the unsubstituted compounds because of the intermolecular C–F$\cdots\pi$ interaction, which controls the structural packing motif and the thermal stability of the crystal through crystal structures.[40]

Bacchi et al. have used the diol derivatives of general formula HOC–(Ar_2)–L–(Ar_2)–COH, where L is a suitable spacer, for the development of 'wheel-and-axle' systems (Fig. 5) utilizing the clathrating property of the triarylmethanols.[41] They showed that, by introducing a metal center into the spacer, the coordination of the metal at the peripheral region of the TAM molecules could control the electronic properties of the chromophore. They selected $Ag(LOH)_2(OTf)(MeCN)$, which was prepared by reacting LOH with excess Ag(OTf). It was shown that the metal is coordinated to two pyridine rings of the LOH group and two OH groups are connected through the SO_2 group via hydrogen bonding (OH$\cdots$O–S–O$\cdots$HO).

Gibson et al. have shown that alcohol-, halide-, phenol-, aniline-, and carboxylic acid-functionalized TAM can serve as blocking end units in rotaxanes and polyrotaxanes.[42] Two different approaches were employed for the introduction of the spacer into various TAM derivatives. In the first approach via a carbanion, the carbinols were reduced to TAM quantitatively and the anions generated with *n*-butyllithium were alkylated with chloro-alcohols, affording the ω,ω,ω-triarylalcohols. In the second approach, the triarylmethyl cation derived from the triarylcarbinol or the chloride was subjected to electrophilic substitution reactions with phenols and aniline (Scheme 11).



Scheme 10.

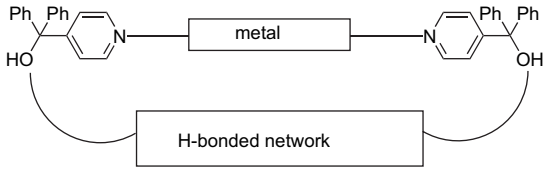
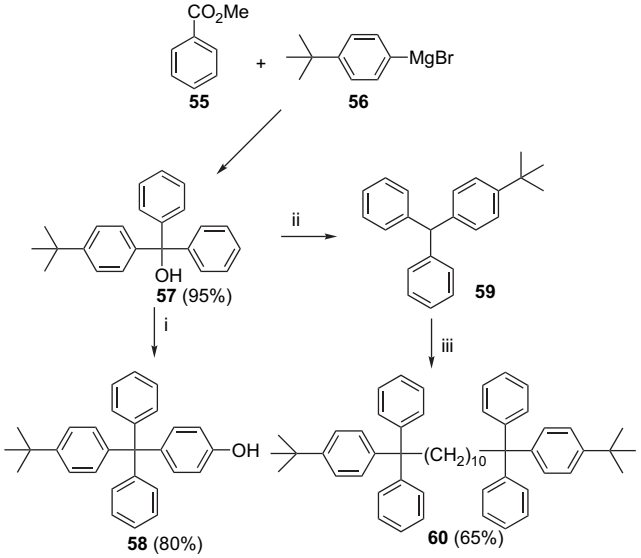


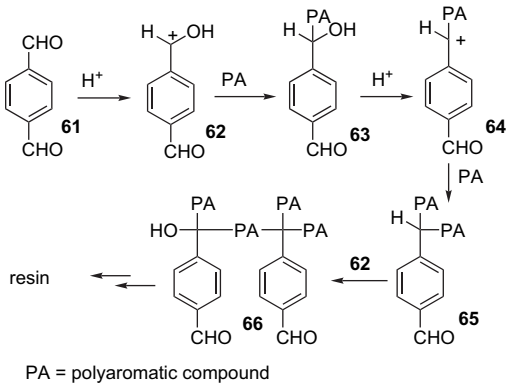
Figure 5.



Scheme 11. (i) H^+, phenol; (ii) HCO_2H; (iii) *n*-BuLi; $Br(CH_2)_{10}Br$.

2.7. TAM in polymer chemistry

Ota's group has developed a thermosetting condensed polynuclear aromatic (COPNA) resin composed of a TAM structure by heating a mixture of a polycyclic aromatic compound and an aromatic aldehyde in the presence of an acid catalyst, as shown in Scheme 12.[43]



Scheme 12.

When the synthesis was carried out in a magnetic field, the resulting resin exhibited ferromagnetic properties with excellent reproducibility.[44] For instance, compound **66′** in Figure 6, which has ferromagnetic properties, was synthesized by heating a mixture of terephthalaldehyde and pyrene with 5 wt % of PTSA at 130–140 °C under a magnetic field of 440 G for 1 h.

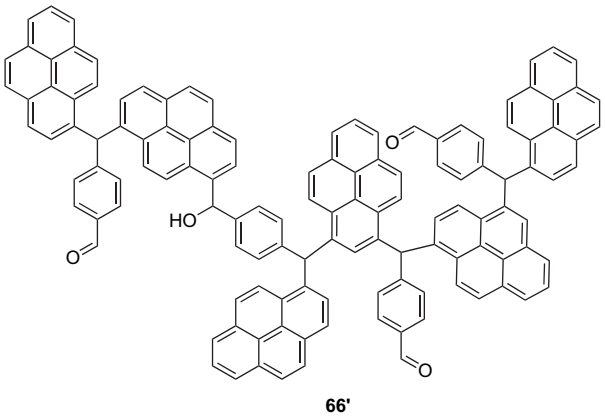


Figure 6.

Polymeric Malachite Green derivatives show complexing ability toward K^+, Cs^+, Li^+, and Na^+ ions and it has been shown that the complexation can be controlled photochemically.[45] Bis-crowned Malachite Green copolymers with styrene and methyl methacrylate as co-monomers and *N*,*N*-dimethylacrylamide and 4-hydroxymethylstyrene as hydrophobic co-monomers were synthesized and studied with respect to their metal-ion-complexing ability. The properties of the polymer having a bis-crowned Malachite Green moiety exhibited a substantial difference from the corresponding monomeric analog. The decoloration reaction of the photocationic form was found to be governed by the rheology of the polymer in solution.[46]

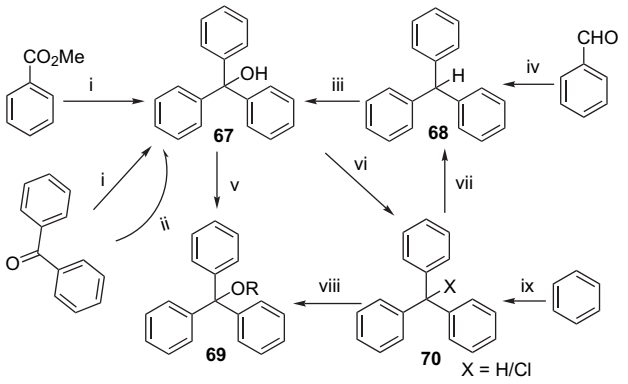
2.8. Miscellaneous

In addition to the applications mentioned above, the trityl group serves as an excellent protective group used in nucleoside, oligonucleoside, peptide, and carbohydrate chemistry.[47] Trityl groups are utilized in multistep syntheses on solid phase, mostly as linkers, and they have found application in medicinal chemistry. Prodrugs with trityl-protected nucleosides were developed and their site-selective delivery was tested in vivo against tumor cells.[48] Because of their easy-ionizing properties, trityl cations can additionally act as a tool for mass-spectrometric analysis.[49]

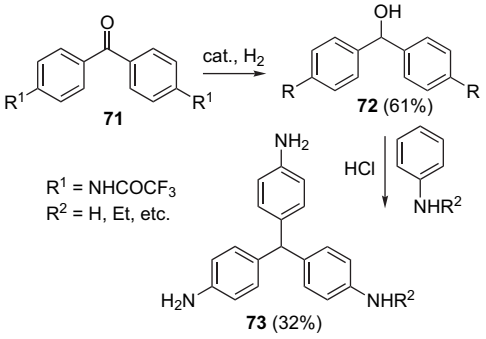
2.9. Synthesis

Two basic approaches to trityl synthesis are (i) the Grignard reaction of various carbonyl compounds like benzophenone, methyl benzoate, etc. and (ii) the aromatic electrophilic substitution reaction in acidic media.[3,50] The alcohol can either be converted into the halogenated compound or into the alkyl ether. Symmetric TAMs were prepared by the treatment of electrophilic reagents such as triethyl orthoformate or chloroform with arene nucleophiles (Scheme 13).[51]

Panda and co-workers have recently reported an efficient synthesis of unsymmetrical triarylmethanes by Friedel–Crafts reaction of aromatic nucleophiles with heteroaryl carbinols.[52] Werbel et al. have reported a facile method for the synthesis of triarylmethane **73** by the catalytic hydrogenation of the diaryl ketone **71** and the subsequent treatment of the carbinol with aryl amines and HCl (Scheme 14).[53]

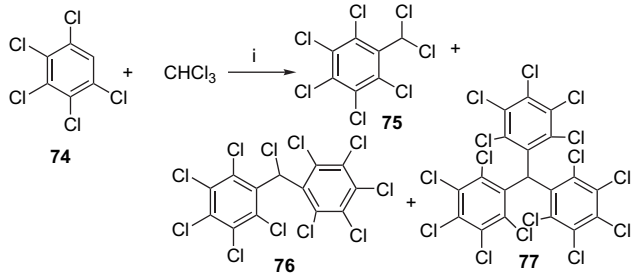


Scheme 13. (i) PhMgBr, (ii) BuLi, bromobenzene; (iii) $PbO/MeCO_2H$, DDO; (iv) $MeCO_2H$, H_2SO_4, benzene; (v) ROH/H^+; (vi) RCOX; (vii) R_2NLi; (viii) ROH/base; (ix) $CCl_3X/AlCl_3$(R=alkyl).



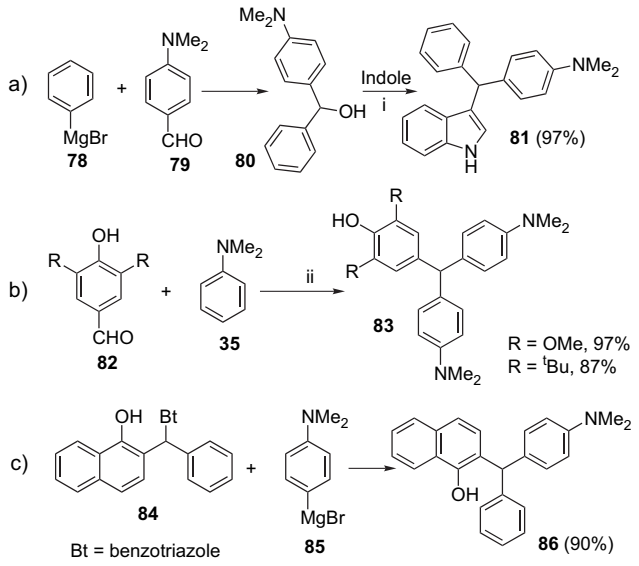
Scheme 14.

A simple, direct, and high-yielding Friedel–Crafts synthesis of highly chlorinated aryl-, diaryl-, and triarylmethanes was reported by Ballaster et al.[54] This involves the condensation reaction between chloroform and the chlorinated benzene, affected using $AlCl_3$ at a temperature ranging from 70 to 150 °C (Scheme 15). It was observed that, according to the amount of halogenated benzene, yields of the products varied. Compounds like **76** and **77** are valuable chemical precursors of inert free radicals.



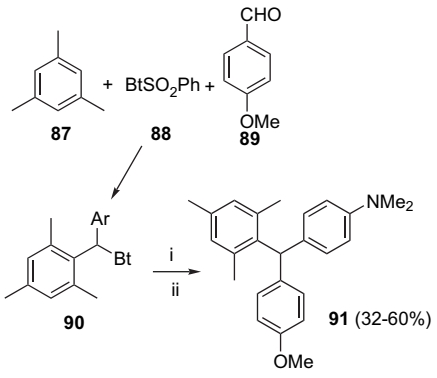
Scheme 15. (i) $AlCl_3$, 70–150 °C.

Katritzky et al. have developed three synthetic routes to access asymmetric TAM: (a) reaction of a benzhydrol with an electron-rich aromatic compound, (b) direct condensation of an aromatic aldehyde with electron-rich arenes, and (c) displacement of benzotriazolyl derivatives by Grignard reagents (Scheme 16).[55]



Scheme 16. (i) Concd HCl, H_2O/MeOH; (ii) concd H_2SO_4, urea, 90 °C.

Recently, a combination of 1-benzenesulfonyl benzotriazole and an aromatic aldehyde has been used in a new protocol for the synthesis of asymmetric TAM (Scheme 17).[56]

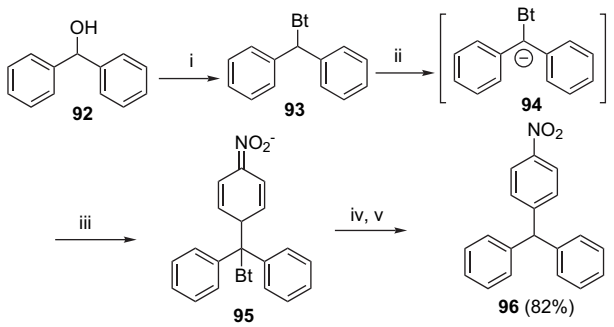


Scheme 17. (i) *N*,*N*-Dimethylaniline; (ii) $ZnCl_2$, CH_2Cl_2.

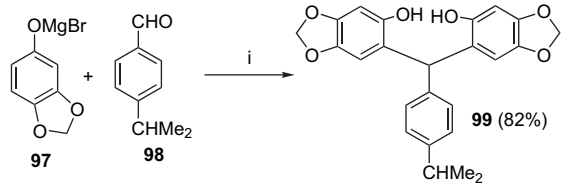
Katritzky's group has reported the synthesis of (*p*-nitroaryl)-diarylmethane, a compound, which has attracted attention because of the easy reduction of the nitro group to an amino group and its subsequent transformations. In this new method, diarylmethanols were treated with benzotriazole under acid catalysis in the presence of perfluorocarbon fluids and, subsequently, with nitrobenzene.[57] Diarylmethyl-benzotriazoles are highly nucleophilic in the presence of strong bases and hence react with electron-poor arenes efficiently (Scheme 18).

The selective condensation of oxophilic metal phenolates with an aromatic aldehyde at the *ortho* position of the starting phenol has been utilized in the synthesis of a 2,2′-dihydroxytriphenylmethane, another TAM derivative (Scheme 19).[58]

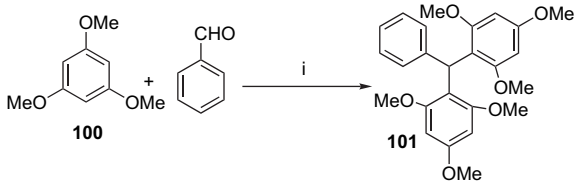
Recently, we have shown that electron-rich arenes can be efficiently condensed with aldehydes under the catalytic influence of $AuCl_3$/AgOTf (Scheme 20).[59]



Scheme 18. (i) BtH; (ii) *t*-BuOK; (iii) nitrobenzene; (iv) *t*-BuOK; (v) H^+.

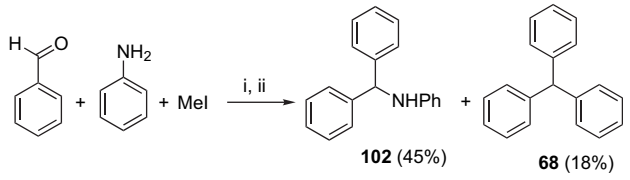


Scheme 19. (i) CH_2Cl_2, reflux.



Scheme 20. (i) 1 mol % [$AuCl_3$/3AgOTf], MeCN, argon, 50 °C, 85%.

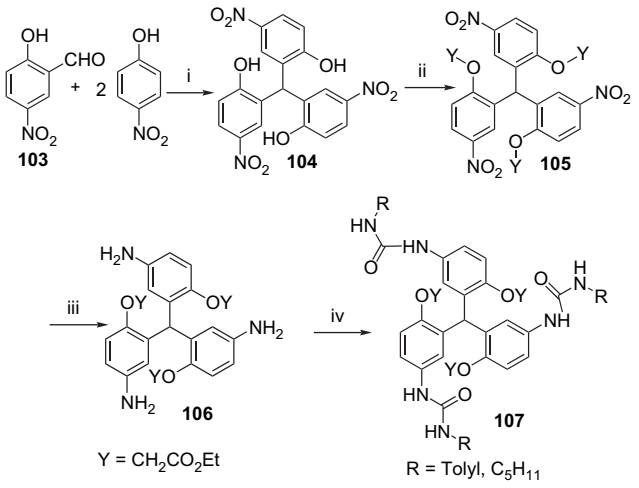
Triarylmethanes are formed, albeit in low yields, in the alkyl radical addition to a mixture of ketone and aniline (Scheme 21).[60]



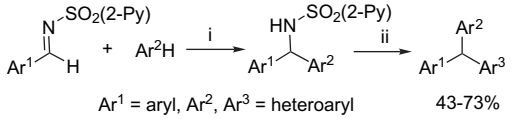
Scheme 21. (i) PhN_2^+, Ti(III); (ii) H^+, H_2O.

Tri-(2-alkoxy-5-ureido-phenyl)methanes were prepared by the acid-catalyzed condensation of 2-hydroxy-5-nitro-benzaldehyde with a two-fold excess of *p*-nitrophenol. The product was *O*-alkylated and subsequent reduction of the nitro group by catalytic hydrogenation and amidation led to the compound **107** (Scheme 22). A single-crystal X-ray analysis of the compound **106** showed that it had a three-bladed propeller conformation in which both the *O*-alkyl group and the amide functionality were found in a *syn* arrangement relative to each other.[61]

Esquivias et al. have recently reported an efficient synthesis of di- and triarylmethanes involving an aza-Friedel–Crafts reaction of *N*-(2-pyridyl)sulfonyl aldimines catalyzed by a $Cu(OTf)_2$/binap system (Scheme 23).[62]



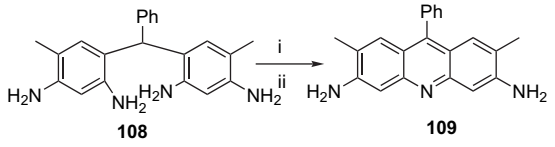
Scheme 22. (i) H_2SO_4, 155 °C; (ii) YBr, K_2CO_3, acetone; (iii) H_2, Raney-Ni, THF/EtOH; (iv) RNCO, CH_2Cl_2.



Scheme 23. (i) $Cu(OTf)_2$ (10 mol %), (±) binap (10 mol %), CH_2Cl_2, reflux; (ii) Ar^3H, 40 °C, 20–120 min.

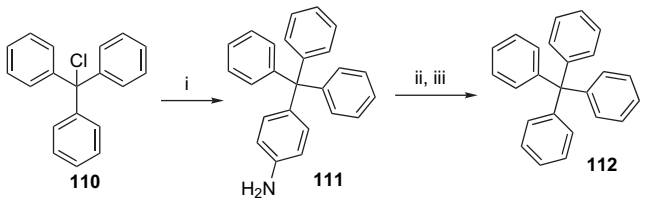
2.10. Reactions of TAM derivatives

Snyder and Konecky have demonstrated that tetraaminoaryl compounds like **108** undergo cyclization and subsequent aromatization to diaminoacridine derivative **109** on treatment with polyphosphoric acid at 165 °C (Scheme 24).[63]



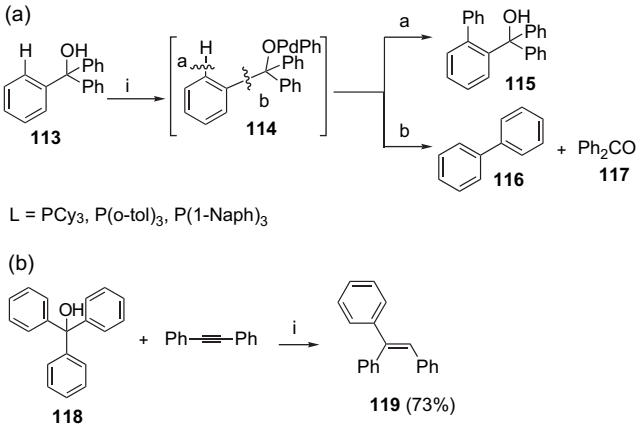
Scheme 24. (i) PPA, 165 °C; (ii) NaOH.

Zimmermann et al. have synthesized tetraphenylmethane by the condensation of triphenylmethyl chloride and aniline, followed by reductive elimination via diazotization and subsequent reduction of the azo compound (Scheme 25).[64]



Scheme 25. (i) Aniline, 220 °C, neat; (ii) H_2SO_4, $C_5H_{11}ONO$, DMF; (iii) aq H_3PO_2.

Terao et al. have shown that a number of α,α-disubstituted arylmethanols react with aryl halides in the presence of palladium acetate to give biaryls. The reaction proceeds through a Pd(II) alcoholate intermediate **114**, which on β-carbon elimination, furnishes the product (path b in Scheme 26). A competitive reaction is the *o*-C–H bond cleavage of the triarylmethanols (path a in Scheme 26). It has been revealed that hydroarylation of some unsaturated compounds like alkynes or α,β-unsaturated compounds occurred when $P(1\text{-Naph})_3$ is employed as the ligand in this reaction.[65] This reaction may also be utilized as a method for the deprotection of diaryl ketones.



Scheme 26. (a) (i) $Pd(OAc)_2$, L, PhBr, Cs_2CO_3; (b) (i) $Pd(OAc)_2$, $P(1\text{-Naph})_3$, PhBr, Cs_2CO_3.

2.11. Stereochemistry

Finocchiaro et al. have separated two diastereomers of the TAM of the type Ar_3Z (e.g., compound **120**, Fig. 7) with three different aryl groups lacking C_2 axes. The compound was prepared by the condensation reaction of 1-(2-methoxynaphthyl)-1-(2-methylnaphthyl)methanol and 2,4,6-trimethoxytoluene in nitromethane with H_2SO_4 as the catalyst. The interconversion between the two forms is due to a one-ring-flip mechanism.[66] Variable temperature NMR studies also confirmed the presence of the diastereomers.

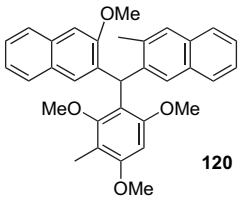


Figure 7.

3. Symmetric triheteroarylmethanes

3.1. Tri-2-thienylmethane

In the context of the wide-ranging applications of triphenylmethyl derivatives,[47] triheteroarylmethanes like tri-2-thienylmethane **121** (Fig. 8), have attracted the attention of several chemists because of their potential applications. The thiophene derivative is expected to have wider applications in view of the ease of functionalization of thiophene at the C-2 position by either an electrophilic substitution reaction or lithiation followed by reaction with electrophiles. The potential use of thiophene as a synthon equivalent of *n*-butane via Raney-nickel reduction is also noteworthy.

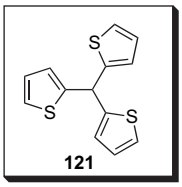


Figure 8.

Noack et al. have synthesized several heterocyclic analogs of the trityl cation containing thiophene and thiazole rings.[67] For application in non-linear optics and conducting polymers, a series of thienylic and oligothienylic trityl analogs was prepared.[68]

3.1.1. Trithienylmethyl radicals. Although the triphenylmethyl radical had been known since 1900, its heterocyclic analogs were brought to light only in 1968, by the detection of tri-2-thienyl- and tri-3-thienylmethyl radicals **122** and **123**, respectively (Fig. 9).[69]

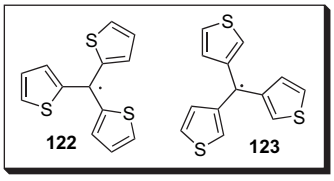


Figure 9.

Bernardi et al. have carried out an INDO study on the tri-2-thienylmethyl radical, which proved that the radical exists as a pair of diastereomeric conformers, a cis form with C_3 symmetry and a trans form with C_1 symmetry, as shown in Figure 10.[70]

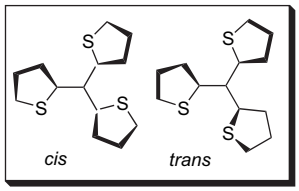
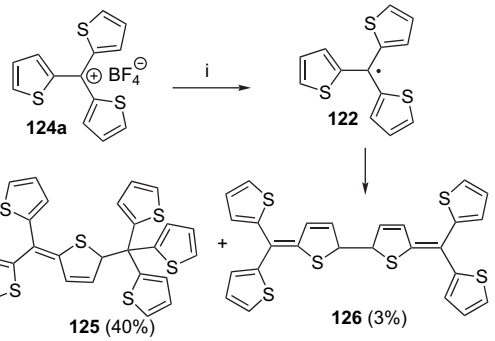


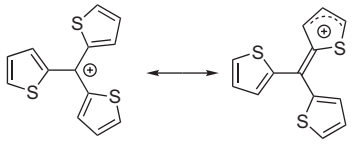
Figure 10.

No structural proposal for the dimer of the tri-2-thienylmethyl radical was attempted until Nakayama et al. carried out some investigations on the dimerization of the radical in 1990. They reported the formation, structure elucidation, and interconversion of the two isomeric dimers of **124a** (Scheme 27).[71] Based on extensive studies, the authors have concluded that the dimerization of the radical between the radical center and the sterically less-hindered 5-position of the 2-thienyl ring initially afforded the kinetically favored product **125**, which then thermally equilibrated to another dimer **126**.

3.1.2. Tri-2-thienylmethyl cation and tri-2-thienylmethyl anion. Taddei et al. in 1970 reported the proton NMR spectral parameters for the isomeric trithienylcarbenium ions,[72] while their ^{13}C NMR characteristics were investigated in some detail by Abarca et al. (Scheme 28).[73]

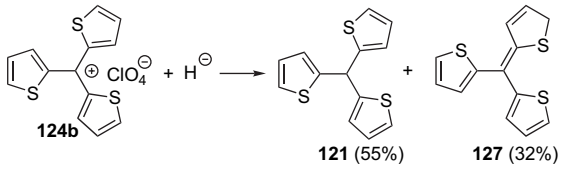


Scheme 27. (i) Zn, DME, 65 °C.



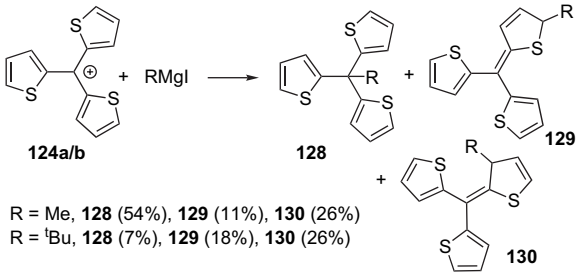
Scheme 28.

The reactivity profile of the carbenium ion toward hydride and carbon nucleophiles was studied by Ishii et al. and it was observed that addition occurs not only at the carbenium ion center, but also on the thiophene ring.[74] Thus, the addition of LAH to an ethereal solution of tri-2-thienylcarbenium perchlorate **124b** at room temperature results in rapid hydride addition, both at the carbenium ion center and at the 5-position of the thiophene ring, affording the compounds **121** and **127** (Scheme 29).



Scheme 29.

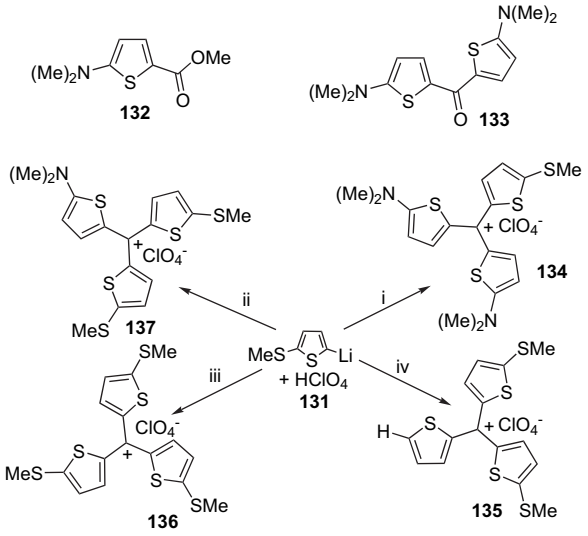
In order to understand the reaction of carbon nucleophiles toward **124**, a variety of organometallic reagents have been employed. This reaction, giving rise to compounds **128**, **129**, and **130**, is illustrated in Scheme 30. As the alkyl group becomes bulky, addition at the central carbon becomes less prominent.



Scheme 30.

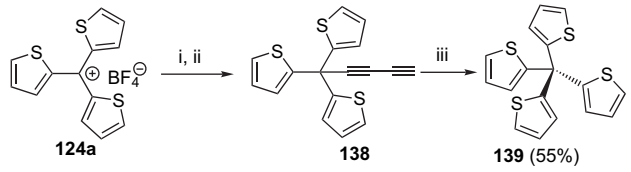
Deeply colored 5-methylmercapto- and 5-methylamino-substituted tri-2-thienylmethinium perchlorates **134–137**

were prepared by the reaction of 2-methylmercapto-5-lithiothiophene **131** with methyl 2-dimethylamino-5-thiophenecarboxylate **132**, bis(2-dimethylaminothiophen-5-yl)ketone **133** or diethyl carbonate and perchloric acid (Scheme 31).[75]



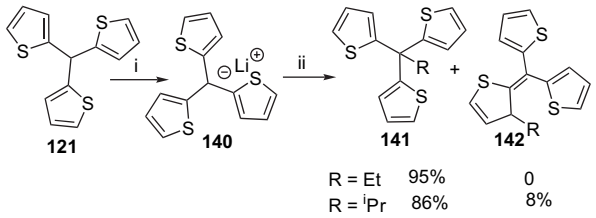
Scheme 31. (i) **133**; (ii) **132**; (iii) $(EtO)_2CO$; (iv) $C_4H_3SCO_2Me$.

The synthetically and structurally important molecule, tetrakis(2-thienyl)methane **139**, was synthesized for the first time by Matsumoto et al. using sodium sulfide-induced cyclization of 5,5,5-tri-2-thienylpentadiyne **138**, which in turn, was obtained from tri-2-thienylmethyl cation **124a** (Scheme 32).[76]



Scheme 32. (i) Li—≡—≡—TMS; (ii) K_2CO_3, MeOH; (iii) Na_2S–NaOH, DMSO, 120 °C.

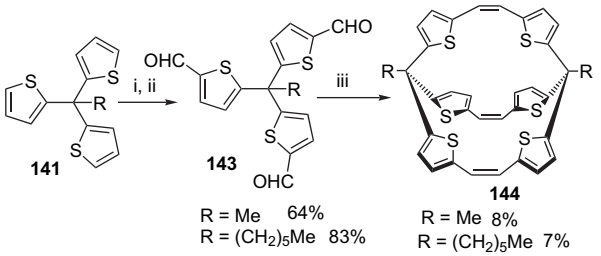
Nakayama et al. explored the reactivity of the corresponding anion, the tri-2-thienylmethyl anion **140**, toward carbon electrophiles.[77] They found that the optimum conditions for addition of electrophiles to the methine carbon involve the use of butyllithium (1.3 equiv) in the presence of TMEDA (1.5 equiv) in THF at −78 °C and subsequent addition of excess alkyl halide (5 equiv) to the red suspension of the anion formed (Scheme 33). Although the reaction of the



Scheme 33. (i) BuLi, TMEDA, THF, −78 °C; (ii) RBr.

tri-2-thienylmethyl anion with primary alkyl halides occurs exclusively at the carbanion center, its reaction with bulkier secondary and tertiary alkyl halides occurs both at the carbanion center and at the less-hindered thiophene ring.

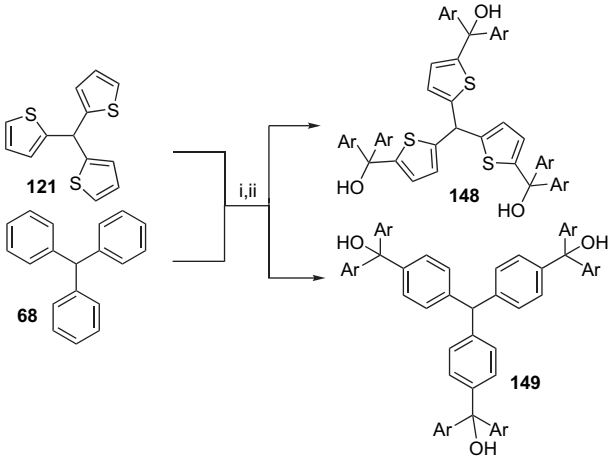
The synthesis of cage molecules (e.g., compound **144**, Scheme 34) bicapped with tri-2-thienylmethane was achieved by Kurata et al. in 2001.[78] The target molecule was obtained via a McMurry coupling reaction of tri-5-formyl-2-thienylmethanes. All the sulfur atoms of the six thienyl groups are directed inwards and, hence, the molecule has a three-dimensional cavity, which can potentially encapsulate metal ions.



Scheme 34. (i) BuLi-THF, 0 °C; (ii) excess DMF; (iii) $TiCl_4$, Zn, CuI-DME, rt.

Compound **144** on further reaction led to the formation of the cage-molecular monocation **145**, dication **146**, and dianion **147** of substantial stability, the first examples of fully conjugated ionic cyclophane cage molecules (Fig. 11).[79]

Oda et al. have synthesized hexaaryl-substituted trimethylenemethane dications and dianions extended with thiophene or benzene, which in turn, were prepared from the corresponding alcohols (Scheme 35).[80] These molecules have tetrapolar properties and long-wavelength absorption in the near-IR region. Thiophene-extended dications and dianions are stable, compared to benzene-extended dications, the latter being sensitive to acids.



Scheme 35. (i) LDA, Ar_2CO; (ii) H_2SO_4.

The dications were prepared by dissolving the alcohols **148** or **149** in CF_3CO_2H and a detailed NMR study revealed that the dication has tetrapolar properties. The corresponding

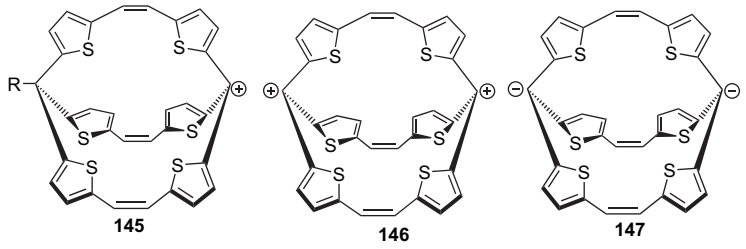
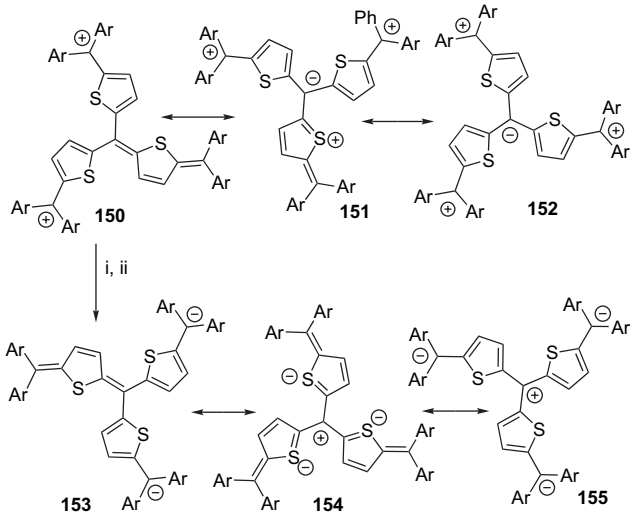


Figure 11.

anion was obtained from the dication by LAH reduction, followed by deprotonation using NaH (Scheme 36).



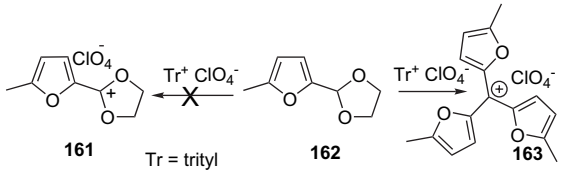
Scheme 36. (i) LAH; (ii) NaH, DMSO.

3.1.3. Electrophilic substitution reactions. Investigations by our group have shown that tri-2-thienylmethane underwent electrophilic substitution reactions such as Friedel–Crafts reaction, formylation reaction, bromination, and reactions with electrophilic carbenes (Scheme 37).[81]

3.2. Tri-2-furylmethane

Conventional methods for the synthesis of symmetric tri-2-furylmethanes consist of the condensation of 2-furaldehyde with the corresponding furan substrates or the reaction between furan compounds and chloroform.[82]

During the synthesis of the dioxolanium salt **161** from 2-[5-methylfur-2-yl]-1,3-dioxolane **162**, accidentally, Stroganova et al. have observed the formation of trifurylcarbenium perchlorate **163** in trace amounts (Scheme 38).[83]

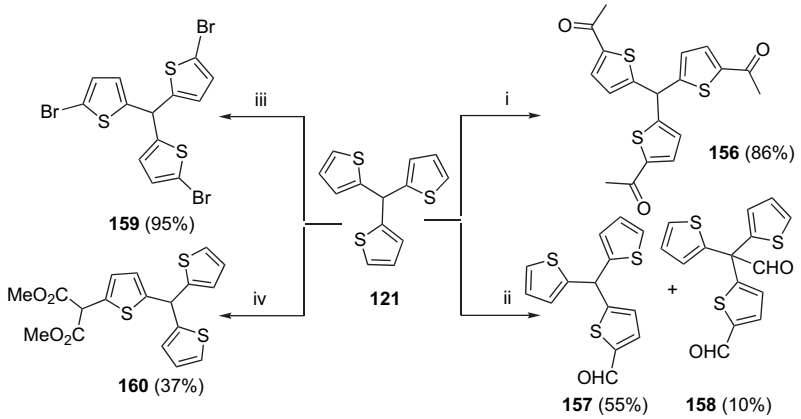


Scheme 38.

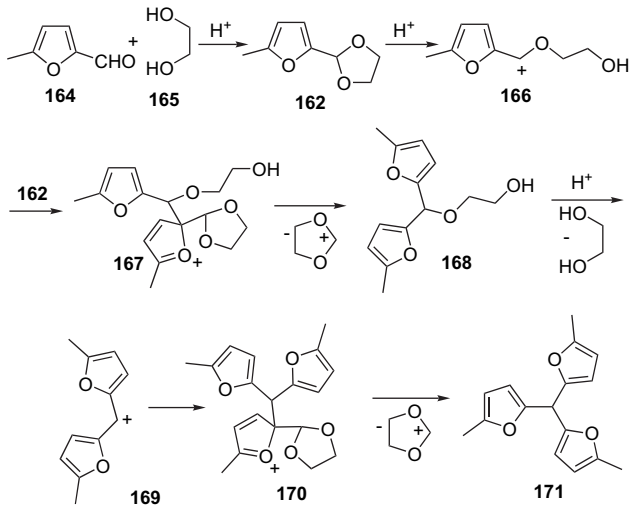
The probable mechanism for the formation of the product is shown in Scheme 39. In another attempt, Riad et al. have synthesized tri-2-furylmethane by the condensation of furfural with furan using macroporous ion-exchange resins as catalysts.[84]

The influence of various acid catalysts on the selectivity of the reaction of furfural and ethylene glycol has been studied and it was concluded from the experimental observations that weakly acidic catalysts like ion-exchange resins favor the formation of the dioxolane **162**, while strong catalysts favor the formation of the trifurylmethane **171** (Scheme 40).

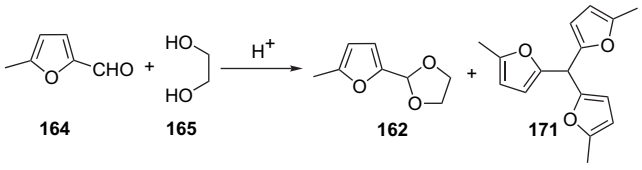
A similar strategy was employed for the synthesis of tri-(5-aryl-2-furyl)methanes **173**, which is important because of the fact that 2-arylfurans are not easily accessible by traditional methods (Scheme 41).



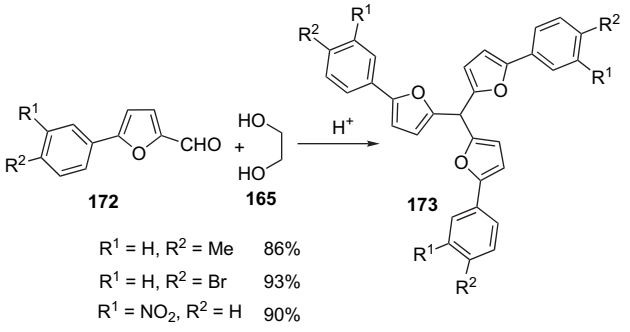
Scheme 37. (i) MeCOCl, $AlCl_3$; (ii) DMF, $POCl_3$; (iii) NBS; (iv) $(CO_2Me)_2CN_2$, Rh(II).



Scheme 39.

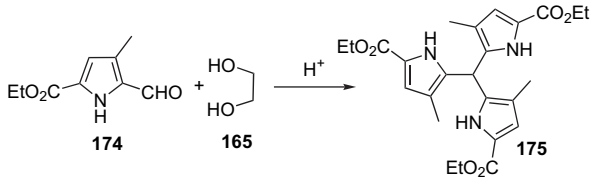


Scheme 40.



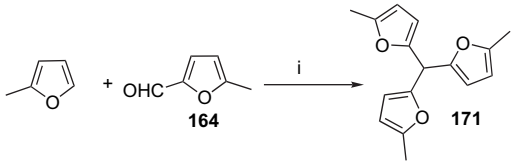
Scheme 41.

A similar conversion of a pyrrolic aldehyde is also known. When the pyrrolic aldehyde **174** was treated with ethylene glycol in the presence of catalytic amount of *p*-TsOH, the product obtained was tripyrrolylmethane **175**, instead of the expected acetal (Scheme 42).[85]



Scheme 42.

A gold(III) chloride-promoted condensation can be effectively utilized for the synthesis of a range of symmetrical and unsymmetrical triheteroarylmethanes.[59] Thus, tri(5-methylfur-2-yl)methane **171** was obtained in 90% yield by the condensation of 2-methylfuran with the corresponding aldehyde in presence of $AuCl_3$ (Scheme 43).



Scheme 43. (i) $AuCl_3$ (1 mol %), MeCN, argon, rt, 12 h, 93%.

Other heterocycles like indole, 2-methylthiophene, etc. also reacted efficiently under the above reaction conditions. A high-yield synthesis of complex molecules like **176** in one pot has been accomplished by the participation of poly-aldehydic substrates in this reaction (Fig. 12).

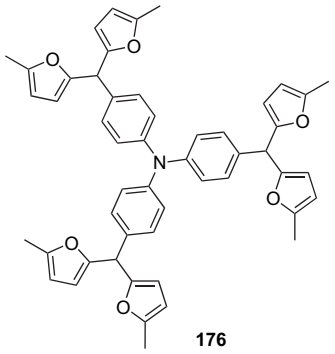
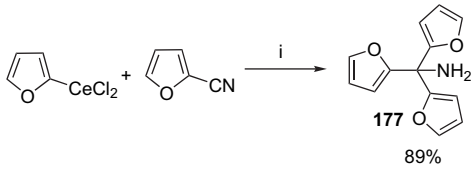


Figure 12.

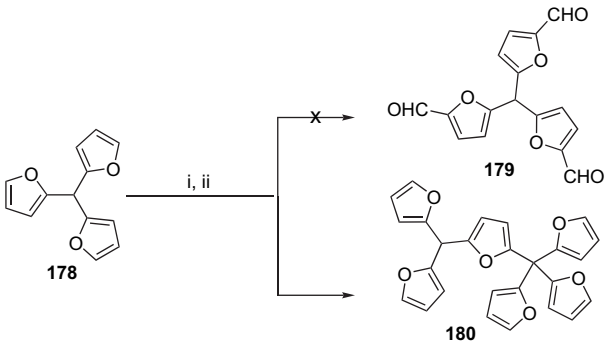
Ciganek has reported the preparation of tri-2-furylmethylamine **177**, via the cerium-mediated addition to 2-cyanofuran (Scheme 44).[86]



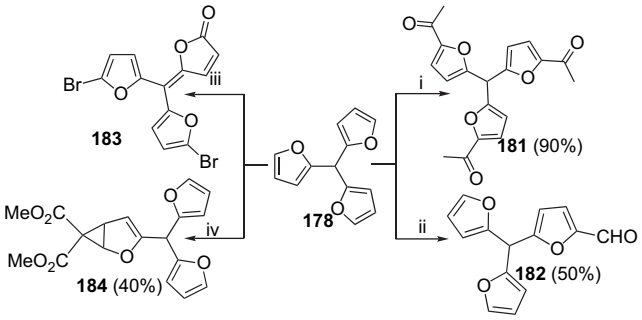
Scheme 44. (i) THF, −65 °C—rt.

3.2.1. Reactions of tri-2-furylmethane. In an experiment designed to synthesize a trisaldehyde of tri-2-furylmethane, serendipitously we observed the formation of 1,1-bisfuryl-1-[5-(tri-2-furylmethyl)]furylmethane **180**, a dimer of the tri-2-furylmethane radical (Scheme 45).[87]

The reactivity of tri-2-furylmethane **178** toward a number of electrophilic reagents was investigated in detail. A Friedel–Crafts reaction on **178** afforded the tris-acetylated compound **181**, while formylation gave only monoaddition. Bromination with NBS under different conditions afforded the butenolide **183** with two bromofuran substituents. Reaction of **178** with electrophilic carbenes yielded cyclopropanated and α-substituted products. Similarly, attempts toward the synthesis of tri-2-furylmethyl bromide resulted in the formation of the furanone derivative **183** (Scheme 46).[81]



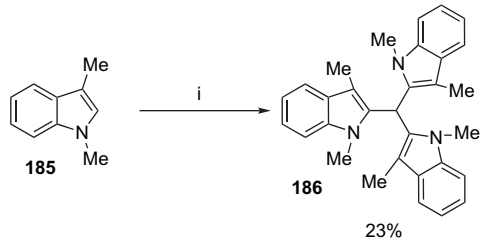
Scheme 45. (i) BuLi, TMEDA, THF, −78 °C; (ii) DMF.



Scheme 46. (i) MeCOCl, $AlCl_3$; (ii) DMF, $POCl_3$; (iii) NBS; (iv) $(CO_2Me)_2CN_2$, Rh(II).

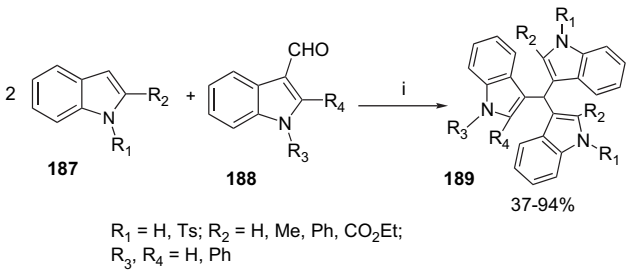
3.3. Triindolylmethane

Triindolylmethanes were usually prepared by the reaction of indoles with the corresponding aldehyde or with triethyl orthoformate.[88–90] Avendano et al. have synthesized tris-[(1,3-dimethyl)-2-indolyl]methanol by the reaction of the 2-lithio derivative of 1,3-dimethylindole with diethyl carbonate (Scheme 47).[91]



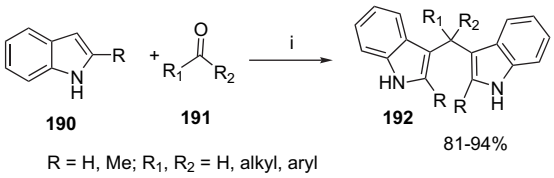
Scheme 47. (i) BuLi, $(EtO)_2CO$.

Chakrabarty et al. have developed a clay-mediated eco-friendly route toward symmetrical and unsymmetrical triindolylmethanes.[92,93] Recently, Jun et al. have shown that solid acids would also catalyze the condensation reaction efficiently.[94] A high-yield synthesis of triindolylmethanes as well as their *N*-acetyl derivatives was reported by Li et al. The reaction involved treatment of indoles with indole aldehydes in presence of acetic acid and acetic anhydride (Scheme 48).[95] The cytotoxic properties of these compounds were also reported by the same authors.



Scheme 48. (i) $MeCO_2H$, $(MeCO)_2O$.

Cerium(IV) ammonium nitrate has been shown to be an efficient catalyst for the synthesis of di- and triindolylmethanes in high yields (Scheme 49).[96]



Scheme 49. (i) CAN, MeCN, rt.

Triindolylmethane with trityl perchlorate or $FeCl_3$ oxidation gave the corresponding triindolyl cation, which was reversibly converted into the carbinol by treatment with alkali.[97,98] Because of the steric bulk and heterocyclic nature, 2,2′,2″-triindolylmethane was used as a good ligand for monometallic complexation, which in turn, would increase the Lewis acidity at the metal centers.[99]

4. Conclusions

Triaryl- and triheteroarylmethanes have found wide-ranging applications in different areas of chemistry. The classical dyestuff chemistry relies mainly on the triarylmethyl core, whereas the trityl group represents an important protective group for a range of functionalities. The medicinal applications of TAM derivatives are also well documented. Again, the photolabile nature of TAM leuco-derivatives made them useful in many photodevices. The present report gives an overview of the major synthetic routes to TAM derivatives, along with an account of their potential applications and reactions. From the ongoing discussion, it is clear that the chemistry of triaryl- and triheteroarylmethanes is witnessing a rapid growth. The development of novel synthetic methodologies enabling higher conversions under mild reaction conditions will definitely provide an added momentum to research in this area. It is anticipated that the present report will attract the attention of many scientists toward the potential applications of triaryl- and triheteroarylmethanes and will lead to important developments in this area.

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Biographical sketch

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CMPO-substituted calix[6]- and calix[8]arene extractants for the separation of An^{3+}/Ln^{3+} from radioactive waste

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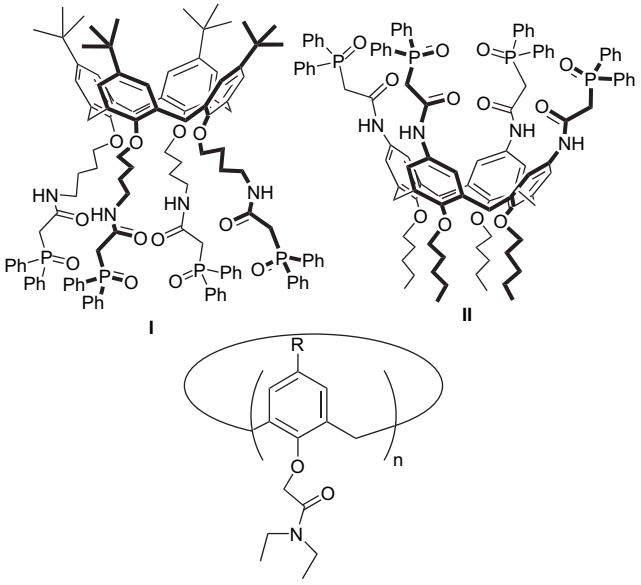
Abstract—Three calix[6]arene derivatives (**1a**–**c**) and two calix[8]arene derivatives (**2a**,**b**), with six and eight CMPO residues, respectively, attached to the narrow/lower rim via ether links, were synthesised. Preliminary liquid–liquid extraction studies for Eu(III) and Am(III) from aqueous nitric acid to *o*-nitrophenylhexyl ether reveal remarkable properties with respect to efficiency and selectivity, especially for the *tert*-butylcalix[6]arene derivative with a $-(CH_2)_3-$ spacer.

1. Introduction

The separation of actinides (An) from lanthanides (Ln) is an urgent and important problem for the management of waste resulting from the reprocessing of fuels of nuclear plants. In fact, spent nuclear fuel contains moderate amounts of long-lived minor actinides (Np, Am, Cm) together with many fission products, among which lanthanides represent one of the major components.[1] The transmutation of actinides into short-lived radionuclides has been proposed for the conditioning of such waste,[1–3] which requires their separation from lanthanides. Organophosphorous ligands like CMPO (carbamoylmethyl-phosphine oxide), used in the TRUEX process, phosphine oxides or dialkylphosphoric acids (TALSPEAK) are quite efficient extractants for An from acidic media. The introduction of CMPO binding groups onto the lower[4] or upper[5,6] rim of calix[4]arenes (**I**, **II**), as well as onto other polyvalent scaffolds,[7,8] increases not only the extraction efficiency of trivalent metal ions by more than 2 orders of magnitude, but also improves the selectivity, in some cases.[9] The original idea of pre-organising four CMPO functional groups on a calix[4]arene fixed in the cone conformation[4,5] was further strengthened by the fact that blocking the calix[4]arene platform in a 'rigid cone' led to an increase (about 10-fold) in extraction ability, although not in selectivity.[6] On the other hand, we have recently shown for a series of calixarene-based amide ligands, that not only the efficiency of alkali and alkaline-earth metal ion complexation greatly changes, passing from the calix[4]arene to the calix[6]- and calix[8]arene scaffold but also the selectivity



III$_4$: n = 4; R = *t*-Bu; cone
III$_6$: n = 6; R = H, t-Bu; OBn, OMe
III$_8$: n = 8; R = H, t-Bu; OBn, OMe

Keywords: Actinides/lanthanides separation; CMPO; Calixarenes; Conformational properties; Radioactive waste treatment; Actinides complexation.
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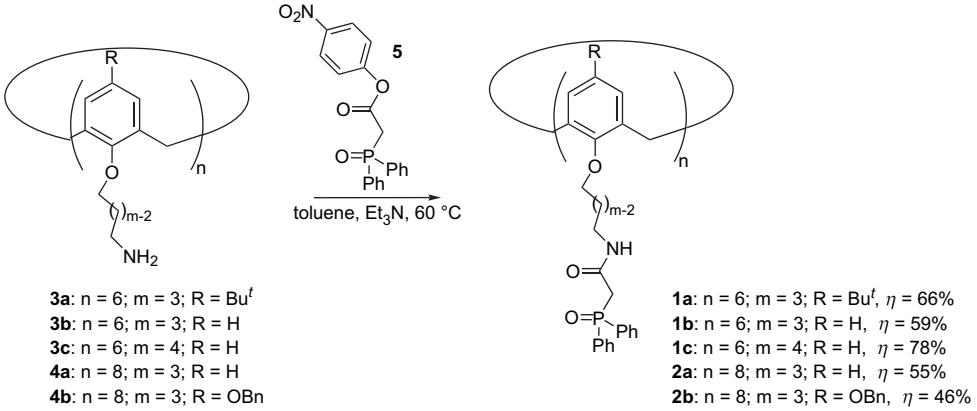
within and between group IA and IIA cations is affected.[10,11] For example, while the tetramide of *p*-*tert*-butylcalix[4]arene (**III$_4$**) has a slight preference for Sr^{2+} over Na^+ complexation, the amides of *p*-*tert*-butylcalix[6]arenes (**III$_6$**) and –calix[8]arenes (**III$_8$**) present a high selectivity[11,12] for Sr^{2+} over Na^+, which makes them promising candidates for the selective removal of strontium radionuclides from radioactive wastes.[13] Therefore, it seems attractive to introduce CMPO binding sites/groups on the larger and more flexible calix[6]- and calix[8]arene scaffolds in order to study how the enlargement of the macrocyclic scaffold and the increase of the number of ligating functions influence the efficiency in trivalent metal ion complexation and the An^{3+}/Ln^{3+} selectivity. We report in this paper the synthesis of the new ligands and results of An(III) liquid–liquid extraction experiments.

2. Synthesis and structures of the ligands

In order to attach CMPO binding groups at the lower rim of calix[6]- and calix[8]arenes we needed the hexaamino- (**3**) and octaamino- (**4**) calixarene derivatives. These compounds were synthesised according to known procedures.[14] Reaction of compounds **3** and **4** with the *p*-nitrophenyl active ester **5** in toluene at 60 °C gives the CMPO ligands **1** and **2** in 46–78% yields (Scheme 1). The introduction of the CMPO units on the calixarene skeleton was confirmed by the presence of the correct molecular peaks in the mass spectra (ESI-MS), of the typical doublet at δ 3.5–3.4 (J=10–14 Hz) for the $POCH_2CO$ protons in the ^1H NMR spectra and by elemental analyses. In the mass spectra, clear peaks of the mono- and/or di-sodium complexes were obtained. The ^1H NMR spectra of these compounds in $CDCl_3$ are usually very broad at rt. Only octamer **2b** shows a sharp spectrum under these conditions with the appearance of several singlets and doublets for the methylene bridges ($ArCH_2Ar$) and for the aromatic protons of the calixarene, indicating the presence of different conformations slowly interconverting on the NMR time-scale. On the other hand, **2b** shows, in DMSO-d_6, even at rt a rather sharp spectrum, which becomes even sharper at 80 °C showing a singlet around δ 3.85 for the methylene bridges, which indicates a fast interconversion between different conformers. This indicates that the presence of several slowly interconverting conformers in $CDCl_3$ at rt, is due, not only to the presence of bulky groups both at the upper and lower rim, but also to the formation of NH···O=P (O=C) hydrogen bonds between the CMPO functions.[15] All the spectra of compounds **1**–**2** were therefore recorded in CD_3OD or DMSO-d_6 (see Section 5). Moreover, the spectrum of *p*-*tert*-butyl hexamer **1a** is much broader than those of its *p*-H-analogues **1b** or **1c** even in DMSO at 100 °C, indicating that the presence of large substituents at both rims of the calixarene skeleton decreases the rate of conformational interconversion especially for this compound.

3. Liquid–liquid extraction studies

Aqueous solutions of Am^{3+} and Eu^{3+} with different concentrations of HNO_3 were extracted with 10^{-3} M solutions of the various ligands in NPHE (*o*-nitrophenylhexyl ether). Since in the case of *p*-H calixarenes **1b**, **1c** or **2a** a precipitate formed at the interphase, extraction experiments, with these compounds, were performed at a ligand concentration of 10^{-4} M. For ligand **1b**, however, at $c(HNO_3)$=2–4 M a precipitate was still observed. This seems to indicate that *tert*-butyl or benzyloxy groups at the upper rim of these large macrocycles considerably help to solubilise the complexes. The distribution coefficients ($D_M=[M]_{org}/[M]_{aq}$) for the extraction experiments are reported in Table 1 and compared with those of compounds **I** and **II**. For all the newly synthesised CMPO ligands **1** and **2**, both D_{Eu} and D_{Am} remarkably increase with increasing HNO_3 concentration in the water layer, in agreement with what was observed with the lower rim substituted calix[4]arene-based CMPO ligands **I**, while the *D*-values for upper rim derivatives of type **II** usually show a maximum for $c(HNO_3)$=2 M. This increase can be explained by the assumption that the nitrate anion is co-extracted together with Am(III) and Eu(III) thus increasing the D_M according to the equation $D_M=K_{ex}[L]^m[NO_3^-]^n$. Interestingly, the data also show that for all the ligands D_{Am} is higher than D_{Eu}. The most efficient ligand seems to be **1a**, but considering that **1b** and **2a** were studied at 10 times higher dilution than **1a**, their D_{Am} values are also rather high. Although these preliminary tests do not allow to determine D_{Am} (and consequently $S_{Am/Eu}$) precisely at $c(HNO_3)>1$ M, it is evident that compound **1a** shows an interesting Am^{3+} over Eu^{3+} selectivity ($S_{Am/Eu}>3$), which seems to increase with



3a: n = 6; m = 3; R = Bu^t
3b: n = 6; m = 3; R = H
3c: n = 6; m = 4; R = H
4a: n = 8; m = 3; R = H
4b: n = 8; m = 3; R = OBn

1a: n = 6; m = 3; R = Bu^t, η = 66%
1b: n = 6; m = 3; R = H, η = 59%
1c: n = 6; m = 4; R = H, η = 78%
2a: n = 8; m = 3; R = H, η = 55%
2b: n = 8; m = 3; R = OBn, η = 46%

Scheme 1. Synthesis of ligands **1** and **2**.

Table 1. D_M values and selectivity $S_{Am/Eu}$ for the extraction of Am^{3+} and Eu^{3+} from an aqueous solution into a NPHE solution of ligands **1** and **2** and **I–II** at different HNO_3 concentrations (T=25 °C)

Ligand	[L] (M)		[HNO_3] (M)						
			0.001	0.01	0.1	1	2	3	4
1a	10^{-3}	D_{Eu}	0.29	0.56	5.1	26.4	27.7	35.5	26.6
		D_{Am}	0.44	0.82	11.1	>100	>100	>100	>100
		$S_{Am/Eu}$	1.5	1.5	2.2	>3	>3	>3	>3
1b	10^{-4}	D_{Eu}	1.6	0.6	1.2	5.9	[a]	[a]	[a]
		D_{Am}	2.8	0.9	2.1	20.6	[a]	[a]	[a]
		$S_{Am/Eu}$	1.7	1.5	1.7	3.5			
1c	10^{-4}	D_{Eu}	0.47	0.25	0.56	3.0	nd	nd	10.6
		D_{Am}	0.85	0.34	0.95	4.5	nd	nd	20.7
		$S_{Am/Eu}$	1.8	1.4	1.7	1.5			1.9
2b	10^{-3}	D_{Eu}	[b]	7.8	9.2	21.9	nd	nd	nd
		D_{Am}	[b]	14.6	18.3	32.8	nd	nd	nd
		$S_{Am/Eu}$		1.9	2.0	1.5			
2a	10^{-4}	D_{Eu}	3.8	5.6	4.2	10.7	12.5	14.7	32
		D_{Am}	3.8	7.1	12.6	12.6	18.0	>100	[b]
		$S_{Am/Eu}$	1	1.3	3.0	1.2	1.4	>6	
I[6]	10^{-3}	D_{Eu}	nr	nr	28	33	44	48	nr
		D_{Am}	nr	nr	48	51	61	63	nr
		$S_{Am/Eu}$			1.7	1.5	1.4	1.3	
II[9]	10^{-3}	D_{Eu}	nr	nr	2.3	30	52	37	19
		D_{Am}	nr	nr	19	195	275	150	100
		$S_{Am/Eu}$			8.3	6.5	5.3	4.0	5.26

nd: not determined; nr: not reported.
[a] Important precipitation at the interphase.
[b] Content of the aqueous layer too low to allow a precise determination of D.

an increase of the HNO_3 concentration. Calix[6]arene-CMPO ligand **1a** is slightly more selective than the analogous derivative based on the calix[4]arene scaffold **I**, having a spacer, which is longer by one methylene group, while the extraction efficiency of the calix[4]arene derivative with the same C_3 spacer is distinctly lower.[4] Interestingly, ligand **1a** having six CMPO arms or the octameric CMPO ligands **2** are much more efficient than dendritic octa-CMPO derivatives.[16] The efficiency and selectivity obtained with the novel calix[6]arene-CMPO ligand **1a** seem close to the upper rim substituted calix[4]arene **II**.

Extraction experiments for compound **1a** were also performed in the presence of the lipophilic dicarbollide anion (BrCosan), which is known to facilitate cation extraction.[17,18]

The results reported in Table 2 indicate that dicarbollide highly facilitates the extraction of M^{3+} ions into the organic phase. Especially at low nitric acid concentration ([HNO_3]≤0.1 M) the D_M values increased by up to 2 orders of magnitude (cf. Tables 1 and 2). Surprisingly, D_{Eu} slightly decreases under these conditions at high nitric acid concentration ([HNO_3]≥0.1 M).

4. Conclusions and outlook

Calix[6]- and calix[8]arenes, bearing at their lower rim six or eight CMPO functions attached via alkylether groups, are easily prepared in reasonable to good yields using well-established synthetic protocols. Although the ligands are less preorganised than calix[4]arene derivatives, the increased number of CMPO functions leads to remarkable properties for the extraction of Eu and Am from aqueous nitric acid to *o*-nitrophenylhexyl ether. Such properties are superior to those shown by dendritic octa-CMPO-derivatives of calix[4]arenes. The extraction studies are presently extended to other calixarene scaffolds and to other organic solvents.

5. Experimental

5.1. General remarks

Melting points were determined with an electrothermal apparatus in sealed capillaries under nitrogen. ^1H and ^{13}C NMR spectra were recorded with Bruker spectrometers AC300 (^1H: 300 MHz, ^{13}C: 75 MHz) or AMX400 (^1H: 400 MHz) with TMS as an internal standard. Mass

Table 2. D_M values and $S_{Am/Eu}$ for the extraction of Am^{3+} and Eu^{3+} from an aqueous solution ([BrCosan]=3×10^{-3} M) into a NPHE solution of ligand **1a** at 25 °C at different [HNO_3]

	[HNO_3] (M)						
	0.001	0.01	0.1	1	2	3	4
D_{Eu}	31.9	46.0	40.8	33.6	39.6	36.8	28.5
D_{Am}	>100	>100	>100	>100	>100	>100	>100
$S_{Am/Eu}$	>3	>2	>2	>3	>2	>2	>3

spectra were obtained in the ESI mode with a Micromass 4LCZ spectrometer (capillary voltage = 3 kV, cone voltage = 60 V, extractor voltage = 3 V, source block temperature = 80 °C, desolvation temperature = 150 °C, cone and desolvation gas (N_2) flow rates = 1.6 and 8 l/min, respectively). TLC was performed on precoated silica gel Merck 60 F_{254}. All the reactions were carried out under nitrogen.

5.2. General procedure for the synthesis of CMPO derivatives

To a solution of amino calix[*n*]arene (0.2 mmol) in dry toluene (40 ml) were added *p*-nitrophenyl (diphenylphosphoryl)-acetate (1.5 equiv per amino group) and triethylamine (1.5 equiv per amino group). The mixture was stirred overnight at 60 °C. Then toluene was evaporated under reduced pressure and dichloromethane (50 ml) was added. The organic phase was washed several times with 10% NaOH (5×40 ml) and dried with anhydrous magnesium sulfate. The dichloromethane was evaporated under reduced pressure to obtain the pure product.

5.2.1. 37,38,39,40,41,42-Hexakis{3-[(diphenylphosphoryl)-acetamide]-propoxy}-*p*-*tert*-butylcalix[6]arene (1a). Yield: 66%. Mp: 133–134 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, 373 K): δ=7.89–7.13 (m, 60H, PhH), 6.89 (br s, 12H, ArH), 3.80 (br s, ArCH_2Ar), 3.45 (d, J=14.0 Hz, 12H, POCH_2CO), 3.40 (br s, 12H, OCH_2CH$_2$CH$_2$), 3.12 (br s, 12H, OCH$_2$CH$_2$CH_2), 1.37 (br s, 12H, OCH$_2$CH_2CH$_2$), 1.05 (br s, 54H, C(CH_3)$_3$). ^{13}C{^{1}H} NMR (DMSO-d_6, 75 MHz, 300 K): δ=164.2 (*C*O), 156.3 (Ar$_{ipso}$), 144.4 (Ar$_{para}$), 134.0–128.3 (Ph and Ar), 70.1 (O*C*H$_2$CH$_2$CH$_2$), 39.1 (OCH$_2$CH$_2$*C*H$_2$), 30.9 (C(*C*H$_3$)), 34.8 (C(*C*H$_3$)), 33.5 (CH$_2$P), 29.4 (Ar*C*H$_2$Ar), 29.3 (OCH$_2$*C*H$_2$CH$_2$). ESI-MS (+): m/z=2790 (100%) [M+Na]$^+$. $C_{168}H_{192}N_6O_{18}P_6$ (2769.26): calcd C 72.87, H 6.99, N 3.03; found C 72.80, H 6.96, N 3.08.

5.2.2. 37,38,39,40,41,42-Hexakis{3-[(diphenylphosphoryl)-acetamide]-propoxy}calix[6]arene (1b). Yield: 59%. Mp: 136–138 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, 363 K): δ=7.78–7.42 (m, 60H, PhH), 6.82 (d, J=7.56 Hz, 12H, ArH$_{meta}$), 6.70 (t, J=7.56 Hz, 6H, ArH$_{para}$), 4.07 (br s, 12H, ArCH_2Ar), 3.45 (d, J=10.1 Hz, 12H, POCH_2CO), 3.38 (br s, 12H, OCH$_2$CH$_2$CH_2), 2.48 (br s, 12H, OCH_2CH$_2$CH$_2$), 1.47 (br s, 12H, OCH$_2$CH_2CH$_2$). ^{13}C{^{1}H} NMR (DMSO-d_6, 75 MHz, 300 K): δ=165.2 (*C*O), 158.6 (Ar$_{ipso}$), 133.9–128.2 (Ph and Ar), 122.4 (Ar$_{para}$), 73.1 (O*C*H$_2$CH$_2$CH$_2$), 39.7 (OCH$_2$CH$_2$*C*H$_2$), 32.3 (CH$_2$P), 30.6 (Ar*C*H$_2$Ar), 30.5 (OCH$_2$*C*H$_2$CH$_2$). ESI-MS (+): m/z=2454 (100%) [M+Na]$^+$. $C_{144}H_{144}N_6O_{18}P_6$ (2432.61): calcd C 71.10, H 5.97, N 3.45, P 7.64; found C 71.08, H 5.94, N 3.49, P 7.61.

5.2.3. 37,38,39,40,41,42-Hexakis{4-[(diphenylphosphoryl)-acetamide]-butoxy}calix[6]arene (1c). Yield: 78%. Mp: 146–147 °C. ^{1}H NMR (CD$_3$OD, 400 MHz, 300 K): δ=7.8–7.4 (m, 60H, PhH), 7.2–6.8 (m, ArH), 3.90 (br s, 12H, ArCH_2Ar), 3.44 (br s, 24H, POCH_2CO and OCH_2CH$_2$CH$_2$CH$_2$), 3.18 (br s, 12H, OCH$_2$CH$_2$CH$_2$CH_2), 1.40–1.31 (m, 24H, OCH$_2$CH_2CH_2CH$_2$). ^{13}C{^{1}H} NMR (DMSO-d_6, 75 MHz, 300 K): δ=164.02 (*C*O), 156.3 (Ar$_{ortho}$), 134.0–128.1 (Ph and Ar), 123.2 (Ar$_{para}$), 71.9 (O*C*H$_2$CH$_2$CH$_2$CH$_2$), 38.6 (OCH$_2$CH$_2$CH$_2$*C*H$_2$), 31.2 (CH$_2$P), 30.5 (Ar*C*H$_2$Ar), 26.9 and 25.6 (OCH$_2$*C*H$_2$-*C*H$_2$CH$_2$). ESI-MS (+): m/z=2538 (100%) [(M+Na)]$^+$. $C_{150}H_{156}N_6O_{18}P_6$ (2516.77): calcd C 71.59, H 6.25, N 3.34; found C 71.65, H 6.32, N 3.45.

5.2.4. 49,50,51,52,53,54,55,56-Octakis{3-[(diphenylphosphoryl)acetamide]-propoxy}calix[8]arene (2a). Yield: 55%. Mp: 180 °C (dec). ^{1}H NMR (CD$_3$OD, 400 MHz, 300 K): δ=7.70–7.31 (m, 80H, PhH), 7.25–7.11 (m, 16H, ArH$_{meta}$), 6.82–6.70 (m, 8H, ArH$_{para}$), 3.93 (br s, 16H, ArCH_2Ar), 3.51 (br s, 16H, POCH_2CO), 3.30 (s, 16H, OCH_2CH$_2$CH$_2$), 3.13 (br s, 16H, OCH$_2$CH$_2$CH_2), 1.60 (br s, OCH$_2$CH_2CH$_2$). ^{13}C{^{1}H} NMR (CD$_3$OD, 75 MHz, 300 K): δ=167.2 (*C*O), 156.7 (Ar$_{ipso}$), 135.7–129.0 (Ph and Ar), 125.5 (Ar$_{para}$), 72.5 (O*C*H$_2$CH$_2$CH$_2$), 38.6 (OCH$_2$CH$_2$*C*H$_2$), 32.3 (CH$_2$P), 31.4 (Ar*C*H$_2$Ar), 31.0 (OCH$_2$*C*H$_2$CH$_2$). ESI-MS (+): m/z=1643.6 (100%) [M+2Na]$^{2+}$. $C_{192}H_{192}N_8O_{24}P_8$ (3243.48): calcd C 71.10, H 5.97, N 3.45; found C 71.18, H 5.90, N 3.54.

5.2.5. 5,11,17,23,29,35,41,47-Octakis[phenylmethoxy]-49,50,51,52,53,54,55,56-octakis{3-[(diphenylphosphoryl)-acetamide]-propoxy}calix[8]arene (2b). Yield: 46%. Mp: 129–130 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, 353 K): δ=7.75–7.36 (m, 80H, PhH), 7.03–7.01 (m, 40H, OCH$_2$Bn*H*), 6.17 (m, 16H, ArH$_{meta}$), 4.56 (br s, 16H, OCH_2Bn), 3.85 (br s, 16H, ArCH_2Ar), 3.63 (t, J=6.1 Hz, 16H, OCH_2CH$_2$CH$_2$), 3.41 (d, J=14.0 Hz, 16H, POCH_2CO), 3.13–3.10 (m, 16H, OCH$_2$CH$_2$CH_2), 1.71 (t, J=6.4 Hz, 16H, OCH$_2$CH_2CH$_2$). ^{13}C{^{1}H} NMR (DMSO-d_6, 75 MHz, 300 K): δ=164.2 (*C*O), 153.8 (Ar$_{ipso}$), 136.7–127.2 (Ar, Ph and Bn), 114.2 (Ar$_{para}$), 71.2 (O*C*H$_2$-CH$_2$CH$_2$), 68.7 (O*C*H$_2$Bn), 39.1 (OCH$_2$CH$_2$*C*H$_2$), 32.5 (CH$_2$P), 30.9 (Ar*C*H$_2$Ar), 30.0 (OCH$_2$*C*H$_2$CH$_2$). ESI-MS (+): m/z=2068 (100%) [M+2Na]$^{2+}$. $C_{248}H_{240}N_8O_{32}P_8$ (4092.47): calcd C 72.79, H 5.91, N 2.74; found C 72.70, H 5.98, N 2.82.

5.3. Extraction experiments

Liquid–liquid extraction experiments were performed by dissolving the ligand at the desired concentration (10^{-4} or 10^{-3} M) in NPHE (*o*-nitrophenylhexyl ether) and mixing the organic phase with an aqueous phase containing Am(III) and Eu(III) nitrates at different HNO_3 concentrations. Am(III) and spiked Eu(III) nitrate aqueous solutions were prepared in order to have a radioactivity in the range 1500–2000 kBq/l. Liquid–liquid extraction experiments were performed by shaking the same volumes of organic and aqueous phases, at the appropriate HNO_3 concentration, inside closed tubes placed in a thermostated cell (25±0.2 °C) for 1 h. Complete separation of the phases was ensured by spinning the tubes in a centrifuge for 5 min. Then, aliquots of aqueous and organic phases were removed for analysis by γ-spectrometry (Eurysis Mesures, Strasbourg). The measurement times were adapted to obtain a reproducibility of ±5%. The distribution coefficients, D_M, were determined as the ratio of cation γ-activity in the organic phase to cation γ-activity in the aqueous phase. The selectivity for Am(III) over Eu(III) is expressed as $S_{Am/Eu} = D_{Am}/D_{Eu}$.

Acknowledgements

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A way to manage the thermal flexibility of ligand candidates for bioassays

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Abstract—An original way to manage both stereochemistry and conformational constraints in ligand candidates for bioassays is presented with reference to a group of model *N*,*N*′-tetrasubstituted *o*-phthalamides and thioamides. The study shows that a scale of thermal flexibility in solution can be envisaged, the divisions of which are represented by compounds sharing quite similar geometrical features. NMR spectroscopy and powder X-ray analysis were used for the physical chemical investigation. An attempt to exploit the conformational instability of a model thioamide in the medium of a bioassay was also performed.

1. Introduction

Recently, we reported our interest in chemical libraries for broad screening purposes, designed using symmetry considerations to control the internal motions of the library members. The properties of certain derivatives of piperidine[1] and *o*-phthalic acid[2] (Fig. 1) were studied in the solid phase and computed in the gas phase. Nitrogen inversion and ring flipping characterize the internal motion of the model piperidine diol **1**. Nitrogen oxidation precludes inversion and unbiases the ring flipping equilibrium in a manner, which can be modulated by the choice of the substituents at positions 3 and 5. Symmetry is broken along the series of models **1**–**3** and other substituents can be imagined to become more or less restricted in their own local motion on the basis of their ability as hydrogen bond donors. As for compounds **4**–**7**, rotation around C_{Ar}–CO(S) and CO(S)–N bonds constituted the focus of the computational study. Experimentally, single crystal X-ray analysis was accomplished for compounds **6** and **7**, the melting behavior of which was also studied.

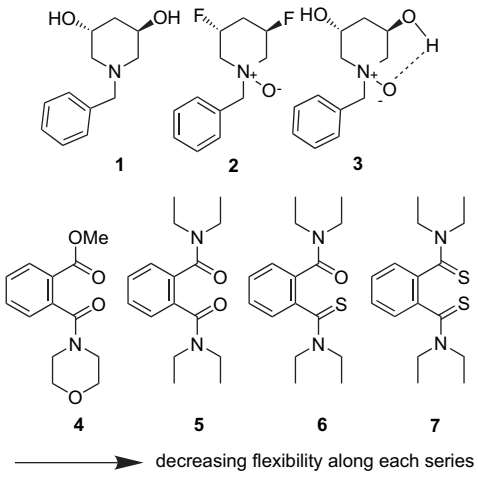

Figure 1.

Many compounds with general formula **I** (Fig. 2) had previously been found to possess nanomolar potency as antagonists of Neurokinin A at the human tachykinin NK-2 receptor.[3]

During this research we found,[4] in agreement with previous literature,[5,6] that amides of type **I** possess expanded features when compared with other *o*-substituted tertiary aromatic amides.[7] So, for instance, once obtained by double amidation of *o*-phthalic acid with the same achiral, differentially substituted, secondary amine, they exist in solution as mixtures of three conformational isomers, one asymmetric (**Ib**) and two dissymmetric (**Ia** and **Ic**). The amide groups are preferentially antiparallel to each other and their planes tend to be orthogonal to the aromatic ring. Interconversion occurs through rotation around the C_{Ar}–CO and CO–$N(CH_2R_1)CH_2R_2$ bonds. Taking the prominent biological

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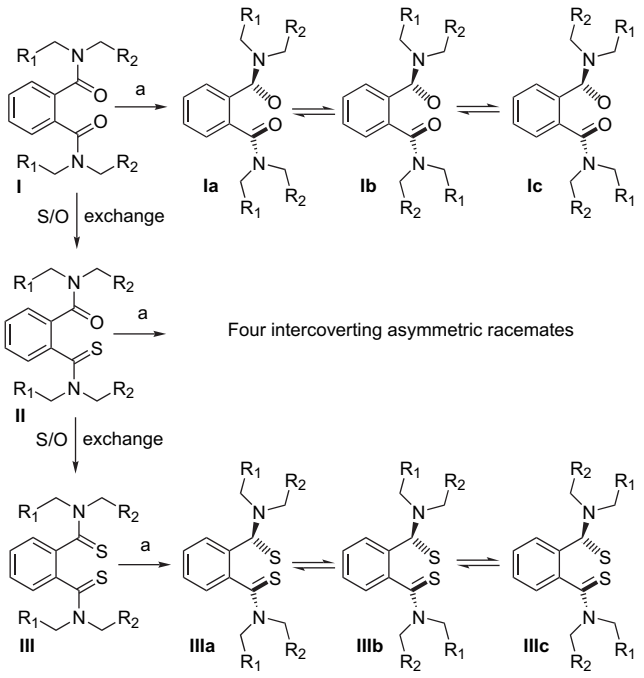
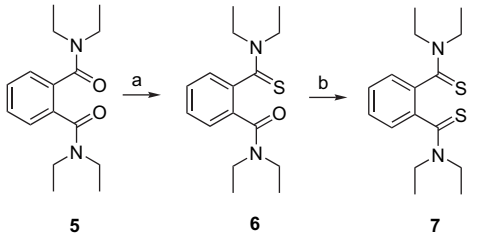


Figure 2. (a) Dissolution.

activity of many compounds of type **I** as a kind of validation, the idea of exploiting their internal motion to build a scale of thermal flexibility was boosted when we became aware that the thioamide replacement of *o*-substituted tertiary benzamides not only slows rotation around the CS–NR_1R_2 bond, but also around the C_{Ar}–CS bond, without major geometrical changes.[8] Indeed, the main difference between compounds **1**–**3** and compounds **4**–**7** (Fig. 1) is just that the piperidine derivatives are enantiomerically stable at any reasonable pharmaceutical and pharmacological time scale,[9] while the same is untrue for the *o*-phthalic acid derivatives. So, in principle, a library obtained by the proper decoration of compounds **4**–**7** could permit to observe the emergence of chirality along the corresponding flexibility scale and to investigate the role of absolute stereochemistry on the bio-performance of the library members.[10,11] On this basis, we wish to show here that compounds of general formulas **I**–**III** are suitable to define a scale of thermal flexibility in solution, the divisions of which are represented by compounds sharing very similar geometrical properties. The result was achieved by studying the behavior of compounds **5**–**7** and **9a**–**9c** in dilute solution by NMR spectroscopy. In addition, the practical conversion of the conformational mixture **9a**–**9c** into the sole isomer **9a** is also described and discussed. Eventually, we disclose that the double thionation of **8**, a tachykinin receptor ligand of type **I**, to give the corresponding compound of type **III** depletes the affinity toward the receptor. This information was obtained while assessing whether the instability of **9a**, after dissolution, could usefully match the bioassay time scale.

2. Results

The synthesis of compounds **5**–**7** is outlined in Scheme 1. Diethyl amine was chosen to yield models of compounds of general formulas **I**, **II**, and **III** in which R_1=R_2, suitable for the ^{1}H NMR spectroscopic investigation.



Scheme 1. (a) LR, 1.04 equiv, THF, rt overnight, then chromatography, 40%. (b) LR, 1.17 equiv, refluxing THF, 8 h, then chromatography, 61%.

We planned to study the conformational interconversion by observing the possible anisochronicity of the methylenic protons within each ethyl group. Phthalamide **5** was converted consecutively into the mono-thioamide **6** and into the bis-thioamide **7**, by means of Lawesson's reagent[12] (LR hereinafter) under two different reaction conditions. Compound **6** was obtained in 40% yield, after chromatographic separation, by reacting compound **5** with a slight molar excess of the thionating agent in THF at rt. This reaction also yielded 10% of compound **7**. This product in turn, was obtained from purified compound **6** in 61% yield after chromatography, by reacting the substrate with an overall excess of LR, added portionwise to the reaction mixture in refluxing THF.

2.1. NMR investigation on compounds 5–7

The rt ^{1}H NMR spectra in $CDCl_3$ at 600 MHz of compounds **5**, **6**, and **7** are stacked in Figure 3. The spectrum of compound **7** (upper spectrum) reveals the dissymmetry of the solute. The aromatic protons give rise to an AA′BB′ spin system. As for the aliphatic protons, the triplets resonating at 1.15 and 1.30 ppm, each integrating to 1.5 times the total area of the aromatic spin system, indicate that a sole cis/trans relationship concerns the amide groups. The eight methylenic protons resonate as four multiplets each integrating to half the total area of the aromatic spin system. The fact that the protons, which belong to the same methylenic group are anisochronous is proved by bidimensional analysis and reflects the existence of a stereogenic element. The fact that there are only four different chemical shifts for the methylenic protons suggests the existence of an internal binary axis of rotation. Aside from residual ethyl acetate, the spectrum of compound **6** is essentially the combination of two different copies (with different chemical shifts) of the spectrum of **7**, thus suggesting that the single O/S replacement solely removed the rotational symmetry from compound **7**. The two multiplets centered at 3.71 and 4.61 ppm in the spectrum of **7** show a slightly more complex fine structure than those centered at 3.46 and 3.30 ppm. A similar appearance also affects four out of eight multiplets in the spectrum of **6** (see insets in Fig. 3). To explain this evidence, we considered compound **7** and found, using the bidimensional analysis and computational simulation, that a long range ^4J of 1.3 Hz correlates two hydrogens at the extrema of each H–C(H)(Me)–N(CS)–C(Me)(H)–H fragment. Although initially surprised to observe such a correlation at rt for an acyclic spin system, we reasoned that the

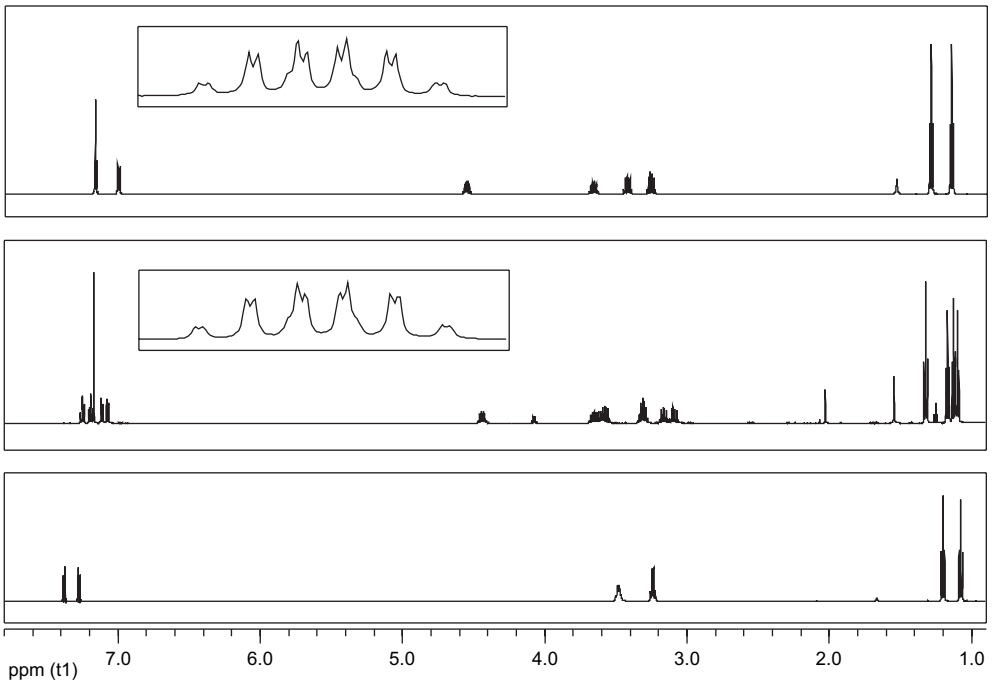


Figure 3. Upper spectrum: compound **7**, inset: multiplet centered at 4.61 ppm. Middle spectrum: compound **6**, inset: multiplet centered at 4.49 ppm. Lower spectrum: compound **5**.

conformational equilibrium related to the fast rotation around N–CH_2 bonds could privilege conformers possessing the proper W arrangement of atoms.[13] To this regard, it is worth noting that one such conformer had been effectively observed in the crystal, while the diasteroisomer obtainable from that by inverting both C_{Ar}–CS bonds was computed (ab initio) to be 5 kcal/mol higher in energy.[2] As for the survey of flexibility differences amongst compounds **5**–**7** in solution, the rt spectrum of compound **5** (lower spectrum in Fig. 3) serves well to introduce the discussion. In fact, the different broadening of the signals centered at 3.23 and 3.47 ppm indicates the geometrical similarity between this compound and compound **7**. At lower temperatures (Fig. 4), each methylenic signal splits into two signals. All four signals integrate to the same value and those originating from the low field signal show a higher difference of chemical shifts than those originating from the high field quartet. In other words, the spectrum of compound **5** at low temperature approaches that of compound **7** at rt. Actually, the rt spectrum of **5** reveals the expected fast interconversion of the enantiomeric conformers existing in the $CDCl_3$ solution. To estimate the rate of enantiomerization at different temperatures, we focussed our attention on the methylenic group resonating at 3.47 ppm (rt spectrum), while irradiating the corresponding methyl group at 1.20 ppm, and successfully simulated this dynamic behavior by computation.

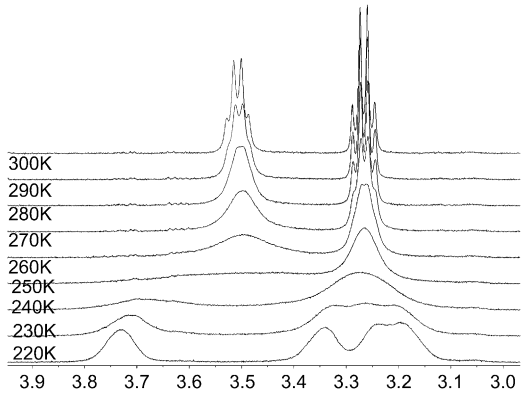


Figure 4.

Eventually, we extrapolated that interconversion occurs with a frequency of about 35,000 s^{-1} at rt, in $CDCl_3$.[6] Since the $CDCl_3$ spectra of **6** and **7** suggested that rotation around the bonds of our interest could be frozen, even C_{Ar}–CO rotation in **6**, we moved to DMSO-d_6 solutions to acquire spectra at higher values of temperature. While assigning the signals in this solvent, we found in the NOESY spectrum (mixing time=0.4 s) of compound **6**, that the two ethyl signals assigned to the oxygenated half of the molecule gave exchange correlation cross-peaks (same sign as diagonal peaks), while the other two ethyl signals (the thionated half) only had NOE type cross-peaks (opposite sign). An indication of the fact that the rotation around the CO–NEt_2 bond, but not that around CS–NEt_2 bond, takes place on the time scale of the NOESY experiment. The finding was confirmed by observing (Fig. 5) the selective broadening of the peaks originating from the oxygenated half of the molecule in the mono-dimensional spectrum of **6** acquired at 340 K. Eventually, for compound **7**, we tried to speed up the rotations under inspection by increasing the temperature to 380 K (DMSO-d_6) without however obtaining any hint of a dynamic process taking place. Compound **7** therefore seems to be the best candidate for attempting the separation and isolation of the enantiomers, a task, which is also in our schedule. By comparing the dynamic properties of compounds **5** and **6**, we can state that, at rt, rotation around CO–NEt_2 bonds is slow in both cases. As for the rotation around C_{Ar}–CO bonds, we have seen that enantiomerization is fast in compound **5**. For this isomerization to occur, it is

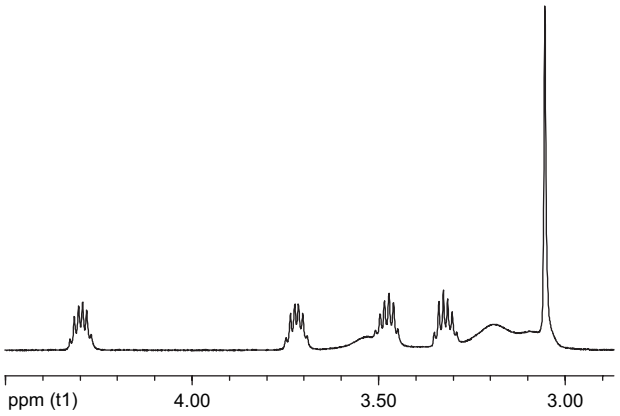
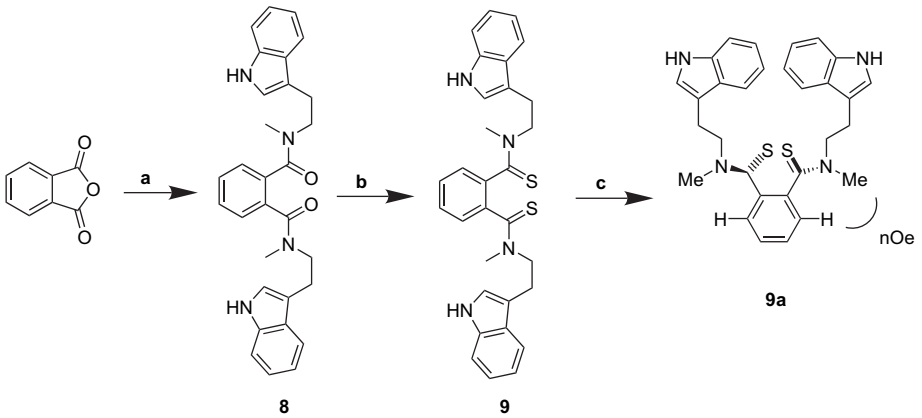


Figure 5. Selective broadening of amide signals in **6**.

necessary that both amide groups invert their orientation with respect to the aromatic plane. Albeit a single step mechanism suffices to interpret DNMR data for compound **5**, literature suggests the existence of a *syn*/*anti* conformational equilibrium strongly privileging the *anti* conformer.[5,6] In a previous study,[2] the stability differences between *syn* and *anti* conformers had been calculated ab initio to be 6.0 kcal/mol for compound **5** and 10.4 kcal/mol for compound **6**. Therefore, if the *syn*/*anti* oscillation occurred in **6** due to C_{Ar}–CO bond rotational lability, no consequence would be easily detected by conventional NMR spectroscopy. The stereochemical properties of the solute would be dictated by the thionated moiety, the rigidity of which is apparent when the spectrum acquired at 340 K is considered.

2.2. Synthesis of compounds 8–9a

The synthesis of compounds **8**–**9a** is outlined in Scheme 2. *N*-Methyltryptamine was chosen to give models of compounds of general formulas **I**, **II**, and **III** in which $R_1 \neq R_2$, suitable for managing different aspects of the problem. The use of an aromatic appending moiety was thought to be interesting in the perspective of the library generation, while the methyl group was chosen to facilitate the understanding of the ^{1}H NMR spectra. Indole was selected as the aromatic group, both for a possible investigation on the mode of binding of the corresponding tachykinin receptor ligands, or to make the thionations more easily followed by TLC analysis. In our experience, 3-indolylmethyl-containing products give violet spots on silica after exposure to *p*-anisaldehyde and heat. Moreover, indole itself is considered as a biologically validated sub-structure.[14] So, our study could furnish an example, maybe the first one, of double use of the indole group in a well defined, yet modulatable, tridimensional space. Compound **8** was obtained in 89% yield, after aqueous work-up, making phthalic anhydride react with a slight excess of *N*-methyltryptamine in DMF at rt, before the addition of EDCI, DIPEA, and HOBT. Compound **8** gave a single spot when analyzed by TLC on silica using 5% of methanol in ethyl acetate as eluent. The thionation of **8** by LR in refluxing toluene proved difficult to monitor by TLC, with the plates being crowded with many spots, soon after the beginning of substrate conversion. Aware that the use of LR usually results in the appearance of many spots on the TLC plate, we monitored the reaction progress using exposure to *p*-anisaldehyde to reveal the TLC spots, HPLC analysis, and MS spectrometry. The combination made us realize that the four diasteroisomers expected for the mono-thionated product and the three diasteroisomers expected for the bis-thionated derivatives could give spots of different R_f values. For the sake of simplicity, we decided to concentrate on the supposed bis-thionated products at this stage of the study. We therefore used an excess of thionating agent and focussed our attention only on the fast moving, indole revealing spots. When we decided that these spots had accumulated enough, we stopped heating and purified the crude material by chromatography. In a previous experience, we had observed that different chromatographic behavior on silica of similar labile diasteroisomers does not ensure their isolation.[4] Therefore, we decided to collect together the three bis-thionated products (overall yield of 49%), despite each one having a specific R_f value on silica, using 3% ethyl acetate in dichloromethane as eluent (R_f=0.32, 0.26, 0.17). This mixture could be readily evaluated in DMSO-d_6, but proved to be sparingly soluble in $CDCl_3$, an occurrence which enabled us to get an unexpected result. In practice, after the filtration of the $CDCl_3$ suspension, the DMSO-d_6 spectrum of the solid was essentially that of only one dissymmetric isomer whose configuration was later assessed as **9a** (Scheme 2) from the NOESY spectrum. The serendipitous isolation of **9a** had two consequences. First, as described below in the text, we were able to follow the kinetics of the conversion into the



Scheme 2. (a) *N*-Methyltryptamine, 2.3 equiv, DMF, rt 1 h, then DIPEA, 2.0 equiv, EDCI, 1.2 equiv, HOBT, 1.2 equiv, rt, overnight, 89%. (b) LR, 2.3 equiv, refluxing toluene, 1 h, then chromatography, 49%. (c) $CHCl_3$, 6.7 equiv, 40 °C, 8 h, 98%.

thermodynamic mixture in DMSO-d_6. Second, we succeeded to convert the mixture of the three isomeric bis-thionated products, as obtained from the chromatographic separation, directly into **9a** in quantitative yield. Indeed, after some attempts, we obtained 464 mg of essentially pure isomer **9a**, by heating 473 mg of dried eluate, containing less than 40% of the same compound, at 40 °C for 8 h in a corked HPLC vial with 500 μl of chloroform (molar ratio 1/6.7). Although serendipitous in origin, this transformation would be looked at as an example of phase transition driven reaction, namely a reaction the selectivity of which should be due to the stability difference amongst the possible isomers in the solid phase, while their interconversion occurs smoothly in the liquid, on the experimental time scale.[15,16]

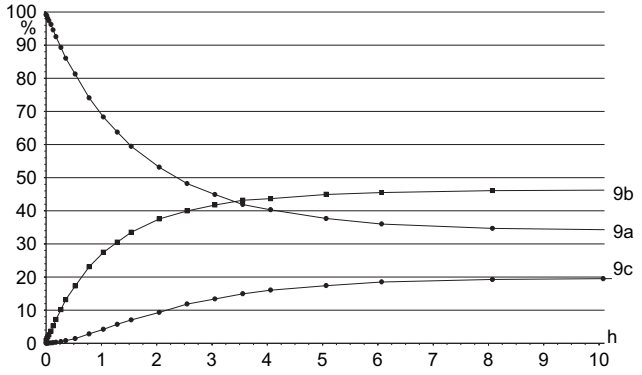


Figure 7.

2.3. Physical–chemical investigation on compound 9a

Compound **9a** was quite stable as a solid and could be stored at rt on the shelf for months. In order to ascertain this observation, we examined an aliquot of the crude reaction product by X-ray powder diffraction analysis. This allowed us to realize that **9a** had accumulated as a crystalline powder during the reaction and that its thermal stability as a solid is effectively high. After the rt analysis, we acquired spectra at 333 K every 2 h for a day without observing any change with respect to the first spectrum. In Figure 6, the first (a) and the eleventh (b) spectra are reported.

Nevertheless, the conversion into the mixture could be monitored by NMR at rt, after dissolution in DMSO-d_6 (Fig. 7). While peaks belonging to the asymmetric form **9b** appeared as soon as the solution was prepared and inserted into the magnet, the other symmetric conformer **9c** appeared, in traces, only after 4 min. The growing curve for these two conformers have exponential and sigmoidal shapes, respectively, supporting the idea by which each symmetric conformer can convert (in one step) only into asymmetric ones and vice versa. Calculating the percentage of each isomer at each stage was complicated by the mutual overlap of signals. We chose to monitor the intensity of the methyl singlets because of their higher sensitivity. Two singlets of interest fall just on top of some methylenic multiplets and we therefore adopted the following protocol.

We integrated each cluster of overlapping peaks as a whole and, separately, one or more other methylenic multiplets originating from the same isomers and comprising the same number of protons, so that the difference between the chosen integrals was equal to the area of the methyl singlet alone. To avoid any complication that may have arisen from the different saturation of different signals, each spectrum was acquired in a single scan. Plotting the calculated percentage, instead of the area, compensated for any change in instrumental sensitivity over the course of the experiment. What we discovered in this way is that, even at rt, the rotation around the thioamide bond is so slow that it takes hours before the equilibrium mixture is reached. A stationary state is achieved after 13 h (**9a**: 34.3%, **9b**: 46.2%, **9c**: 19.5%), while **9a** still accounts for about 60% of the mixture 90 min after dissolution.

2.4. Binding affinity tests

In the light of the pharmaceutical and pharmacological time scale definitions,[9] compound **9a** could be an example of a pharmaceutically stable substance, whose pharmacological stability would depend upon the consideration of either in vitro, or in vivo experiments. It is apparent that **9a**, independently from any consideration of its racemic nature, could not be looked at as a sole substance with respect to an in vivo experiment.[17] Conversely, its homogeneity could be challenged on the time scale of an in vitro assay. Before the present study, we had synthesized compound **8** during our efforts to obtain antagonists of Neurokinin A at the human tachykinin NK-2 receptor.[4] In Figure 8, it is shown that compound **8** inhibits the binding of iodinated NKA to human NK-2 receptor in concentration-dependent manner, with sub-micromolar potency.

With compound **9a** at hand and the knowledge of its behavior in DMSO-d_6 solution, we reasoned that there was the possibility to investigate the binding mode of **8** by using

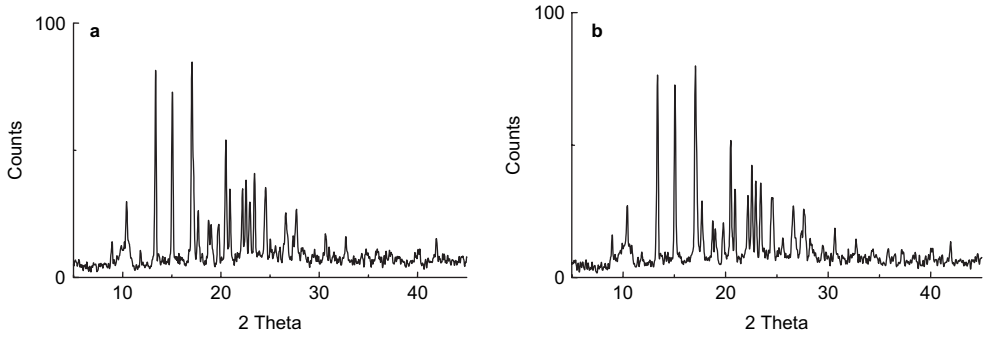


Figure 6. X-ray powder diffraction spectra of **9a** at 333 K. (a) Time zero. (b) After 22 h.

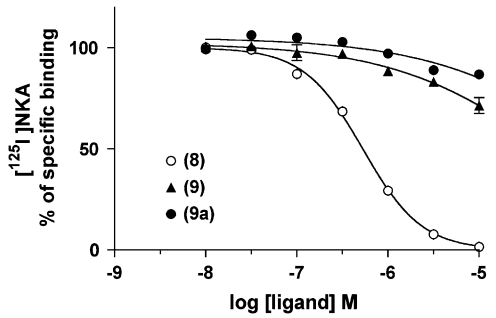


Figure 8.

separately compound **9a** and its equilibrium mixture. In principle, only the inspection of the preferred absolute stereochemistry would have been precluded, **9a** being reasonably a racemate. Unfortunately, the thionated mixture **9**, as well as compound **9a** was far less potent than **8** in the same assay (Fig. 8). Worst, the possibility to discriminate **9a** from **9** by the careful reduction of the equilibration time of the bioassay at higher concentrations of the thionated candidates was hampered by their insolubility in the medium of the bioassay. Overall, the only information we gained from these pharmacological experiments was that the double thionation of **8** provokes a marked affinity loss at the receptor. On the other hand, since the overall tridimensional shape is conserved when passing from **8** to the mixture **9a**–**9c**, it is not unreasonable to admit that the removal of at least one critically positioned hydrogen bond acceptor from **8** is responsible for affinity depletion. The interpretation would agree also with similar results obtained in a previous study with other ligands at the same receptor.

3. Conclusion

In this paper, we have shown that compounds of general formulas **I**–**III** can define a scale of thermal flexibility in solution, the divisions of which are represented by compounds sharing very similar geometrical properties. Although we have not shown the effective emergence of chirality along the series of the investigated model compounds, that of a homogenous and rt thermally stable substance from a conformational mixture was demonstrated. The resolution of compound **7** into its enantiomers and the assessment of their lifetimes is under investigation. In addition we are working to synthesize a pool of compound **8** congeners. The aim is to investigate whether the scope of the solidification driven conversion from **9a**–**9c** into **9a** is wider, and also to use the entire pool for challenging other biological targets than human NK-2 receptor. The results of these efforts will be reported in due course.[18]

4. Experimental

4.1. General

Anhydrous solvents were purchased from Fluka. TLC monitoring: Merck silica gel 60 F_{254} plates, detection by UV light or *p*-anisaldehyde and heat. HPLC monitoring: Waters 2690 Separation Module equipped with a Waters 996 photodiode array detector. Separations were obtained by a Symmetry300™ C_{18} 5 μm column (4.6×250 mm), eluting at a flow rate of 1 ml/min with a mobile phase consisting of A—water+0.1% TFA, B—acetonitrile+0.1% TFA. Gradient elutions were performed from 20% to 80% B in 20 min. Melting points were determined in capillary tubes and are uncorrected. IR spectra were collected with a Nicolet Avatar 360 FTIR E.S.P. spectrophotometer. ^{1}H NMR spectra were acquired at 500 or 600 MHz on Bruker Avance instruments and referenced against the residual solvent peak (DMSO-d_6 at 2.50 ppm and $CDCl_3$ at 7.25 ppm). For variable temperature studies, we assumed that the temperature of the probe (reported by the spectrometer) was the same as that of the solution. NOESY spectra were typically acquired with a mixing time of 0.4 s, 2K points along f_2 and 512 points along f_1, in the phase-sensitive mode according to the States-TPPI protocol. The f_1 dimension was extended four times with Linear Prediction. The baseplane of the transformed spectrum was further processed with polynomial corrections of the fifth order in both dimensions. ^{13}C NMR spectra were acquired on a Varian Gemini 200 spectrometer, at the operating frequency of 50 MHz. All the processing was performed using the program SwaN-MR.[19] Mass spectra were obtained with a Finnigan LCQ ion trap mass spectrometer, operated in positive-ion electrospray ionization. The samples were analyzed by full-scan MS and product ion MS/MS of the protonated quasi-molecular ions, at 30% relative collision energy, using helium as the collision gas. The high resolution mass spectrum of compound **8** was obtained with a Thermo Electron LTQ Orbitrap spectrometer, equipped with nano-electrospray ion source, operated in positive-Electrospray. The instrument was externally calibrated with a mixture of caffeine, MRFA, and Ultramark 1621. The sample (about 10 μg/mL in 1:1 acetonitrile/10 mM ammonium acetate) was introduced by infusion at 1 μL/min, and analyzed in full-scan MS mode at resolution=50,000. X-ray powder diffraction data were collected on a Bruker D8 Advance powder diffractometer, equipped with a Debye–Scherrer transmission θ–θ geometry, using the Cu Kα radiation. The Sol-X solid state Si(Li) detector was used. C/Ni Goebel–Spiegel mirrors in the incident beam were used as monochromator; 1.0 mm divergence, 0.2 scatter and 0.1 for the receiving slits were used. The sample was prepared by pressing the thin layer of the sample on a Pt–Rh fine foil. The sample was heated under high-temperature (HT) chamber (mri wide range) from rt to 333 K with a step of 0.2 °C/min. The spectra were recorded in the 2θ range 5–45 °C. Binding affinity experiments: Inhibition binding curves were performed with membrane preparations (150 μg/ml) of stably transfected CHO cells expressing the human tachykinin NK_2 receptor, using [^{125}I]NKA (0.13 nM) as radioligand.[20] Binding data were fitted by nonlinear regression using GraphPad Prism 4.0.

4.1.1. Synthesis of 2-diethylthiocarbamoyl-*N*,*N*-diethylbenzamide. Compound 6. LR (1.17 g, 2.88 mmol) was suspended in 10 ml of anhydrous THF under a nitrogen atmosphere. A solution of compound **5** (762 mg, 2.76 mmol) in anhydrous THF (12 ml) was added through a dropping funnel, while magnetically stirring. The reaction mixture was left under stirring overnight at rt. The volatiles were removed under reduced pressure and the residue (2.21 g as a yellow oil) was purified by Flash-Master chromatography.

The column was conditioned with *n*-hexane. Compound **7** was eluted first using 50% of CH_2Cl_2 in *n*-hexane (31 mg as a yellow solid; yield=10%). Compound **6** was then eluted using 20% of ethyl acetate in *n*-hexane (314 mg as a white solid; yield=40%).

4.1.1.1. 2-Diethylthiocarbamoyl-*N*,*N*-diethyl-benzamide. Compound 6. Mp: 81 °C–82 °C (*n*-hexane). R_f=0.1, CH_2Cl_2/*n*-hexane, 1:1. IR (KBr) ν (cm^{-1}): 2990, 2968, 2930, 2880, 2867, 1626, 1510, 1429. ESI$^+$-MS: *m*/*z*= 293 (MH^+), 220, 192, 164, 130, 121, 105. $C_{16}H_{24}N_2OS$ (292.44): calcd C, 65.71; H, 8.27; N, 9.58; found C 65.43, H 8.42, N 9.44. ^{1}H NMR (DMSO-d_6, 300 K, 600 MHz): 7.39 δ (1H, t), 7.33 δ (1H, t), 7.25 δ (1H, d), 7.15 δ (1H, d), 4.32 δ (1H, m), 3.61 δ (1H, m), 3.57 δ (1H, m), 3.43 δ (1H, m), 3.28 δ (1H, m), 3.16 δ (1H, m), 3.09 δ (1H, m), 3.01 δ (1H, m), 1.19 δ (3H, t), 1.07 δ (3H, t), 1.05 δ (3H, t), 1.04 δ (3H, t). ^{13}C NMR ($CDCl_3$) δ: 196.69, 169.38, 140.90, 132.76, 128.51, 127.23, 125.53, 124.81, 48.44, 45.46, 43.42, 38.81, 13.86, 13.45, 12.32, 10.82.

4.1.1.2. *N*,*N*,*N*′,*N*′-Tetraethyl-benzene-1,2-dicarbothioic acid. Compound 7. Mp: 118 °C–119 °C (ethyl acetate). R_f=0.1, ethyl acetate/*n*-hexane, 1:4. IR (KBr) ν (cm^{-1}): 3076, 3058, 3007, 2974, 2953, 2872, 1507, 1429. ESI$^+$-MS: *m*/*z*=309 (MH^+), 236, 208, 180. $C_{16}H_{24}N_2S_2$ (308.51): calcd C, 62.29; H, 7.84; N, 9.08; found C 62.50, H 7.99, N 9.01. ^{1}H NMR (DMSO-d_6, 300 K, 600 MHz): 7.25 δ (2H, m), 7.08 δ (2H, m), 4.60 δ (2H, m), 3.70 δ (2H, m), 3.46 δ (2H, m), 3.29 δ (2H, m), 1.29 δ (6H, t, *J*=7.1 Hz), 1.14 δ (6H, t, *J*=7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 196.27, 138.38, 127.52, 124.48, 48.96, 45.12, 13.52, 10.51.

4.1.2. Synthesis of compound 7 from compound 6. LR (476 mg, 1.17 mmol) and thioxoamide **6** (292 mg, 1.0 mmol) were suspended in 8 ml of anhydrous THF under magnetic stirring and a nitrogen atmosphere. The solvent was refluxed for 8 h, and then the mixture was left to stand overnight. LR (202 mg, 0.50 mmol) was added and the solvent was refluxed for 3 h. LR (203 mg, 0.50 mmol) was added and the solvent refluxed for 5 h, then the mixture was left on standing overnight. LR (203 mg, 0.50 mmol) was added and the solvent refluxed for 1 h. The volatiles were removed under reduced pressure and the residue (2.06 g) was purified by Flash-Master chromatography. Compound **7** (188 mg as a white solid, yield=61%) was eluted using 50% of CH_2Cl_2 in *n*-hexane.

4.1.3. Synthesis of *N*,*N*′-bis-[2-(1*H*-indol-3-yl)-ethyl]-*N*,*N*′-dimethyl-phthalamide. Compound 8. Phthalic anhydride (325 mg, 2.60 mmol) and *N*-methyltryptamine (1.00 g, 5.74 mmol) were dissolved in 8 ml of anhydrous DMF under a nitrogen atmosphere. The mixture was magnetically stirred for 1 h at rt. DIPEA (672 mg, 5.20 mmol), EDCI (598 mg, 3.12 mmol), and HOBt (422 mg, 3.12 mmol) were added and the mixture was left overnight under stirring. The solution was poured into 1 M aqueous HCl under vigorous stirring. The white precipitated material was collected by filtration, washed with water, and dried in the air. Compound **8** (1.11 g; yield=89%) was obtained as a white solid and used for thionation without further purification. R_f=0.31 (ethyl acetate as eluent). The sample for bioassays and analysis was purified by chromatography on silica eluting with ethyl acetate. Mp: 97–102 °C. ESI$^+$-MS: *m*/*z*=479 (MH^+), 305, 144. IR (KBr) ν (cm^{-1}): 3400, 3288, 3047, 2929, 2853, 1623. ^{1}H NMR (DMSO-d_6, 300 K, 500 MHz): 10.82 δ (1H, br s), 10.80 δ (1H, br s), 10.79 δ (1H, br s), 10.76 δ (1H, br s), 7.60 δ (1H, t), 7.44 δ (1H, dd), 7.42 δ (1H, t), 7.35–7.21 δ (5H, m), 7.09–6.91 δ (6H, m), 6.79 δ (1H, m), 3.66 δ (2H, m), 3.33 δ (2H, m), 2.99–2.91 δ (4H, m), 3.01, 3.00, 2.83 and 2.77 δ (6H, singlets). ^{13}C NMR (DMSO-d_6) δ: 169.26, 169.20, 168.84, 168.80, 136.42, 136.02, 135.16, 134.87, 134.72, 134.64, 128.41, 128.33, 128.25, 128.16, 127.08, 126.86, 126.34, 126.20, 125.99, 122.71, 120.75, 118.46, 118.00, 117.94, 111.28, 111.25, 111.18, 110.68, 51.56, 47.46, 47.26, 32.01, 23.60, 22.32. HRMS (MH^+) calcd for $C_{30}H_{31}N_4O_2$: 479.2442; found: 479.2419.

4.1.4. Synthesis of *N*,*N*′-bis-[2-(1*H*-indol-3-yl)-ethyl]-*N*,*N*′-dimethyl-benzene-1,2-dicarbothioic acid. Compound 9. LR (694 mg, 1.71 mmol) and amide **8** (362 mg, 0.75 mmol) were suspended in 11 ml of anhydrous toluene under magnetic stirring and a nitrogen atmosphere. The solvent was made to reflux for 1 h. During this time, a dark orange solution formed. The volatiles were removed under reduced pressure and the residue (1.45 g) was purified by Flash-Master chromatography using CH_2Cl_2 as eluent. Compound **9** was obtained (188 mg as a pale yellow solid, yield=49%) by collecting together the fractions corresponding to three spots on the TLC plate: R_f=0.17, 0.26, 0.32 (ethyl acetate/CH_2Cl_2, 3:97 as eluent). ESI$^+$-MS: *m*/*z*=511 (MH^+), 337, 144. $C_{30}H_{30}N_4S_2$ (510.72): calcd C, 70.55; H, 5.92; N, 10.97; found C 70.07, H 5.99, N 10.67. ^{1}H NMR (DMSO-d_6, 300 K, 600 MHz) of the major (*E*,*Z*) conformer: 10.86 δ (1H, br s), 10.83 δ (1H, br s), 7.73 δ (1H, d, *J*=8.0 Hz), 7.36–6.85 δ (13H, m), 4.45 δ (1H, m), 4.00 δ (1H, m), 3.81 δ (1H, m), 3.51 δ (3H, s), 3.50 δ (1H, m), 3.25–3.16 δ (2H, m), 3.12 δ (3H, s), 3.00 δ (1H, m). After the identification, **9** was renamed as mixture **9a**–**9c**. ^{13}C NMR (DMSO-d_6) δ: 196.66, 196.54, 196.31, 196.06, 139.21, 138.70, 138.65, 138.23, 136.12, 135.98, 127.41, 127.09, 127.00, 126.82, 126.78, 124.96, 124.74, 124.40, 124.27, 122.96, 122.78, 120.87, 118.43, 118.151, 111.26, 110.84, 110.74, 110.16, 110.10, 57.47, 57.34, 54.53, 54.07, 42.42, 42.38, 23.71, 20.52. The acquisition of ^{13}C NMR spectrum started several hours after compound **9a** dissolution and must be considered as the spectrum of the thermodynamic mixture **9a**–**9c** in DMSO-d_6.

4.1.5. Conversion to thioamide 9a. The mixture of thioamides **9a**–**9c**, obtained as described above (473 mg, 0.93 mmol) and $CHCl_3$ (500 µl, 6.2 mmol) were placed in a stoppered HPLC vial, which was heated at 40 °C for 8 h in a sand bath. The mixture was filtered with the help of few milliliters of pre-cooled $CHCl_3$. Compound **9a** (464 mg) was recovered as a white solid. R_f=0.32 (ethyl acetate/CH_2Cl_2, 3:97 as eluent). ESI$^+$-MS: *m*/*z*=511 (MH^+), 337, 144. The composition of this solid was not altered after standing for a few months on the shelf, at rt. On the other hand, it melted in a capillary tube, after 30 min at 130 °C. IR (KBr) ν (cm^{-1}): 3275, 3057, 3000, 2930, 2852, 1514, 1457. ^{1}H NMR (DMSO-d_6, 300 K, 600 MHz): 10.84 δ (1H, br s), 8.32 δ (1H, s), 7.73 δ (1H, d, *J*=7.9 Hz), 7.33 δ (4H, m), 7.22 δ (2H, s), 7.10 δ (2H, dd, *J*=5.6, 3.2 Hz),

7.05 δ (2H, t, J=7.6 Hz), 6.91 δ (2H, t, J=7.4 Hz), 4.61 δ (2H, td, J=11.6, 5.2 Hz), 3.96 δ (2H, td, J=11.6, 5.2 Hz), 3.29 δ (2H, m), 3.21 δ (6H, s), 3.08 δ (2H, m).

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Phosphoramidites and solid supports based on *N*-substituted 2,4-dihydroxybutyramides: universal reagents for synthesis of modified oligonucleotides

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Abstract—A general and convenient method for synthesis of modified oligonucleotides by use of new non-nucleoside phosphoramidites is reported. A chiral 1,3-diol backbone of the modifying reagents is generated either from (*R*)-(+)-α-hydroxy-γ-butyrolactone or (*R*)-(−)-pantolactone. Aliphatic amines were acylated with the lactones to give the corresponding *N*-substituted 2,4-dihydroxybutyramides. After protection of a side chain, if necessary, the diols were converted into phosphoramidites or solid supports suitable for use in oligonucleotide synthesis. The reagents allow single, multiple or combined introduction of various functions (e.g., alkylamine, imidazole and pyrene residues) into synthetic oligonucleotides. The structures of the conjugates were confirmed by MALDI-TOF mass spectrometry.

1. Introduction

Although modified oligonucleotides have diverse use in chemistry, biology and medicine, the emergence of new areas of their application is a current trend.[1] Solid-phase phosphoramidite oligonucleotide synthesis, a highly efficient and reliable technique developed in the 1980s,[2] is still a mainstay of chemical DNA preparation. Numerous modifying phosphoramidite reagents for oligonucleotide synthesis also have been developed[3] and many of them are commercially available. The potential of phosphoramidites for combinatorial chemistry has been recognised.[4] Our aim was to develop reagents applicable for the preparation of oligonucleotide conjugates in combinatorial mode, for instance, for synthesis of artificial ribonucleases that have an oligonucleotide part for complementary recognition and a variable catalytic part carrying active functional groups, e.g., amine, imidazole, etc.

A 1,3-diol system with one primary and one secondary hydroxyl group mimicking the 5′- and 3′-OH groups of nucleosides is an obvious structural element in the design of non-nucleoside reagents suitable for machine-aided assembly of modified oligonucleotides. We recently reported that it can be obtained easily by acylation of amines with α-hydroxy-γ-butyrolactones.[5] In the procedure, a suitable compound with an aliphatic primary or even secondary amino group can be converted into the corresponding *N*-substituted 2,4-dihydroxybutyramide. The precursor is then subjected to successive steps of side-chain protection (if required), 4,4′-dimethoxytritylation of the primary hydroxyl group, and conversion into the phosphoramidite or attachment to a solid support via the secondary hydroxyl group. Here we describe the synthesis of several new reagents that allow the introduction of reactive (amine), catalytically active (imidazole) and fluorescent (pyrene) residues into oligonucleotides as well as the preparation and characterisation of various oligonucleotide conjugates.

2. Results and discussion

2.1. Synthesis of phosphoramidites

α-Hydroxy-γ-butyrolactones **1a**,**b** are known to react smoothly with amines.[6] No racemisation at the α-carbon

Keywords: Modified oligonucleotides; Phosphoramidites; 2,4-Dihydroxybutyramides; Imidazole; Pyrene.
Abbreviations: DIC, 1,3-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; Dmt, 4,4′-dimethoxytrityl; Fmoc, 9-fluorenylmethoxycarbonyl; FmocOSu, 9-fluorenylmethyl *N*-succinimidyl carbonate; LCAA-CPG, long-chain alkylamino controlled pore glass; Py, pyridine; RP-HPLC, reversed-phase HPLC; Tfa, trifluoroacetyl.
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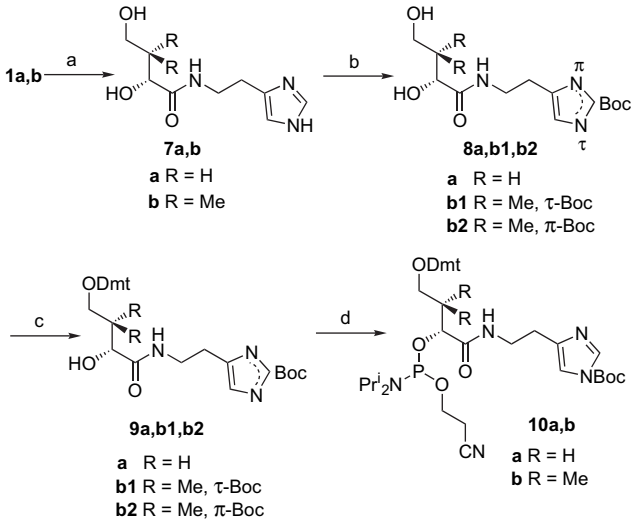
atom was reported. We found that in the case of α-hydroxy-γ-butyrolactones **1a** and **1b** a simple un-catalysed reaction with primary amines at a slightly elevated temperature gives very good yields of the corresponding 2,4-dihydroxybutyramides after 24–48 h. Primary amines with a bulky substituent, e.g., pyrene or secondary amine, e.g., *N*,*N*′-dimethylethylenediamine require much longer reaction times.

α,ω-Diamines served as precursors for aminolinker reagents. Lactones **1a** and **1b** were treated with an excess (5.0 equiv) of ethylenediamine or *N*,*N*′-dimethylethylenediamine at 55 °C for 1–7 days to give mono-acylated products **2** (Scheme 1).

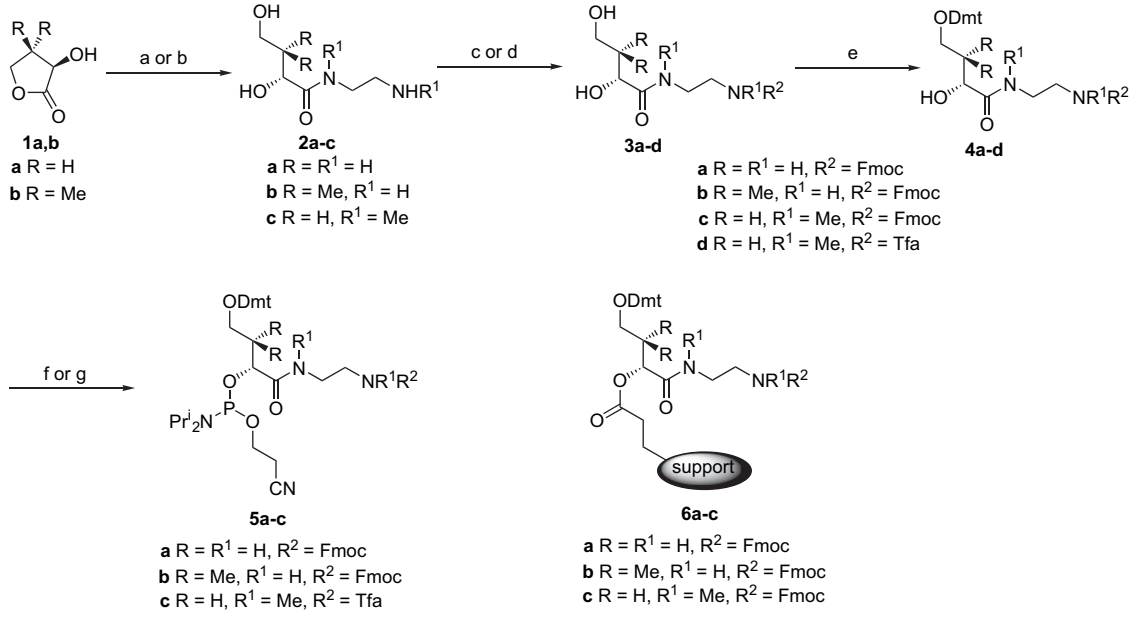
The remaining amino function was masked with Fmoc or Tfa, common amino protecting groups compatible with oligonucleotide synthesis,[3] affording **3a–d**. Compound **3a** precipitated readily from the reaction mixture. By contrast, the presence of two geminal 3-methyl groups in the pantolactone-derived compound **3b** or N-methylation in **3c**,**d** allow their purification by column chromatography. The hydroxyl groups in compounds **3** were functionalised by known procedures of nucleoside chemistry:[7] the primary hydroxyl group was 4,4′-dimethoxytritylated[8] selectively, and the resulting compounds **4** were converted into phosphoramidites **5a–c**[9] and solid-supported reagents **6a–c**.[10] The loading of the supports was estimated to be in the range of 35–52 μmol/g. Phosphoramidites were found to decompose significantly on silica gel during column chromatography and thus were isolated by precipitation into hexane. Reagents **5a–c** are white solids that can be stored at −20 °C for at least several months without any substantial loss of reactivity. Phosphoramidite **5b** is appreciably more stable upon storage compared to **5a**, which indicates a positive shielding effect of the two methyl groups. The reagents allow introduction of one or several primary or secondary amino groups into any position within an oligonucleotide sequence.

Imidazole residues of histidines are present in catalytic centres of DNA- and RNA-cleaving enzymes.[11] Many syntheses of imidazole–oligonucleotide conjugates as potential target-addressed RNA cleavers are reported.[12] It was found that the Boc group is suitable for histidine N^{Im}-protection during phosphoramidite DNA synthesis, undergoing smooth cleavage upon standard ammonolytic deprotection conditions.[13] This prompted us to investigate its use for imidazole protection in phosphoramidite reagents **10a**,**b**.

The synthesis of the imidazole reagents is outlined in Scheme 2. The primary amino group of histamine was acylated with lactones **1a** or **1b** to give dihydroxybutyramide **7a**



Scheme 2. Synthesis of imidazole phosphoramidites. Reaction conditions and yields: (a) histamine, 55 °C, 83% (**7a**); (b) Boc_2O, 76% (**8a**), 38% and 17% (**8b1** and **8b2** from **1b**); (c) DmtCl, Py, 0 °C to rt, 1–3 h, 86% (**9a**), 55% and 37% (**9b1** and **9b2** from **8b1**); (d) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, 80% (**10a**), 60% (**10b**).



Scheme 1. Synthesis of amino-modifier reagents and solid supports. Reaction conditions and yields: (a) $(CH_2NH_2)_2$, 55 °C, overnight, quant. (**2a–b**); (b) $(CH_2NHMe)_2$, 55 °C, 7 days, quant. (**2c**); (c) FmocOSu, aq MeCN, 88% (**3a**), 75% (**3b**), 64% (**3c**); (d) CF_3CO_2Me, rt, 30 min; (e) DmtCl, Py, 0 °C to rt, 1–3 h, 81% (**4a**), 97% (**4b**), 89% (**4c**), 93% (**4d** from **2c**); (f) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, rt, 2 h, 85% (**5a**), 67% (**5b**), 77% (**5c**); (g) succinylated LCAA-CPG, DIC, DMAP, Py, rt, loading (μmol/g): 35 (**6a**), 52 (**6b**), 44 (**6c**).

or **7b**, respectively. These were treated with Boc_2O to afford imidazole-protected compounds **8**. Only the τ-Boc isomer was isolated in the case of **8a**, whilst **7b** gave a mixture of τ- and π-isomers (**8b1** and **8b2**), easily separable by column chromatography. Dimethoxytritylation of **8** followed by phosphitylation yielded phosphoramidites **10**. Only the major isomer **8b1** was used in the reaction with DmtCl in pyridine.

Surprisingly, two separable products **9b1** and **9b2** were again isolated after a usual workup and column chromatography. This indicates that partial τ→π migration of the Boc group takes place during dimethoxytritylation, possibly catalysed by pyridine hydrochloride formed in the reaction. π-Isomers (**8b2** and **9b2**) were found to be unstable: after storage of the compounds as dried solids for one month at +4 °C, **8b2** gave ca. 1:1 mixture of **8b1** and **8b2**, and **9b2** was completely converted into **9b1**. The structures of compounds **8b1**, **8b2** and **9b1** were determined using Rapoport's NMR criteria.[14,15] Moreover, a ROESY experiment for **9b1** (spectral width 10 ppm×10 ppm, 16,000×512 of complex points) showed similar cross-peaks of medium intensity between Boc protons and both H-2 and H-5 of the imidazole moiety.

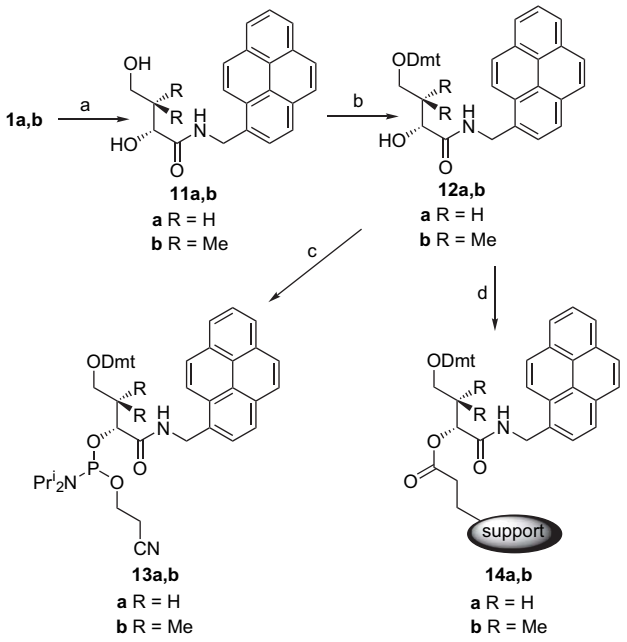
Interestingly, the less stable π-Boc isomers and their rearrangement to thermodynamically more stable τ-Boc compounds were detected in the '**b**' series only. The influence of the remote *gem*-dimethyl group in **7b** on regioselectivity of imidazole acylation in comparison with **7a** seems to be unlikely. The observed difference could be explained rationally by the following assumptions: (1) π-Boc isomer is also formed from **7a**, but converts rapidly into the stable τ-Boc compound; (2) the substantial difference in π→τ-migration rates between **a** and **b** series is caused by spatial hindrance of the hydroxyl(s) that probably facilitates the rearrangement.

Compound **9b1** was then phosphitylated to give **10b**, a pair of diastereomers arising from the phosphoramidite group, as a single product. No migration of the Boc group was observed during the reaction.

Of course, the exact position of the Boc group in the reagents **10a**,**b** does not influence the structure of the resultant imidazole–oligonucleotide conjugates, since both τ- and π-Boc isomers should be deprotected in concd aq ammonia.

To the best of our knowledge, amongst pyrene non-nucleoside reagents described to date, only a few examples contain a 1,3-diol backbone.[16] Multistep syntheses of deoxyribosyl pyrene *C*-nucleosides are complex, time consuming and include separation of a mixture of α- and β-anomers.[17] Scheme 3 depicts a straightforward synthesis of new pyrene phosphoramidite reagents **13a**,**b** and supports **14a**,**b** from 1-pyrenemethylamine.

The structures of 2,4-dihydroxybutyramide derivatives **2a**, **3a**,**b**, **7a**, **8a**,**b1** and **11a**,**b** were confirmed by ^{1}H–^{13}C NMR correlations. The assignment of signals in the ^{13}C NMR spectra was performed using HMQC (cross-peaks from proton-bound carbon atoms) and HMBC (cross-peaks from interaction through two, three and four bonds) spectra.



Scheme 3. Synthesis of pyrene phosphoramidites. Reaction conditions and yields: (a) 1-pyrenemethylamine, 55 °C, 100% (**11a**), 92% (**11b**); (b) DmtCl, Py, 62% (**12a**), 83% (**12b**); (c) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, 70% (**13a**), 92% (**13b**); (d) succinylated LCAA-CPG, DIC, DMAP, pyridine, rt, loading (μmol/g): 20 (**14a**), 16 (**14b**).

2.2. Synthesis and characterisation of modified oligonucleotides

All the phosphoramidites and supports obtained were tested in machine-assisted solid-phase oligonucleotide synthesis. The coupling time for the modified phosphoramidites **5a**–**c**, **10a**,**b** and **13a**,**b** was extended to 10 min. The coupling yields were comparable to those for nucleoside phosphoramidites (determined by the released Dmt cation). The conjugates prepared were cleaved from their supports, deprotected by concd aq ammonia treatment at 55 °C overnight, analysed by reversed-phase HPLC and MALDI-TOF MS and purified by PAGE for thermal denaturation studies. Some loss of the 5′-terminal modified unit(s) was observed when the Dmt group was removed prior to ammonolysis, possibly resulting from the attack of the 5′-terminal hydroxyl on the neighbouring phosphodiester bond. To obtain uniformly high yields of the 5′-modified oligonucleotides these were synthesised routinely in 'Dmt On' mode, and the Dmt protecting group was cleaved after ammonia treatment. Sequences and some properties of the modified oligodeoxyribonucleotides are summarised in Table 1. Examples of HPLC traces are shown in Figure 1. Even in the crude mixtures after synthesis, the content of the desired conjugates was usually more than 90% with almost no detectable amount of any side products.

As expected, the incorporation of the hydrophobic pyrene moiety into oligonucleotides increases the retention time of conjugates on the HPLC column whilst other 2,4-dihydroxybutyramide substituents influence the chromatographic mobility of the corresponding oligomers only negligibly.

Thermal stability experiments show, as expected,[18] an increase in T_m for duplexes with pyrene 2,4-dihydroxybutyramide in

Table 1. Properties of oligodeoxyribonucleotides bearing (*R*)-2,4-dihydroxybutyramide non-nucleoside units

#	Sequence, 5′ to 3′[a]	Amidite or support source of X	MALDI-TOF, found/calcd	RP-HPLC retention time, min[b]
ON01	CTCCCAGGCTCAAAT**X**p	**5a**	4801.1/4796.8	15.20
ON02	CTCCCAGGCTCAAAT**X**p	**5b**	4824.8/4826.9	12.68
ON03	**X**CTCCCAGGCTCAAAT	**5b**	4750.8/4749.2	12.48
ON04	CTCCCAGGCTCAAAT**X**	**6a**	4718.7/4719.2	12.75
ON05	CTCCCAGGCTCAAAT**XX**	**6b**, **5b**	5078.8/5078.9	13.23
ON06	CTCCCAGGCTCAAAT**X**p	**13a**	4970.1/4969.9	22.21
ON07	**X**CTCCCAGGCTCAAAT**X**	**13b**, **14b**	5341.7/5341.0	34.80
ON08	CTCCCAGGCTCAAAT**X**	**14a**	4890.5/4889.9	24.85
ON09	CTCCCAGGCTCAAAT**X**	**14b**	4920.6/4920.3	27.71
ON10	ATTTGAGCCTGGGAG**X**	**14a**	5041.9/5040.9	21.42
ON11	ATTTGAGCCTGGGAG**X**	**14b**	5070.4/5071.4	25.30
ON12	CTCCCAGGCTCAAAT**X**	**6b**	4747.9/4749.2	12.51
ON13	CTCCCAGGCTCAAAT**X**p	**10a**	4851.8/4850.8	12.79
ON14	**X**CTCCCAGGCTCAAAT	**10a**	4773.5/4772.1	12.63
ON15	CTCCCAGGCTCAAAT**X**p	**10b**	4888.9/4881.2	12.55
ON16	**X**CTCCCAGGCTCAAAT	**10b**	4804.7/4801.2	13.17
ON17	CTCCCAGGCTCAAAT**XX**p	**10a**	5124.6/5124.9	12.52
ON18	**XX**CTCCCAGGCTCAAAT	**10a**	5044.2/5044.9	12.54
ON19	CTCCCAGGCTCAAAT**XX**p	**10b**	5182.8/5183.4	13.02
ON20	**XX**CTCCCAGGCTCAAAT	**10b**	5102.5/5103.5	14.14
ON21	CTCCCAGGCTCAAAT**X**	**6c**	4750.3/4749.2	13.78
ON22	CTCCCAGGCTCAAAT**X**p	**5c**	4847.3/4844.1	12.90
ON23	**X**CTCCCAGGCTCAAAT	**5c**	4748.0/4749.2	12.64
ON24	CTCCCAGGCTCAAAT**XX**p	**5c**	5081.7/5080.0	13.26
ON25	**XX**CTCCCAGGCTCAAAT	**5c**	5001.7/5001.4	12.71
ON26	**X**CTCCCAGGCTCAAAT**X**p	**5c**	5079.0/5080.0	12.59
ON27	**CUCCCAXGCUCA**[c]	(**5a**+**10a**+dG)	3731.7/3729.1 3784.5/3780.0 3836.7/3833.2	—[d]
ON28	**CUCCCAXGCUCA**[c]	(**5b**+**10b**+2′OMe-rG)	3761.5/3762.5 3812.6/3814.1 3868.6/3873.3	—[d]

[a] p—3′-phosphate.
[b] HPLC conditions are given in Section 4.
[c] 2′-*O*-Methylribonucleotides are in bold.
[d] Broad unresolved peak.

a dangling position and a slight destabilisation for duplexes with the 3′-phosphate group (Table 2). Other terminal modifications influence the T_m values insignificantly.

Next, we tested the suitability of 2,4-dihydroxybutyramide reagents for the generation of combinatorial libraries of oligonucleotides. We used a 1:1:1 (mol) mixture of **5a** and **10a** with dG phosphoramidite for synthesis of **ON27** and **5b** and **10b** with 2′-OMe-rG phosphoramidite in the same ratio for synthesis of **ON28**. The sum of the concentrations of phosphoramidites was 0.1 M in acetonitrile. Oligonucleotides were synthesised in 'Dmt On' mode and purified by RP-HPLC (for conditions see Section 4). The purified Dmt-containing oligomers were treated with 80% acetic acid at room temperature for 30 min, isolated by 1 M $LiClO_4$–acetone precipitation, and analysed by RP-HPLC and MALDI-TOF MS. In both cases, HPLC showed only one broad peak at ca. 15 min (data not shown). In the mass spectra, three main signals corresponding to individual library members were present (Fig. 2).

The spectra indicate that the reactivity of phosphoramidites derived from 2,4-dihydroxybutyramide is similar to 2′-deoxyribonucleoside phosphoramidites, and of those from 2,4-dihydroxy-3,3-dimethylbutyramide to 2′-*O*-methylribonucleoside phosphoramidites.

3. Conclusions

Our results demonstrate that various primary and secondary aliphatic amines may be easily converted into 2,4-dihydroxybutyramide phosphoramidites and solid supports and used for the preparation of modified oligonucleotides carrying diverse functional groups, e.g., amine, imidazole or pyrene. When a mixture of modifying phosphoramidites is used, a mixture of conjugates is produced that shows the potential of the reagents for application in combinatorial chemistry.

4. Experimental

4.1. General

500 MHz ^{1}H, 125.7 MHz ^{13}C and 202.4 MHz ^{31}P NMR spectra were recorded on a Bruker DRX-500 spectrometer and referenced to DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C), MeCN-d_3 (1.96 ppm for ^{1}H), MeOH-d_3 (3.34 ppm for ^{1}H and 49.00 ppm for ^{13}C) and 85% aq H_3PO_4 (0.00 ppm for ^{31}P). ^{1}H–^{13}C gradient-selected HMQC and HMBC spectra were obtained by using 2048 (t_2)×256 (t_1) complex point data sets, zero filled to 2048 (F_2)×1024 (F_1) points. The spectral widths were 13 and 200 ppm for ^{1}H and ^{13}C dimensions, respectively. HMBC spectra were measured with 50 ms delay for evolution of long-range couplings. Varian

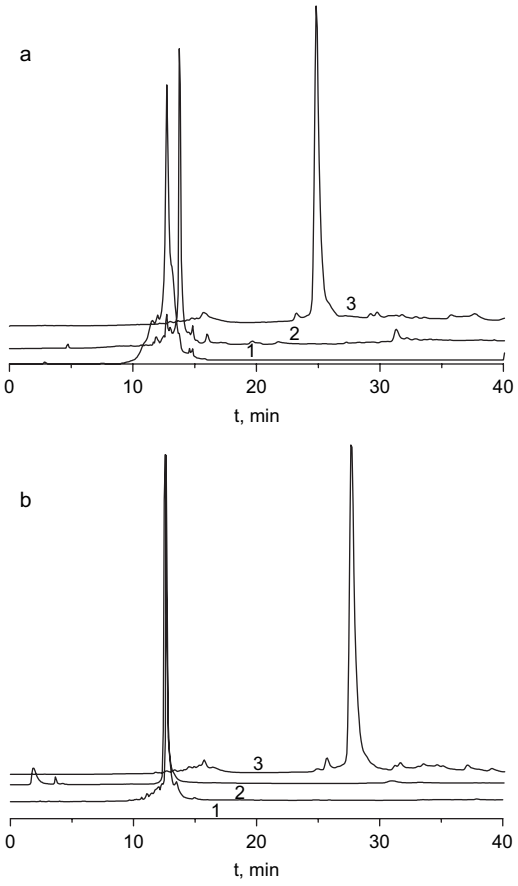


Figure 1. (a) Typical RP-HPLC traces of crude (*R*)-2,4-dihydroxybutyramide-containing oligodeoxyribonucleotides: (1) 2-aminoethyl **ON04**, (2) *N*,*N*′-dimethyl-2-aminoethyl **ON21** and (3) 1-pyrenemethyl **ON08** (Table 1). (b) Typical RP-HPLC traces of crude (*R*)-2,4-dihydroxy-3,3-dimethylbutyramide-containing oligodeoxyribonucleotides: (1) 2-aminoethyl **ON02**, (2) 2-(imidazol-4′-yl)ethyl **ON15** and (3) 1-pyrenemethyl **ON09** (Table 1). For HPLC conditions, see Section 4.

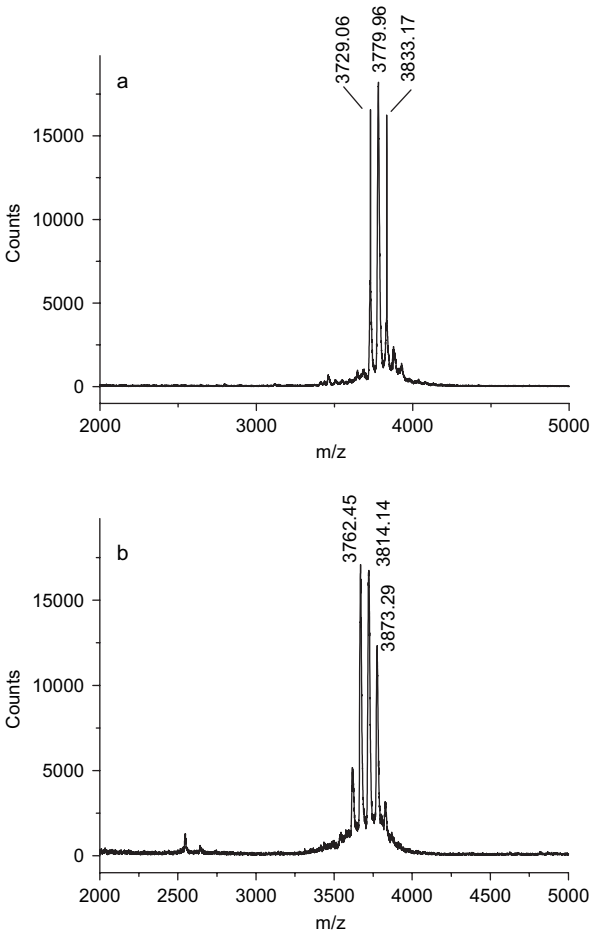


Figure 2. MALDI-TOF mass spectra of combinatorial mixtures **ON27** (a) and **ON28** (b).

Unity NMR spectrometer (600 MHz) was used for measuring DQF-COSY and ROESY spectra. ^{1}H NMR coupling constants are reported in Hertz and refer to apparent multiplicities. ESI-TOF HRMS spectra in positive ion mode were recorded on Micromass LCT reflection TOF mass spectrometer. IR spectra were recorded using Bruker Vector 22 spectrometer. Melting points were determined using a Boetius heating table and are uncorrected. Analytical thin-layer chromatography was performed on Kieselgel 60 F_{254} precoated aluminium plates (Merck), spots were visualised under UV light (254 nm). Silica gel column chromatography was performed using Merck Kieselgel 60 0.040–0.063 mm.

Table 2. Stability of various (*R*)-2,4-dihydroxybutyramide-modified oligodeoxyribonucleotides as duplexes with an unmodified complementary DNA strand

#	Modifying reagent	Position of modification	T_m, °C	ΔT_m/mod, °C
ON04	**6a**	3′-	57.3	−0.3
ON08	**14a**	3′-	61.6	+4.0
ON09	**14b**	3′-	62.0	+4.4
ON10	**14a**	3′-	59.1	+1.5
ON11	**14b**	3′-	58.5	+0.9
ON13	**10a**	3′-	57.4	−0.2
ON15	**10b**	3′-	57.3	−0.3
ON16	**10b**	5′-	58.9	+1.3
ON17	**10a**	3′-	56.9	−0.4
ON18	**10a**	5′-	57.9	+0.2
ON19	**10b**	3′-	56.9	−0.4
ON20	**10b**	5′-	58.0	+0.2
ON22	**5c**	3′-	57.0	−0.6
ON23	**5c**	5′-	58.1	+0.5
ON24	**5c**	3′-	56.7	−0.5
ON25	**5c**	5′-	58.4	+0.4
ON26	**5c**	5′- and 3′-	56.7	−0.5

Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. Diisopropylammonium tetrazolide was prepared as described.[19] DCM was used freshly distilled from CaH_2. THF was freshly distilled from powdered $LiAlH_4$ and stored over 4 Å molecular sieves under argon. Other solvents were used as received.

Oligonucleotide synthesis was carried out on a ABI 380B DNA/RNA synthesiser either on 0.2 or 1 μmol scale using 2′-deoxynucleoside phosphoramidites from Cruachem (Scotland). The molecular mass of each oligonucleotide was checked by MALDI-TOF MS on a Perseptive Biosystems Voyager DE workstation in positive ion mode using a mixture (1:1 v/v) of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aq diammonium hydrogen citrate (80 mg/mL) as a matrix premixed just before loading the samples onto a plate. Thermal denaturation experiments were performed on a Perkin–Elmer Lambda 40 UV/vis

Spectrometer with PTP 6 (Peltier Temperature Programmer) in a buffer containing 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0.

4.1.1. (2*R*)-*N*-(2-Aminoethyl)-2,4-dihydroxybutyramide (2a). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) in ethylenediamine (3.5 mL, 50 mmol) was kept at 55 °C overnight, evaporated, co-evaporated with toluene (30 mL) and *N*,*N*-diisopropylethylamine (1.8 mL, 10 mmol). The residue was triturated in Et_2O under argon and dried in vacuo to yield amide **2a** (1.63 g, 100%) as a white solid. ESI-TOF HRMS: *m*/*z*=163.1074 $[M+H]^+$, calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077. ^1H NMR (DMSO-d_6) δ 7.81 (br s, 0.19H), 7.67 (br s, 0.81H) (CON*H*, exchangeable with D_2O), 3.93 (dd, 1H, J_1=3.6 Hz, J_2=8.6 Hz, C*H*O), 3.49 (m, 2H, CH_2O), 3.40–3.00 (br, 2H,‡ NH_2, exchangeable with D_2O), 3.07 (apparent q, 2H, *J*=6.3 Hz, CH_2NH), 2.57 (t, 2H, *J*=6.3 Hz, CH_2NH$_2$), 1.83 (m, 1H), 1.55 (m, 1H) (CH$_2$CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.63 (*C*=O), 69.07 (*C*HOH), 57.92 (*C*H$_2$OH), 41.97, 41.65 (*C*H$_2$*C*H$_2$), 38.07 (C*C*H$_2$C).

4.1.2. (2*R*)-*N*-(2-Aminoethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (2b). Compound **2b** was prepared in a similar manner as **2a** from (*R*)-(−)-pantolactone (3.25 g, 25 mmol) and ethylenediamine (9 mL, 125 mmol). After co-evaporation with toluene (100 mL) and *N*,*N*-diisopropylethylamine (4.4 mL, 25 mmol), trituration in Et_2O and drying, the desired amide **2b** (4.76 g, 100%) was obtained as a white solid. ESI-TOF HRMS: *m*/*z*=191.1388 $[M+H]^+$, calcd for $[C_8H_{18}N_2O_3+H]^+$ 191.1390. IR (KBr): 3342 cm^{-1}, ν (OH, NH); 2963 cm^{-1}, ν (sp^3-CH); 1654 cm^{-1}, ν (C=O); 1540 cm^{-1}, ν (NH). ^1H NMR (DMSO-d_6) δ 7.81 (br s, 0.08H), 7.67 (br s, 0.92H) (CON*H*, exchangeable with D_2O), 3.71 (s, 1H, C*H*O), 3.29 (d, 1H), 3.18 (d, 1H) (^2J=10.4 Hz, CH_2O), 3.20–2.80 (br, 2H, NH_2, exchangeable with D_2O), 3.07 (m, 2H, J_{HCCH}=J_{HNCH}=6.4 Hz, ^2J=12.8 Hz, CH_2NH), 2.57 (m, 2H, J_{HCCH}=6.4 Hz, ^2J=12.6 Hz, CH_2NH$_2$), 0.81 (s, 3H), 0.80 (s, 3H) (C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ 173.84 (*C*=O), 75.20 (*C*HOH), 66.18 (*C*H$_2$OH), 42.12, 41.53 (*C*H$_2$*C*H$_2$), 38.39 (C*C*Me$_2$C), 21.42, 20.81 (*C*H$_3$).

4.1.3. (2*R*)-*N*-Methyl-*N*-(2-methylaminoethyl)-2,4-dihydroxybutyramide (2c). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) in *N*,*N*′-dimethylethylenediamine (5.2 mL, 50 mmol) was kept at 55 °C for 7 days, then evaporated, co-evaporated with *N*,*N*-diisopropylethylamine (1.8 mL, 10 mmol) and then toluene (3×30 mL) to give diol **2c** as a yellowish viscous oil (1.90 g, quant.). ESI-TOF HRMS: *m*/*z*=191.1381 $[M+H]^+$, calcd for $[C_8H_{18}N_2O_3+H]^+$ 191.1390. The compound was used without further purification in the next steps.

4.1.4. (2*R*)-*N*-[2-(9-Fluorenylmethoxycarbonylamino)-ethyl]-2,4-dihydroxybutyramide (3a). To a stirred solution of crude amine **2a** (1.63 g, 10 mmol) in 50% aq MeCN (20 mL), a solution of FmocOSu (3.38 g, 10 mmol) in MeCN (20 mL) was added in one portion and stirring continued overnight at ambient temperature. The precipitate formed was filtered off, washed with MeCN (10 mL), Et_2O (30 mL) and dried in vacuo to yield diol **3a** (2.50 g, 65%) as a white crystalline powder. An additional amount of product (0.90 g) was obtained by evaporation of combined filtrate, dissolving the residue in EtOAc (100 mL), washing with water (2×70 mL), drying (Na_2SO_4), evaporation and crystallisation from ^{i}PrOH. Total yield 3.40 g (88%), mp 133.5–134.0 °C (^{i}PrOH). R_f 0.13 ($CHCl_3$–MeOH 9:1 v/v). ESI-TOF HRMS: *m*/*z*=385.1759 $[M+H]^+$, calcd for $[C_{21}H_{24}N_2O_5+H]^+$ 385.1758. IR (KBr): 3321 cm^{-1}, ν (OH, NH); 2944 cm^{-1}, ν (sp^3-CH); 1694 cm^{-1}, ν (C=O); 1536 cm^{-1}, ν (NH); 1451 cm^{-1}, ν (sp^3-CH). ^1H NMR (DMSO-d_6) δ 7.88 (m, 2H, Ar*H* (H-4, H-5)), 7.77 (br s, 1H, CHCON*H*), 7.68 (m, 2H, Ar*H* (H-1, H-8)), 7.40 (m, 2H, Ar*H* (H-3, H-6)), 7.33 (m, 2H, Ar*H* (H-2, H-7)), 7.28 (br s, 1H, OCON*H*), 5.40 (d, 1H, *J*=5.4 Hz, CHO*H*), 4.40 (t, 1H, *J*=5.1 Hz, CH$_2$O*H*) (both exchangeable with D_2O), 4.30 (m, 2H, CH_2OCO), 4.21 (t, 1H, *J*=6.9 Hz, fluorene H-9), 3.94 (m, 1H, C*H*O) (in the presence of D_2O gives dd, J_1=3.7 Hz, J_2=8.4 Hz), 3.49 (m, 2H, CH_2OH), 3.16 (m, 2H), 3.07 (m, 2H) (NCH_2CH_2), 1.83 (m, 1H), 1.55 (m, 1H) (CH$_2$CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.91 (HN*C*=O), 156.64 (O*C*=O), 144.35 (2C), 141.18 (2C), 128.05 (2C), 127.51 (2C), 125.57 (2C), 120.55 (2C) (Ar, Fmoc), 69.04 (*C*HOH), 65.81 (*C*H$_2$OCO), 57.91 (*C*H$_2$OH), 47.18 (fluorene C-9), ~40‡ N*C*H$_2$), 38.66 (N*C*H$_2$), 38.04 (C*C*H$_2$C).

4.1.5. (2*R*)-*N*-[2-(9-Fluorenylmethoxycarbonylamino)-ethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (3b). Compound **3b** was prepared by treatment of amine **2b** (1.90 g, 10 mmol) in 50% aq MeCN (20 mL) by a solution of FmocOSu (3.38 g, 10 mmol) in MeCN (20 mL) at room temperature for 48 h. The mixture was filtered, evaporated, the residue was dissolved in EtOAc (200 mL), washed with water (150 mL), 5% $NaHCO_3$ (2×150 mL), then dried (Na_2SO_4), evaporated, co-evaporated with toluene (3×30 mL) and chromatographed on silica gel column using stepwise gradient elution with 0.5→1→2→3→5→8% MeOH in $CHCl_3$ (v/v). Fractions containing product were combined, evaporated and the residue was dried in vacuo to afford pure **3b** as a white solid (3.10 g, 75%), mp 64.0–65.0 °C. R_f 0.17 ($CHCl_3$–MeOH 9:1 v/v). ESI-TOF HRMS: *m*/*z*=435.1909 $[M+Na]^+$, calcd for $[C_{23}H_{28}N_2O_5+Na]^+$ 435.1890. IR (KBr): 3329 cm^{-1}, ν (OH, NH); 2957 cm^{-1}, ν (sp^3-CH); 1697 cm^{-1}, ν (C=O); 1537 cm^{-1}, ν (NH); 1450 cm^{-1}, ν (sp^3-CH bending). ^1H NMR (DMSO-d_6) δ 7.88 (d, 2H, *J*=7.3 Hz, Ar*H* (H-4, H-5)), 7.74 (br s, 1H, CHCON*H*, exchangeable with D_2O), 7.67 (d, 2H, *J*=7.5 Hz, Ar*H* (H-1, H-8)), 7.41 (m, 2H, Ar*H* (H-3, H-6)), 7.33 (m, 2H, Ar*H* (H-2, H-7)), 7.25 (br s, 1H, OCON*H*, exchangeable with D_2O), 5.33 (d, 1H, *J*=5.5 Hz, CHO*H*), 4.44 (t, 1H, *J*=5.5 Hz, CH$_2$O*H*) (both exchangeable with D_2O), 4.29 (m, 2H, CH_2OCO), 4.20 (m, 1H, fluorene H-9), 3.71 (d, 1H, *J*=5.5 Hz, C*H*O), 3.29 (m, 1H,‡ CH*H*OH), 3.25–3.03 (m, 5H, NCH_2CH_2, CH*H*OH), 0.80 (s, 3H), 0.78 (s, 3H) (C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ 173.66 (HN*C*=O), 156.63 (O*C*=O), 144.35 (2C), 141.18 (2C), 128.05 (2C), 127.50 (2C), 125.56 (2C), 120.55 (2C) (Ar, Fmoc), 75.63 (*C*HOH), 68.48 (*C*H$_2$OCO), 65.80 (*C*H$_2$OH), 47.17 (fluorene C-9), ~40 (2C,‡ N*C*H$_2$*C*H$_2$), 38.53 (C*C*Me$_2$C), 21.40, 20.79 (*C*H$_3$).

4.1.6. (2*R*)-*N*-Methyl-*N*-(2-[methyl(9-fluorenylmethoxycarbonyl)amino]ethyl)-2,4-dihydroxybutyramide (3c). The crude amine **2b** (1.14 g, 6 mmol) in 50% aq MeCN

‡ Calculated value; the signal of water or a solvent is also present in the region.

(15 mL) was treated with a solution of FmocOSu (2.03 g, 6 mmol) in MeCN (10 mL) for 48 h. The workup was similar to the above procedure. After column chromatography in 0.5→1→1.5→2→2.5% MeOH in $CHCl_3$ (v/v) the desired compound **3c** was isolated as a white foam (1.58 g, 64%), R_f 0.36 ($CHCl_3$–MeOH 9:1 v/v). ESI-TOF HRMS: *m/z*= 435.1878 $[M+Na]^+$, calcd for $[C_{23}H_{28}N_2O_5+Na]^+$ 435.1890. ^1H NMR (MeCN-d_3) δ 7.85 (d, 2H, *J*=7.3 Hz, Ar*H*, H-4, H-5), 7.79 (d, 0.57H, *J*=7.5 Hz, Ar*H*), 7.65 (d, 1.43H, *J*=7.3 Hz, Ar*H*) (H-1, H-8), 7.43 (m, 2H, Ar*H*, H-3, H-6), 7.36 (m, 2H, Ar*H*, H-2, H-7), 4.59–4.25 (m, 4H, C*H*O, C*H*CH_2OCO), 3.85–2.33 (m, 12H, CH_2OH, N(CH_3)CH_2CH_2NCH_3), 1.89–1.26 (m, 2H, CH_2CH_2CH). ^{13}C NMR (MeCN-d_3) δ 172.24 (MeN*C*=O), 155.80 (O*C*=O), 144.16 (2C), 141.09 (2C), 128.01 (2C), 127.35 (2C), 125.46 (2C), 120.47 (2C) (Ar, Fmoc), 68.88 (*C*HOH), 68.42 (*C*H_2OCO), 65.74 (*C*H_2OH), 47.10 (fluorene C-9), 39.55, 39.16 (N*C*H_2*C*H_2), 38.44 (C*C*H_2C), 35.10, 34.52 (*C*H_3).

4.1.7. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxybutyramide (4a). Diol **3a** (2.50 g, 6.5 mmol) was co-evaporated with pyridine (3×20 mL), dissolved in dry pyridine (50 mL), cooled in an ice bath, and DmtCl (2.40 g, 7.1 mmol) was added in three portions within 1 h. After disappearance of the starting material, the excess of DmtCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with $CHCl_3$ (300 mL), washed with 2.5% $NaHCO_3$ (3×200 mL), water (2×200 mL), 20% NaCl (200 mL), then dried (Na_2SO_4), evaporated, co-evaporated with toluene (3×30 mL) and the residue was chromatographed on silica gel column using stepwise gradient $CHCl_3$–toluene 1:2→1:1→2:1→$CHCl_3$+1% Py (v/v/v). Fractions containing the product were combined, evaporated and the residue was dried in vacuo to afford **4a** as a white foam (3.6 g, 81%), R_f 0.16 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=709.2869 $[M+Na]^+$, calcd for $[C_{42}H_{42}N_2O_7+Na]^+$ 709.2884. ^1H NMR (DMSO-d_6) δ 7.88 (d, 2H, *J*=7.5 Hz, Ar*H* (fluorene H-4, H-5)), 7.78 (br s, 1H CHCON*H*), 7.67 (d, *J*=7.3 Hz, Ar*H*, fluorene, H-1, H-8), 7.41–7.12 (m, 14H, Ar*H* (Dmt+fluorene H-2, H-3, H-6, H-7), OCON*H*), 6.86 (d, 4H, *J*=8.9 Hz, Ar*H* (Dmt)), 5.37 (d, 1H, *J*=5.3 Hz, CHO*H*, exchangeable with D_2O), 4.29 (d, 2H, *J*=6.7 Hz, CH_2OCO), 4.18 (t, 1H, *J*=6.7 Hz, fluorene H-9), 3.96 (m, 1H, C*H*O) (in the presence of D_2O gives dd J_1=3.5 Hz, J_2=8.4 Hz), 3.72 (s, 6H, OCH_3), 3.16–2.98 (m, 6H, NCH_2CH_2, CH_2ODmt), 1.98 (m, 1H), 1.70 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.17, 174.11 (HN*C*=O), 158.02, 158.00, 156.20 (O*C*=O), 149.69, 149.66, 149.64, 145.31, 143.95, 142.63, 140.79, 139.47, 137.48, 136.16, 136.11, 129.67, 128.97, 128.95, 128.25, 127.79, 127.73, 127.65, 127.34, 127.31, 127.11, 126.57, 125.36, 125.17, 123.94, 123.92, 121.42, 121.40, 120.15, 120.07, 120.05, 113.15, 109.76, 85.35, 68.65, 59.77, 55.05 (OMe), 46.79, 34.74, 21.08.

4.1.8. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide (4b). Diol **3b** (2.10 g, 5 mmol) was tritylated in a similar manner with DmtCl (1.90 g, 5.5 mmol). Yield 3.50 g (97%), white foam, R_f 0.22 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=737.3205 $[M+Na]^+$, calcd for $[C_{44}H_{46}N_2O_7+Na]^+$ 737.3197. ^1H NMR (DMSO-d_6) δ 7.88 (d, 2H, *J*=7.3 Hz, Ar*H* (fluorene H-4, H-5)), 7.71 (br s, 1H, CHCON*H*), 7.66 (d, 2H, *J*=7.3 Hz, Ar*H* (fluorene H-1, H-8), 7.43–7.16 (m, 14H, Ar*H* (fluorene H-2, H-3, H-6, H-7, Dmt), OCON*H*), 6.86 (d, 4H, *J*=8.9 Hz, Ar*H* (Dmt)), 5.36 (d, 1H, *J*= 5.3 Hz, O*H*, exchangeable with D_2O), 4.28 (m, 2H, CH_2OCO), 4.21 (m, 1H, fluorene H-9), 3.80 (d, 1H, *J*= 5.3 Hz, C*H*O), 3.72 (s, 6H, OCH_3), 3.17–2.98 (m, 5H, NCH_2CH_2, CH*H*ODmt), 2.75 (d, 1H, ^2J=8.3 Hz, CH*H*ODmt), 0.92 (s, 3H), 0.78 (s, 3H) (C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ 172.73 (HN*C*=O), 157.97, 157.95, 157.93, 156.22 (O*C*=O), 149.66, 145.49, 143.94, 140.78, 136.21, 136.16, 136.11, 129.87, 129.83, 128.96, 128.94, 128.24, 127.88, 127.69, 127.65, 127.33, 127.09, 126.50, 125.36, 125.15, 123.94, 121.42, 120.16, 113.03, 84.95, 75.27, 68.36, 65.40, 55.05, 55.03, 55.00 (OMe), 46.78, 38.60, 38.12, 22.21, 21.08, 20.51.

4.1.9. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-methyl-*N*-(2-[methyl-*N*′-(9-fluorenylmethoxycarbonyl)amino]-ethyl)-2,4-dihydroxybutyramide (4c). Compound **4c** was prepared from **3c** (1.48 g, 3.6 mmol) and DmtCl (1.37 g, 3.95 mmol). The product was purified by column chromatography in $CHCl_3$–toluene→1:4→1:1→2:1→$CHCl_3$+1% Py (v/v/v). Yield 2.28 g (89%), white foam, R_f 0.27 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=737.3202 $[M+Na]^+$, calcd for $[C_{44}H_{46}N_2O_7+Na]^+$ 737.3197. ^1H NMR (DMSO-d_6) δ 7.87 (m, 2H, Ar*H* (fluorene H-4, H-5)), 7.58 (m, 2H, Ar*H* (fluorene H-1, H-8)), 7.42–7.12 (m, 13H, Ar*H* (fluorene H-2, H-3, H-6, H-7, Dmt)), 6.84 (m, 4H, Ar*H* (Dmt)), 4.79 (m, 1H, O*H*, exchangeable with D_2O), 4.63–4.20 (m, 4H, C*H*O, C*H*CH_2OCO), 3.72 (s, 6H, OCH_3), 3.55–2.55 (m, 12H,‡ N(CH_3)CH_2CH_2NCH_3, CH_2ODmt), 1.83–1.43 (m, 2H, CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 173.00, 172.93 (MeN*C*=O), 158.03, 157.68 (O*C*=O), 143.97, 143.90, 143.85, 142.63, 140.81, 139.47, 137.48, 136.06, 136.00, 129.65, 128.96, 128.94, 128.24, 127.80, 127.70, 127.65, 127.33, 127.12, 126.61, 125.35, 124.95, 121.41, 120.11, 120.06, 120.04, 113.15, 113.11, 109.76, 85.46, 65.06, 64.98, 59.87, 55.06, 55.05, 55.02 (OMe), 34.45, 21.08.

4.1.10. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-methyl-*N*-(2-[*N*′-methyl-*N*′-trifluoroacetylamino]ethyl)-2,4-dihydroxybutyramide (4d). The crude diol **2c** (0.75 g, 4 mmol) was co-evaporated with dry MeOH (20 mL), dissolved in the mixture of MeOH (10 mL), Et_3N (0.6 mL, 4 mmol) and CF_3CO_2Me (2.0 mL 10 mmol). The mixture was stirred at ambient temperature for 30 min and evaporated to give crude **3d**. This was co-evaporated with pyridine and tritylated with DmtCl (2.02 g, 6.0 mmol) as above. The product **4d** was chromatographed on silica gel eluting with $CHCl_3$–toluene 1:2→1:1→$CHCl_3$+0.25% Et_3N (v/v/v). Yield 1.44 g (93%) as a white foam, R_f 0.23 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=611.2331 $[M+Na]^+$, calcd for $[C_{31}H_{35}F_3N_2O_6+Na]^+$ 611.2339. ^1H NMR (DMSO-d_6) δ 7.38–7.21 (m, 9H, Ar*H* (Dmt)), 6.87 (d, 4H, *J*=8.7 Hz, Ar*H* (Dmt)), 5.07 (d, 0.04H, *J*=7.8 Hz), 4.91 (d, 0.16H, *J*=7.8 Hz), 4.75 (d, 0.19H, *J*=7.8 Hz), 4.59 (d, 0.61H, *J*=8.0 Hz) (O*H*, exchangeable with D_2O), 4.42 (m, 1H, C*H*O), 3.73 (s, 6H, OCH_3), 3.80–3.26 (m, 4H,‡ CH_2O, CH_2NTfa), 3.15–2.78 (m, 8H, N(CH_3)CH_2CH_2N(CH_3)Tfa),

1.86–1.50 (m, 2H, CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 173.90, 173.58, 172.96 (MeNC=O), 158.05, 156.13, 155.85, 155.57, 145.24, 145.18, 136.03, 129.62, 128.93, 128.24, 127.81, 127.77, 127.72, 127.66, 126.62, 125.35, 117.49, 115.18, 113.17, 113.13, 85.46, 65.13, 64.98, 59.86, 55.05 (OMe), 45.94, 45.76, 43.76, 40.19, 40.02, 39.86, 35.39, 35.03, 34.85, 34.82, 34.80, 34.53, 34.44, 34.36, 33.46.

4.1.11. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxybutyramide (5a). Compound **4a** (1.38 g, 2 mmol) was co-evaporated with dry DCM (2×20 mL), dissolved in dry DCM, diisopropylammonium tetrazolide (0.51 g, 3 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (1 mL, 3 mmol) were added, and the mixture was stirred under argon for 2 h. After conversion of the starting compound is complete (monitoring by TLC, $CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v) the mixture was diluted with EtOAc and washed with 5% $NaHCO_3$ (2×100 mL) and 20% NaCl (100 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and the residue was dissolved in 30 mL DCM and precipitated into cold (−10 °C) hexane (500 mL), the solid was filtered off, co-evaporated with dry DCM (3×20 mL) and dried in vacuo to afford phosphoramidite **5a** (1.51 g, 85%) as a white foam, R_f 0.34, 0.43 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m*/*z*=909.4066 $[M+Na]^+$, calcd for $[C_{51}H_{59}N_4O_8P+Na]^+$ 909.3963. ^{31}P NMR (MeCN-d_3) δ 151.13, 148.73 (diastereomers, ∼1:2). ^1H NMR (MeCN-d_3) δ 7.85–7.19 (m, 18H, Ar*H* (fluorene, Dmt), CCON*H*), 6.94 (m, 1H, OCON*H*), 6.84 (m, 4H, Ar*H* (Dmt)), 4.35–4.01 (m, 4H, C*H*CH_2OCO, C*H*O), 3.76 (m, 6H, OCH_3), 3.65–2.48 (m, 12H, NCH_2CH_2, $POCH_2CH_2$, NC*H*, CH_2ODmt), 2.18–2.00 (m, 2H, CH_2CH_2CH), 1.30–0.96 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 173.57, 173.27, 173.16 (HNC=O), 159.49, 157.49 (OC=O), 146.37, 145.14, 142.08, 137.31, 130.86, 128.96, 128.67, 128.63, 128.06, 127.60, 126.34, 126.10, 120.91, 113.91, 86.88, 86.77, 72.09, 66.98, 60.55, 60.26, 59.74, 59.59, 59.12, 58.07, 55.81 (OMe), 55.24, 48.09, 47.19, 45.96, 43.99, 43.89, 41.49, 39.73, 35.29, 34.84, 24.87, 23.12, 23.05, 21.91, 20.86, 20.51, 19.99.

4.1.12. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide (5b). Compound **4b** (1.87 g, 2.6 mmol) was phosphitylated as above with diisopropylammonium tetrazolide (0.67 g, 3.9 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (1.27 mL, 3.9 mmol) to afford **5b** (1.60 g, 67%) as a white foam. ESI-TOF HRMS: *m*/*z*=937.4284 $[M+Na]^+$, calcd for $[C_{53}H_{63}N_4O_8P+Na]^+$ 937.4276. ^{31}P NMR (MeCN-d_3) δ 152.38, 149.04 (diastereomers, ∼1:2). ^1H NMR (MeCN-d_3) δ 7.84 (d, 2H, *J*=7.4 Hz, Ar*H* (fluorene H-4, H-5)), 7.66 (m, 2H, Ar*H* (fluorene H-1, H-8)), 7.48–7.22 (m, 15H, Ar*H* (fluorene H-2, H-3, H-6, H-7, Dmt), CHCON*H*, OCON*H*), 6.86 (m, 4H, Ar*H* (Dmt)), 4.37–4.31 (m, 2H, CH_2OCO), 4.23 (m, 1H, fluorene H-9), 4.03–3.92 (m, 1H, C*H*O), 3.77 (c, 6H, OCH_3), 3.73–2.70 (m, 10H, NCH_2CH_2, $POCH_2$, NC*H*, CH_2ODmt), 2.46 (t, 0.8H, *J*=6.0 Hz), 2.29 (t, 1.2H, *J*=6.0 Hz) (CH_2CN, diastereomers), 1.28–0.95 (m, 18H, $CH_2C(CH_3)_2CH$, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 171.71 (HNC=O), 159.46, 157.51 (OC=O), 146.53, 145.17, 142.08, 137.23, 131.21, 131.13, 129.19, 129.08, 128.61, 128.05, 127.55, 126.05, 120.91, 119.41, 113.78, 86.54, 78.96, 69.24, 68.85, 66.99, 66.87, 59.79, 59.64, 59.06, 55.81 (OMe), 55.24, 48.06, 47.27, 45.91, 44.18, 44.04, 43.94, 41.48, 40.06, 39.78, 25.03, 24.83, 23.05, 22.83, 22.24, 21.41, 20.87, 20.19.

4.1.13. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-methyl-*N*-(2-[methyl(trifluoroacetyl)amino]ethyl)-2,4-dihydroxybutyramide (5c). Compound **4d** (1.18 g, 2.0 mmol) was phosphitylated with diisopropylammonium tetrazolide (510 mg, 3.0 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (1.0 mL, 3 mmol) under argon for 2 h. The conversion of the starting material was monitored by TLC ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). The compound was precipitated from DCM (30 mL) into cold hexane. Yield 1.18 g (77%), white foam, R_f 0.29, 0.39 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m*/*z*=811.3442 $[M+Na]^+$, calcd for $[C_{40}H_{52}F_3N_4O_7P+Na]^+$ 811.3418. ^{31}P NMR (MeCN-d_3) δ 150.42 (0.19P), 150.03 (0.36P), 149.86 (0.11P), 149.30 (0.34P). ^1H NMR (MeCN-d_3) δ 7.49–7.20 (m, 9H, Ar*H* (Dmt)), 6.89 (m, 4H, Ar*H* (Dmt)), 4.88–4.64 (m, 0.44H), 4.20–4.02 (m, 0.56H) (C*H*O), 3.79 (m, 6H, OCH_3), 3.71–2.48 (m, 18H, $POCH_2CH_2$, NC*H*, CH_2ODmt, $CH_3NCH_2CH_2NCH_3$), 2.14–1.81 (m, 2H,‡ CH_2CH_2CH), 1.29–1.03 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 172.59 (MeNC=O), 159.57, 146.41, 146.11, 137.23, 130.85, 128.87, 128.72, 127.68, 119.49, 113.95, 87.04, 86.88, 69.15, 60.88, 60.49, 59.61, 59.45, 59.28, 59.12, 55.83, 55.24 (OMe), 47.18, 46.72, 45.97, 45.22, 43.95, 43.85, 35.83, 35.68, 34.95, 24.90, 24.80, 24.74, 23.13, 23.06, 20.88.

4.1.14. (2*R*)-*N*-[2-(Imidazol-4-yl)ethyl]-2,4-dihydroxybutyramide (7a). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) and histamine (1.17 g, 10 mmol) in EtOH (10 mL) was kept at 55 °C for 24 h. The solid formed was filtered off, washed with EtOH (5 mL), Et_2O (2×5 mL) and dried in vacuo to afford **7a** (1.77 g, 83%) as white crystals, mp 149.0–149.5 °C (EtOH). ESI-TOF HRMS: *m*/*z*=214.1187 $[M+H]^+$, calcd for $[C_9H_{15}N_3O_3+H]^+$ 214.1186. IR (KBr): 3392 cm^{-1}, ν (OH); 3164 cm^{-1}, ν (NH); 2904 cm^{-1}, ν (sp^3-CH); 1644 cm^{-1}, ν (C=O); 1533 cm^{-1}, ν (NH). ^1H NMR (CD_3OD) δ 7.61 (m, 1H, imidazole H-2), 6.89 (br s, 1H, imidazole H-5), 4.14 (dd, 1H, J_1=3.7 Hz, J_2=8.6 Hz, C*H*O), 3.71 (m, 2H, CH_2O), 3.51 (t, 2H, *J*=7.0 Hz, NCH_2), 2.82 (t, 2H, *J*=7.0 Hz, NCH_2CH_2), 2.01 (m, 1H), 1.74 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (CD_3OD) δ 177.21 (*C*O), 136.13 (3C, imidazole C-2, C-4, C-5), 70.56 (*C*HOH), 59.57 (CH_2OH), 39.88, 38.23 (NCH_2CH_2), 27.80 (CH_2CH_2CH).

4.1.15. (2*R*)-*N*-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxybutyramide (8a). To a stirred solution of diol **7a** (0.745 g, 3.5 mmol) in 50% aq dioxan (50 mL), Boc_2O (0.839 g, 3.8 mmol) was added at room temperature and stirring was continued for 5 h. After completion of the reaction (monitored by TLC, $CHCl_3$–MeOH 4:1 v/v), the solvent was evaporated, and the residue was chromatographed on a silica gel column using stepwise gradient elution with 0→2→4→6→8% MeOH in $CHCl_3$. Yield 0.83 g (76%), white crystals, mp 92.0–93.0 °C. R_f 0.37

($CHCl_3$–MeOH 5:1 v/v). ESI-TOF HRMS: m/z=314.1715 $[M+H]^+$, calcd for $[C_{14}H_{23}N_3O_5+H]^+$ 314.1710. IR (KBr): 3428 cm^{-1}, ν (OH); 3316 cm^{-1}, 3103 cm^{-1}, ν (NH); 2938 cm^{-1}, ν (sp^3-CH); 1653 cm^{-1}, ν (C=O); 1536 cm^{-1}, ν (NH). ^1H NMR (DMSO-d_6) δ 8.10 (br s, 1H, imidazole H-2), 7.79 (m, 1H, CON*H*, exchangeable with D_2O), 7.28 (br s, 1H, imidazole H-5), 5.41 (d, 1H, J=5.5 Hz, CHO*H*), 4.39 (t, 1H, J=5.3 Hz, CH_2O*H*) (both exchangeable with D_2O), 3.91 (m, 1H, C*H*O), 3.48 (m, 2H, CH_2O), 3.33 (m, 2H,‡ NCH_2), 2.63 (t, 2H, J=7.0 Hz, NCH_2CH_2), 1.81 (m, 1H, CH_2CH*H*CH), 1.52 (m, 10H, CH_3 (Boc), CH_2CH*H*CH). ^{13}C NMR (DMSO-d_6) δ 174.53 (HN*C*=OC), 147.19 (N*C*OO), 141.45 (imidazole C-4), 137.14 (imidazole C-2), 113.86 (imidazole C-4), 85.48 ($C(CH_3)_3$), 69.01 (*C*HOH), 57.91 (CH_2OH), 37.97 (2C, NCH_2CH_2), 28.22 (CH_2CH_2CH), 27.85 (3C, CH_3).

4.1.16. (2*R*)-*N*-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (8b1) and (2*R*)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-5-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (8b2). A solution of (*R*)-(−)-pantolactone (4.3 g, 33 mmol) and histamine (3.3 g, 30 mmol) in abs EtOH (20 mL) was kept at 55 °C for 72 h, then evaporated, co-evaporated with toluene to afford (2*R*)-*N*-[2-(imidazol-4-yl)ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide **7b** as a yellow oil. The product was used without further purification. ESI-TOF HRMS: m/z=242.1501 $[M+H]^+$, calcd for $[C_{11}H_{19}N_3O_3+H]^+$ 242.1499. The crude **7b** was treated with Boc_2O (7.20 g, 33 mmol) in 50% aq dioxane (50 mL) as above to afford two isomers, **8b1** (3.88 g, 38%) and **8b2** (1.75 g, 17%) as white solids, R_f 0.54 and 0.48, correspondingly ($CHCl_3$–MeOH 4:1 v/v). 'Fast moving' product **8b1**: ESI-TOF HRMS: m/z=342.2019 $[M+H]^+$, calcd for $[C_{16}H_{27}N_3O_5+H]^+$ 342.2023. ^1H NMR (DMSO-d_6) δ 8.10 (br s, 1H, imidazole H-2), 7.75 (m, 1H, CON*H*, exchangeable with D_2O), 7.29 (br s, 1H, imidazole H-5), 5.31 (d, 1H, J=5.5 Hz, CHO*H*), 4.43 (t, 1H, J=5.4 Hz, CH_2O*H*) (both exchangeable with D_2O), 3.69 (d, 1H, J=5.5 Hz, C*H*O), 3.44–3.14 (m, 4H, CH_2O (^2J=10.3 Hz, J_{HCOH}=5.4 Hz), NCH_2), 2.64 (m, 2H, NCH_2CH_2), 1.55 (s, 9H, CH_3 (Boc)), 0.77 (s, 3H), 0.75 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (DMSO-d_6) δ 173.30 (HN*C*=OC), 147.19 (N*C*OO), 141.47 (imidazole C-4), 137.13 (imidazole C-2), 113.94 (imidazole C-4), 85.43 ($C(CH_3)_3$), 75.58 (*C*HOH), 68.52 (CH_2OH), ~40,‡ 37.97 (NCH_2CH_2), 28.23 (CH_2CCH), 27.85 (3C, CH_3), 21.29, 20.75 ($C(CH_3)_2$). 'Slow moving' product **8b2**: ESI-TOF HRMS: m/z= 342.2013 $[M+H]^+$, calcd for $[C_{16}H_{27}N_3O_5+H]^+$ 342.2023. The compound purified by column chromatography was kept at 4 °C for one month before recording its NMR spectrum. NMR (as well as TLC) showed considerable $\pi\rightarrow\tau$ Boc migration (conversion >50%). ^1H NMR (DMSO-d_6) of 'slow moving' product **8b2** (deduced from NMR spectrum of the mixture of **8b1** and **8b2**) δ 8.08 (br s, 1H, imidazole H-2), 7.76 (m, 1H, CON*H*, exchangeable with D_2O), 6.79 (br s, 1H, imidazole H-4), 5.31 (d, 1H, J=5.5 Hz, CHO*H*), 4.44 (t, 1H, J=5.4 Hz, CH_2O*H*) (both exchangeable with D_2O), 3.70 (d, 1H, J=5.5 Hz, C*H*O), 3.45–3.13 (m, 4H,‡ CH_2O, NCH_2), 2.91 (m, 2H, NCH_2CH_2), 1.57 (s, 9H, CH_3 (Boc)), 0.77 (s, 3H), 0.76 (s, 3H) ($C(CH_3)_2$).

4.1.17. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxybutyramide (9a). Diol **8a** (0.78 g, 2.5 mmol) was tritylated with DmtCl (0.93 g, 2.75 mmol) according to the procedure for **4a**. The product was purified by chromatography using a stepwise gradient $CHCl_3$–toluene 1:2→1:1→2:1→$CHCl_3$+ 0.25% Py (v/v/v), then 10%→50% EtOAc in $CHCl_3$+ 0.25% Py (v/v/v). Yield 1.31 g (86%), white foam, R_f 0.46 ($CHCl_3$–MeOH 20:1+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=638.2766 $[M+Na]^+$, calcd for $[C_{35}H_{41}N_3O_7+Na]^+$ 638.2837. ^1H NMR (DMSO-d_6) δ 8.10 (br s, 1H, imidazole H-2), 7.80 (m, 1H, CON*H* (exchangeable with D_2O)), 7.37–7.22 (m, 10H, Ar*H* (Dmt), imidazole H-5), 6.87 (d, 4H, J=8.9 Hz, Ar*H* (Dmt)), 5.40 (d, 1H, J=5.7 Hz, CHO*H*, exchangeable with D_2O), 3.98 (m, 1H, C*H*O), 3.72 (s, 6H, OCH_3), 3.30 (m, 2H‡), 3.04 (m, 2H) (CH_2N, CH_2O), 2.58 (t, 2H, J=7.0 Hz, NCH_2CH_2), 1.96 (m, 3H), 1.69 (m, 3H) (CH_2CH_2CH), 1.55 (s, 9H, CH_3 (Boc)). ^{13}C NMR (DMSO-d_6) δ 173.76 (HN*C*=O), 158.01, 146.80, 145.29, 141.03, 136.74, 136.10, 136.07, 129.66, 128.94, 128.24, 127.78, 127.72, 126.56, 125.36, 113.42, 113.14, 85.35, 85.08, 68.60, 59.73, 55.05 (OMe), 37.60, 34.77, 27.81, 27.49, 27.48, 27.45, 27.43, 27.41 (CMe_3), 21.08.

4.1.18. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (9b1) and (2*R*)-O^4-(4,4′-dimethoxytrityl)-*N*-{2-[1-(*tert*-butyloxycarbonyl)imidazol-5-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (9b2). The 'fast moving' product **8b1** (3.7 g, 11 mmol) was tritylated with DmtCl (4.15 g, 12 mmol) as described above and isolated from silica gel column eluted with $CHCl_3$–toluene 1:2→1:1→2:1→$CHCl_3$+0.25% Py (v/v/v), then 2.5%→5% EtOAc in $CHCl_3$+0.25% Py (v/v/v). Fractions containing products were combined, evaporated and the residues were dried in vacuo to afford **9b1** (3.95 g, 55%) and **9b2** (2.60 g, 37%) as white foams, R_f 0.53 and 0.47, correspondingly ($CHCl_3$–MeOH 20:1+1% Et_3N v/v/v). 'Fast moving' product **9b1**: ESI-TOF HRMS: m/z=666.3163 $[M+Na]^+$, calcd for $[C_{37}H_{45}N_3O_7+Na]^+$ 666.3150. ^1H NMR (MeCN-d_3) δ 7.96 (m, 1H, imidazole H-2), 7.48–7.15 (m, 10H, Ar*H* (Dmt) imidazole H-5), 6.94 (br s, 1H, CON*H* (exchangeable with D_2O)), 6.86 (d, 4H, J=8.8 Hz, Ar*H* (Dmt)), 3.94 (d, 1H, J=5.7 Hz, O*H* (exchangeable with D_2O)), 3.78 (s, 6H, OCH_3), 3.67 (d, 1H, J=5.7 Hz, C*H*O), 3.37 (m, 2H, CH_2N), 3.07 (d, 1H, ^2J=8.7 Hz), 2.85 (d, 1H, ^2J=8.7 Hz) (CH_2O), 2.61 (m, 2H, NCH_2CH_2), 1.60 (s, 9H, CH_3 (Boc)), 0.96 (s, 3H), 0.88 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (MeCN-d_3) δ 172.96 (HN*C*=O), 158.47, 145.98, 145.46, 141.37, 136.89, 136.58, 136.39, 129.90, 128.78, 128.55, 127.83, 127.72, 126.91, 125.40, 113.88, 113.69, 86.55, 86.38, 68.85, 59.37, 55.03 (OMe), 38.43, 34.87, 28.01, 27.87, 27.63, 27.57, 27.39, 27.31 (CMe_3), 21.29, 21.18, 19.95. Compound **9b2** was stored at 4 °C for one month before recording its NMR spectrum. NMR and TLC showed complete conversion **9b2**→**9b1** during storage.

4.1.19. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxybutyramide (10a). Compound **9a** (1.23 g, 2.0 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.95 mL, 3 mmol) and diisopropylammonium tetrazolide (510 mg, 3.0 mmol) to afford phosphoramidite **10a** (1.29 g, 80%) as a white foam, R_f 0.37, 0.26 ($CHCl_3$–EtOAc

1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=838.3908 $[M+Na]^+$, calcd for $[C_{44}H_{58}N_5O_8P+Na]^+$ 838.3915. ^{31}P NMR (MeCN-d_3) δ 150.61, 148.64 (diastereomers, ~9:10). ^1H NMR (MeCN-d_3) δ 8.01 (br s, 1H, imidazole H-2), 7.46–7.17 (m, 10H, Ar*H* (Dmt), imidazole H-5), 7.07 (m, 0.59H), 7.01 (m, 0.41H) (CON*H*, diastereomers), 6.87 (m, 4H, Ar*H* (Dmt)), 4.31 (m, 0.41H), 4.24 (m, 0.59H) (C*H*O, diastereomers), 3.78 (s, 6H, OCH_3), 3.67–2.51 (m, 12H, CH_2O, NCH_2CH_2, $POCH_2CH_2$, NC*H*), 2.18–2.00 (m, 2H, CH_2CH_2CH), 1.61 (s, 9H, CH_3 (Boc)), 1.27–0.98 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 170.23 (HN*C*=O), 158.87, 147.16, 145.97, 141.67, 137.12, 136.19, 130.27, 129.71, 128.47, 127.98, 127.36, 126.87, 119.99, 113.88, 113.11, 85.44, 77.77, 68.32, 59.12, 58.84, 55.14 (OMe), 44.88, 42.52, 42.35, 38.05, 37.86, 27.54 (CMe_3), 24.78, 24.17, 23.06, 22.24, 22.08, 19.97.

4.1.20. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (10b). Compound **9b1** (3.22 g, 5.0 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (2.38 mL, 7.5 mmol) and diisopropylammonium tetrazolide (1.28 g, 7.5 mmol) to give compound **10b** as a white foam (2.36 g, 60%), R_f 0.38, 0.30 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=866.4238 $[M+Na]^+$, calcd for $[C_{46}H_{62}N_5O_8P+Na]^+$ 866.4228. ^{31}P NMR (MeCN-d_3) δ 152.29, 149.11 (diastereomers, ~9:10). ^1H NMR (MeCN-d_3) δ 7.97 (br s, 1H, imidazole H-2), 7.49–7.18 (m, 11H, Ar*H* (Dmt), imidazole H-5, CON*H*), 6.87 (m, 4H, Ar*H* (Dmt)), 3.97 (d, 0.55H, J_{HCOP}=12.9 Hz), 3.93 (d, 0.45H, J_{HCOP}=9.9 Hz) (C*H*O, diastereomers), 3.79 (m, 6H, OCH_3), 3.75–2.51 (m, 12H, CH_2O, NCH_2CH_2, $POCH_2CH_2$, NC*H*), 1.59 (s, 9H, CH_3 (Boc)), 1.27–0.94 (m, 18H, $C(CH_3)_2$, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 169.16 (HN*C*=O), 157.99, 146.76, 145.27, 140.91, 136.72, 135.88, 129.94, 129.81, 127.93, 127.80, 127.67, 126.54, 118.79, 113.58, 112.98, 85.08, 77.31, 67.91, 58.62, 58.23, 55.03 (OMe), 44.58, 42.82, 42.55, 37.76, 37.53, 27.43 (CMe_3), 24.27, 24.08, 22.66, 22.16, 21.96, 21.10, 20.67, 19.81, 19.42.

4.1.21. (2*R*)-*N*-(Pyren-1-ylmethyl)-2,4-dihydroxybutyramide (11a). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) and 1-pyrenemethylamine (2 g, 9 mmol) in ethanol (5 mL) was kept at 55 °C for one week, evaporated and co-evaporated with toluene. The residue was triturated in EtOAc–toluene and dried in vacuo to yield amide **11a** (2.62 g, 88%) as white crystals, mp 162.0–164.0 °C (EtOAc–toluene). ESI-TOF HRMS: *m/z*=356.1260 $[M+Na]^+$, calcd for $[C_{21}H_{19}NO_3+Na]^+$ 356.1257. IR (KBr): 3330 cm^{-1}, ν (OH); 3273 cm^{-1}, ν (NH); 3040 cm^{-1}, ν (sp^2-CH); 1642 cm^{-1}, ν (C=O); 1537 cm^{-1}, ν (NH); 1044 cm^{-1}, ν (C–O); 839 cm^{-1}, ν (ArH). ^1H NMR (DMSO-d_6) δ 8.42 (m, 2H, Ar*H* (H-10), N*H*), 8.33–8.04 (m, 8H, Ar*H*), 5.52 (d, 1H, *J*=5.1 Hz, CHO*H*, exchangeable with D_2O), 5.05 (d, 2H, *J*=5.1 Hz, NCH_2), 4.43 (m, 1H, CH_2OH, exchangeable with D_2O), 4.07 (m, 1H, C*H*O) (in the presence of D_2O gives dd with J_1=3.1 Hz and J_2=7.1 Hz), 3.53 (m, 2H, CH_2O), 1.92 (m, 1H), 1.64 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.68 (CO), 133.61, 131.25, 130.76, 130.44, 128.41, 127.90, 127.83, 127.39, 126.90, 126.65, 125.62, 125.56, 125.11, 124.44 (2C), 123.69 (pyrene), 69.19 (*C*HOH), 57.94 (CH_2OH), ~40‡ (NCH_2), 38.22 (CCH_2C).

4.1.22. (2*R*)-*N*-(Pyren-1-ylmethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (11b). A solution of (*R*)-(−)-pantolactone (3.25 g, 25 mmol) and 1-pyrenemethylamine (4.6 g, 20 mmol) in acetone (50 mL) was kept at 55 °C for 7 days, then evaporated and chromatographed on silica gel column using stepwise gradient $CHCl_3$–toluene 1:1→2:1→$CHCl_3$ (v/v), then 0.5%→1%→2% MeOH in $CHCl_3$ (v/v). Fractions containing the product were combined, evaporated and the residue was dried in vacuo to afford pure **11b** (6.64 g, 92%) as a yellow foam, R_f 0.24 ($CHCl_3$–MeOH 4:1 (v/v)). ESI-TOF HRMS: *m/z*=384.1577 $[M+Na]^+$, calcd for $[C_{23}H_{23}NO_3+Na]^+$ 384.1570. IR (KBr): 3396 cm^{-1}, ν (OH); 3041 cm^{-1}, ν (sp^2-CH); 1649 cm^{-1}, ν (C=O); 1529 cm^{-1}, ν (NH); 1041 cm^{-1}, ν (C–O); 841 cm^{-1}, ν (Ar-H). ^1H NMR (DMSO-d_6) δ 8.49 (d, 1H, *J*=8.1 Hz, Ar*H* (H-10)), 8.38 (m, 1H, N*H*, exchangeable with D_2O), 8.32–8.06 (m, 8H, Ar*H*), 5.43 (d, 1H, *J*=4.1 Hz, CHO*H*, exchangeable with D_2O), 5.04 (m, 2H, J_{HNCH}=5.1 Hz, ^2J=12.2 Hz, NCH_2), 4.45 (m, 1H, CH_2OH, exchangeable with D_2O), 3.85 (d, *J*=4.1 Hz, C*H*O), 3.30 (m, 1H‡), 3.20 (m, 1H) (CH_2O, in the presence of D_2O gives two d, ^2J=9.2 Hz), 0.82 (s, 3H), 0.79 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (DMSO-d_6) δ 173.38 (CO), 133.67, 131.24, 130.78, 130.47, 128.47, 127.83 (2C), 127.40, 126.84, 126.63, 125.60 (2C), 125.04, 124.45 (2C), 123.93 (pyrene), 75.71 (*C*HOH), 68.46 (CH_2OH), ~40‡ (NCH_2), 37.21 ($C(CH_3)_2$), 21.51, 20.83 (CH_3).

4.1.23. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxybutyramide (12a). Diol **11a** (1.29 g, 3.9 mmol) was tritylated with DmtCl (1.40 g, 4.1 mmol) as described for **4a**. The product was purified by chromatography in $CHCl_3$–toluene 1:21:1→2:1→$CHCl_3$+0.25% Py (v/v/v), then 10%→50% EtOAc in $CHCl_3$+0.25% Py (v/v/v). Yield 1.33 g (62%), white foam, R_f 0.28 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=658.2572 $[M+Na]^+$, calcd for $[C_{42}H_{37}NO_5+Na]^+$ 658.2564. ^1H NMR (DMSO-d_6) δ 8.42–7.99 (m, 10H, N*H*, Ar*H* (pyrene)), 7.36–7.13 (m, 9H, Ar*H* (Dmt)), 6.81 (m, 4H, Ar*H* (Dmt)), 5.50 (d, 1H, *J*=4.1 Hz, O*H*, exchangeable with D_2O), 5.01 (m, 2H, J_{HNCH}=5.1 Hz, ^2J=12.2 Hz, NCH_2), 4.12 (m, 1H, C*H*O) (in the presence of D_2O gives dd with J_1=3.1 Hz and J_2=6.1 Hz), 3.69 (s, 6*H*, CH_3), 3.07 (m, 2H, CH_2O), 2.04 (m, 1H), 1.82 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 173.80 (CO), 157.98, 145.33, 137.39, 136.13, 136.07, 133.11, 130.85, 130.35, 130.05, 129.65, 128.94, 128.24, 128.03, 127.78, 127.69, 127.49, 127.43, 127.01, 126.60, 126.55, 126.25, 125.35, 125.24, 125.17, 124.68, 124.06, 124.01, 123.29, 113.10, 85.36, 68.73 (*C*HOH), 59.68, 55.01 (OMe), 34.88 (CCH_2C).

4.1.24. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (12b). Diol **11b** (6.48 g, 18 mmol) was tritylated with DmtCl (6.80 g, 20 mmol) as above. The compound was chromatographed on a silica gel column eluted with 2.5%→5%→7.5%→10%→12.5%→15% EtOAc in toluene+0.25% Et_3N (v/v/v). Yield 10.0 g (83%), yellow foam, R_f 0.35

($CHCl_3$+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=686.2872 $[M+Na]^+$, calcd for $[C_{44}H_{41}NO_5+Na]^+$ 686.2877. ^1H NMR (DMSO-d_6) δ 8.46–8.02 (m, 10H, N*H*, Ar*H* (pyrene)), 7.40–7.16 (m, 9H, ArH (Dmt)), 6.83 (d, 4H, J=7.1 Hz, Ar*H* (Dmt)), 5.46 (d, 1H, J=4.1 Hz, O*H*, exchangeable with D_2O), 4.99 (m, 2H, NCH_2), 3.92 (m, 1H, C*H*O), 3.70 (s, 6H, OCH_3), 3.01 (d, 1H, ^2J=7.1 Hz), 2.78 (d, 1H, ^2J=7.1 Hz) (CH_2O), 0.93 (s, 3H), 0.82 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (DMSO-d_6) δ 172.47 (CO), 157.93, 145.50, 136.19, 136.12, 133.25, 130.84, 130.36, 130.04, 129.85, 128.94, 128.24, 128.02, 127.85, 127.69, 127.42, 126.29, 126.89, 126.48, 126.23, 125.35, 125.21, 125.15, 124.63, 124.05, 124.01, 123.46, 113.01, 84.95, 75.31, 68.42 (*C*HOH), 55.01 (OMe), 38.91 ($CCMe_2C$), 22.30, 20.60 ($CC(CH_3)_2C$).

4.1.25. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxybutyramide (13a). Compound **12a** (0.95 g, 1.5 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.73 mL, 2.25 mmol) and diisopropylammonium tetrazolide (0.38 g, 2.25 mmol) to afford **13a** (0.945 g, 70%) as a white foam, R_f 0.54, 0.62 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=858.3640 $[M+Na]^+$, calcd for $[C_{51}H_{54}N_3O_6P+Na]^+$ 858.3642. ^{31}P NMR (MeCN-d_3) δ 150.71, 149.03 (diastereoisomers, ~1:1). ^1H NMR (MeCN-d_3) δ 8.29–7.90 (m, 9H, Ar*H* (pyrene)), 7.42–7.12 (m, 10H, N*H*, Ar*H* (Dmt)), 6.81 (m, 4H, Ar*H* (Dmt)), 5.10–5.04 (m, 2H, NCH_2), 4.44 (m, 0.5H), 4.37 (m, 0.5H) (C*H*O, diastereomers), 3.70 (c, 6*H*, CH_3), 3.57–2.83 (m, 6H, $POCH_2$, NC*H*, CH_2O), 2.46 (t, 1H, J=6.0 Hz), 2.29 (t, 1H, J=6.0 Hz) (CH_2CN, diastereomers), 2.18 (m, 2H, CH_2CH_2CH), 1.98–0.72 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 172.25, 172.03 (CO), 132.17, 131.79, 131.68, 130.84, 129.49, 128.88, 128.80, 128.68, 128.34, 128.19, 128.11, 128.04, 127.92, 127.57, 127.18, 126.23, 125.70, 125.56, 125.47, 125.33, 124.05, 123.98, 113.83, 86.78, 72.52, 72.40, 71.87, 60.17, 60.07, 59.98, 59.76, 59.54, 59.38, 59.10, 58.94, 55.75, 55.24 (OMe), 46.66, 45.91, 43.89, 43.79, 41.75, 41.61, 34.81, 34.38, 32.24, 24.61, 24.43, 23.28, 23.11, 23.04, 21.91, 20.85, 20.53, 19.62, 14.31.

4.1.26. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (13b). The precursor **12b** (2.65 g, 4.0 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (2.0 mL, 6 mmol) and diisopropylammonium tetrazolide (1.02 g, 6 mmol) to give phosphoramidite **13b** as a white foam (3.16 g, 92%), R_f 0.30, 0.39 (hexane–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=886.3949 $[M+Na]^+$, calcd for $[C_{53}H_{58}N_3O_6P+Na]^+$ 886.3955. ^{31}P NMR (MeCN-d_3) δ 152.49, 149.36 (diastereomers, ~9:10). ^1H NMR (MeCN-d_3) δ 8.32–7.90 (m, 9H, Ar*H* (pyrene)), 7.45–7.18 (m, 9H, Ar*H* (Dmt)), 6.94 (m, 1H, N*H*), 6.79–6.68 (m, 4H, Ar*H* (Dmt)), 5.02 (m, 2H, NCH_2), 4.04 (d, 0.55H, J_{HCOP}=12.6 Hz), 3.99 (d, 0.45H, J_{HCOP}=9.9 Hz) (C*H*O, diastereomers), 3.70 (m, 6*H*, OCH_3), 3.57–2.75 (m, 6H, $POCH_2$, NC*H*, CH_2O), 2.47 (t, 1.1H, J=6.0 Hz), 2.33 (t, 0.9H, J=6.0 Hz) (CH_2CN, diastereomers), 1.26–0.70 (m, 18H, $CH_2C(CH_3)_2CH$, CH_3 (^{i}Pr)). ^{13}C NMR (DMSO-d_6) δ 169.29 (CO), 129.79, 128.21, 128.16, 127.90, 127.75, 127.70, 127.65, 127.59, 127.40, 127.16, 127.08, 127.04, 126.51, 126.31, 126.24, 125.32, 125.25, 125.21, 125.17, 124.68, 124.57, 124.09, 123.98, 123.95, 123.49, 123.25, 118.76, 118.57, 112.96, 85.13, 85.11, 68.01, 67.66, 58.68, 58.23, 58.19, 55.00 (OMe), 54.96, 44.58, 44.53, 42.69, 42.65, 42.59, 42.55, 24.35, 24.28, 24.23, 24.13, 24.04, 23.98, 22.65, 22.30, 22.22, 21.12, 20.70, 19.84, 19.79, 19.64, 19.42, 19.37.

4.1.27. Solid supports (6a–c) and (14a,b). The solid supports were prepared by a modification of the published method.[10] A solution of succinic anhydride (300 mg, 3 mmol) and DMAP (80 mg, 0.66 mmol) in 10 mL of dry pyridine was added to 300 mg of LCAA-CPG-500 Å, and the mixture was left at room temperature for 24 h with occasional swirling. After filtration, successive washes with 10 mL portions of pyridine, CH_2Cl_2, and Et_2O, and drying, the succinylated LCAA-CPG was suspended in 4 mL of DMF–pyridine (1:1 v/v); compound **4a–c** or **12a**,**b** (0.25 mmol), 1,3-diisopropylcarbodiimide (DIC) (0.28 mL, 1.8 mmol) and DMAP (20 mg) were added; and the slurry was left for 48 h at room temperature. To block the remaining carboxylic acid groups, methanol (0.25 mL) (supports **6a–c**) or a solution of pentachlorophenol (100 mg) in pyridine (1 mL) (supports **14a**,**b**) was added, and the mixture was kept at room temperature for the next 12 h. Then, the support was filtered and for **14a**,**b** only, re-suspended in 5% v/v solution of piperidine in pyridine (3 mL), reacted for 10 min and filtered again. All the supports were washed successively with 10 mL portions of $CHCl_3$, MeOH, MeCN and Et_2O, and dried in vacuo. Loadings were determined by treatment of a portion of the support (5 mg) with 3% w/v CCl_3CO_2H in 1,2-dichloroethane (1 mL) and measuring the absorbance of Dmt^+ at 504 nm (ε=76 mL cm^{-1} μmol^{-1})[10] and were found to be (in μmol/g): 35 (**6a**), 52 (**6b**), 44 (**6c**), 20 (**14a**) and 16 (**14b**).

4.1.28. Synthesis of oligodeoxynucleotides. Oligonucleotide synthesis was carried out on a DNA/RNA synthesiser using standard manufacturer's protocols. Modified phosphoramidites **5a–c**, **10a**,**b** and **13a**,**b** were used in solid-phase oligodeoxyribonucleotide synthesis with their coupling time increased to 10 min. After assembly, the CPG-bound oligonucleotides were treated with concd ammonium hydroxide at 55 °C overnight, evaporated and precipitated from 1 M $LiClO_4$ (0.4 mL) by dilution with acetone (1.6 mL). Oligonucleotides were isolated by electrophoresis in 20% denaturing (7 M urea) polyacrylamide gel in Tris–borate buffer, pH 8.3, and desalted using NAP-10 column and standard purification procedure.

RP-HPLC of oligonucleotides was carried out using a Phenomenex RP-C18 column (3.90×300 mm) and dual-wavelength (215 and 254 nm) UV detection using gradient of acetonitrile in 0.1 M aq triethylammonium acetate (0–5%, 5 min, 5–15%, 10 min, 15–40%, 30 min, 40–80%, 10 min, 80–0%, 10 min).

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Microwave-assisted one-pot U-4CR and intramolecular O-alkylation toward heterocyclic scaffolds☆

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Abstract—The one-pot U-4CR and intramolecular O-alkylation sequence starting from 2-aminophenols in combination with α-bromoalkanoic acids, aldehydes, and isocyanides under controlled microwave heating has been established for a rapid access to highly functionalized 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines. With appropriate substitutions on the 1,4-benzoxazines, a microwave-assisted Cu-catalyzed intramolecular amidation was performed to furnish a novel class of heterocyclic conjugates of 1,4-benzoxazines with a 2-oxindole linked through a C–N single bond.

1. Introduction

Multicomponent reactions (MCRs)[1–3] are referred to as the one-pot processes, where multiple bonds are formed among three or more starting materials to furnish the product with essentially all of the atoms of the reactants. As a special subclass, the isocyanide-based MCRs (IMCRs)[3] offer a number of advantages originating from the unique reactivity of an isocyanide, which acts as a nucleophile and an electrophile at the same time. The most popular IMCRs are the Passerini three-component reaction (P-3CR) and the Ugi four-component reaction (U-4CR), which produce linear α-acyloxycarboxamides and the peptide-like α-acylaminoamides, respectively.[3a] In recent years, a number of bifunctional components, such as oxo acids, amino acids, amino aldehydes, and isocyanoacetamides,[4] have been used for the synthesis of drug-like cyclic scaffolds.[3f] Various strategies for post-U-4CR modifications have been developed,[1a,3] including Armstrong's work on convertible isocyanides,[5] Hulme's UDC (Ugi/De-Boc/Cyclize) methodology,[6] and the combinations of U-4CRs with Wittig,[7] Heck,[8] RCM,[9] IMDA,[10] S_N2,[11] S_NAr,[12] and so on.[1a,3f] In Hulme's synthesis of benzimidazoles[6d] and quinoxalines[6e] via UDC, mono-*N*-Boc-protected phenylene diamines were used as the amine components. We envisaged that 2-aminophenols **1** could be used in U-4CRs as the amine component without protection of the phenolic hydroxyl group.[13] Therefore, a subsequent cyclization of **5a** or **5b** via a base-mediated O-alkylation or O-arylation (paths *a* and *b*) could be assumed (Chart 1). We report here our original results on the syntheses of heterocyclic scaffolds **6** via microwave-assisted one-pot U-4CR and intramolecular O-alkylation sequence. A further post-modification on **6** possessing R^2=*O*-BrC_6H_4 through the Cu-catalyzed intramolecular amidation (path *c*) has been successfully explored, providing a novel class of heterocyclic conjugates **8** linked through a C–N single bond.

2. Results and discussion

2-Aminophenols are commercially available and inexpensive building blocks and have been used in the syntheses of indoles,[14] benzofurans,[15] and 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines[16] in our previous studies. To the best of our knowledge, the parent 2-aminophenol was used in one example of U-4CR in combination with 4-fluoro-3-nitrobenzoic acid and the resultant adduct was subjected to a base-mediated intramolecular S_NAr reaction to furnish a 2-nitrobenz[*b*,*f*][1,4]oxazepin-11(10*H*)-one in 25% isolated yield.[12a] We examined the U-4CRs by using five substituted 2-aminophenols (**1**), five aromatic aldehydes (**9**), two α-bromoalkanoic acids (**10**), and two isocyanides (**11**) at both room temperature and under controlled microwave heating,[17,18] respectively. The structures and yields of the

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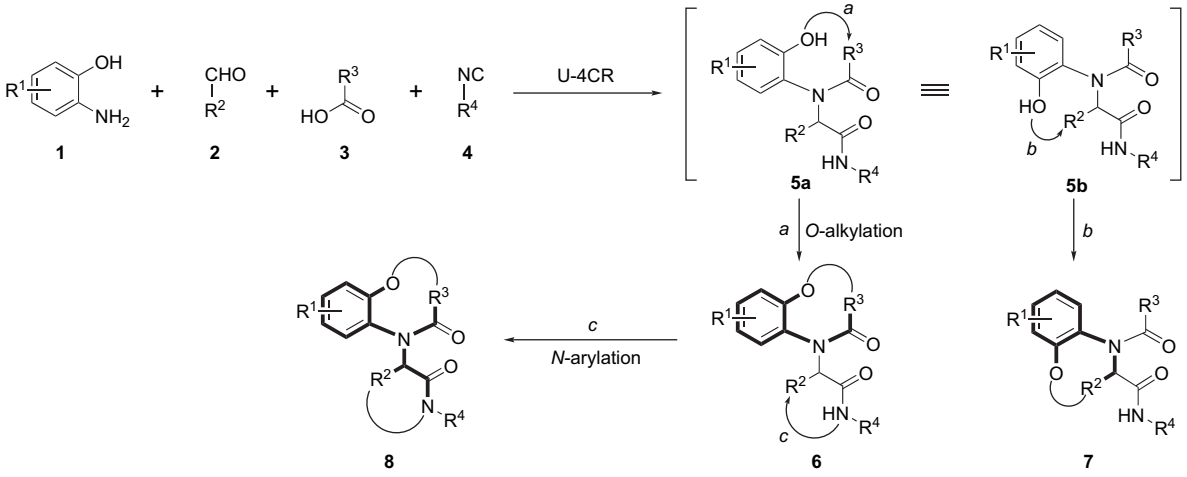
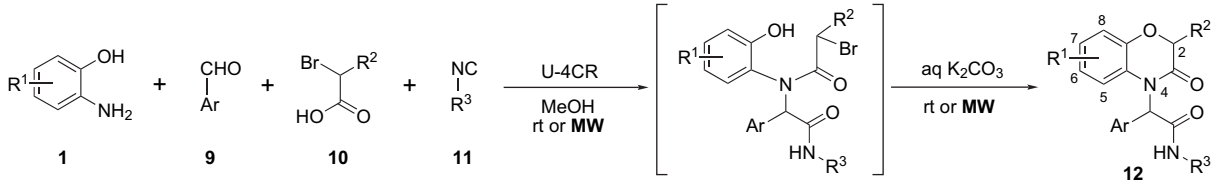


Chart 1. One-pot U-4CR-intramolecular O-alkylation and intramolecular amidation sequence toward heterocyclic scaffolds **6**–**8**.

products are summarized in Table 1. The U-4CR was carried out in MeOH and then, without isolation of the acyclic product, an aqueous solution of K_2CO_3 was added for promoting the intramolecular O-alkylation. A collection of 14 highly functionalized 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines **12a**–**n** were isolated in 61–95% yields for the two-step reactions carried out at room temperature. Initially, the base-promoted cyclization was stirred at room temperature for overnight (8–12 h) (Table 1, entries 1–11). We found that the cyclization could complete in 20–45 min (Table 1, entries 12–14). Moreover, a prolonged reaction time generally improved the yield of U-4CR. It is not rare that some U-4CRs took up to 7 days at room temperature as in the cases of entries 12 and 13 of Table 1.

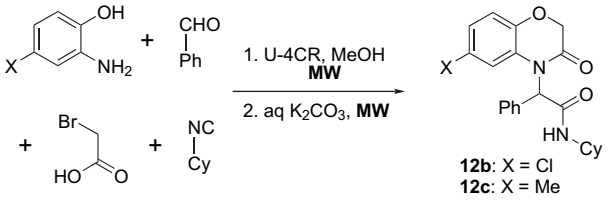
Table 1. One-pot synthesis of 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines **12** via U-4CR and intramolecular O-alkylation[a]



Entry	**1** (R^1)[b]	**9** (Ar)	**10** (R^2)	**11** (R^3)	Room temp	MW[f]
					t_U (h),[c] t_A (h);[d] **12** (%)[e]	**12** (%)[e]
1	H	Ph	H	Cy	36, 11; **12a**: 79	81 (83)[g]
2	6-Cl	Ph	H	Cy	30, 11; **12b**: 75	82
3	6-Me	Ph	H	Cy	29, 12; **12c**: 85	85
4	6,7-$(CH_2)_4$–	Ph	H	Cy	36, 12; **12d**: 65	63
5	H	4-$MeOC_6H_4$	H	Cy	36, 12; **12e**: 77	72
6	H	Ph	Me	Cy	37, 12; **12f**: 65[h]	88[h]
7	H	2-Furyl	H	Cy	36, 12; **12g**: 61	56
8	H	2-FC_6H_4	H	Cy	37, 9; **12h**: 95	90
9	H	Ph	H	Bn	23, 8; **12i**: 90	83
10	6-Me	Ph	Me	Bn	24, 12; **12j**: 78[h]	84[h]
11	H	2-FC_6H_4	H	Bn	25, 12; **12k**: 79	81
12	7-Me	2-BrC_6H_4	H	Bn	168, 0.33; **12l**: 86	63
13	6-Me	2-BrC_6H_4	H	Bn	168, 0.33; **12m**: 84	64
14	6-Cl	2-BrC_6H_4	H	Bn	48, 0.75; **12n**: 73	52[i]

[a] An equal molar ratio of the four reagents was used with MeOH as the solvent.
[b] The numbering system of 1,4-benzoxazine is used.
[c] The reaction time for U-4CR at room temperature. A longer reaction time usually gave a higher yield.
[d] The reaction time for base-promoted cyclization at room temperature. In most cases, the reaction mixture was stirred for overnight. The cyclization should complete within 20–45 min as seen in entries 12–14.
[e] Isolated overall yields of **12** for the one-pot synthesis.
[f] All microwave-heated reactions were carried out in MeOH in closed pressurized vials with the reaction temperature measured by an IR sensor. For U-4CR step, the reaction mixture was heated at 80 °C for 20 min. After the addition of 1.2 equiv of aqueous K_2CO_3, the same vial was heated at 120 °C for 15 min for cyclization.
[g] The yield in the parentheses was obtained from the same reaction mixture heated in an oil bath instead of microwave irradiation.
[h] A ca. 60:40 mixture of diastereomers was obtained.
[i] Heated for 20 min for O-alkylation.

Table 2. Optimization of microwave-assisted one-pot synthesis of **12**[a]

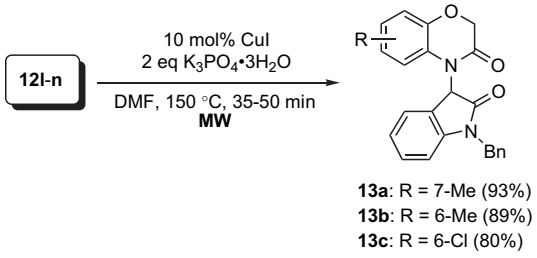


Entry	U-4CR T (°C); t (min)	K_2CO_3 (equiv)	O-Alkylation T (°C); t (min)	Yield (%)[b]
1	100; 20	2.0	150; 15	**12c**: 70
2	80; 20	1.2	120; 15	**12c**: 85
3	80; 20	2.0	150; 15	Black
4	80; 20	2.0	120; 15	**12b**: 71
5	80; 25	2.0	120; 15	**12b**: 64
6	80; 20	1.2	150; 15	**12b**: 64
7	80; 20	1.2	120; 15	**12b**: 82

[a] An equal molar ratio of the four reagents was used with MeOH as the solvent. The same microwave reaction as described in Table 1 was used.
[b] Isolated yield.

We attempted to accelerate the one-pot U-4CR and intramolecular O-alkylation for a high-throughput synthesis of **12** by using controlled microwave irradiation. With the purpose for an automated synthesis in mind, we optimized a set of unified reaction conditions by balancing between the reaction time and the product yield. Table 2 shows some results of the reactions under microwave irradiation, which were carried out in a closed vial, allowing heating above the boiling point of the solvent. We found that the U-4CR was not favorable at the temperature higher than 80 °C or with the reaction time longer than 20 min at 80 °C (entry 1 vs entry 2; entry 4 vs entry 5). Also, it was not advantageous to promote the O-alkylation at the temperature higher than 120 °C or with an excess base than 1.2 equiv (entry 3 vs entry 4; entry 6 vs entry 7; entry 4 vs entry 7). The optimized reaction conditions for the microwave-assisted one-pot process (entries 2 and 7 of Table 2) were then used for the one-pot synthesis of the 1,4-benzoxazines **12a–n** in 52–90% yields (Table 1). According to the data in Table 1, we can conclude that the product yields obtained from the microwave-assisted versions of the one-pot synthesis are generally comparable or slightly lower than the room temperature versions, except for several cases that produced the products **12l–n** in ca. 20% higher yields at room temperature (Table 1, entries 12–14). In terms of efficiency of the synthesis, the throughput is much higher for the microwave-assisted synthesis, i.e., 35 min (MW) versus 32–168 h (rt) for each compound. We also carried out the synthesis of **12a** by using a pre-heated oil bath and obtained the same result (Table 1, entry 1). Nevertheless, with the dedicated technical microwave reactor, it is much more easy to set and control the reaction temperature and to design and perform automated sequential synthesis of compound libraries.

For exploration of further annulation within **6** via the path *c* as depicted in Chart 1, we turned our attention to intramolecular amidation (N-arylation) of **12l–n** (Scheme 1). The CuI-catalyzed arylation of amines and amides has received growing interest in recent years.[19] We used controlled microwave heating for the intramolecular amidation[20,21] of **12l–n** in the presence of 10 mol % CuI and 2 equiv of $K_3PO_4 \cdot 3H_2O$ in DMF at 150 °C for 35–50 min. The



Scheme 1. CuI-catalyzed amidation under microwave heating.

expected 2-oxindoles **13a–c** were obtained in 80–93% yields. The structures of **13** represent a novel class of heterocyclic scaffold that features the conjugate of 1,4-benzoxazines with a 2-oxindole linked through a C–N bond. The ^1H and ^{13}C NMR spectra of **13** exhibit two sets of discrete signals (see Supplementary data), suggesting for atropisomers in solution. We performed HPLC analysis of **13a** over a chiral stationary phase and observed a dynamic

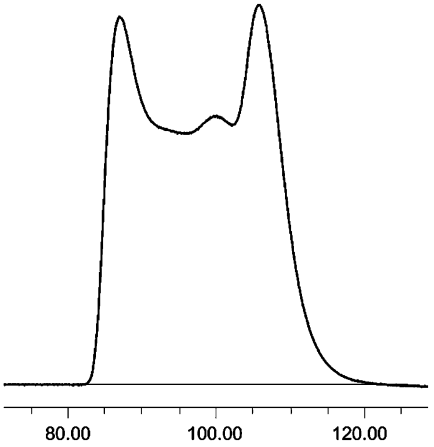


Figure 1. Dynamic HPLC chromatogram of **13a** (HPLC setting: Chiralcel OJ eluted with 90:10 hexane–*i*-PrOH at 10 mL/min and by UV detection at 254 nm). There are four diastereomers for **13a** arising from one stereogenic center and one stereogenic axis. Three out of four diastereomers are resolved.

HPLC chromatogram (Fig. 1). The latter is characteristic to atropisomerism of the C–N bond linked conjugates.

3. Conclusion

We have established a general and high-throughput synthesis of highly functionalized 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines **12** starting from the building blocks, 2-aminophenols (**1**), aromatic aldehydes (**9**), α-bromoalkanoic acids (**10**), and isocyanides (**11**) via microwave-assisted one-pot U-4CR and intramolecular O-alkylation. Further post-modification has been showcased by the microwave-assisted CuI-catalyzed intramolecular amidation within **12l–n** to furnish a novel heterocyclic scaffold **13a–c**, which exhibits atropisomerism through rotation across the C–N single bond.

4. Experimental

4.1. General information and the microwave reactor

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 (400 MHz for ^{1}H and 100 or 125 MHz for ^{13}C, respectively) with $CHCl_3$ or DMSO as the internal reference. IR spectra were taken on an FTIR spectrophotometer. Mass spectra (MS) were measured by the +ESI method. Melting points are uncorrected. Silica gel plates pre-coated on glass were used for thin-layer chromatography using UV light, or 7% ethanolic phosphomolybdic acid and heating as the visualizing methods. Silica gel was used for flash column chromatography. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Methanol was distilled from magnesium before use. Reagents were obtained commercially and used as received. All microwave-assisted reactions were carried out on an Emrys creator from Personal Chemistry AB (now under Biotage AB, Uppsala, Sweden) with temperature measured by an IR sensor. The microwave-assisted reaction time is the hold time at the final temperature.

4.2. General procedure for one-pot synthesis of 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines 12a–n at room temperature

To a 25-mL round bottom flask were added 2-aminophenol **1** (0.25 mmol), aldehyde **9** (0.25 mmol), and MeOH (2 mL), and the mixture was stirred for 15–30 min at room temperature. α-Bromoalkanoic acid **10** (0.25 mmol) was added to the mixture followed by stirring for another 5 min. Finally, isocyanide **11** (0.25 mmol) was added. After the resultant mixture was stirred at room temperature for 23–168 h, solid K_2CO_3 (52 mg, 0.38 mmol) was added. After the resultant mixture was stirred at room temperature for overnight (8–12 h for entries 1–11, Table 1) or 20–45 min (for entries 12–14, Table 1), the reaction mixture was quenched by water and the organic layer was extracted with EtOAc (3×10 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with EtOAc and petroleum ether (60–90 °C) to afford **12a–n**. The structures and yields of the products **12a–n** are given in Table 1.

4.3. General procedure for microwave-assisted one-pot synthesis of 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazines 12a–n

To a 10-mL pressurized process vial were added 0.25 mmol each of 2-aminophenol **1**, aldehyde **9**, α-bromoalkanoic acid **10**, and isocyanide **11**, and MeOH (2 mL). The loaded vial was then sealed with a cap containing a silicon septum, and put into the microwave cavity and heated at 80 °C for 20 min. Then, an aqueous solution of K_2CO_3 (1 mL, 0.30 mmol) was added to the reaction vial through a syringe followed by heating at 120 °C for 15 min in the microwave cavity. Water was added to the reaction mixture and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with EtOAc and petroleum ether (60–90 °C) to afford **12a–n**. The structures and yields of the products are given in Table 1.

4.3.1. *N*-Cyclohexyl-2-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-phenylacetamide (12a). A white crystalline solid; mp 140–142 °C; IR (KBr) 1684, 1655 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.37 (m, 5H), 7.12 (d, *J*=8.0 Hz, 1H), 7.07–7.00 (m, 2H), 6.94–6.90 (m, 1H), 6.19 (s, 1H), 6.12 (br d, *J*=8.0 Hz, 1H), 4.78 and 4.69 (ABq, *J*=15.2 Hz, 2H), 3.95–3.85 (m, 1H), 2.01–1.98 (m, 1H), 1.87–1.84 (m, 1H), 1.75–1.61 (m, 3H), 1.47–1.33 (m, 2H), 1.30–1.05 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.4, 146.0, 134.0, 128.8 (×2), 128.6, 128.2, 127.8 (×2), 124.4, 122.7, 117.6, 117.0, 68.2, 61.4, 48.6, 32.6, 32.5, 25.4, 24.5 (×2); MS (+ESI) *m*/*z* 403 (M+K$^+$, 100), 387 (M+Na$^+$, 26). Anal. Calcd for $C_{22}H_{24}N_2O_3$: C, 72.50; H, 6.64; N, 7.69. Found: C, 72.51; H, 6.58; N, 7.84.

4.3.2. *N*-Cyclohexyl-2-(6-chloro-3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-phenylacetamide (12b). A white crystalline solid; mp 181–183 °C; IR (KBr) 1691, 1651 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.33 (m, 5H), 7.05 (s, 1H), 6.89 (d, *J*=0.4 Hz, 2H), 6.05 (s, 1H), 5.95 (br d, *J*=8.0 Hz, 1H), 4.69 and 4.59 (ABq, *J*=15.2 Hz, 2H), 3.90–3.75 (m, 1H), 1.96–1.92 (m, 1H), 1.84–1.81 (m, 1H), 1.70–1.50 (m, 3H), 1.40–1.23 (m, 2H), 1.20–1.00 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 165.9, 144.6, 133.6, 129.6, 129.1 (×2), 128.6, 127.9 (×2), 1276, 124.1, 117.9, 117.7, 68.1, 61.6, 48.7, 32.6, 32.5, 25.3, 24.5 (×2); MS (+ESI) *m*/*z* 439 (M+2+K$^+$, 33), 437 (M+K$^+$, 100), 423 (M+2+Na$^+$, 10), 421 (M+Na$^+$, 31). Anal. Calcd for $C_{22}H_{23}ClN_2O_3$: C, 66.24; H, 5.81; N, 7.02. Found: C, 66.26; H, 5.83; N, 7.09.

4.3.3. *N*-Cyclohexyl-2-(3,4-dihydro-6-methyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-phenylacetamide (12c). A white crystalline solid; mp 148–150 °C; IR (KBr) 1686, 1663 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.25 (m, 5H), 6.85 (d, *J*=8.0 Hz, 1H), 6.83 (s, 1H), 6.72 (d, *J*=8.8 Hz, 1H), 6.09 (br d, *J*=8.0 Hz, 1H), 6.04 (s, 1H), 4.61 and 4.55 (ABq, *J*=15.2 Hz, 2H), 3.85–3.75 (m, 1H), 2.13 (s, 3H), 1.90–1.86 (m, 1H), 1.79–1.76 (m, 1H), 1.65–1.47 (m, 3H), 1.33–1.20 (m, 2H), 1.15–0.97 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 166.5, 143.8, 134.2, 132.4, 128.8 (×2), 128.6, 128.2, 127.7 (×2), 124.9, 117.9,

116.7, 68.3, 61.8, 48.6, 32.6, 32.4, 25.4, 24.5, 24.5, 20.9; MS (+ESI) *m/z* 401 (M+Na$^+$, 100). Anal. Calcd for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40. Found: C, 73.06; H, 7.07; N, 7.46.

4.3.4. *N*-Cyclohexyl-2-{3,4,6,7,8,9-hexahydro-3-oxo-2*H*-naphtho[2,3-*b*][1,4]oxazin-4-yl}-2-phenylacetamide (12d). A white crystalline solid; mp 138–140 °C; IR (KBr) 1686, 1656 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.35 (m, 5H), 6.78 (s, 1H), 6.76 (s, 1H), 6.16 (br d, J=8.0 Hz, 1H), 6.08 (s, 1H), 4.679 and 4.63 (ABq, J=15.2 Hz, 2H), 3.95–3.83 (m, 1H), 2.72–2.67 (m, 2H), 2.60 (br s, 2H), 2.00–1.90 (m, 1H), 1.90–1.88 (m, 1H), 1.80–1.57 (m, 7H), 1.45–1.31 (m, 2H), 1.25–1.08 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 166.4, 143.6, 134.2, 133.5, 131.5, 128.8 (×2), 128.1, 127.6 (×2), 126.5, 117.3, 116.9, 68.4, 62.0, 48.5, 32.5, 32.4, 28.9, 28.7, 25.4, 24.5, 24.5, 23.0, 22.8; MS (+ESI) *m/z* 457 (M+K$^+$, 41), 441 (M+Na$^+$, 100). Anal. Calcd for $C_{26}H_{30}N_2O_3$: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.53; H, 7.18; N, 6.66.

4.3.5. *N*-Cyclohexyl-2-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(4-methoxyphenyl)acetamide (12e). A white crystalline solid; mp 136–138 °C; IR (KBr) 1685, 1653 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J=8.8 Hz, 2H), 7.05 (d, J=7.6 Hz, 1H), 7.01–6.94 (m, 2H), 6.88 (d, J=8.4 Hz, 3H), 6.02 (s, 1H), 5.97 (br d, J=7.6 Hz, 1H), 4.71 and 4.61 (ABq, J=15.2 Hz, 2H), 3.87–3.79 (m, 1H), 3.79 (s, 3H), 1.96–1.93 (m, 1H), 1.81–1.78 (m, 1H), 1.70–1.53 (m, 3H), 1.40–1.25 (m, 2H), 1.20–1.00 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 166.2, 159.4, 145.9, 129.3 (×2), 128.7, 125.9, 124.4, 122.7, 117.4, 117.0, 114.3 (×2), 68.2, 61.1, 55.2, 48.6, 32.6, 32.5, 25.4, 24.6 (×2); MS (+ESI) *m/z* 433 (M+K$^+$, 100). Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 70.10; H, 6.67; N, 7.12.

4.3.6. *N*-Cyclohexyl-2-(3,4-dihydro-2-methyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-phenylacetamide (12f). A white crystalline solid and a 63:37 mixture of two diastereomers; mp 150–152 °C; IR (KBr) 1685, 1655 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 7.04–6.82 (m, 4H), 6.16 (s, 0.63H, major), 6.12 (s, 0.3H, minor), 5.98 (d, J=7.6 Hz, 1H), 4.75 (q, J=6.8 Hz, 0.37H, minor), 4.65 (q, J=6.8 Hz, 0.63H, major), 3.88–3.77 (m, 1H), 1.97–1.88 (m, 1H), 1.85–1.70 (m, 1H), 1.70–1.50 (m, 3H), 1.63 (d, J=7.2 Hz, 1.89H, major), 1.56 (d, J=6.8 Hz, 1.11H, minor), 1.42–1.23 (m, 2H), 1.20–0.95 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 168.5, 166.8, 166.8, 145.3, 144.8, 134.1, 134.1, 128.8 (×2), 128.7 (×2), 128.7, 128.1, 128.1, 127.8 (×2), 127.6 (×2), 124.5, 124.3, 122.6, 122.5, 117.5, 117.5, 117.2, 117.0, 74.0, 74.0, 61.5, 61.2, 48.5, 48.5, 32.6, 32.6, 32.5, 32.5, 25.4, 25.4, 24.5 (×2), 24.5 (×2), 16.0, 16.0; MS (+ESI) *m/z* 401 (M+Na$^+$, 100). Anal. Calcd for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40. Found: C, 73.04; H, 6.95; N, 7.42.

4.3.7. *N*-Cyclohexyl-2-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(furan-2-yl)acetamide (12g). A white crystalline solid; mp 124–126 °C; IR (KBr) 1684, 1658 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.16 (d, J=7.6 Hz, 1H), 7.00–6.90 (m, 3H), 6.61 (d, J=3.2 Hz, 1H), 6.47 (s, 1H), 6.36 (d, J=2.4 Hz, 1H), 6.09 (br d, J=7.6 Hz, 1H), 4.69 and 4.62 (ABq, J=15.2 Hz, 2H), 3.90–3.75 (m, 1H), 1.95–1.91 (m, 1H), 1.82–1.79 (m, 1H), 1.73–1.52 (m, 3H), 1.43–1.01 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 164.8, 147.1, 145.7, 142.8, 127.8, 124.4, 122.6, 117.0 (×2), 111.2, 110.8, 67.9, 54.0, 48.7, 32.6, 32.5, 25.3, 24.5, 24.5; MS (+ESI) *m/z* 377 (M+Na$^+$, 100). Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.82; H, 6.43; N, 7.84.

4.3.8. *N*-Cyclohexyl-2-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(2-fluorophenyl)acetamide (12h). A white crystalline solid; mp 174–176 °C; IR (KBr) 1696, 1674 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (t, J=7.4 Hz, 1H), 7.39–7.34 (m, 1H), 7.19–7.09 (m, 3H), 7.05–6.92 (m, 3H), 6.34 (s, 1H), 5.94 (br d, J=8.0 Hz, 1H), 4.73 and 4.65 (ABq, J=15.2 Hz, 2H), 3.92–3.80 (m, 1H), 1.99–1.96 (m, 1H), 1.88–1.82 (m, 1H), 1.74–1.55 (m, 3H), 1.44–1.25 (m, 2H), 1.22–1.00 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.5, 160.7 (d, J_{C-F}= 246.9 Hz), 145.9, 130.5 (d, J_{F-C}=8.7 Hz), 129.4 (d, J_{F-C}= 2.5 Hz), 128.6, 124.6, 124.4 (d, J_{F-C}=3.3 Hz), 122.8, 121.7 (d, J_{F-C}=12.9 Hz), 117.1, 117.0, 115.8 (d, J_{F-C}= 18.7 Hz), 68.1, 56.2 (d, J_{F-C}=2.4 Hz), 48.7, 32.6, 32.5, 25.4, 24.6, 24.5; MS (+ESI) *m/z* 405 (M+Na$^+$, 100). Anal. Calcd for $C_{22}H_{23}FN_2O_3$: C, 69.09; H, 6.06; N, 7.33. Found: C, 69.16; H, 6.12; N, 7.47.

4.3.9. *N*-Benzyl-2-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-phenylacetamide (12i). A white crystalline solid; mp 110–112 °C; IR (KBr) 1686, 1657 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.15 (m, 10H), 7.02–6.94 (m, 3H), 6.82 (t, J=6.8 Hz, 1H), 6.58 (t, J=5.6 Hz, 1H), 6.18 (s, 1H), 4.65 and 4.56 (ABq, J=14.8 Hz, 2H), 4.44 (d, J=5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 166.4, 145.9, 137.8, 133.8, 128.9, 128.9, 128.6, 128.5, 128.4, 128.3, 128.0 (×2), 127.5 (×2), 127.4, 124.5, 122.7, 117.5, 117.1, 68.1, 61.2, 43.8; MS (+ESI) *m/z* 395 (M+Na$^+$, 100). Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 74.15; H, 5.52; N, 7.69.

4.3.10. *N*-Benzyl-2-(3,4-dihydro-2,6-dimethyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-phenylacetamide (12j). A white crystalline solid and a 56:44 mixture of two diastereomers; mp 142–144 °C; IR (KBr) 1689, 1662 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.24 (m, 10H), 7.00–6.83 (m, 3H), 6.63–6.60 (m, 1H), 6.27 (s, 0.56H, major), 6.22 (s, 0.44H, minor), 4.77–4.49 (m, 3H), 2.26 (s, 1.32H, minor), 2.22 (s, 1.68H, major), 1.67 (d, J=7.2 Hz, 1.68H, major), 1.62 (d, J=6.8 Hz, 1.32H, minor); ^{13}C NMR (100 MHz, $CDCl_3$) for the major isomer: δ 168.6, 167.8, 143.1, 137.9, 134.0, 132.2, 128.8 (×2), 128.6 (×2), 128.5, 128.2, 127.9 (×2), 127.5 (×2), 127.3, 124.9, 117.6, 117.2, 74.0, 61.4, 43.7, 20.9, 16.0; ^{13}C NMR (100 MHz, $CDCl_3$) for the minor isomer: δ 168.5, 168.0, 142.7, 137.8, 134.0, 132.4, 128.9 (×2), 128.7, 128.6 (×2), 128.2, 127.7 (×2), 127.4 (×2), 127.3, 125.0, 117.6, 117.0, 74.0, 61.8, 43.7, 21.0, 15.9; MS (+ESI) *m/z* 423 (M+Na$^+$, 100). Anal. Calcd for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 74.95; H, 6.05; N, 6.95.

4.3.11. *N*-Benzyl-2-(3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(2-fluorophenyl)acetamide (12k). A white

crystalline solid; mp 133–135 °C; IR (KBr) 1692, 1671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.56 (t, J=7.6 Hz, 1H), 7.38–6.93 (m, 12H), 6.47 (t, J=2.0 Hz, 1H), 6.43 (s, 1H), 4.72 and 4.63 (ABq, J=15.2 Hz, 2H), 4.56 and 4.53 (ABqd, J=14.8, 2.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 166.1, 160.7 (d, J_{F-C}= 246.9 Hz), 145.9, 137.6, 130.7 (d, J_{F-C}=8.6 Hz), 129.5 (d, J_{F-C}=3.0 Hz), 128.6 (×2), 128.5, 127.5 (×2), 127.4, 124.7, 124.6 (d, J_{F-C}=3.3 Hz), 122.8, 121.6 (d, J_{F-C}=13.2 Hz,), 117.2, 116.8, 115.9 (d, J_{F-C}=21.5 Hz), 68.1, 56.0 (d, J_{F-C}=2.5 Hz), 43.9; MS (+ESI) m/z 429 (M+K^+, 100). Anal. Calcd for $C_{23}H_{19}FN_2O_3$: C, 70.76; H, 4.91; N, 7.18. Found: C, 70.63; H, 4.92; N, 7.09.

4.3.12. *N*-Benzyl-2-(3,4-dihydro-7-methyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(2-bromophenyl)acetamide (12l). A white crystalline solid; mp169–170 °C (EtOAc–hexane); R_f=0.24 (20% EtOAc in hexane); IR (KBr) 3347, 1677, 1654, 1508 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, J=8.0, 1.2 Hz, 1H), 7.55 (dd, J=8.0, 1.6 Hz, 1H), 7.33–7.23 (m, 6H), 7.20 (td, J=8.0, 1.6 Hz, 1H), 6.82 (s, 1H), 6.81 (d, J=8.0 Hz, 1H), 6.71–6.68 (m, 1H), 6.18 (t, J=4.8 Hz, 1H), 6.14 (s, 1H), 4.70 and 4.59 (ABq, J=15.2 Hz, 2H), 4.54 and 4.51 (ABqd, J=13.2, 4.8 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 166.1, 145.8, 137.7, 134.8, 133.6, 133.5, 130.4, 129.9, 128.6 (×2), 128.1, 127.6 (×2), 127.4, 126.5, 124.7, 123.3, 117.7, 116.4, 68.3, 62.9, 44.0, 20.7; MS (+ESI) m/z 489 (M+2+Na^+, 98), 487 (M+Na^+, 100). Anal. Calcd for $C_{24}H_{21}BrN_2O_3$: C, 61.95; H, 4.55; N, 6.02. Found: C, 61.98; H, 4.54; N, 6.09.

4.3.13. *N*-Benzyl-2-(3,4-dihydro-6-methyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(2-bromophenyl)acetamide (12m). A white crystalline solid; mp163–165 °C (CH_2Cl_2–hexane); R_f=0.24 (20% EtOAc in hexane); IR (KBr) 3298, 1685, 1670, 1508 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (td, J=8.0, 1.6 Hz, 2H), 7.33–7.23 (m, 6H), 7.20 (td, J=7.6, 1.6 Hz, 1H), 6.89 (d, J=8.0 Hz, 1H), 6.78 (d, J=8.0 Hz, 1H), 6.77 (s, 1H), 6.30 (br s, 1H), 6.16 (s, 1H), 4.67 and 4.56 (ABq, J=15.2 Hz, 2H), 4.55 and 4.49 (ABqd, J=14.8, 6.4 Hz, 2H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 166.4, 143.8, 137.7, 133.6, 113.6, 132.5, 130.4, 130.2, 128.7, 128.6 (×2), 128.0, 127.6 (×2), 127.4, 125.1, 124.7, 117.3, 116.8, 68.4, 63.0, 43.9, 21.0; MS (+ESI) m/z 489 (M+2+Na^+,100), 487 (M+Na^+, 92). Anal. Calcd for $C_{24}H_{21}BrN_2O_3$: C, 61.95; H, 4.55; N, 6.02. Found: C, 61.54; H, 4.68; N, 5.94.

4.3.14. *N*-Benzyl-2-(3,4-dihydro-6-chloro-3-oxo-2*H*-1,4-benzoxazin-4-yl)-2-(2-bromophenyl)acetamide (12n). A white crystalline solid; mp 216–217 °C (CH_2Cl_2–hexane); R_f=0.22 (20% EtOAc in hexane); IR (KBr) 3312, 1691, 1672, 1496 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (t, J=7.2 Hz, 2H), 7.35–7.19 (m, 7H), 6.97 (s, 1H), 6.91 (s, 2H), 6.18 (t, J=5.6 Hz, 1H), 6.13 (s, 1H), 4.72 and 4.58 (ABq, J=15.2 Hz, 2H), 4.53 (d, J=5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 165.9, 144.7, 137.4, 133.8, 132.8, 130.8, 130.3, 129.7, 128.7 (×2), 128.2, 127.7, 127.6 (×2), 127.5, 124.7, 124.4, 118.0, 117.3, 68.2, 62.8, 44.0; MS (+ESI) m/z 511 (M+4+Na^+,100), 509 (M+2+Na^+,100), 507 (M+Na^+, 77). Anal. Calcd for $C_{23}H_{18}BrClN_2O_3$: C, 56.87; H, 3.73; N, 5.77. Found: C, 56.88; H, 3.76; N, 5.73.

4.4. General procedure for microwave-assisted CuI-catalyzed intramolecular amidation of 12l–n

To a 10-mL pressurized process vial were added CuI (1.5×10^{-2} mmol, pre-washed by dry THF), the substrate **12l–n** (0.15 mmol), and $K_3PO_4\cdot3H_2O$ (0.30 mmol). The loaded vial was then sealed with a cap containing a silicon septum. The vial was evacuated and backfilled with nitrogen several times by using a needle through the septum. The degassed DMF (5 mL) was then added by syringe through the septum. The resultant mixture was heated in the microwave cavity at 150 °C for 35 min (for **12l**) or 50 min (for **12m** and **12n**). The reaction mixture was then filtrated through Celite with washing by EtOAc. The combined filtrate was dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with EtOAc and petroleum ether (60–90 °C) to afford **13a–c**. The structures and yields of the products are given in Scheme 1.

4.4.1. *N*-Benzyl-3-(3,4-dihydro-7-methyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)oxindole (13a). A white crystalline solid and a 57:43 mixture of two atropisomers in $CDCl_3$; mp 113–114 °C (EtOAc–hexane); R_f=0.31 (20% EtOAc in hexane); IR (KBr) 1727, 1690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major atropisomer: δ 7.41–6.87 (m, 9H), 6.81 (s, 1H), 6.75 (s, 1H), 6.27 (d, J=7.6 Hz, 1H), 5.73 (d, J=8.0 Hz, 1H), 5.11 and 4.79 (ABq, J=15.6 Hz, 2H), 4.79 and 4.69 (ABq, J=15.2 Hz, 2H), 2.13 (s, 3H); ^1H NMR (400 MHz, $CDCl_3$) for the minor atropisomer: δ 7.41–6.87 (m, 11H), 6.66 (d, J=7.6 Hz, 1H), 5.32 (s, 1H), 5.09 and 4.87 (ABq, J=15.6 Hz, 2H), 4.60 and 4.43 (ABq, J= 15.2 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major atropisomer: δ 171.9, 166.0, 145.4, 142.2, 135.3, 134.4, 129.4, 128.8 (×3), 128.1 (×2), 127.5, 124.1, 123.6, 123.1, 122.7, 117.9, 115.2, 109.9, 67.7, 53.2, 44.4, 20.6; ^{13}C NMR (100 MHz, $CDCl_3$) for the minor atropisomer: δ 171.7, 164.0, 145.4, 143.4, 135.4, 134.8, 129.4, 128.7 (×3), 128.0, 127.2 (×2), 124.3, 123.7, 123.3, 123.0, 118.1, 114.2, 109.6, 67.9, 56.2, 44.2, 20.7; MS (−ESI) m/z 383 (M−H^+, 100). Anal. Calcd for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29. Found: C, 74.89; H, 5.20; N, 7.33.

4.4.2. *N*-Benzyl-3-(3,4-dihydro-6-methyl-3-oxo-2*H*-1,4-benzoxazin-4-yl)oxindole (13b). A white crystalline solid and a 52:48 mixture of two atropisomers in DMSO-d_6; mp 151–152 °C (EtOAc–hexane); R_f=0.30 (20% EtOAc in hexane); IR (KBr) 1730, 1685 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) for the major atropisomer: δ 7.47–6.86 (m, 11H), 6.73 (d, J=8.0 Hz, 1H), 5.43 (s, 1H), 5.15 and 4.95 (ABq, J=16.0 Hz, 2H), 4.64 and 4.48 (ABq, J=15.2 Hz, 2H), 2.39 (s, 3H); ^1H NMR (400 MHz, DMSO-d_6) for the minor atropisomer: δ 7.47–6.86 (m, 11H), 6.68 (d, J=8.4 Hz, 1H), 5.75 (s, 1H), 5.22 and 4.82 (ABq, J= 15.6 Hz, 2H), 4.82 and 4.76 (ABq, J=15.2 Hz, 2H), 1.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for the major atropisomer: δ 172.1, 164.6, 143.7, 143.7, 135.7, 132.5, 130.0, 129.6, 129.0 (×2), 127.8, 127.5 (×2), 125.2, 124.4, 123.5, 123.0, 117.2, 115.3, 109.9, 68.2, 56.5, 44.5, 21.4; ^{13}C NMR (125 MHz, $CDCl_3$) for the minor atropisomer: δ 172.0, 166.6, 143.8, 142.5, 135.7, 133.3, 129.7, 129.2 (×2), 128.2, 128.2 (×2), 126.8, 124.9, 124.3, 123.7, 123.2, 117.5, 116.2, 110.0, 68.1, 53.5, 44.7, 21.0; MS (+ESI) m/z

407 ($M+Na^+$, 100), 791 ($2M+Na^+$, 77). Anal. Calcd for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29. Found: C, 74.79; H, 5.24; N, 7.27.

4.4.3. *N*-Benzyl-3-(3,4-dihydro-6-chloro-3-oxo-2*H*-1,4-benzoxazin-4-yl)oxindole (13c). A white crystalline solid and a 51:49 mixture of two atropisomers in $CDCl_3$; mp 193–195 °C (EtOAc–hexane); R_f=0.31 (20% EtOAc in hexane); IR (KBr) 1731, 1691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) for the major atropisomer: δ 7.45–6.88 (m, 11H), 6.73 (d, J=8.4 Hz, 1H), 6.02 (d, J=2.0 Hz, 1H), 5.13 and 4.91 (ABq, J=16.0 Hz, 2H), 4.65 and 4.49 (ABq, J=15.2 Hz, 2H); ^1H NMR (400 MHz, $CDCl_3$) for the minor atropisomer: δ 7.45–6.88 (m, 12H), 5.33 (s, 1H), 5.19 and 4.86 (ABq, J=15.6 Hz, 2H), 4.84 and 4.78 (ABq, J=15.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) for the major atropisomer: δ 171.4, 165.8, 144.3, 142.2, 135.1, 131.1, 129.9, 129.2 (×2), 127.9, 127.6, 127.5 (×2), 124.1, 123.6, 123.5, 122.8, 118.4, 115.5, 110.3, 67.6, 53.3, 44.6; ^{13}C NMR (100 MHz, $CDCl_3$) for the minor atropisomer: δ 171.4, 163.8, 144.2, 143.3, 135.3, 129.6, 128.8 (×2), 128.3, 127.9, 127.6, 127.2 (×2), 124.3, 124.0, 122.9, 122.7, 118.6, 114.8, 109.8, 67.8, 56.4, 44.2; MS (+ESI) m/z 831.0 ($2M+Na^+$, 45); 429 ($M+2+Na^+$, 33), 427 ($M+Na^+$, 100). Anal. Calcd for $C_{23}H_{17}ClN_2O_3$: C, 68.23; H, 4.23; N, 6.92. Found: C, 68.14; H, 4.19; N, 6.75.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2006.05.001.

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DABCO catalyzed reaction of various nucleophiles with activated alkynes leading to the formation of alkenoic acid esters, 1,4-dioxane, morpholine, and piperazinone derivatives

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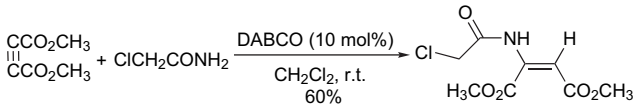
Abstract—The reaction of acids, alcohols, acylamides, 1,2-diols, 1,2-diamines or amino alcohols with activated alkynes catalyzed by 1,4-diazabicyclo[2.2.2]octane (DABCO) was systematically investigated. A series of unsaturated alkenoic acid esters or heterocycles were formed in the reaction of monobasic or dibasic nucleophiles in excellent yields, respectively.

1. Introduction

The organocatalytic carbon–carbon bond-forming reaction involving the addition of nucleophiles to activated alkynes has been drawing increasing attention.[1] Several organic catalysts have been investigated in detail in recent years.[2] Among them, DABCO, a tertiary amine base with weak basicity and moderate hindrance, has been widely used as a potential catalyst in a broad range of organic reactions, such as Hillman–Baylis reaction,[3] cyclopropanation,[4] heterocycle formation,[5] and other transformations. In our recent investigations on DABCO catalyzed reaction of α-halo carbonyl compounds with dimethyl acetylenedicarboxylate (DMAD), it was found that the Michael addition product rather than desired cyclization product was formed in moderate yield using 2-chloroacetamide as the substrate (Scheme 1).[5e]



Scheme 1.

On the basis of this observation and previous investigations,[6] we considered DABCO as a potential catalyst for the reaction of nucleophiles with activated alkynes. As a part of our continuing investigation on DABCO catalyzed organic reactions, we decided to look closer into this type of the reaction.[5d,e] A series of nucleophiles, such as alcohols, acylamides, acids, 1,2-diols, 1,2-diamines, and amino alcohols were selected to demonstrate the scope of the reaction and excellent results were obtained. On one hand, the reaction between DMAD and alcohols, acylamides or acids proceeded smoothly and rapidly at room temperature to give α,β-unsaturated carbonyl compounds in excellent yields. On the other hand, using dibasic nucleophiles, such as 1,2-diols, 1,2-diamines, and amino alcohols as the substrates, different cycloaddition products were obtained under the same conditions. Herein the full details of our research including the scope of the reaction, suitable reaction conditions, and selectivity optimization, along with the corresponding possible reaction mechanisms were reported.

2. Results and discussion

In an initial experiment, the reaction of alcohols with DMAD in the presence of a catalytic amount of DABCO (10 mol %) was examined. In a typical procedure, DMAD (0.5 mmol), DABCO (0.05 mmol), and alcohols (0.5 mmol) in CH_2Cl_2 were stirred for 10 min at room temperature and gave unsaturated alkenoic acid esters facilely by flash on chromatography on silica column. The reaction appeared to be general with a number of alcohols affording conjugate addition products in excellent yields (Table 1, entries 1–5), but it suffered from a drawback of stereoselectivity. Most of the reactions gave the final products as a pair of (*E*)-

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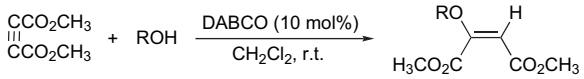
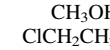
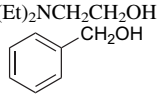
Table 1. Reaction of alcohols with DMAD[a]



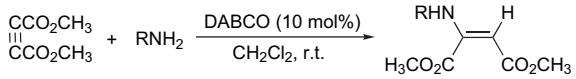
Entry	ROH	Product	Yield/%[b]	*E*/*Z*[c]
1	CH_3OH	**1**	88	20:80
2	$ClCH_2CH_2OH$	**2**	95	30:70
3		**3**	91	45:55
4	$(Et)_2NCH_2CH_2OH$	**4**	92	*E*
5		**5**	90	40:60

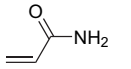
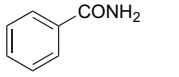
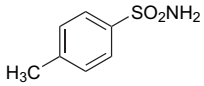
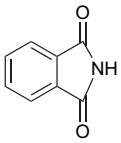
[a] All the reactions were performed at room temperature and completed within 10 min.
[b] Isolated yields.
[c] The (*E*)- and (*Z*)-isomers were separated by silica gel column chromatography.

and (*Z*)-isomers (Table 1, entries 1–3 and 5). These two isomers can be easily separated by column chromatography. An exception appeared when $Et_2NCH_2CH_2OH$ was used as the substrate. The product **4** was obtained with dominant (*E*)-selectivity (Table 1, entry 4). The structure and stereochemistry of the products were determined on the basis of their elemental analysis, mass spectrometry (MS), ^{1}H NMR, ^{13}C NMR, and IR data. The ^{1}H NMR spectral data of the products exhibited two isomers (except for product **4**) and both of them displayed characteristic resonance pattern with appropriate chemical shift. For example, the ^{1}H NMR spectrum of product **1** showed three single sharp lines at 3.76, 3.85, and 3.95 ppm for three methoxy groups in the (*Z*)-isomer and three singlets at 3.71, 3.75, and 3.89 ppm for the (*E*)-isomer. The olefinic proton in the (*Z*)-isomer resonated at 6.19 ppm, while the olefinic proton in the (*E*)-isomer resonated at 5.21 ppm. It was in agreement with the values reported in the previous literature.[7] In all the ^{1}H NMR spectra, (*E*)-isomer displayed a signal as a single sharp resonance at about 5.21–5.37 ppm for the olefinic proton. The ^{1}H NMR chemical shift of the (*Z*)-form was similar to that of the (*E*)-isomer, except for the olefinic proton, which was at about 6.19–6.36 ppm (see Section 4).

Although amine reacted with activated alkynes quickly and no base catalyst was needed,[8] we found that the same reaction of acylamides was very sluggish in the absence of a base catalyst. However, in the presence of a catalytic amount of DABCO (10 mol %), various acylamides could undergo a smooth reaction with activated alkynes at room temperature and gave the corresponding unsaturated alkenoic acid esters in moderate yields with excellent stereoselectivity. Only the (*E*)-form isomers were isolated in all the reactions of various acylamides with DMAD (Table 2, entries 1–5). Though the reaction showed optimal stereoselectivity, most of the yields were lower comparing to that of alcohols. It could be attributed to the following two reasons: (i) The reaction could not proceed completely even longer reaction time (24 h) and higher temperature (reflux) were adopted; (ii) complex side products were formed in company with the generation of unsaturated alkenoic acid esters. In each ^{1}H NMR spectrum of the products, a singlet was observed at about 5.54–5.74 ppm for the olefinic proton (except for product **10**). These shifts were similar to those observed in the previous literature.[7]

Table 2. Reaction of acylamides with DMAD[a]



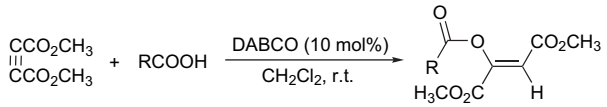
Entry	RNH_2 (or NH)	Product	Yield/%[b]	*E*/*Z*[c]
1	$ClCH_2CONH_2$	**6**	60	*E*
2		**7**	30	*E*
3		**8**	51	*E*
4		**9**	71	*E*
5		**10**	85	*E*

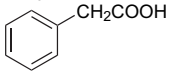
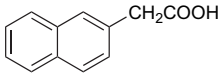
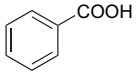
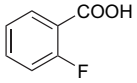
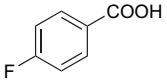
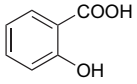
[a] All the reactions were performed at room temperature and the reaction time was extended to 12 h.
[b] Isolated yields.
[c] Determined by ^{1}H NMR (300 MHz) spectra.

With an intention to expand the scope of this conjugate addition, we tested various acids in this reaction and got excellent results surprisingly. In spite of longer reaction time required, the present method seemed to be perfect in terms of stereoselectivity. Only the (*Z*)-form isomers were isolated in all the reactions of various acids with DMAD (Table 3, entries 1–8). Moreover, the reaction had perfect selectivity in cases of more than one nucleophilic groups presented in the acids. For example, in the reaction of 2-hydroxybenzoic acid, only COOH group participated and gave the desired product with dominate (*Z*)-form (Table 3, entry 8). It should be an acidity-controlled process and the selectivity was determined by the pK_a value of the nucleophile. Nucleophilic groups with lower pK_a value will participate in the reaction first. The structure of the product was characterized by spectroscopic analysis. In each ^{1}H NMR spectrum of the products, the olefinic proton resonated at 6.66–6.82 ppm corresponding to the proposed (*Z*)-isomer. Furthermore, the structure and stereoselectivity of the products were established unambiguously by X-ray analysis of **17** as representative example (Fig. 1).[9] To the best of our knowledge, there is no such nucleophilic conjugate addition process involving acids to activated alkynes that has been reported in the literature so far.

The reaction of other activated alkynes was also investigated, such as benzyl propiolate, methyl propiolate with acids, alcohols or acylamides. It was found that excellent results were obtained under the same conditions (0.5 mmol of benzyl propiolate, 0.5 mmol of nucleophiles, and 0.05 mmol of DABCO, CH_2Cl_2, at room temperature). The reaction proceeded smoothly and rapidly (within 10 min) to give conjugate addition products in excellent yields with better stereoselectivities than that of DMAD (Table 4). Most of the reaction gave (*E*)-form alkenoic acid esters as the main product. The ^{1}H NMR spectrum of (*E*)-form isomer displayed two doublets with J=12.3 Hz for the two olefinic

Table 3. Reaction of acids with DMAD[a]



Entry	RCOOH	Product	Yield /%[b]	*E*/*Z*[c]
1	CH_3COOH	**11**	94	*Z*
2	$CH_3(CH_2)_2COOH$	**12**	99	*Z*
3		**13**	91	*Z*
4		**14**	85	*Z*
5		**15**	88	*Z*
6		**16**	78	*Z*
7		**17**	88	*Z*
8		**18**[d]	76	*Z*

[a] All the reactions were performed at room temperature and completed within 6 h.
[b] Isolated yields.
[c] Determined by ^{1}H NMR (300 MHz) analysis.
[d] The reaction time need to be extended to 24 h.

protons, while the chemical shift of the (*Z*)-form appeared with J=6.6 Hz. It was in agreement with the proposed (*E*)- and (*Z*)-isomers, respectively.

A proposed mechanism for this reaction was outlined in Scheme 2 based on the previous investigation of Nozaki Kyoko et al.[10] Initially, the zwitterionic intermediate **a**,[11] formed from DABCO and DMAD, deprotonated the nucleophile to form the corresponding intermediates **b** and **c**. Subsequent Michael addition of **b** to **c** gave intermediate **d**, which then eliminated DABCO to afford the final product. According to the proposed reaction mechanism, ethyl

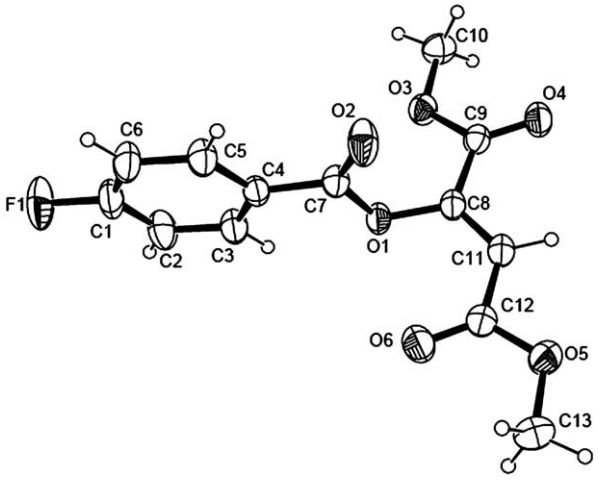
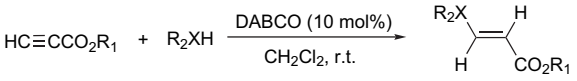
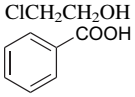
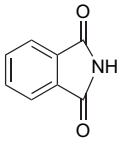


Figure 1. X-ray structure of **17**.

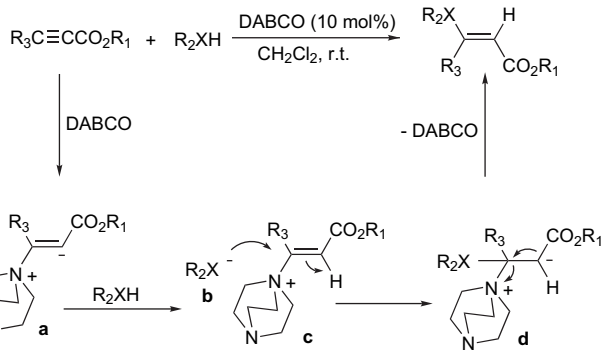
Table 4. Reaction of various nucleophiles with methyl or benzyl propiolate[a]



Entry	R_1	R_2XH	Product	Yield /%[b]	*E*/*Z*[c]
1	Bn	$ClCH_2CH_2OH$	**19**	99	95:5
2	Bn		**20**	98	*E*
3	Bn		**21**	99	*E*
4	CH_3	$ClCH_2CH_2OH$	**22**	90	90:10

[a] All the reactions were performed at room temperature and completed within 10 min.
[b] Isolated yields.
[c] Determined by ^{1}H NMR (300 MHz) spectra.

3-phenylpropiolate should also perform the same reaction with alcohols, acylamides or acids. However, the reaction did not take place even when the reaction mixture was heated to reflux or the reaction time was prolonged to 48 h.

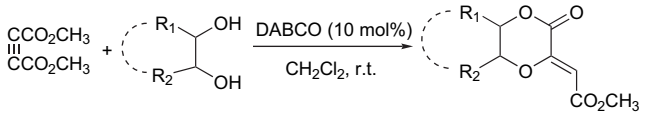


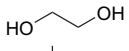
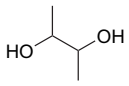
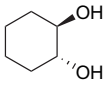
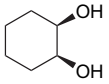
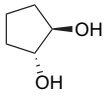
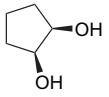
Scheme 2. Plausible mechanism for the reaction of various nucleophiles with activated alkynes.

Further scope exploration revealed that 1,2-diols were another class of suitable nucleophiles for this reaction. Interestingly, 1,2-diols on treatment with DMAD in the presence of a catalytic amount of DABCO (10 mol %) in CH_2Cl_2 afforded 1,4-dioxane derivatives in good to excellent yields. The reaction appeared to be general with a number of 1,2-diols. The results using this mild and straightforward procedure were shown in Table 5. The structure of the product was revealed by ^{1}H and ^{13}C NMR analyses. In addition, the NMR-based structure was confirmed by X-ray crystallographic analysis of **23** as representative example (Fig. 2).[12] All the reactions proceeded extremely fast and completed within 10 min. The formation of 1,4-dioxane derivatives involved a Michael addition of 1,2-diol to DMAD and a subsequent intramolecular esterification. Further study revealed that this reaction did not take place in the absence of DABCO, which suggested that the tertiary amine was required as a catalyst in this procedure.

On the basis of the above encouraging results, we used this concept to devise a new one-step approach to the synthesis

Table 5. Reaction of 1,2-diols with DMAD[a]



Entry	1,2-Diol	Product	Yield/%[b]
1		**23**	80
2		**24**	96
3		**25**	60
4		**26**	74
5		**27**	58
6		**28**	60

[a] All the reactions were performed between DMAD (0.5 mmol), 1,2-diols (3 mmol), and DABCO (0.05 mmol) at room temperature and completed within 10 min.
[b] Isolated yields.

of morpholine derivatives from amino alcohols. As we expected, excellent result was obtained in the reaction of equimolar amount of 2-aminoethanol with DMAD under above conditions (Table 6, entry 1). The structure of the product was deduced from its elemental analysis, mass spectrometry (MS), and ^{1}H and ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum showed a sharp singlet at 3.71 ppm for three methoxy group protons and a broad peak at 8.37 ppm for NH group proton. The olefinic proton resonated at 5.66 ppm corresponding to the proposed (*Z*)-isomer. On top of this, a triplet at 4.50–4.54 ppm and a multiplet at 3.50–3.55 ppm were readily attributable to CH_2 bonding to oxygen and CH_2 bonding to NH group, respectively. It was worthy of note that this reaction could take place in the absence of DABCO. It was demonstrated that the NH_2 group in the amino alcohol played the same role as DABCO in this

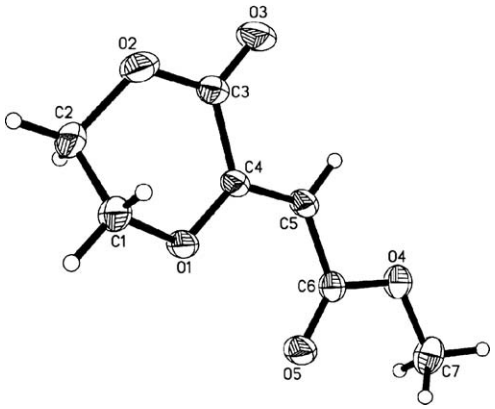
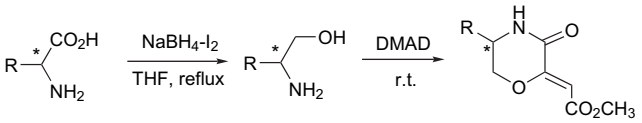
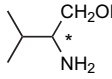
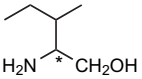
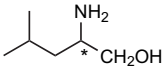
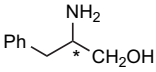
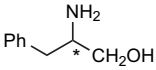
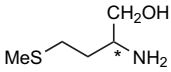
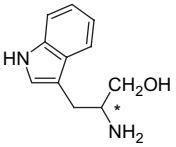
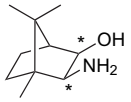


Figure 2. X-ray structure of **23**.

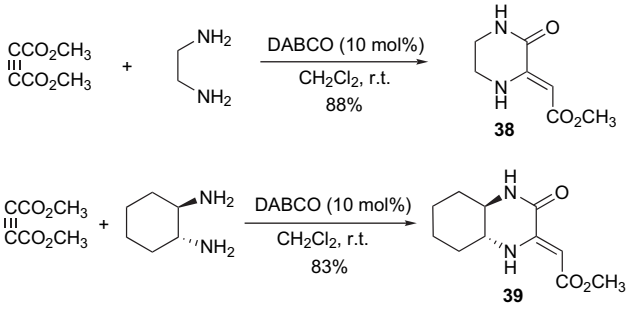
Table 6. Reaction of amino alcohols with DMAD[a]



Entry	Amino acid	Amino alcohol	Product Yield/%[b]	$[\alpha]_D^{20c}$
1	—	$NH_2CH_2CH_2OH$	**29** (80)	—
2	D-Valine		**30** (96)	−58
3	L-Isoleucine		**31** (60)	+109
4	L-Leucine		**32** (74)	+70
5	L-Phenylalanine		**33** (58)	+7
6	D-Phenylalanine		**34** (60)	−7
7	D-Methionine		**35** (80)	−56
8	DL-Tryptophane		**36** (90)	—
9	—		**37** (61)	−38

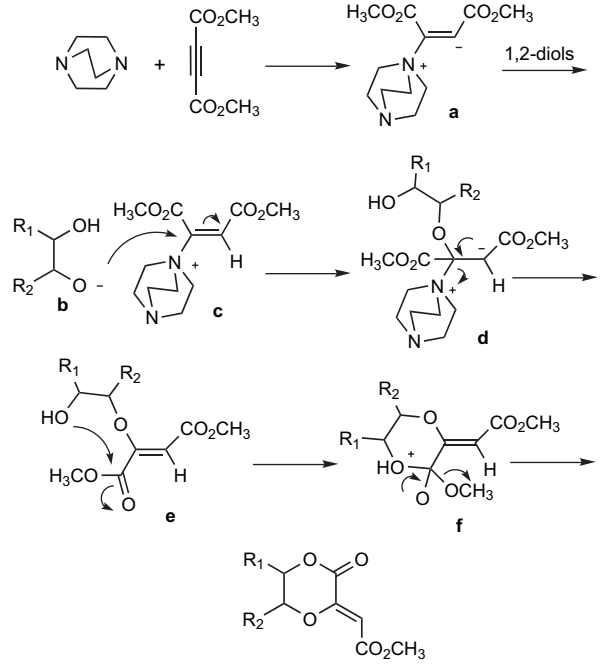
[a] All the reactions were performed between equimolar DMAD and amino alcohols at room temperature and completed within 10 h.
[b] Isolated yields.
[c] $[\alpha]_D$ in $CHCl_3$ (c=1).

reaction. It served not only as a reactant, but also as a catalyst in this reaction. A series of amino alcohols, which were readily prepared from commercial available amino acids,[13] were tested in this process to afford the corresponding morpholines (Table 6, entries 2–8). The reaction was found to be general with respect to various amino alcohols and morpholine derivatives were obtained in high yields. Moreover, we were pleased to find that this technique could also be extended to provide bicyclic morpholine in good yield (Table 6, entry 9). An attractive feature of this synthetic approach was that the incorporation of morpholine C3-substitution was facilitated by the aide availability of natural and unnatural α-amino acids. Similarly, 1,2-diamines, such as ethane-1,2-diamine and cyclohexane-1,2-diamine, could also react with DMAD in the absence of DABCO under the identical conditions to afford piperazinone derivatives in excellent yields (88% and 83%, respectively, Scheme 3). The present process was more efficient with primary 1,2-diamine or amino alcohol having primary NH_2 group, while secondary 1,2-diamine or amino alcohol with secondary NH_2 group did not work in this manner. For example, treatment of *N*1,*N*2-dimethylethane-1,2-diamine or L-prolinol with DMAD did not give the expected products under the same conditions.

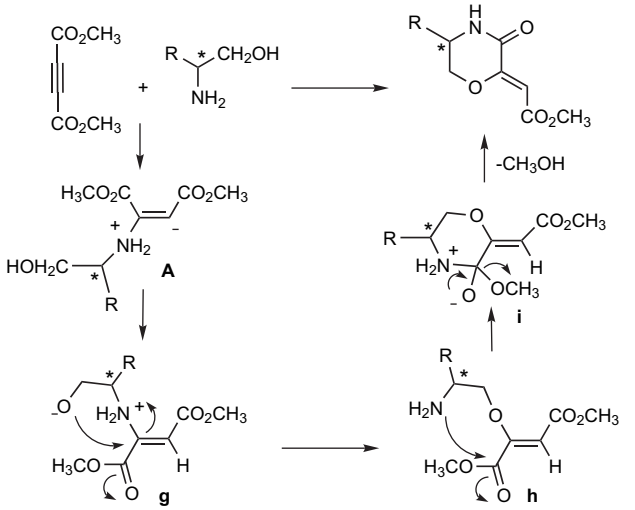


Scheme 3. Reaction of 1,2-diamines with DMAD.

The mechanisms of these reactions have not been unequivocally established, but two plausible explanations were proposed in Schemes 4 and 5. In the reaction of 1,2-diol or 1,2-diamines with DMAD, it might also involve the initial generation of a zwitterionic intermediate **a** between DABCO and DMAD (Scheme 4), which was readily protonated by one of the two protons of the nucleophile to yield intermediates **b** and **c**. Subsequent Michael addition of **b** to **c** formed intermediate **d**. It underwent the elimination of DABCO to form intermediate **e**, which was followed by intramolecular esterification in the presence of DABCO to produce the corresponding 1,3-dioxane derivatives and regenerate DABCO to accomplish the catalytic cycle. In the reaction of amino alcohol with DMAD, similarly, zwitterionic intermediate **A** generated from the addition of amino alcohol to DMAD (Scheme 5) underwent a second Michael addition of the OH group, which was followed by the leaving of NH_2 group. Subsequent intramolecular amidization led to the final morpholine derivatives. The slow transformation step of **h** to **i** was assumed to be the rate determining step, accounting for the longer reaction time required for the final product.



Scheme 4. Plausible mechanism for the reaction of 1,2-diols or 1,2-diamines with DMAD.



Scheme 5. Plausible mechanism for the reaction of amino alcohols with DMAD.

Further comparison studies demonstrated that DABCO was the optimal catalyst for the addition of acids and cyclization of 1,2-diols. Triphenylphosphine, which was commonly employed in the traditional conjugate addition reaction, was ineffective in promoting these reactions under the same conditions. Triethylamine and 4-methylmorpholine gave the lowered yields.

3. Conclusion

In the present work, we reported an excellent catalyst, DABCO, for the reaction of nucleophiles with some activated alkynes. The procedure provided an easy access to unsaturated alkenoic acid ester derivatives and various heterocycles in good to excellent yields under mild reaction conditions. This process expanded not only the scope of organocatalyst catalyzed carbon–carbon bond-forming reaction but also unprecedented approaches to heterocycle formations.

4. Experimental

4.1. General

All reagents were used directly as obtained commercially unless otherwise noted. Melting points were determined on a microscopic apparatus and were uncorrected. Column chromatography was carried out on silica gel. ^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ and ^{13}C NMR spectra were recorded at 75 MHz in $CDCl_3$ using TMS as internal standard. Mass spectra were recorded by the EI method. Nicolet AVATAR 360 FTIR spectrometer was used for IR spectra. HRMS spectra were obtained with a Bruker APEX II instrument.

4.2. Typical procedure for the reaction of various nucleophiles with DMAD. Using alcohol as an example

DMAD (0.5 mmol), DABCO (0.05 mmol), and alcohol (0.5 mmol, 3 mmol for 1,2-diol, 0.5 mmol for other nucleophiles) were stirred in CH_2Cl_2 (4 ml) at room temperature for 10 min. (The reaction time needed to be extended

to 10 h for the reaction of amino alcohols and 6 h for acids.) The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica column to give final products.

4.2.1. Dimethyl 2-methoxyfumarate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.19 (s, 1H), 3.95 (s, 3H), 3.85 (s, 3H), 3.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.9, 163.4, 155.0, 107.9, 61.2, 53.0, 51.9. MS (EI, 70 eV): *m/z* (%)=174 (M^+, 2.58), 159 (20.46), 143 (48.67), 115 (67.41), 69 (100). Known compound.[7a]

4.2.2. Dimethyl 2-methoxymaleate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.21 (s, 1H), 3.89 (s, 3H), 3.75 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.2, 163.9, 162.4, 93.0, 56.9, 52.9, 51.5. MS (EI, 70 eV): *m/z* (%)=174 (M^+, 4.7), 159 (17.2), 143 (51.1), 115 (55), 69 (100). Known compound.[7a]

4.2.3. Dimethyl 2-(2-chloroethoxy) fumarate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.30 (s, 1H), 4.34–4.38 (t, *J*=6 Hz, 2H), 3.85 (s, 3H), 3.77–3.81 (t, *J*=6 Hz, 2H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.3, 162.9, 153.3, 110.6, 73.2, 52.8, 51.7, 42.2. MS (EI, 70 eV): *m/z* (%)=222 (M^+, 0.64), 207 (0.45), 191 (15.05), 163 (41.83), 127 (36.58), 101 (61.28), 69 (100). Anal. Calcd for $C_8H_{11}ClO_5$: C, 43.16; H, 4.98. Found: C, 43.34; H, 4.54. IR (KBr): 2956, 2852, 1728, 1643, 1269.

4.2.4. Dimethyl 2-(2-chloroethoxy) maleate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.22 (s, 1H), 4.09–4.13 (t, *J*=6 Hz, 2H), 3.90 (s, 3H), 3.76–3.79 (t, *J*=5.7 Hz, 2H), 3.71 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.0, 163.5, 160.9, 94.2, 69.4, 53.0, 51.6, 40.3. MS (EI, 70 eV): *m/z* (%)=222 (M^+, 0.49), 207 (0.15), 191 (8.71), 163 (22.19), 127 (16.43), 101 (49.94), 69 (100). Anal. Calcd for $C_8H_{11}ClO_5$: C, 43.16; H, 4.98. Found: C, 43.12; H, 4.68. IR (KBr): 2956, 2851, 1751, 1717, 1630, 1212, 1154.

4.2.5. Dimethyl 2-(prop-2-ynyloxy) fumarate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.36 (s, 1H), 4.91 (s, 2H), 3.85 (s, 3H), 3.77 (s, 3H), 2.58 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.4, 163.1, 151.9, 111.5, 77.1, 77.0, 60.2, 53.0, 51.8. MS (EI, 70 eV): *m/z* (%)=198 (M^+, 1.25), 183 (0.63), 167 (12.10), 139 (74.73), 111 (60.55), 69 (100). Anal. Calcd for $C_9H_{10}O_5$: C, 54.55; H, 5.09. Found: C, 54.32; H, 5.34. IR (KBr): 2958, 2852, 1733, 1633, 1256, 1116.

4.2.6. Dimethyl 2-(prop-2-ynyloxy) maleate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.37 (s, 1H), 4.60 (s, 2H), 3.89 (s, 3H), 3.72 (s, 3H), 2.66 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=165.8, 163.3, 159.7, 95.1, 77.8, 75.4, 57.4, 52.9, 51.6. MS (EI, 70 eV): *m/z* (%)=198 (M^+, 0.60), 183 (0.43), 167 (18.84), 139 (66.80), 111 (52.50), 69 (100). Anal. Calcd for $C_9H_{10}O_5$: C, 54.55; H, 5.09. Found: C, 54.57; H, 5.04. IR (KBr): 2954, 2853, 1748, 1714, 1630, 1144.

4.2.7. Dimethyl 2-(2-(diethylamino) ethoxy) maleate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.21 (s, 1H), 3.90–3.95 (t, *J*=6.6 Hz, 2H), 3.88 (s, 3H), 3.70 (s, 3H), 2.81–2.85 (t, *J*=6.6 Hz, 2H), 2.54–2.61 (q, *J*=7.2 Hz, 4H), 1.01–1.05 (t, *J*=7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.1, 163.8, 161.7, 93.1, 68.7, 52.6, 51.3, 50.5, 47.6. MS (EI, 70 eV): *m/z* (%)=259 (M^+, 0.44), 244 (0.92), 100 (15.08), 86 (100). Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.37; H, 8.02; N, 5.56. IR (KBr): 2956, 2926, 2851, 1754, 1719, 1627, 1209, 1150.

4.2.8. Dimethyl 2-(benzyloxy) fumarate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.31–7.45 (m, 5H), 6.25 (s, 1H), 5.19 (s, 2H), 3.81 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.5, 163.3, 153.6, 136.0, 128.4, 128.3, 128.1, 110.2, 74.9, 52.7, 51.6. MS (EI, 70 eV): *m/z* (%)=250 (M^+, 0.55), 218 (0.63), 191 (1.33), 159 (2.20), 91 (100). Anal. Calcd for $C_{13}H_{14}O_5$: C, 62.39; H, 5.64. Found: C, 62.10; H, 5.22. IR (KBr): 2953, 2849, 1729, 1640, 1266, 742, 698.

4.2.9. Dimethyl 2-(benzyloxy) maleate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.36–7.38 (m, 5H), 5.29 (s, 1H), 4.91 (s, 2H), 3.88 (s, 3H), 3.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.2, 163.8, 161.5, 134.0, 128.7, 127.8, 94.0, 71.8, 52.9, 51.6. MS (EI, 70 eV): *m/z* (%)=250 (M^+, 0.14), 218 (0.44), 191 (1.35), 159 (3.24), 91 (100). Anal. Calcd for $C_{13}H_{14}O_5$: C, 62.39; H, 5.64. Found: C, 62.39; H, 5.52. IR (KBr): 2954, 1752, 1725, 1637, 1203, 1131, 826.

4.2.10. Dimethyl 2-(2-chloroacetamido) maleate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=11.03 (s, 1H), 5.64 (s, 1H), 4.14 (s, 2H), 3.83 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.0, 164.8, 163.9, 142.4, 104.3, 53.4, 52.4, 42.4. MS (EI, 70 eV): *m/z* (%)=235 (M^+, 5.36), 237 (1.85), 204 (11.46), 206 (3.76), 176 (100), 178 (30.74). Anal. Calcd for $C_8H_{10}ClNO_5$: C, 40.78; H, 4.28; N, 5.94. Found: C, 40.61; H, 4.01; N, 5.65. IR (KBr): 3285, 2956, 2851, 1746, 1694, 1638, 1295, 1223.

4.2.11. Dimethyl 2-(acrylamido) maleate. White powder, mp 57–58 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=10.45 (s, 1H), 5.87–6.46 (m, 3H), 5.54 (s, 1H), 3.88 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.4, 164.1, 162.9, 143.8, 130.2, 129.4, 101.5, 53.1, 51.9. MS (EI, 70 eV): *m/z* (%)=213 (M^+, 3.39), 182 (3.63), 154 (35.25), 55 (100). Anal. Calcd for $C_9H_{11}NO_5$: C, 50.70; H, 5.20; N, 6.57. Found: C, 50.51; H, 5.13; N, 6.65. IR (KBr): 3302, 2954, 2852, 1745, 1692, 1633, 1283, 1220.

4.2.12. Dimethyl 2-(benzamido) maleate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=11.24 (s, 1H), 7.47–7.96 (m, 5H), 5.60 (s, 1H), 3.92 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.8, 164.5, 144.5, 133.1, 131.8, 128.9, 127.8, 101.2, 53.1, 51.9. MS (EI, 70 eV): *m/z* (%)=263 (M^+, 5.55), 232 (4.71), 204 (82.65), 105 (100). Anal. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.19; H, 5.28; N, 5.20. IR (KBr): 3291, 2954, 2851, 1746, 1684, 1631, 1289, 1220, 774, 708.

4.2.13. Dimethyl 2-(tosylamino) maleate. White powder, mp 65–66 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=10.25 (s, 1H), 7.80–7.83 (d, *J*=8.1 Hz, 2H), 7.32–7.35 (d, *J*=8.4 Hz, 2H), 5.74 (s, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.2, 162.9, 144.2, 143.6, 137.2, 129.4, 127.1, 103.4, 53.1, 52.1, 21.5.

MS (EI, 70 eV): *m/z* (%)=313 (M^+, 0.85), 282 (0.13), 189 (5.19), 164 (19.42), 155 (30.23), 91 (100). Anal. Calcd for $C_{13}H_{15}NO_6S$: C, 49.83; H, 4.83; N, 4.47. Found: C, 49.61; H, 4.76; N, 4.17. IR (KBr): 3116, 2955, 2844, 1747, 1682, 1627, 1272, 1230, 1161, 837.

4.2.14. Dimethyl 2-(1,3-dioxoisoindolin-2-yl) maleate. White powder, mp 104–105 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.78–7.95 (m, 4H), 7.18 (s, 1H), 3.87 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=165.5, 163.1, 162.3, 134.5, 132.0, 131.6, 128.6, 124.1, 53.5, 52.4. MS (EI, 70 eV): *m/z* (%)=289 (M^+, 8.61), 258 (5.89), 230 (100), 104 (97.83), 76 (81.30). Anal. Calcd for $C_{14}H_{11}NO_6$: C, 58.13; H, 3.83; N, 4.84. Found: C, 58.36; H, 4.10; N, 4.55. IR (KBr): 2956, 2924, 2853, 1732, 1659, 720.

4.2.15. Dimethyl 2-acetoxy fumarate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.69 (s, 1H), 3.86 (s, 3H), 3.77 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.7, 163.1, 161.4, 146.5, 116.8, 53.1, 51.9, 20.2. MS (EI, 70 eV): *m/z* (%)=202 (M^+, 0.07), 171 (4.40), 143 (46.92), 101 (77.55), 69 (55.10), 43 (100). Anal. Calcd for $C_8H_{10}O_6$: C, 47.53; H, 4.99. Found: C, 47.59; H, 4.73. IR (KBr): 2957, 2852, 1779, 1732, 1662, 1280, 1162.

4.2.16. Dimethyl 2-(butyryloxy) fumarate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.68 (s, 1H), 3.84 (s, 3H), 3.76 (s, 3H), 2.54–2.59 (t, *J*=6.9 Hz, 2H), 1.74–1.81 (m, 2H), 1.01–1.06 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 163.1, 161.5, 146.6, 116.7, 53.0, 51.9, 35.3, 17.9, 13.3. MS (EI, 70 eV): *m/z* (%)=230 (M^+, 0.09), 199 (1.24), 171 (1.97), 129 (1.76), 71 (100). Anal. Calcd for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13. Found: C, 51.93; H, 5.72. IR (KBr): 2961, 2880, 1773, 1732, 1664, 1279, 1102.

4.2.17. 13 Dimethyl 2-(2-phenylacetoyloxy) fumarate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.29–7.36 (m, 5H), 6.68 (s, 1H), 3.90 (s, 2H), 3.78 (s, 3H), 3.67 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.5, 163.1, 161.4, 146.4, 132.5, 129.5, 128.5, 127.3, 117.1, 53.1, 52.0, 40.3. MS (EI, 70 eV): *m/z* (%)=278 (M^+, 0.02), 247 (0.38), 219 (0.20), 118 (17.35), 91 (100). Anal. Calcd for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07. Found: C, 60.39; H, 5.32. IR (KBr): 2953, 2924, 2853, 1773, 1729, 1664, 1279, 1102, 789, 689.

4.2.18. Dimethyl 2-(2-(naphthalen-6-yl) acetoyloxy) fumarate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.40–8.08 (m, 7H), 6.66 (s, 1H), 4.32 (s, 2H), 3.68 (s, 3H), 3.58 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.4, 163.1, 161.4, 146.4, 133.7, 132.0, 129.1, 128.6, 128.3, 126.3, 125.8, 125.4, 123.8, 117.2, 53.0, 51.9, 38.2. MS (EI, 70 eV): *m/z* (%)=328 (M^+, 9.61), 297 (0.30), 168 (100), 141 (73.02), 115 (67.61). Anal. Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.88; H, 4.95. IR (KBr): 2954, 2849, 1774, 1730, 1664, 1279, 1105, 789, 689.

4.2.19. Dimethyl 2-(benzoxy) fumarate. White powder, mp 40–42 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.14–8.16 (d, *J*=7.2 Hz, 2H), 7.61–7.65 (t, *J*=7.5 Hz, 1H), 7.47–7.52 (t, *J*=7.5 Hz, 2H), 6.79 (s, 1H), 3.84 (s, 3H), 3.70 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=163.6, 163.0, 161.5, 146.8, 133.9, 130.3, 128.5, 128.0, 117.0, 53.1, 52.0. MS (EI, 70 eV): *m/z* (%)=264 (M^+, 0.49), 2.33 (0.74), 205 (0.11), 105 (100), 77 (42.36). Anal. Calcd for $C_{13}H_{12}O_6$: C, 59.09; H, 4.58. Found: C, 59.37; H, 4.34. IR (KBr): 2956, 2848, 1736, 1664, 1279, 1104, 707, 669.

4.2.20. Dimethyl 2-(2-fluorobenzoxy) fumarate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.17–8.13 (m, 4H), 6.80 (s, 1H), 3.87 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.1, 163.0, 161.4, 160.7, 146.4, 135.8, 135.7, 132.6, 124.2, 117.3, 116.9, 116.6, 116.5, 53.2, 52.1. MS (EI, 70 eV): *m/z* (%)=282 (M^+, 0.27), 251 (0.59), 223 (0.36), 123 (100), 95 (19.92). HRMS (EI) calcd for $C_{13}H_{11}FO_6$: (M+Na) 305.0432. Found: 305.0431. IR (KBr): 2957, 1733, 1279, 756.

4.2.21. Dimethyl 2-(4-fluorobenzoxy) fumarate. White powder, mp 69–70 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.15–8.20 (m, 2H), 7.15–7.21 (t, *J*=8.7 Hz, 2H), 6.79 (s, 1H), 3.86 (s, 3H), 3.72 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=168.0, 164.6, 163.0, 162.6, 161.5, 146.7, 133.2, 133.0, 124.3, 117.2, 116.0, 115.7, 53.2, 52.0. MS (EI, 70 eV): *m/z* (%)=282 (M^+, 0.35), 251 (0.58), 223 (0.15), 123 (100), 95 (29.54). HRMS (EI) calcd for $C_{13}H_{11}FO_6$: (M+Na) 305.0432. Found: 305.0437. IR (KBr): 2964, 1732, 1273, 856, 759.

4.2.22. Dimethyl 2-(2-hydroxybenzoxy) fumarate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=9.99 (s, 1H), 6.94–8.01 (m, 4H), 6.82 (s, 1H), 3.88 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.1, 162.9, 162.0, 161.3, 146.0, 136.9, 130.8, 119.7, 117.8, 110.9, 53.4, 52.3. MS (EI, 70 eV): *m/z* (%)=280 (M^+, 0.37), 249 (0.68), 221 (0.90), 121 (100). Anal. Calcd for $C_{13}H_{12}O_7$: C, 55.72; H, 4.32. Found: C, 55.83; H, 4.48. IR (KBr): 3239, 2957, 2851, 1732, 1699, 1653, 1293, 1156, 759.

4.2.23. (*E*)-Benzyl 3-(2-chloroethoxy) acrylate. White powder, mp 40–41 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.59–7.63 (d, *J*=12.3 Hz, 1H), 7.30–7.36 (m, 5H), 5.26–5.30 (d, *J*=12.3 Hz, 1H), 5.15 (s, 2H), 4.03–4.07 (t, *J*=6 Hz, 2H), 3.66–3.70 (t, *J*=5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.0, 161.8, 136.2, 128.4, 128.0, 97.1, 70.5, 65.6, 41.2. MS (EI, 70 eV): *m/z* (%)=240 (M^+, 0.44), 195 (21.69), 160 (32.72), 133 (100), 91 (99.32). Anal. Calcd for $C_{12}H_{13}ClO_3$: C, 59.88; H, 5.44. Found: C, 59.69; H, 5.66. IR (KBr): 1709, 1627, 1132, 963, 751, 698.

4.2.24. (*Z*)-Benzyl 3-(2-chloroethoxy) acrylate. White powder, mp 50–51 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.26–7.40 (m, 5H), 6.56–6.59 (d, *J*=6.9 Hz, 1H), 5.15 (s, 2H), 4.93–4.96 (d, *J*=7.2 Hz, 1H), 4.24–4.28 (t, *J*=6 Hz, 2H), 3.72–3.76 (t, *J*=6.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.7, 158.9, 136.3, 128.4, 128.0, 127.9, 96.5, 96.4, 74.9, 65.4, 42.2. MS (EI, 70 eV): *m/z* (%)=240 (M^+, 3.17), 195 (7.77), 160 (4.66), 133 (96.92), 91 (100). Anal. Calcd for $C_{12}H_{13}ClO_3$: C, 59.88; H, 5.44. Found: C, 59.67; H, 5.56. IR (KBr): 1709, 1634, 1157, 1134, 745, 697.

4.2.25. (*E*)-2-((Benzyloxy) carbonyl) vinyl benzoate. White powder, mp 79–81 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.56–8.60 (d, *J*=12.3 Hz, 1H), 7.32–8.11 (m, 10H), 5.93–5.97 (d, *J*=12.6 Hz, 1H), 5.22 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=165.9, 162.4, 150.2, 135.8,

134.3, 130.3, 128.7, 128.5, 128.2, 127.6, 106.0, 66.2. MS (EI, 70 eV): *m/z* (%)=282 (M^+, 0.02), 177 (1.49), 160 (2.76), 105 (100). Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.15; H, 5.11. IR (KBr): 1744, 1708, 1655, 1261, 1106, 959, 755, 700, 679.

4.2.26. (*E*)-Benzyl 3-(1,3-dioxoisoindolin-2-yl) acrylate. White powder, mp 108–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.23–7.97 (m, 10H), 6.97–7.02 (d, J=15 Hz, 1H), 5.22 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.9, 165.4, 135.9, 135.2, 131.4, 128.5, 128.2, 124.2, 108.3, 66.3. MS (EI, 70 eV): *m/z* (%)=307 (M^+, 3.59), 261 (36.67), 200 (98.52), 91 (100). Anal. Calcd for $C_{18}H_{13}NO_4$: C, 70.35; H, 4.26; N, 4.56. Found: C, 70.18; H, 4.39; N, 4.27. IR (KBr): 2956, 1751, 1717, 1630, 1212, 1153, 971, 764, 676.

4.2.27. (*E*)-Methyl 3-(2-chloroethoxy) acrylate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.58–7.62 (d, J=12.9 Hz, 1H), 5.23–5.28 (d, J=12.6 Hz, 1H), 4.09–4.13 (t, J=5.7 Hz, 2H), 3.73–3.77 (t, J=5.7 Hz, 2H), 3.71 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.7, 161.6, 97.1, 70.5, 51.2, 41.3. MS (EI, 70 eV): *m/z* (%)=164 (M^+, 17.0), 133 (100). Anal. Calcd for $C_6H_9ClO_3$: C, 43.78; H, 5.51. Found: C, 43.91; H, 5.11. IR (KBr): 2952, 1712, 1629, 1143.

4.2.28. (*Z*)-Methyl 2-(3-oxo-1,4-dioxan-2-ylidene) acetate. White powder, mp 105–106 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.19 (s, 1H), 4.60–4.63 (m, 2H), 4.37–4.40 (m, 2H), 3.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.2, 158.5, 150.0, 104.7, 66.5, 64.2, 51.4. MS (EI, 70 eV): *m/z* (%)=172 (M^+, 8.01), 141 (70.01), 69 (100). Anal. Calcd for $C_7H_8O_5$: C, 48.84; H, 4.68. Found: C, 48.79; H, 4.81. IR (KBr): 2960, 1742, 1715, 1639, 1287, 1236, 1092.

4.2.29. (*Z*)-Methyl 2-(5,6-dimethyl-3-oxo-1,4-dioxan-2-ylidene) acetate. White powder, mp 77–78 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.14 (s, 1H), 4.41–4.46 (m, 1H), 4.06–4.11 (m, 1H), 3.72 (s, 3H), 1.37–1.42 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.7, 159.5, 150.2, 104.2, 78.7, 76.0, 51.7, 17.2, 16.5. MS (EI, 70 eV): *m/z* (%)=200 (M^+, 23.1), 169 (53.7), 69 (100). Anal. Calcd for $C_9H_{12}O_5$: C, 54.00; H, 6.04. Found: C, 54.24; H, 6.26. IR (KBr): 2959, 2917, 2850, 1734, 1701, 1646, 1279, 1237, 1070.

4.2.30. (*Z*)-Methyl 2-((4a*R*,8a*R*)-hexahydro-2-oxobenzo-[*b*][1,4]dioxin-3 (2*H*)-ylidene) acetate. White powder, mp 62–63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.12 (s, 1H), 4.21–4.29 (m, 1H), 3.86–4.94 (m, 1H), 3.69 (s, 3H), 1.21–2.32 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.4, 159.3, 150.6, 103.9, 79.5, 77.5, 51.4, 29.5, 29.1, 23.0, 22.8. MS (EI, 70 eV): *m/z* (%)=226 (M^+, 4.80), 195 (8.33), 101 (54.37), 69 (100). Anal. Calcd for $C_{11}H_{14}O_5$: C, 58.40; H, 6.24. Found: C, 58.64; H, 5.91. IR (KBr): 2947, 2865, 1742, 1712, 1641, 1259, 1188, 1072.

4.2.31. (*Z*)-Methyl 2-((4a*S*,8a*R*)-hexahydro-2-oxobenzo-[*b*][1,4]dioxin-3(2*H*)-ylidene) acetate. Sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.17 (s, 1H), 4.67–4.71 (m, 1H), 4.42–4.46 (m, 1H), 3.75 (s, 3H), 1.42–1.97 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.6, 159.2, 149.2, 103.9, 76.0, 73.0, 51.5, 28.3, 27.4, 21.0, 20.3. MS (EI, 70 eV): *m/z* (%)=226 (M^+, 9.81), 195 (19.09), 101 (46.37), 69 (100). Anal. Calcd for $C_{11}H_{14}O_5$: C, 58.40; H, 6.24. Found: C, 58.28; H, 5.94. IR (KBr): 2949, 2869, 1720, 1643, 1237, 1174, 1073.

4.2.32. (*Z*)-Methyl 2-((4a*R*,7a*R*)-tetrahydro-2-oxo-2*H*-cyclopenta[*b*][1,4]dioxin-3(4a*H*)-ylidene) acetate. White powder, mp 68–70 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.16 (s, 1H), 4.48–4.57 (m, 1H), 4.19–4.28 (m, 1H), 3.73 (s, 3H), 1.69–2.24 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.4, 160.0, 150.7, 105.2, 80.6, 79.4, 51.6, 24.3, 24.0, 17.4. MS (EI, 70 eV): *m/z* (%)=212 (M^+, 26.09), 181 (72.38), 69 (100). Anal. Calcd for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.58; H, 5.75. IR (KBr): 2924, 1721, 1642, 1233, 1105.

4.2.33. (*Z*)-Methyl 2-((4a*S*,7a*R*)-tetrahydro-2-oxo-2*H*-cyclopenta[*b*][1,4]dioxin-3(4a*H*)-ylidene) acetate. White powder, mp 94–96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.27 (s, 1H), 4.85–4.88 (m, 1H), 4.67–4.70 (m, 1H), 3.75 (s, 3H), 1.76–2.18 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=164.4, 158.4, 148.0, 105.3, 80.1, 76.1, 51.5, 29.0, 27.8, 19.2. MS (EI, 70 eV): *m/z* (%)=212 (M^+, 24.02), 181 (100), 69 (59.28). Anal. Calcd for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.64; H, 5.90. IR (KBr): 2956, 1709, 1642, 1239, 1087.

4.2.34. (*Z*)-Methyl 2-(3-oxomorpholin-2-ylidene) acetate. White powder, mp 79–80 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.37 (s, 1H), 5.66 (s, 1H), 4.50–4.54 (t, J=4.8 Hz, 2H), 3.71 (s, 3H), 3.50–3.55 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 160.3, 144.4, 89.9, 67.3, 51.0, 38.4. MS (EI, 70 eV): *m/z* (%)=171 (M^+, 41.6), 140 (52.9), 68 (100). Anal. Calcd for $C_7H_9NO_4$: C, 49.12; H, 5.30; N, 8.18. Found: C, 49.22; H, 5.75; N, 8.24. IR (KBr): 3326, 2923, 1744, 1663, 1619, 1287, 1233.

4.2.35. (*Z*)-Methyl 2-((*R*)-5-isopropyl-3-oxomorpholin-2-ylidene) acetate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.51 (s, 1H), 5.56 (s, 1H), 4.26–4.46 (m, 2H), 3.66 (s, 3H), 3.22–3.29 (m, 2H), 1.76–1.88 (m, 1H), 0.97–1.03 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 160.3, 144.0, 89.1, 69.4, 53.7, 50.8, 29.6, 18.6, 18.4. MS (EI, 70 eV): *m/z* (%)=213 (M^+, 16.3), 182 (8.9), 170 (100), 138 (97.9). Anal. Calcd for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.43; H, 7.20; N, 6.19. IR (KBr): 3310, 2965, 1747, 1664, 1620, 1265, 1237.

4.2.36. (*Z*)-Methyl 2-((*S*)-5-*sec*-butyl-3-oxomorpholin-2-ylidene) acetate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.55 (s, 1H), 5.61 (s, 1H), 4.33–4.51 (m, 2H), 3.71 (s, 3H), 3.38–3.43 (m, 1H), 1.57–1.70 (m, 2H), 1.25–1.34 (m, 1H), 0.94–1.01 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 160.3, 144.0, 88.9, 69.3, 52.3, 50.8, 35.8, 25.1, 14.6, 10.8. MS (EI, 70 eV): *m/z* (%)=227 (M^+, 16.0), 196 (7.8), 170 (100), 138 (92.0). Anal. Calcd for $C_{11}H_{17}NO_4$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.23; H, 7.58; N, 5.82. IR (KBr): 3309, 2966, 1748, 1664, 1619, 1254, 1233.

4.2.37. Methyl 2-((*S*)-5-isobutyl-3-oxomorpholin-2-ylidene) acetate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.46 (s, 1H), 5.61 (s, 1H), 4.17–4.46 (m, 2H), 3.71 (s, 3H), 3.66–3.69 (m, 1H), 1.32–1.80 (m, 3H), 0.97–1.01 (t,

J=6.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 160.2, 143.9, 89.2, 71.3, 50.8, 46.2, 39.7, 24.4, 22.8, 21.7. MS (EI, 70 eV): m/z (%)=227 (M^+, 15.43), 196 (7.72), 170 (100), 138 (93.98). Anal. Calcd for $C_{11}H_{17}NO_4$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.47; H, 7.15; N, 5.97. IR (KBr): 3310, 2957, 1748, 1666, 1619, 1253, 1227.

4.2.38. (*Z*)-Methyl 2-((*S*)-5-benzyl-3-oxomorpholin-2-ylidene) acetate. White powder, mp 83–84 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.36 (s, 1H), 7.20–7.39 (m, 5H), 5.65 (s, 1H), 4.22–4.43 (m, 2H), 3.74–3.80 (m, 1H), 3.68 (s, 3H), 2.85–2.87 (d, J=7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.1, 160.3, 143.4, 135.3, 129.0, 128.9, 127.4, 90.2, 70.2, 51.0, 49.7, 38.0. MS (EI, 70 eV): m/z (%)=261 (M^+, 6.51), 230 (2.83), 170 (100), 138 (89.10). Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.61; H, 5.88; N, 5.09. IR (KBr): 3315, 2949, 1740, 1667, 1622, 1238, 772, 698.

4.2.39. (*Z*)-Methyl 2-((*R*)-5-benzyl-3-oxomorpholin-2-ylidene) acetate. White powder, mp 74–75 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.36 (s, 1H), 7.20–7.39 (m, 5H), 5.65 (s, 1H), 4.22–4.42 (m, 2H), 3.77–3.79 (m, 1H), 3.68 (s, 3H), 2.84–2.87 (d, J=7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.0, 160.3, 143.4, 135.3, 129.0, 128.9, 127.4, 90.1, 70.2, 50.9, 49.6, 38.0. MS (EI, 70 eV): m/z (%)=261 (M^+, 5.17), 230 (2.67), 170 (91.84), 138 (100). Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.52; H, 5.76; N, 4.97. IR (KBr): 3321, 2947, 1740, 1667, 1622, 1238, 772, 699.

4.2.40. (*Z*)-Methyl 2-((*R*)-5-(2-(methylthio) ethyl)-3-oxomorpholin-2-ylidene) acetate. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.47 (s, 1H), 5.59 (s, 1H), 4.21–4.50 (m, 2H), 3.68–3.78 (m, 1H), 3.66 (s, 3H), 2.50–2.65 (m, 2H), 2.09 (s, 3H), 1.77–1.95 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.2, 160.1, 143.4, 89.9, 70.5, 50.9, 46.9, 30.5, 29.9, 15.4. MS (EI, 70 eV): m/z (%)=245 (M^+, 12.60), 214 (5.26), 170 (19.14), 138 (84.38), 61 (100). Anal. Calcd for $C_{10}H_{15}NO_4S$: C, 48.96; H, 6.16; N, 5.71. Found: C, 49.25; H, 6.39; N, 5.44. IR (KBr): 3307, 2949, 1745, 1666, 1619, 1269, 1240.

4.2.41. (*Z*)-Methyl 2-(5-((1*H*-indol-3-yl) methyl)-3-oxomorpholin-2-ylidene) acetate. Yellow powder, mp 59–61 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.40 (s, 1H), 8.31 (s, 1H), 7.54–7.56 (d, J=7.8 Hz, 1H), 7.36–7.39 (d, J=8.4 Hz, 1H), 7.13–7.25 (m, 3H), 5.65 (s, 1H), 4.27–4.49 (m, 2H), 3.88–3.91 (m, 1H), 3.67 (s, 3H), 2.90–3.11 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.3, 160.5, 143.6, 136.4, 126.7, 123.1, 122.5, 119.8, 118.2, 111.5, 109.3, 89.8, 70.8, 51.0, 48.4, 27.7. MS (EI, 70 eV): m/z (%)=300 (M^+, 3.02), 269 (0.42), 241 (2.00), 138 (4.20), 130 (100). Anal. Calcd for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.76; H, 5.55; N, 9.12. IR (KBr): 3407, 2948, 1742, 1664, 1616, 1260, 1237, 744.

4.2.42. (*Z*)-Methyl 1,7,7-trimethylbicyclo[2.2.1]hepta-[*c*]2-((4a*S*,8a*R*)-3-oxomorpholin-2-ylidene) acetate. White powder, mp 87–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.44 (s, 1H), 5.75 (s, 1H), 4.42–4.45 (d, J=8.7 Hz, 1H), 3.71 (s, 3H), 3.59–3.62 (dd, J=8.4 Hz, J=3 Hz, 1H), 0.82–1.91 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.5, 159.1, 142.2, 89.7, 85.6, 55.4, 51.5, 50.9, 50.3, 47.5, 32.4, 25.4, 22.1, 19.7, 10.7. MS (EI, 70 eV): m/z (%)=279 (M^+, 17.22), 248 (3.55), 169 (17.17), 95 (100). Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.23; H, 8.01; N, 5.34. IR (KBr): 3292, 2954, 1738, 1664, 1628, 1258.

4.2.43. (*Z*)-Methyl 2-(3-oxopiperazin-2-ylidene) acetate. White powder, mp 169–170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.29 (s, 1H), 7.87 (s, 1H), 5.59 (s, 1H), 3.70 (s, 3H), 3.42–3.55 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.8, 161.6, 148.9, 86.3, 50.7, 40.1, 38.9. MS (EI, 70 eV): m/z (%)=170 (M^+, 92.2), 139 (100), 110 (75.4). Anal. Calcd for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.18; H, 5.85; N, 16.12. IR (KBr): 3328, 3203, 3073, 2948, 1698, 1657, 1621, 1218.

4.2.44. (*Z*)-Methyl 2-((4a*R*,8a*R*)-hexahydro-3-oxopiperazin-2(1*H*,2*H*,4*H*)-ylidene) acetate. White powder, mp 188–189 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.08 (s, 1H), 7.63 (s, 1H), 5.59 (s, 1H), 3.69 (s, 3H), 3.10–3.25 (m, 2H), 1.37–1.98 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.8, 161.6, 149.1, 86.0, 55.4, 54.9, 50.7, 29.5, 29.4, 23.7, 23.4. MS (EI, 70 eV): m/z (%)=224 (M^+, 83.9), 193 (41.3), 41 (100). Anal. Calcd for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: C, 59.01; H, 7.57; N, 12.26. IR (KBr): 3296, 3191, 3072, 2933, 1693, 1657, 1615, 1212.

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References and notes

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One-step construction of carbazoles by way of the palladium-catalyzed double N-arylation reaction and its application to the total synthesis of murrastifoline-A

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Abstract—The one-step construction of *N*-substituted carbazoles by way of the Pd-catalyzed double N-arylation reaction of primary amines with 2,2′-dibromobiphenyl is described. Aryl and aliphatic amines including *tert*-butylamine and a protected glucopyranosylamine were effectively transformed into the corresponding *N*-substituted carbazoles. The first total synthesis of murrastifoline-A, a biscarbazole alkaloid, based on this methodology is also presented.

1. Introduction

Carbazole alkaloids are known to show a wide range of biological properties, such as antitumor, antibiotic, psychotropic, antiinflammatory, and antihistaminic activities.[1] Carbazoles are also useful organic materials, as they possess photorefractive, photoconductive, and light-emitting properties. Due to the interesting and important properties of carbazoles, a number of methodologies for the construction of the carbazole ring have been reported.[2] Dehydrogenation of tetrahydrocarbazoles prepared by the Fischer–Borsche synthesis is one of the most classical methods.[3a] Coupling of the metal-coordinated cyclohexadienylium ion with an electron-rich arylamine, followed by metal-mediated oxidative cyclization and aromatization,[3b] the Pd(0) catalyzed intramolecular cyclization of 2-amino-2′-halobiphenyl,[3c] and the Pd(II) mediated oxidative cyclization of a diarylamine[3d] have also been developed. Diels–Alder reaction,[3e] electrocyclic reaction,[3f] and acid catalyzed cyclization of ketosulfoxide[3g] are methods used for the construction from indole derivatives. Cyclization of 2-arylacetanilides by the action of $Pd(OAc)_2$ and $Cu(OAc)_2$ in the presence of O_2,[3h] and the anionic [4+2] cycloaddition of furoindolones[3i] were reported in 2005. These methods, however, sometimes encounter difficulties in controlling the regioselectivities during the preparation of multi-substituted carbazoles.

Recently, Nozaki and co-workers reported a new synthetic methodology; the Pd-catalyzed double N-arylation of primary amines with biphenyls possessing leaving groups (Br, I, and OTf) at C-2 and 2′.[4] This method is an important extension of the Buchwald–Hartwig N-arylation reaction,[5] and proved to be an excellent protocol for the regioselective construction of unsymmetrical multi-substituted carbazoles in one-step. By this reaction, a variety of primary amines, such as aryl amines and protected amines (*O*-alkyl carbamates) were successfully transformed into the corresponding *N*-substituted carbazoles, however, lower yield has been observed when an aliphatic primary amine (*n*-octylamine) was employed as the substrate under the conditions using $Pd_2(dba)_3$, *t*-Bu_3P, and NaO*t*-Bu in toluene.[4a] The Nozaki group also reported the successful synthesis of mukonine, a carbazole alkaloid, using this novel methodology.[4b]

Our group has an interest in the application of the Buchwald–Hartwig N-arylation reaction to the natural products' synthesis, and reported the first total synthesis of spicamycin,[6] a novel nucleoside antibiotic possessing a unique N-glycoside structure, by way of the Pd-catalyzed coupling of a heptopyranosylamine with a protected 6-chloropurine derivative.[6a,d] To extend the N-arylation methodology to the synthesis of a variety of natural products, we have independently studied the possibility of the Pd-catalyzed double N-arylation reaction of primary amines with 2,2′-dibromobiphenyl. In this paper, we report our results of the Pd-catalyzed double N-arylation reaction, which generated *N*-aryl-, *N*-alkyl-, and *N*-(glucopyranosyl)carbazoles in moderate to high yields in one-pot reactions. The first total synthesis

Keywords: *N*-Substituted carbazole; Double N-arylation; One-pot synthesis; Murrastifoline-A.
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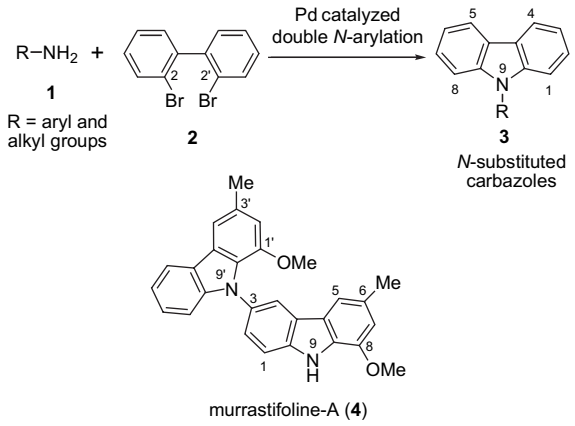


Figure 1.

of murrastifoline-A (**4**), a biscarbazole alkaloid, utilizing this methodology is also disclosed[7] (Fig. 1).

2. Results and discussion

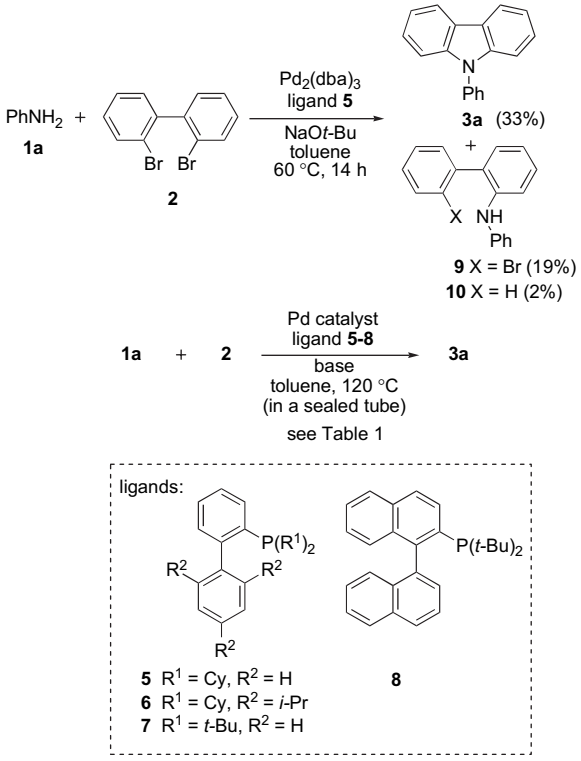
2.1. Double N-arylation of aniline

The double N-arylation of aniline (**1a**) with 2,2′-dibromobiphenyl[8] (**2**) was first attempted under the conditions reported by the Buchwald group for the N-arylation reaction of primary amines[5] with aryl halides after slight modification. When a mixture of **1a** (1 equiv) and **2** (1.1 equiv) in toluene in the presence of $Pd_2(dba)_3$ (10 mol % to **1a**), 2-(dicyclohexylphosphino)biphenyl (**5**)[5c] (30 mol % to **1a**), and NaO*t*-Bu (3 equiv) was heated at 60 °C for 14 h in a sealed tube, the desired product, *N*-phenylcarbazole (**3a**) was isolated in 33% yield (Scheme 1). A mixture of the mono N-arylation products (**9** and **10**) was also obtained in 21% yield (**9**:**10**=10:1, determined by ^{1}H NMR). To our delight, the same reaction at higher temperature (120 °C) significantly improved the yield of the desired product, and carbazole **3a** was obtained in 79% yield. In this case, a small amount of the mono N-arylation products (**9** and **10**) were detected by TLC, but could not be isolated. Although the Buchwald group reported that the mono N-arylation of aniline with 2′-chloroacetophenone proceeded in 81% yield with $Pd_2(dba)_3$ (1 mol %) and ligand **5** (2 mol %),[5c] the double N-arylation of aniline with **2** was found to be very slow with less than 10 mol % Pd catalyst. It was also found that the molar ratio of ligand/$Pd_2(dba)_3$ (2–3:1) was important for higher yields of **3a**. The dependence on the various reaction parameters was then examined. For the ligands, **5** as well as other dialkylphosphinobiaryls (**6**,[5d] **7**,[5c] and **8**[5e]), which have been reported by the Buchwald group being excellent ligands for the N-arylation, were tested. These results are listed in Table 1, which showed that (i) $Pd_2(dba)_3$ was the Pd source of choice (entries 2–4, when $Pd(OAc)_2$ was employed, the formation of Pd metal precipitates was observed during the course of the reaction), (ii) **5** and 2-(dicyclohexylphosphino)-2′,4′,6′-(triisopropyl)biphenyl (**6**) were effective ligands (entries 5–8), and (iii) the use of NaO*t*-Bu as a base gave good results whereas Cs_2CO_3 or K_3PO_4 significantly decreased the yields (entries 5, 9, and 10). As a result, *N*-phenylcarbazole (**3a**) was obtained in 85% yield under the conditions noted in entry 5.

Table 1. The double N-arylation of aniline (**1a**) with 2,2′-dibromobiphenyl (**2**)[a]

Entry	Pd Source	Ligand	Base	Time (h)	Yield of **3a** (%)[b]
1	$Pd_2(dba)_3$	**5**	NaO*t*-Bu	14	79
2[c]	$Pd_2(dba)_3$	**5**	NaO*t*-Bu	24	84
3	$Pd(PPh_3)_4$	**5**	NaO*t*-Bu	24	69
4[d]	$Pd(OAc)_2$	**5**	NaO*t*-Bu	24	62
5	$Pd_2(dba)_3$	**5**	NaO*t*-Bu	24	85
6	$Pd_2(dba)_3$	**6**	NaO*t*-Bu	24	82
7	$Pd_2(dba)_3$	**7**	NaO*t*-Bu	24	51
8	$Pd_2(dba)_3$	**8**	NaO*t*-Bu	13	22
9	$Pd_2(dba)_3$	**5**	Cs_2CO_3	24	42
10	$Pd_2(dba)_3$	**5**	K_3PO_4	24	32

[a] Reaction conditions: A mixture of **1a** (1.0 equiv), **2** (1.1 equiv), Pd catalyst (10 mol %), ligand (30 mol %), and base (3.0 equiv) in toluene was heated at 120 °C in a sealed tube.
[b] Isolated yield after chromatographic purification.
[c] $Pd_2(dba)_3$ (5 mol %), **5** (15 mol %).
[d] $Pd(OAc)_2$ (20 mol %), **5** (50 mol %).

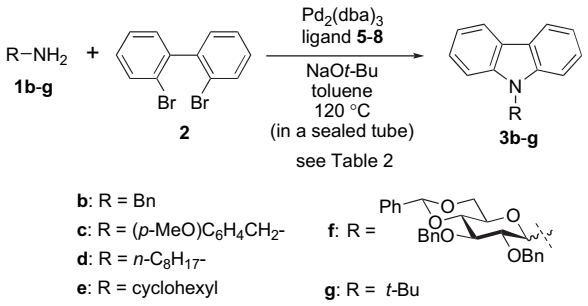


Scheme 1. dba=Dibenzylideneacetone, Cy=cyclohexyl.

2.2. Double N-arylation of aliphatic amines

The successful preparation of *N*-phenylcarbazole (**3a**) by the double N-arylation reaction led us to explore the reaction of aliphatic primary amines with dibromobiphenyl **2** (Scheme 2 and Table 2).

Although ligands **5** and **6**, which were found to be effective for the reaction of aniline, gave less satisfactory results when benzylamine was employed (entries 1 and 2), the use of 2-(di-*tert*-butylphosphino)binaphthyl (**8**) brought about a significant improvement, giving the desired product, *N*-benzylcarbazole (**3b**), in 60% yield (entry 4). The phosphine group in **8** is more electron-rich and sterically bulky than that in **5** and **6**. These electronic and steric factors play an important role in suppressing any undesired side reactions, such as



Scheme 2. Bn=–CH_2Ph.

Table 2. The double N-arylation of aliphatic amines with 2,2′-dibromobiphenyl (**2**)[a]

Entry	Amine	Ligand	Product	Time (h)	Yield (%)[b]
1	**1b**	**5**	**3b**	13	20
2	**1b**	**6**	**3b**	13	9
3	**1b**	**7**	**3b**	13	42
4	**1b**	**8**	**3b**	13	60
5	**1c**	**8**	**3c**	13	71
6	**1d**	**8**	**3d**	24	67
7	**1e**	**8**	**3e**	24	80
8[c]	**1f**[d]	**8**	**3f**[e]	24	52
9	**1g**	**8**	**3g**	13	17
10	**1g**	**6**	**3g**	13	42

[a] Reaction conditions: A mixture of **1** (1.0 equiv), **2** (1.1 equiv), $Pd_2(dba)_3$ (10 mol %), ligand (30 mol %), and NaO*t*-Bu (3.0 equiv) in toluene was heated at 120 °C in a sealed tube.
[b] Isolated yield after chromatographic purification.
[c] **2** (300 mol % to **1f**), $Pd_2(dba)_3$ (100 mol %), and **8** (300 mol %) at 60 °C.
[d] β-Anomer.
[e] Obtained as an anomeric mixture (α/β=1:1.7).

the formation of unreactive Pd bis-amine complexes and/or β-hydride elimination of the Pd-amido intermediates,[5a,g] in the double N-arylation process. Compound **8** was also found to work well for other aliphatic primary amines (entries 5–7). It is important to note that the *N*-alkylcarbazoles, which were prepared in poor yields under Nozaki's conditions,[4a] were obtained in moderate to good yields by the double N-arylation reactions when **8** was employed as the ligand. Under similar conditions, glucopyranosylamine derivative **1f**, a structurally complex and chemically unstable amine, could be converted into *N*-(glucopyranosyl)carbazole derivative **3f**[9] in 52% yield, although excess amounts of the Pd catalyst and ligand **8** were required (entry 8). The reaction of *tert*-butylamine (**1g**) with ligand **8** (entry 9), however, resulted in a low yield of the desired product. It was found that the use of ligand **6** instead of **8** produced a better result by generating *N*-(*tert*-butyl)carbazole (**3g**) in moderate (42%) yield (entry 10). The steric bulk of ligand **8** would prevent the sterically hindered *tert*-butylamine from approaching the catalyst. While the reasons for the better yield by the double N-arylation of *tert*-butylamine with ligand **6** are not clear, the effectual combination of the electronic (**6** should be electron richer than **5**, but electron poorer than **8**) and steric (**6** is smaller than **8**) factors in **6** would contribute to its effectiveness.

Based on these experiments, it was shown that the double N-arylation methodology is effective for the one-step synthesis of various *N*-substituted carbazoles. For aniline, the combination of $Pd_2(dba)_3$, NaO*t*-Bu, and ligand **5** provided the product in good yield. For aliphatic primary amines with sterically unencumbered structures, the use of ligand **8** gave favorable results, whereas the use of ligand **6** proved to be effective for the sterically hindered *tert*-butylamine (**1g**).

2.3. Total synthesis of murrastifoline-A

We next tried to extend this methodology to the synthesis of the structurally more complicated carbazole alkaloid, murrastifoline-A (**4**). Murrastifoline-A was isolated by Furukawa and co-workers from the root bark of *Murraya euchrestifolia* (Rutaceae) collected in Taiwan.[10] The structure elucidation study by spectral analyses revealed that murrastifoline-A is a new biscarbazole possessing the dimeric structure of 1′-methoxy-3′-methylcarbazole (murrayafoline-A, murrastifoline-A numbering), where the nitrogen in one carbazole unit (at the 9′-position) is connected to a carbon atom at the 3-position of another carbazole unit. While many monomeric carbazoles have been isolated from higher plants,[2] much attention has been recently focused on such biarylic biscarbazole alkaloids[11,12] due to their interesting structures and expected biological activities. The *C*,*N*-bonded biaryl biscarbazole structure found in **4** is very unique among the biscarbazole alkaloids,[11] however, reports on the synthetic approach to the *C*,*N*-bonded biaryl biscarbazoles are limited.[11d,12] In 2001, Bringmann disclosed the total synthesis of murrastifoline-F, an isomer of murrastifoline-A (**4**) in which the nitrogen in a carbazole unit at the 9′-position is bonded to another carbazole at C-5 (murrastifoline-A numbering), by the lead tetraacetate-mediated oxidative coupling of 1′-methoxy-3′-methylcarbazole.[12b]

Our retrosynthetic analysis of murrastifoline-A (**4**) is shown in Figure 2. The biscarbazole structure of murrastifoline-A (**4**) could be constructed by the key double N-arylation of the bottom-half segment, carbazolamine **12**, with the top-half segment, dibromobiphenyl derivative **11**. For preparation of both the top and bottom segments (**11** and **12**), we chose 2-amino-5-methylphenol (**13**) as the common starting material.

The synthesis of top-half segment (**11**) commenced from the known *O*-tosylate (**14**),[13] prepared from commercially available **13** in 89% yield (Scheme 3). The conventional iodination with *N*-iodosuccinimide (NIS) of **14** afforded **15** (69%), whose Suzuki–Miyaura cross-coupling reaction[14] with 2-bromophenylboronic acid in the presence of $Pd(PPh_3)_4$

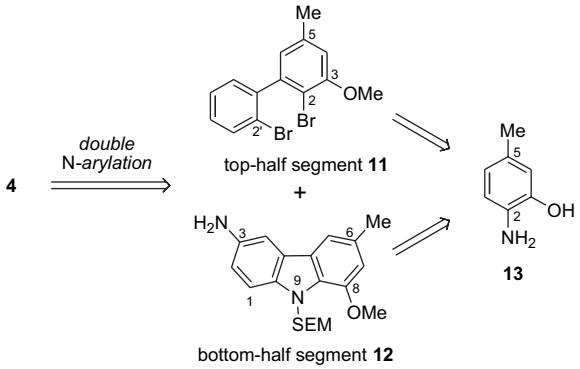
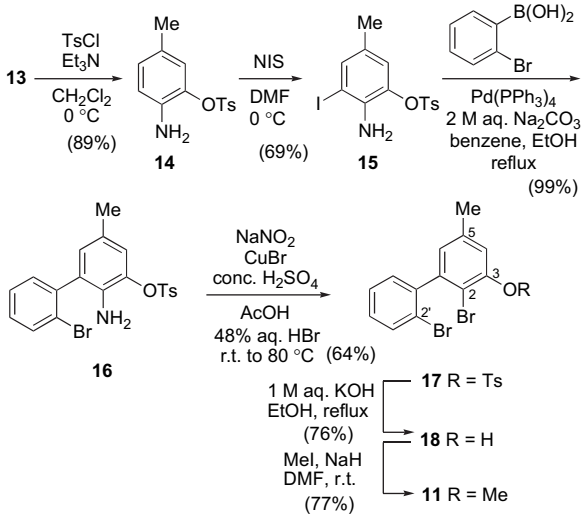


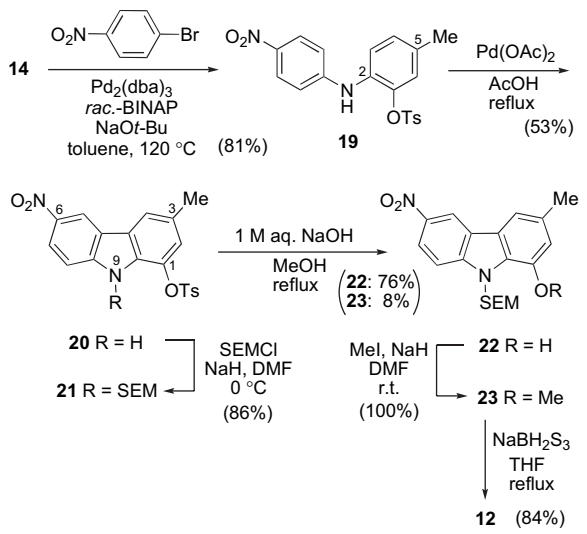
Figure 2. SEM=–$CH_2OCH_2CH_2SiMe_3$.

in EtOH–benzene–2 M aqueous Na_2CO_3 cleanly afforded **16** in 99% yield. Sandmeyer reaction of **16** gave dibromobiphenyl **17** in 64% yield. In this reaction, the use of AcOH as a co-solvent was essential for the effective diazotization. The *O*-Ts protecting group in **17** was removed by basic hydrolysis to give **18**, whose O-methylation furnished the top-half segment **11** in 59% yield from **17**.



Scheme 3. Ts=–$SO_2C_6H_4$(*p*-Me).

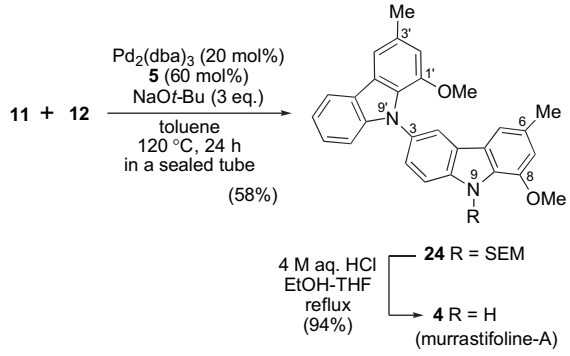
The bottom-half segment **12** was synthesized as shown in Scheme 4. Thus, the Buchwald–Hartwig N-arylation[5f] of **14** with 4-bromonitrobenzene afforded diarylamine **19** in 81% yield. The treatment of **19** with excess $Pd(OAc)_2$ in AcOH induced the cyclization[3d,13b] to provide carbazole **20** in 53% yield. After protection of the nitrogen function in **20** with the 2-trimethylsilylethoxymethyl (SEM) group (86% yield), the product **21** was treated with NaOH in MeOH-H_2O to provide de-*O*-tosyl derivative **22** along with its methyl ether **23** in 76 and 8% isolated yields, respectively. Methyl ether **23** would be formed by the nucleophilic aromatic substitution reaction of compound **21** with the methoxide ion.[15] The O-methylation of **22** quantitatively



Scheme 4. BINAP=2,2′-bis(diphenylphosphino)-1,1′-binaphthyl.

afforded **23**. Although the attempted reduction of the nitro function in **23** by catalytic hydrogenation (H_2 in the presence of 5% Pd on carbon) resulted in the formation of many unidentified products, the treatment of **23** with $NaBH_2S_3$[16] cleanly provided the bottom-half segment **12** in 84% yield.

With both the top- and bottom-half segments in hand, the crucial double N-arylation reaction was explored (Scheme 5). From the observations of the coupling reactions of aniline with 2,2′-dibromobiphenyl (Table 1), it was expected that the use of **5** as the ligand would be the most effective for the reaction. Indeed, when a mixture of segments **11** and **12** in toluene was heated at 120 °C in the presence of $Pd_2(dba)_3$, NaO*t*-Bu, and **5**, the double N-arylation successfully took place to provide the desired *N*-protected biscarbazole **24** in 58% yield. It was found, as anticipated, the use of ligands **6** and **8** gave less satisfactory results (28% yield with **6** and 23% yield with **8**). Finally, the *N*-SEM group was removed under acidic conditions to furnish murrastifoline-A (**4**) in 94% yield. The spectral data of synthetic **4** were fully identical with those of the natural product.[10]



Scheme 5.

3. Conclusion

In summary, we described the Pd-catalyzed double N-arylation reaction of primary amines with 2,2′-dibromobiphenyls, which provided *N*-substituted carbazoles in a one-step reaction. By the choice of ligands, both the aryl and aliphatic amines including *tert*-butylamine and an *O*-protected glucopyranosylamine could be transformed into the corresponding carbazoles. Based on this methodology, the first total synthesis of murrastifoline-A (**4**) has been accomplished. This synthesis fully confirmed the proposed structure of the natural product and revealed that the double N-arylation methodology is highly effective for the one-step construction of the structurally complex, unsymmetrical multisubstituted carbazole derivatives.

4. Experimental

4.1. General

Melting points (mp) were determined on a Mitamura-riken micro hot stage and are uncorrected. ^{1}H NMR spectra were measured with a JEOL JNM-*Lambda* 300 (300 MHz) or a Varian MVX-300 (300 MHz) spectrometer, with

tetramethylsilane as the internal standard for solutions in $CDCl_3$ at rt, unless otherwise noted. Chemical shifts are reported as δ values in ppm. ^{13}C NMR spectra were taken on a 75 MHz spectrometer. Mass spectra were measured by a JEOL GC-Mate spectrometer with EI mode (70 eV), unless otherwise noted. Optical rotations were measured with a JASCO DIP-370 instrument with 1-dm tube and values of $[\alpha]_D$ are recorded in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were taken with a JASCO FTIR-200 spectrometer. Organic extracts were dried over anhydrous Na_2SO_4 and concentrated below 40 °C under reduced pressure. Solvents were dried over 3 Å molecular sieves after distillation. Benzene, toluene, and DMF were distilled from CaH_2. MeOH was distilled from $CaSO_4$ (DRIERITE®). AcOH was distilled from Ac_2O and $KMnO_4$. EtOH (95%, dried over 3 Å molecular sieves), Et_2O (dehydrated), THF (dehydrated, stabilizer free), and CH_2Cl_2 (dehydrated) were purchased from Kanto Chemical Co., INC. For column chromatography, Merck silica gel 60 (230–400 mesh) was used, unless otherwise noted. For TLC analysis, Merck precoated TLC plates (silica gel 60 F_{254} on glass plates, 0.25 mm) were used. For preparative TLC, Merck precoated TLC plates (silica gel 60 F_{254} on glass plates, 0.5 mm) were used.

4.2. General procedure for the double N-arylation reaction (Tables 1 and 2)

Ar was bubbled into a mixture of amine (**1**) (0.250 mmol), dibromobiphenyl (**2**) (85.8 mg, 0.275 mmol), $Pd_2(dba)_3$ (22.9 mg, 0.0250 mmol), ligand (**5**, **6**, **7** or **8**) (0.0750 mmol), and NaO*t*-Bu (72.1 mg, 0.750 mmol) in toluene (1 mL) for 15 min, unless otherwise noted. The mixture was then heated at 120 °C in a sealed tube for 13–24 h (see Tables 1 and 2). After cooling, the mixture was filtrated through a pad of silica gel (3 g, toluene). The filtrate was concentrated to give a residue, which was purified by column chromatography (silica gel: 6 g, toluene/hexane) to afford carbazole **3**.

4.2.1. *N*-Phenylcarbazole (3a)[4a,17] (Table 1, entry 5). The general procedure using 2-(dicyclohexylphosphino)biphenyl (**5**) gave *N*-phenylcarbazole (**3a**) (51.9 mg, 85%) as a colorless solid: R_f=0.37 (toluene/hexane=1:5); mp 89–90 °C (lit.[17] mp 89–90 °C); ^1H NMR δ 7.23–7.31 (m, 2H), 7.37–7.41 (m, 4H), 7.42–7.48 (m, 1H), 7.54–7.62 (m, 4H), 8.14 (d, *J*=7.8 Hz, 1H); ^{13}C NMR δ 109.9, 120.0, 120.4, 123.5, 126.0, 127.3, 127.6, 130.0, 137.9, 141.0; IR (KBr) ν 3020, 1595 cm^{-1}; MS *m*/*z* 243 (M$^+$, 100%), 139 (12), 121 (9); HRMS Calcd for $C_{18}H_{13}N$ (M$^+$): 243.1048. Found: 243.1040. Anal. Calcd for $C_{18}H_{13}N$: C, 88.86; H, 5.39; N, 5.76%. Found: C, 88.89; H, 5.32; N, 5.76%.

4.2.2. *N*-[2-(2′-Bromobiphenyl)]aniline (9) and *N*-(2-biphenyl)aniline (10)[5e]. The general procedure using 2-(dicyclohexylphosphino)biphenyl (**5**) at 60 °C gave *N*-phenylcarbazole (**3a**) (20.1 mg, 33%) and a mixture of mono N-arylation products **9** and **10** (10:1, determined by ^1H NMR, 16.8 mg, 21%). A small amount of the mixture was separated by HPLC (Finepak SIL, JASCO Corp., 4.6 mm i.d., 250 mmL, EtOAc/hexane=1:40, 1.0 mL min^{-1}) to provide compounds **9** (retention time 5.40 min) and **10** (retention time 6.95 min) in pure forms and for use as analytical samples. Data for **9**: R_f=0.53 (toluene/hexane=1:1); mp 84–87 °C; ^1H NMR δ 5.27 (s, 1H), 6.92 (ddd, *J*=7.5, 7.2, <1 Hz, 1H), 6.99 (ddd, *J*=7.8, 7.2, 1.5 Hz, 1H), 7.04 (dd, *J*=7.5, <1 Hz, 1H), 7.15 (dd, *J*=7.8, 1.7 Hz, 1H), 7.20–7.39 (m, 7H), 7.69 (dd, *J*=7.7, 0.9 Hz, 1H); ^{13}C NMR δ 116.6, 119.1, 120.5, 121.6, 124.6, 128.0, 129.0, 129.4, 129.5, 130.5, 130.8, 132.1, 133.3, 139.8, 141.0, 143.0; IR (neat) ν 3010 cm^{-1}; MS *m*/*z* 325 [M$^+$(^{81}Br), 22%], 323 [M$^+$(^{79}Br), 20], 244 (81), 167 (32), 64 (100); HRMS Calcd for $C_{18}H_{14}N^{79}Br$ (M$^+$): 323.0310. Found: 323.0311. Data for **10**: R_f=0.53 (toluene/hexane=1:1); ^1H NMR δ 5.52 (s, 1H), 6.83 (ddd, *J*=7.3, 7.3, 1.2 Hz, 1H), 6.88–6.97 (m, 3H), 7.14–7.19 (m, 4H), 7.29–7.36 (m, 6H); ^{13}C NMR δ 117.6, 118.4, 121.2, 121.2, 127.6, 128.4, 129.0, 129.5, 131.0, 131.7, 139.2, 140.3, 143.5; IR (neat) ν 3405 cm^{-1}; MS *m*/*z* 245 (M$^+$, 100%), 167 (32); HRMS Calcd for $C_{18}H_{15}N$ (M$^+$): 245.1204. Found: 245.1198.

4.2.3. *N*-Benzylcarbazole (3b)[4b,18] (Table 2, entry 4). The general procedure using 2-(di-*tert*-butylphosphino)binaphthyl (**8**) gave *N*-benzylcarbazole (**3b**) (39.0 mg, 60%) as a colorless solid: R_f=0.53 (toluene/hexane=1:1); mp 119–120 °C (lit.[4b] mp 118–120 °C); ^1H NMR δ 5.50 (s, 2H), 7.11–7.14 (m, 2H), 7.22–7.27 (m, 5H), 7.35 (d, *J*=7.3 Hz, 2H), 7.42 (dd, *J*=7.3, 0.9 Hz, 2H), 8.13 (dd, *J*=7.6, 0.9 Hz, 2H); ^{13}C NMR δ 46.7, 109.0, 119.4, 120.5, 123.2, 126.0, 126.6, 127.6, 128.9, 137.3, 140.8; IR (KBr) ν 3030, 2930, 1595, 1450 cm^{-1}; MS *m*/*z* 257 (M$^+$, 100%), 166 (24), 109 (17), 91 (92); HRMS Calcd for $C_{19}H_{15}N$ (M$^+$): 257.1204. Found: 257.1203. Anal. Calcd for $C_{19}H_{15}N\cdot 0.1H_2O$: C, 88.07; H, 5.91; N, 5.41%. Found: C, 88.08; H, 5.89; N, 5.40%.

4.2.4. *N*-(4-Methoxybenzyl)carbazole (3c)[19] (Table 2, entry 5). The general procedure using 2-(di-*tert*-butylphosphino)binaphthyl (**8**) gave *N*-(4-methoxybenzyl)carbazole (**3b**) (51.2 mg, 71%) as a colorless solid: R_f=0.32 (toluene/hexane=1:1); mp 122–123 °C; ^1H NMR δ 3.73 (s, 3H), 5.46 (s, 2H), 6.78 (d, *J*=8.6 Hz, 2H), 7.08 (d, *J*=8.6 Hz, 2H), 7.24 (dd, *J*=7.8, 7.4 Hz, 2H), 7.37 (d, *J*=7.6 Hz, 2H), 7.42 (dd, *J*=7.6, 7.4 Hz, 2H), 8.12 (d, *J*=7.8 Hz, 2H); ^{13}C NMR δ 46.2, 55.4, 109.1, 114.3, 119.3, 120.5, 123.1, 125.9, 127.8, 129.4, 140.8, 159.1; IR (KBr) ν 3050, 2835, 1595, 1460 cm^{-1}; LRMS *m*/*z* 287 (M$^+$, 30%), 166 (11), 121 (100), 77 (12); HRMS Calcd for $C_{20}H_{17}NO$ (M$^+$): 287.1310. Found: 287.1300. Anal. Calcd for $C_{20}H_{17}NO$: C, 83.59; H, 5.96; N, 4.87%. Found: C, 83.43; H, 5.95; N, 4.85%.

4.2.5. *N*-Octylcarbazole (3d)[20] (Table 2, entry 6). The general procedure using 2-(di-*tert*-butylphosphino)binaphthyl (**8**) gave *N*-octylcarbazole (**3d**) (46.5 mg, 67%) as a colorless syrup: R_f=0.50 (toluene/hexane=1:5); ^1H NMR δ 0.86 (t, *J*=6.7 Hz, 3H), 1.24–1.40 (m, 10H), 1.86 (tt, *J*=7.3, 7.3 Hz, 2H), 4.28 (t, *J*=7.3 Hz, 2H), 7.21 (ddd, *J*=7.6, 7.6, 1.2 Hz, 2H), 7.39 (dd, *J*=7.8, 1.2 Hz, 2H), 7.45 (ddd, *J*=7.8, 7.6, 1.0 Hz, 2H), 8.09 (dd, *J*=7.6, 1.0 Hz, 2H); ^{13}C NMR δ 14.2, 22.7, 27.4, 29.1, 29.3, 29.5, 31.9, 43.2, 108.8, 118.8, 120.5, 122.9, 125.7, 140.5; IR (neat) ν 3055, 2925, 1600, 1455 cm^{-1}; MS *m*/*z* 279 (M$^+$, 79%), 245 (19), 180 (100); HRMS Calcd for $C_{20}H_{25}N$ (M$^+$): 279.1987. Found: 279.1982. Anal. Calcd for $C_{20}H_{25}N$: C, 85.97; H, 9.02; N, 5.01%. Found: C, 85.99; H, 8.93; N, 4.91%.

4.2.6. *N*-Cyclohexylcarbazole (3e)[21] (Table 2, entry 7). The general procedure using cyclohexylamine (**1e**) (0.0229 mL, 0.200 mmol), dibromobiphenyl (**2**) (68.6 mg, 0.220 mmol), $Pd_2(dba)_3$ (18.3 mg, 0.0200 mmol), 2-(di-*tert*-butylphosphino)binaphthyl (**8**) (23.9 mg, 0.0600 mmol), NaO*t*-Bu (57.7 mg, 0.600 mmol), and toluene (0.8 mL) gave *N*-cyclohexylcarbazole (**3e**) (40.0 mg, 80%) as a colorless solid: R_f=0.53 (toluene/hexane=1:5); mp 143–144 °C (lit.[21] mp 143 °C); ^{1}H NMR δ 1.31–1.61 (m, 3H), 1.82–1.87 (m, 1H), 1.94–2.04 (m, 4H), 2.33–2.47 (m, 2H), 4.49 (tt, J=12.3, 3.9 Hz, 1H), 7.20 (dd, J=7.8, 7.6 Hz, 2H), 7.43 (ddd, J=8.1, 7.6, 1.2 Hz, 2H), 7.56 (d, J=8.1 Hz, 2H), 8.10 (dd, J=7.6, 1.2 Hz, 2H); ^{13}C NMR δ 25.8, 26.7, 30.9, 55.5, 110.4, 118.6, 120.4, 123.4, 125.4, 139.8; IR (KBr) ν 3055, 2920, 1590, 1455 cm^{-1}; MS *m*/*z* 249 (M^+, 100%), 206 (43), 167 (92); HRMS Calcd for $C_{18}H_{19}N$ (M^+): 249.1517. Found: 249.1517. Anal. Calcd for $C_{18}H_{19}N$: C, 86.70; H, 7.68; N, 5.62%. Found: C, 86.48; H, 7.60; N, 5.58%.

4.2.7. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosylamine (1f). To a suspension of NaH (39 mg, 1.63 mmol) in DMF (2 mL) was slowly added 4,6-*O*-benzylidene-β-D-glucopyranosylazide[22] (120 mg, 0.409 mmol) at 0 °C. After stirring at rt for 5 min, the reaction mixture was cooled to 0 °C. To this mixture was slowly added benzyl bromide (0.15 mL, 1.26 mmol), and the mixture was stirred at rt for 2 h. After addition of MeOH at 0 °C, the reaction mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 6 g, EtOAc/hexane=1:20) to afford 2,3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosylazide (176 mg, 90%) as a white solid: R_f=0.88 (EtOAc/toluene=1:2); mp 112 °C; $[\alpha]_D^{27}$ +69.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 3.36 (dd, J=8.7, 8.4 Hz, 1H), 3.42 (ddd, J=9.9, 9.6, 4.8 Hz, 1H), 3.64 (dd, J=9.6, 9.3 Hz, 1H), 3.69 (dd, J=10.5, 9.9 Hz, 1H), 3.75 (dd, J=9.3, 8.7 Hz, 1H), 4.32 (dd, J=10.5, 4.8 Hz, 1H), 4.65 (d, J=8.4 Hz, 1H), 4.77 and 4.92 (2d, J=11.4 Hz, each 1H), 4.81 (s, 2H), 5.52 (s, 1H), 7.25–7.33 (m, 13H), 7.45–7.48 (m, 2H); ^{13}C NMR δ 68.1, 68.4, 75.2, 75.7, 81.2, 81.3, 81.4, 90.6, 101.2, 126.0, 127.8, 128.0, 128.1, 128.3, 128.3, 128.4, 128.5, 129.1, 137.1, 137.7, 138.2; IR ν 2115 cm^{-1}; MS *m*/*z* 473 (M^+, 1%), 431 (1), 382 (18), 91 (100); HRMS Calcd for $C_{27}H_{27}N_3O_5$ (M^+): 473.1951. Found: 473.1960. Anal. Calcd for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87%. Found: C, 68.51; H, 5.86; N, 8.64%.

To a solution of 2,3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosylazide (136 mg, 0.287 mmol) in toluene (4 mL) was added Lindlar catalyst (70 mg). The reaction mixture was stirred for 12 h under H_2 atmosphere (1 atm) at rt. Then the catalyst was removed by filtration through Celite and the filtrate was concentrated to give a residue, which was recrystallized from EtOH to afford glucosylamine **1g** (98.5 mg, 76%) as a white solid: R_f=0.34 (EtOAc/toluene=1:2); mp 111–112 °C (decomp.); $[\alpha]_D^{27}$ −38.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.91 (br s, 2H), 3.21 (dd, J=8.6, 8.6 Hz, 1H), 3.42 (ddd, J=9.6, 9.3, 5.0 Hz, 1H), 3.65 (dd, J=9.3, 9.1 Hz, 1H), 3.71 (dd, J=10.4, 9.6 Hz, 1H), 3.80 (dd, J=9.1, 8.6 Hz, 1H), 4.22 (d, J=8.6 Hz, 1H), 4.32 (dd, J=10.4, 5.0 Hz, 1H), 4.80 and 4.94 (2d, J=11.4 Hz, each 1H), 4.84 and 4.92 (2d, J=10.5 Hz, each 1H), 5.56 (s, 1H), 7.26–7.38 (m, 13H), 7.47–7.51 (m, 2H); ^{13}C NMR δ 67.3, 69.0, 75.2, 75.5, 82.1, 82.1, 86.8, 101.1, 126.1, 127.8, 127.9, 128.2, 128.3, 128.3, 128.4, 128.5, 129.0, 137.5, 138.3, 138.6; IR ν 3400, 3335 cm^{-1}; MS *m*/*z* 447 (M^+, 1%), 356 (2), 248 (37), 91 (100); HRMS Calcd for $C_{27}H_{29}NO_5$ (M^+): 447.2046. Found: 447.2056. Anal. Calcd for $C_{27}H_{29}NO_5$: C, 72.46; H, 6.53; N, 3.13%. Found: C, 72.32; H, 6.55; N, 2.74%.

4.2.8. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α- and β-D-glucopyranosylcarbazole (3f) (Table 2, entry 8). Ar gas was bubbled into a mixture of glucosylamine (**1f**) (20 mg, 0.0447 mmol), dibromobiphenyl (**2**) (42.0 mg, 0.135 mmol), $Pd_2(dba)_3$ (41 mg, 0.0447 mmol), 2-(di-*tert*-butylphosphino)-binaphthyl (**8**) (53.0 mg, 0.133 mmol), and NaO*t*-Bu (12.9 mg, 0.134 mmol) in toluene (0.8 mL) for 15 min. The reaction mixture was then heated at 60 °C in a sealed tube for 24 h. After cooling, the mixture was purified by column chromatography (silica gel: 2 g, EtOAc/hexane=1:40) to afford an anomeric mixture of glucosylcarbazole (**3f**). The mixture was separated by preparative TLC using EtOAc/hexane=1:8 as an eluent to give α-anomer (**3fα**)[9] (5.1 mg, 19%) as a colorless syrup and β-anomer (**3fβ**) (8.9 mg, 33%) as a colorless syrup. Data for **3fα**: R_f=0.23 (EtOAc/hexane=1:8); $[\alpha]_D^{21}$ −14.5 (*c* 0.1, $CHCl_3$); ^{1}H NMR (C_6D_6) δ 3.50 (dd, J=10.5, 10.2 Hz, 1H), 3.73 and 3.83 (2d, J=11.9 Hz, each 1H), 3.93 (br d, J=1.8 Hz, 1H), 4.02–4.07 (m, 2H), 4.30 (dd, J=10.5, 5.1 Hz, 1H), 4.42 and 4.53 (2d, J=12.2 Hz, each 1H), 4.60–4.71 (m, 1H), 5.39 (s, 1H), 6.47 (d, J=1.8 Hz, 1H), 6.54 (d, J=6.3 Hz, 2H), 6.81–6.90 (m, 4H), 7.11–7.36 (m, 11H), 7.65 (br d, J=7.8 Hz, 4H), 8.03 (d, J=7.5 Hz, 2H); IR (neat) ν 3030, 2920, 1455 cm^{-1}; MS *m*/*z* 597 (M^+, 13%), 167 (23), 91 (100); HRMS Calcd for $C_{39}H_{35}NO_5$ (M^+): 597.2515. Found: 597.2522. Data for **3fβ**: R_f=0.20 (EtOAc/hexane=1:8); $[\alpha]_D^{25}$ +31.7 (*c* 0.97, $CHCl_3$); ^{1}H NMR δ 3.35 (d, J=10.0 Hz, 1H), 3.79 (m, 1H), 3.93 (dd, J=10.5, 10.2 Hz, 1H), 4.00–4.08 (m, 2H), 4.06 (d, J=10.0 Hz, 1H), 4.40 (dd, J=8.8, 8.8 Hz, 1H), 4.46 (dd, J=10.5, 4.9 Hz, 1H), 4.83 (d, J=11.2 Hz, 1H), 5.00 (d, J=11.2 Hz, 1H), 5.73 (s, 1H), 5.88 (d, J=8.8 Hz, 1H), 6.34 (d, J=7.6 Hz, 2H), 6.93 (dd, J=7.6, 7.6 Hz, 2H), 7.05 (dd, J=7.6, 7.6 Hz, 1H), 7.28–7.64 (m, 16H), 8.09 (d, J=7.6 Hz, 2H); ^{13}C NMR δ 68.9, 69.4, 75.2, 75.6, 78.8, 82.0, 82.4, 85.5, 101.5, 109.8, 112.8, 120.4, 126.2, 127.8, 127.9, 128.1, 128.2, 128.5, 128.6, 129.2, 136.7, 137.4, 138.5; IR (neat) ν 3030, 2875, 1455 cm^{-1}; MS *m*/*z* 597 (M^+, 6%), 167 (12), 91 (100); HRMS Calcd for $C_{39}H_{35}NO_5$ (M^+): 597.2515. Found: 597.2513.

4.2.9. *N*-*tert*-Butylcarbazole (3g) (Table 2, entry 10). The general procedure using 2-(dicyclohexylphosphino)-2′,4′,6′-triisopropylbiphenyl (**6**) gave *N*-(*tert*-butyl)carbazole (**3g**) (23.6 mg, 42%) as a colorless solid: R_f=0.38 (toluene/hexane=1:5); mp 122–123 °C; ^{1}H NMR δ 2.00 (s, 9H), 7.19 (ddd, J=7.8, 7.1, 0.7 Hz, 2H), 7.37 (ddd, J=8.7, 7.1, 1.5 Hz, 2H), 7.86 (dd, J=8.7, 0.7 Hz, 2H), 8.10 (dd, J=7.8, 1.5 Hz, 2H); ^{13}C NMR δ 31.2, 59.2, 113.9, 118.6, 120.0, 124.6, 125.2, 140.6; IR (KBr) ν 3050, 2970, 1590, 1440 cm^{-1}; MS *m*/*z* 223 (M^+, 17%), 167 (100), 140 (16); HRMS Calcd for $C_{16}H_{17}N$ (M^+): 223.1361. Found: 223.1359. Anal. Calcd for $C_{16}H_{17}N\cdot 0.1H_2O$: C, 85.37; H, 7.70; N, 6.22%. Found: C, 85.34; H, 7.63; N, 6.29%.

4.3. Synthesis of murrastifoline-A

4.3.1. 4-Toluenesulfonic acid 2-amino-5-methylphenyl ester[13] (14). To a solution of 2-amino-5-methylphenol (**13**, 3 g, 24.4 mmol) in CH_2Cl_2 (45 ml) were added Et_3N (3.74 ml, 26.8 mmol) and TsCl (5.11 g, 26.6 mmol) at 0 °C. After stirring at 0 °C for 15 min, the reaction mixture was extracted with $CHCl_3$ and washed with H_2O. The organic layer was dried and concentrated to give a residue, which was recrystallized from Et_2O to afford tosylate **14** (6.04 g, 89%) as a brown solid: R_f=0.23 (EtOAc/petroleum ether=1:5); mp 81–82 °C (lit.[13b] 81–82 °C); ^{1}H NMR δ 2.15 (s, 3H), 2.46 (s, 3H), 3.64 (br s, 2H), 6.61 and 6.83 (2d, J=8.0 Hz, each 1H), 6.66 (s, 1H), 7.33 and 7.78 (2d, J=8.3 Hz, each 2H); MS m/z 277 (M^+, 41%), 122 (100), 94 (89); HRMS Calcd for $C_{14}H_{15}NO_3S$ (M^+): 277.0773. Found: 277.0773. Anal. Calcd for $C_{14}H_{15}NO_3S$: C, 60.63; H, 5.45; N, 5.05%. Found: C, 60.46; H, 5.42; N, 4.83%.

4.3.2. 4-Toluenesulfonic acid 2-amino-3-iodo-5-methylphenyl ester (15). To a solution of tosylate **14** (2.00 g, 7.21 mmol) in DMF (40 mL) was slowly added NIS (1.78 g, 7.93 mmol) at 0 °C. The reaction mixture (protected from light) was stirred for 3 h at rt, then diluted with Et_2O, and washed with 30 wt % of aqueous $Na_2S_2O_3$ solution and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 60 g, EtOAc/petroleum ether=1:7) to afford iodide **15** (2.01 g, 69%) as an orange solid: R_f=0.45 (EtOAc/petroleum ether=1:5); mp 141–142 °C; ^{1}H NMR δ 2.13 (s, 3H), 2.47 (s, 3H), 4.06 (br s, 2H), 6.69 (s, 1H), 7.34 (s, 1H), 7.35 and 7.78 (2d, J=8.3 Hz, each 2H); ^{13}C NMR δ 19.9, 21.8, 84.6, 123.7, 128.5, 129.0, 130.0, 132.6, 135.2, 137.7, 138.4, 145.9; IR (neat) ν 3460 cm^{-1}; MS m/z 403 (M^+, 18%), 248 (100), 121 (12); HRMS Calcd for $C_{14}H_{14}NO_3IS$ (M^+): 402.9739. Found: 402.9741. Anal. Calcd for $C_{14}H_{14}NO_3IS$: C, 41.70; H, 3.50; N, 3.47%. Found: C, 41.93; H, 3.66; N, 3.26%.

4.3.3. 2-Amino-2′-bromo-5-methyl-3-(4-toluenesulfonyloxy)-1,1′-biphenyl (16). To a solution of $Pd(PPh_3)_4$ (22.8 mg, 0.0198 mmol) in benzene (1 mL) was added iodide (**15**) (200 mg, 0.495 mmol) in benzene (5 mL) under Ar. Then, 2 M aqueous Na_2CO_3 solution (1.9 mL, 3.96 mmol) and 2-bromophenylboronic acid (120 mg, 0.595 mmol) in EtOH (2.4 mL) were added to the mixture. The reaction mixture was heated at reflux for 2 h under vigorous stirring. After cooling, the mixture was diluted with Et_2O and washed with brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 10 g, EtOAc/hexane=1:10) to afford biphenyl **16** (213 mg, 99%) as a pale yellow syrup; R_f=0.37 (EtOAc/petroleum ether=1:5); ^{1}H NMR δ 2.22 (s, 3H), 2.44 (s, 3H), 3.42 (s, 2H), 6.72 and 6.91 (2d, J=1.2 Hz, each 1H), 7.22 (2ddd, J=8.4, 7.5, 1.2 Hz, each 1H), 7.31 and 7.79 (2d, J=8.4 Hz, each 2H), 7.36 (dd, J=7.5, 1.2 Hz, 1H), 7.63 (dd, J=8.4, 1.2 Hz, 1H); ^{13}C NMR δ 20.2, 21.6, 122.8, 123.7, 126.8, 127.7, 128.4, 128.7, 129.0, 129.4, 129.6, 131.4, 132.5, 132.9, 134.5, 136.7, 138.7, 145.3; IR (neat) ν 3480 cm^{-1}; MS m/z 433 [M(^{81}Br)$^+$, 11%], 431 [M(^{79}Br)$^+$, 11], 278 (58), 276 (59), 197 (100); HRMS Calcd for $C_{20}H_{18}NO_3^{81}BrS$ (M^+): 433.0170. Found: 433.0169. Anal. Calcd for $C_{20}H_{18}NO_3BrS$: C, 55.56; H, 4.20; N, 3.24%. Found: C, 55.33; H, 4.28; N, 3.02%.

4.3.4. 2,2′-Dibromo-5-methyl-3-(4-toluenesulfonyloxy)-1,1′-biphenyl (17). To a solution of aminobromobiphenyl **16** (128 mg, 0.197 mmol) in AcOH (2.5 mL) was slowly added $NaNO_2$ (40.9 mg, 0.593 mmol) in concd H_2SO_4 (0.04 mL) at 0 °C, then the mixture was stirred for 1 h at rt. The reaction mixture was slowly added to CuBr (85.1 mg, 0.593 mmol) in 47 wt % aqueous HBr solution (1.7 mL) at 80 °C, and stirred for 1.5 h at 80 °C. After cooling, the reaction mixture was extracted with Et_2O and washed successively with 1 M aqueous NaOH solution, saturated aqueous $NaHCO_3$ solution, and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 15 g, EtOAc/petroleum ether=1:20) to afford dibromobiphenyl **17** as white crystals: R_f=0.43 (EtOAc/petroleum ether=1:5); mp 163 °C; ^{1}H NMR δ 2.05 (s, 3H), 2.46 (s, 3H), 6.62 (s, 1H), 7.09 (s, 1H), 7.20 (d, J=6.9 Hz, 2H), 7.26–7.43 (m, 4H), 7.65 and 7.81 (2dd, J=8.1, <1 Hz, each 1H); ^{13}C NMR δ 21.4, 21.9, 118.8, 120.2, 123.5, 124.0, 128.1, 128.8, 129.7, 130.5, 130.8, 133.5, 139.9, 141.5, 143.3, 150.0; IR (neat) ν 2920, 1600, 1580 cm^{-1}; MS m/z 498 [M(^{81}Br$_2$)$^+$, 14%], 496 [M(^{81}Br,^{79}Br)$^+$, 24], 494 [M(^{79}Br$_2$)$^+$, 12], 416 (22), 414 (18), 343 (12), 341 (23), 339 (12), 335 (23), 155 (100); HRMS Calcd for $C_{20}H_{16}O_3^{79}Br_2S$ (M^+): 493.9187. Found: 493.9183.

4.3.5. 2,2′-Dibromo-5-methyl-1,1′-biphenyl-3-ol (18). To a solution of tosylate **17** (47.8 mg, 0.0963 mmol) in EtOH (4 mL) was added 1 M aqueous KOH solution (0.3 mL) at rt. The reaction mixture was heated at reflux for 1 h. After cooling, the mixture was extracted with Et_2O and washed with 10 wt % aqueous citric acid solution and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 3 g, Et_2O/petroleum ether=1:10) to afford hydroxybiphenyl **18** (25 mg, 76%) as a light yellow oil; R_f=0.48 (EtOAc/petroleum ether=1:5); ^{1}H NMR δ 2.33 (s, 3H), 5.62 (s, 1H), 6.65 (d, J=1.7 Hz, 1H), 6.90 (d, J=1.7 Hz, 1H), 7.21–7.28 (m, 2H), 7.40 (ddd, J=7.5, 7.5, 1.2 Hz, 1H), 7.66 (dd, J=7.5, 1.2 Hz, 1H); ^{13}C NMR (75 MHz) δ 21.2, 108.7, 116.0, 123.5, 123.6, 127.3, 129.5, 130.9, 132.7, 138.8, 141.9, 142.3, 152.2; IR (neat) ν 3500 cm^{-1}; MS m/z 344 [M(^{81}Br$_2$)$^+$, 49%], 342 [M(^{81}Br,^{79}Br)$^+$, 100], 340 [M(^{79}Br$_2$)$^+$, 51], 263 (58), 261 (58), 182 (93); HRMS Calcd for $C_{13}H_{10}O^{79}Br_2$ (M^+): 339.9099. Found: 339.9102.

4.3.6. 2,2′-Dibromo-3-methoxy-5-methyl-1,1′-biphenyl (11). To a solution of hydroxylbiphenyl **18** (4.7 mg, 0.0137 mmol) in DMF (0.5 mL) were added NaH (1.1 mg, 0.0275 mmol) and MeI (1.7 μL, 0.0275 mmol) at 0 °C. After stirring at 0 °C for 45 min, the reaction mixture was quenched with MeOH. The mixture was extracted with Et_2O and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 0.4 g, EtOAc/petroleum ether=1:50) to give methoxybiphenyl (**11**) (3.7 mg, 77%) as a colorless oil; R_f=0.66 (EtOAc/petroleum ether=1:5); ^{1}H NMR δ 2.01 (s, 3H), 3.27 (s, 3H), 6.32 (d, J=1.6 Hz, 2H), 6.54 (d, J=1.6 Hz, 1H), 6.78 (ddd, J=7.5, 7.4, 1.8 Hz, 1H), 6.96 (ddd, J=7.4, 7.3, 1.2 Hz, 1H), 7.10 (dd, J=7.3, 1.8 Hz, 1H), 7.48 (dd, J=7.5, 1.2 Hz, 1H); ^{13}C NMR δ 21.3, 55.7, 110.7, 112.3, 123.9, 124.1, 127.2, 129.3,

131.3, 132.9, 138.0, 143.1, 144.0, 156.6; IR (neat) ν 2940, 1580 cm^{-1}; MS *m/z* 358 [M($^{81}Br_2$)$^+$, 49%], 356 [M(^{81}Br,^{79}Br)$^+$, 100], 354 [M($^{79}Br_2$)$^+$, 51], 277 (77), 275 (79), 196 (43), 181 (42), 165 (22); HRMS Calcd for $C_{14}H_{12}O_2^{79}Br_2$ (M^+): 353.9255. Found: 353.9254.

4.3.7. 4-Toluenesulfonic acid 5-methyl-2-[(4-nitrophenyl)-amino]phenyl ester (19). Ar gas was bubbled into a mixture of amine (**14**) (125 mg, 0.450 mmol), 4-bromonitrobenzene (137 mg, 0.678 mmol), $Pd_2(dba)_3$ (82 mg, 0.0895 mmol), *rac*.-BINAP (168 mg, 0.269 mmol), and NaO*t*-Bu (64.5 mg, 0.671 mmol) in toluene (5 mL) for 15 min. The reaction mixture was then heated at 120 °C in a sealed tube for 15 h. After cooling, the mixture was filtered through Celite. The filtrate concentrated to give a residue, which was purified by column chromatography (silica gel: 15 g, EtOAc/petroleum ether=1:10) to afford diarylamine **19** (233 mg, 81%) as a yellow solid; R_f=0.31 (EtOAc/petroleum ether=1:5); mp 108–109 °C; ^{1}H NMR δ 2.31 (s, 3H), 2.32 (s, 3H), 6.34 (br s, 1H), 6.68 (d, *J*=8.4 Hz, 2H), 7.00 (s, 1H), 7.08 and 7.25 (2d, *J*=7.5 Hz, each 1H), 7.16 (d, *J*=7.7 Hz, 2H), 7.65 (d, *J*=7.7 Hz, 2H), 8.03 (d, *J*=8.4 Hz, 2H); ^{13}C NMR δ 21.0, 21.8, 113.8, 123.4, 125.1, 126.0, 128.4, 128.8, 129.9, 130.2, 132.0, 135.8, 140.0, 141.7, 146.0, 149.6; IR (neat) ν 3380, 1500, 1325 cm^{-1}; MS *m/z* 398 (M^+, 36%), 243 (100), 226 (38), 197 (57); HRMS Calcd for $C_{20}H_{18}N_2O_5S$ (M^+): 398.0937. Found: 398.0937.

4.3.8. 3-Methyl-6-nitro-1-(4-toluenesulfonyloxy)carbazole (20). To a solution of diarylamine **19** (142 mg, 0.355 mmol) in AcOH (14 mL) was added $Pd(OAc)_2$ (319 mg, 1.42 mmol) at rt. The reaction mixture was heated at reflux for 5 h. After cooling, the mixture was filtered through a pad of Celite. The filtrate was extracted with Et_2O and washed with H_2O, saturated aqueous $NaHCO_3$ solution, and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 14 g, EtOAc/petroleum ether=1:7) to afford carbazole **20** (75 mg, 53%) as a yellow solid: R_f=0.23 (EtOAc/petroleum ether=1:5); mp 224–225 °C; ^{1}H NMR δ 2.41 (s, 3H), 2.47 (s, 3H), 6.71 (s, 1H), 7.35 (d, *J*=8.3 Hz, 2H), 7.47 (d, *J*=9.0 Hz, 1H), 7.78 (d, *J*=8.3 Hz, 2H), 7.80 (s, 1H), 8.37 (dd, *J*=9.0, 2.0 Hz, 1H), 8.94 (br s, 1H), 8.94 (d, *J*=2.0 Hz, 1H); ^{13}C NMR δ 21.4, 21.9, 111.1, 117.6, 119.9, 122.0, 122.5, 122.8, 126.4, 128.8, 130.1, 131.5, 131.6, 131.9, 134.3, 141.6, 143.2, 146.3; IR (neat) ν 3370, 1520, 1320 cm^{-1}; MS *m/z* 396 (M^+, 10%), 348 (11), 330 (31), 241 (34), 197 (100); HRMS Calcd for $C_{20}H_{16}N_2O_5S$ (M^+): 396.0780. Found: 396.0780.

4.3.9. *N*-[2-(Trimethylsilyl)ethoxymethyl]-3-methyl-6-nitro-1-(4-toluenesulfonyloxy)carbazole (21). To a solution of carbazole **20** (779 mg, 1.96 mmol) in DMF (40 mL) was added NaH (70.7 mg, 2.95 mmol) at 0 °C. After stirring at 0 °C for 1 h, to the mixture was added 2-(trimethylsilyl) ethoxymethyl chloride (SEMCl) (0.42 mL, 2.39 mmol) and the mixture was stirred at 0 °C for 1.5 h. After addition of MeOH, the mixture was diluted with EtOAc and washed successively with water, saturated aqueous $NaHCO_3$ solution, and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 50 g, EtOAc/hexane=1:7) to afford SEM protected carbazole **21** (884 mg, 86%) as a yellow solid: R_f=0.37 (EtOAc/petroleum ether=1:5); ^{1}H NMR δ −0.13 (s, 9H), 0.84 (t, *J*=7.6 Hz, 2H), 2.43 (s, 3H), 2.48 (s, 3H), 3.49 (t, *J*=7.6 Hz, 2H), 5.89 (s, 2H), 6.87 (s, 1H), 7.38 (d, *J*=7.7 Hz, 2H), 7.57 (d, *J*=8.6 Hz, 1H), 7.82 (s, 1H), 7.83 (d, *J*=7.7 Hz, 2H), 8.38 (dd, *J*=8.6, 2.0 Hz, 1H), 8.93 (d, *J*=2.0 Hz, 1H); ^{13}C NMR δ −1.4, 17.7, 21.2, 21.9, 66.2, 74.0, 110.4, 117.1, 119.7, 122.2, 122.3, 122.9, 126.7, 128.8, 130.1, 131.4, 131.7, 132.7, 135.1, 141.9, 144.7, 146.2; IR (neat) ν 1520, 1330 cm^{-1}; MS *m/z* 526 (M^+, 27%), 468 (11), 313 (26), 261 (25), 73 (100); HRMS Calcd for $C_{26}H_{30}N_2O_6SSi$ (M^+): 526.1594. Found: 526.1558.

4.3.10. *N*-[2-(Trimethylsilyl)ethoxymethyl]-3-methyl-6-nitrocarbazol-1-ol (22). To a solution of tosylate **21** (38.3 mg, 0.0727 mmol) in MeOH (3.8 mL) was added 1 M aqueous NaOH solution (0.2 mL). The reaction mixture was heated at reflux for 1 h. After cooling, the products were extracted with Et_2O and the organic layer was washed with 10 wt % aqueous citric acid solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 2.5 g, EtOAc/petroleum ether=1:10) to give hydroxycarbazole **22** (20.6 mg, 76%) as a yellow solid and methoxycarbazole **23** (2.2 mg, 8%) as a yellow solid. Data for **22**: R_f=0.28 (EtOAc/petroleum ether=1:5); mp 172 °C; ^{1}H NMR δ −0.04 (s, 9H), 1.02 (t, *J*=8.4 Hz, 2H), 2.50 (s, 3H), 3.73 (t, *J*=8.4 Hz, 2H), 5.81 (s, 2H), 6.94 (s, 1H), 7.43 (d, *J*=9.0 Hz, 1H), 7.51 (s, 1H), 7.73 (s, 1H), 8.36 (dd, *J*=9.0, 2.3 Hz, 1H), 8.93 (d, *J*=2.3 Hz, 1H); ^{13}C NMR δ −1.4, 18.0, 21.5, 66.8, 74.2, 108.4, 113.0, 117.1, 117.6, 122.0, 123.7, 126.0, 128.6, 133.5, 141.4, 142.8, 143.8; IR (neat) ν 3240, 1520, 1320 cm^{-1}; MS *m/z* 372 (M^+, 6%), 314 (14), 254 (23), 75 (100); HRMS Calcd for $C_{19}H_{24}N_2O_4Si$ (M^+): 372.1505. Found: 372.1508.

4.3.11. *N*-[2-(Trimethylsilyl)ethoxymethyl]-1-methoxy-3-methyl-6-nitro-carbazole (23). To a solution of hydroxycarbazole **22** (13.6 mg, 0.0365 mmol) in DMF (1.3 mL) were added NaH (1.8 mg, 0.0750 mmol) and MeI (5 μL, 0.080 mmol) at 0 °C. After stirring for 50 min at 0 °C, the reaction was quenched by addition of MeOH. The mixture was diluted with Et_2O and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 2 g, EtOAc/petroleum ether=1:50) to give methoxycarbazole **23** (14.1 mg, 100%) as a light yellow solid: R_f=0.51 (EtOAc/petroleum ether=1:5); mp 113 °C; ^{1}H NMR δ −0.11 (s, 9H), 0.87 (t, *J*=7.8 Hz, 2H), 2.54 (s, 3H), 3.57 (t, *J*=7.8 Hz, 2H), 4.02 (s, 3H), 6.05 (s, 2H), 6.85 (s, 1H), 7.53 (s, 1H), 7.57 (d, *J*=8.6 Hz, 1H), 8.34 (dd, *J*=8.6, 2.4 Hz, 1H), 8.93 (d, *J*=2.4 Hz, 1H); ^{13}C NMR δ −1.3, 18.0, 21.9, 55.7, 65.9, 74.7, 110.1, 110.8, 113.2, 117.3, 121.6, 123.5, 125.1, 129.1, 132.0, 141.5, 144.4, 146.8; IR (neat) ν 1515, 1330 cm^{-1}; MS *m/z* 386 (M^+, 12%), 309 (7), 75 (100); HRMS Calcd for $C_{20}H_{26}N_2O_4Si$ (M^+): 386.1662. Found: 386.1658.

4.3.12. *N*-[2-(Trimethylsilyl)ethoxymethyl]-8-methoxy-6-methyl-carbazol-3-amine (12). To a solution of nitrocarbazole **23** (18.0 mg, 0.0466 mmol) in THF (1.0 mL) at 0 °C was added a THF solution of $NaBH_2S_3$[16] [prepared by stirring a mixture of $NaBH_4$ (11 mg, 0.279 mmol) and sulfur

(31 mg, 0.978 mmol) in THF (0.8 mL) under Ar at rt for 40 min] under Ar. The reaction mixture was heated at reflux for 30 min. After cooling, the products were extracted with Et_2O and the organic layer was washed with H_2O and 1 M aqueous NaOH solution, and then dried. Removal of the solvent left a residue, which was purified by column chromatography (silica gel: 2 g, EtOAc/petroleum ether=1:3) to give carbazolamine **12** (13.9 mg, 84%) as a light yellow oil: R_f=0.08 (EtOAc/petroleum ether=1:5); ^{1}H NMR δ −0.12 (s, 9H), 0.85 (t, J=8.1 Hz, 2H), 2.49 (s, 3H), 3.20–3.80 (br s, 2H), 3.53 (t, J=8.1 Hz, 2H), 3.97 (s, 3H), 5.95 (s, 2H), 6.72 (s, 1H), 6.87 (dd, J=8.4, 2.1 Hz, 1H), 7.31 (d, J=2.1 Hz, 1H), 7.36 (d, J=8.4 Hz, 1H), 7.37 (s, 1H); ^{13}C NMR δ −1.3, 18.0, 21.8, 55.5, 65.1, 74.3, 105.8, 109.1, 110.9, 112.8, 115.8, 124.5, 125.0, 128.6, 129.2, 136.0, 139.7, 146.7; IR (neat) δ 3350, 2950 cm^{-1}; MS m/z 356 (M^+, 14%), 239 (11), 226 (15), 149 (17), 75 (100); HRMS Calcd for $C_{20}H_{28}N_2O_2Si$ (M^+): 356.1920. Found: 356.1922.

4.3.13. 1′,8-Dimethoxy-3′,6-dimethyl-9-[2-(trimethylsilyl) ethoxymethyl]-3,9′-bi-9*H*-carbazole (*N*-SEM-murrastifoline-A) (24). Ar gas was bubbled into a mixture of dibromobiphenyl **11** (17.2 mg, 0.0483 mmol), carbazolamine **12** (15.2 mg, 0.0426 mmol), $Pd_2(dba)_3$ (7.8 mg, 0.0085 mmol), 2-(dicyclohexylphosphino)biphenyl (**5**) (9.2 mg, 0.0262 mmol), and NaO*t*-Bu (8.2 mg, 0.0852 mmol) in toluene (0.6 mL) for 10 min. The reaction mixture was then heated at 120 °C in a sealed tube for 24 h. After cooling, the mixture was purified by column chromatography (silica gel: 2 g, EtOAc/hexane=1:30) to afford SEM protected murrastifoline-A (**24**) (13.6 mg, 58%) as a colorless syrup: R_f=0.64 (EtOAc/petroleum ether=1:5); ^{1}H NMR δ −0.07 (s, 9H), 0.93 (t, J=7.5 Hz, 2H), 2.50 (s, 3H), 2.55 (s, 3H), 3.55 (s, 3H), 3.65 (t, J=7.5 Hz, 2H), 4.03 (s, 3H), 6.09 (d, J=3.9 Hz, 2H), 6.74 (d, J=0.6 Hz, 1H), 6.81 (d, J=0.6 Hz, 2H), 7.18 (d, J=7.9 Hz, 1H), 7.22 (ddd, J=7.9, 7.9, 1.2 Hz, 1H), 7.32 (ddd, J=7.9, 7.9, 1.2 Hz, 1H), 7.42 (s, 1H), 7.47 (dd, J=8.7, 1.8 Hz, 1H), 7.60 (s, 1H), 7.62 (d, J=8.7 Hz, 1H), 8.04 (d, J=1.8 Hz, 1H), 8.08 (d, J=7.8 Hz, 1H); ^{13}C NMR δ −1.3, 18.1, 21.8, 21.9, 55.7, 56.1, 65.5, 74.5, 109.5, 109.8, 110.1, 110.5, 112.9, 112.9, 119.4, 119.9, 120.1, 123.1, 123.2, 123.6, 125.4, 125.7, 126.4, 128.6, 129.4, 129.7, 130.2, 132.2, 140.4, 143.2, 146.8, 146.9; IR (neat) ν 2950, 1500 cm^{-1}; MS m/z 550 (M^+, 1%), 433 (1), 405 (1), 359 (1), 167 (12), 129 (18), 59 (100); HRMS Calcd for $C_{34}H_{38}N_2O_3Si$ (M^+): 550.2652. Found: 550.2657.

4.3.14. 1′,8-Dimethoxy-3′,6-dimethyl-3,9′-bi-9*H*-carbazole (murrastifoline-A) (4). To a solution of SEM protected murrastifoline-A (**24**, 6.2 mg, 0.011 mmol) in THF (0.2 mL) and EtOH (0.6 mL) was added 4 M aqueous HCl solution (0.3 mL) at rt. The reaction mixture was heated at reflux for 1.5 h. After cooling, the mixture was diluted with Et_2O and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried and concentrated to give a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/petroleum ether=1:10) to give murrastifoline-A (**4**) (4.4 mg, 94%) as a colorless oil: R_f=0.30 (EtOAc/petroleum ether=1:5); ^{1}H NMR (acetone-d_6) δ 2.48 (s, 3H), 2.51 (s, 3H), 3.56 (s, 3H), 4.02 (s, 3H), 6.84 (s, 1H), 6.88 (s, 1H), 7.15 (d, J=8.4 Hz, 1H), 7.20 (ddd, J=8.1, 7.8, 1.2 Hz, 1H), 7.32 (ddd, J=8.4, 7.8, 1.2 Hz, 1H), 7.40 (dd, J=8.4, 2.1 Hz, 1H), 7.54 (s, 1H), 7.62 (s, 1H), 7.66 (d, J=8.4 Hz, 1H), 8.09 (d, J=2.1 Hz, 1H), 8.13 (d, J=8.1 Hz, 1H), 10.45 (s, 1H); ^{13}C NMR (acetone-d_6) δ 21.7, 21.9, 55.9, 56.1, 108.8, 110.7, 111.1, 111.7, 113.4, 113.4, 120.2, 120.5, 120.8, 123.9, 124.0, 125.1, 126.0, 126.4, 126.6, 129.9, 130.0, 130.1, 130.4, 132.0, 140.0, 144.0, 146.7, 147.8; IR (neat) ν 3420 cm^{-1}; MS m/z 420 (M^+, 6%), 270 (14), 252 (2), 58 (100); HRMS Calcd for $C_{28}H_{24}N_2O_2$ (M^+): 420.1838. Found: 420.1838.

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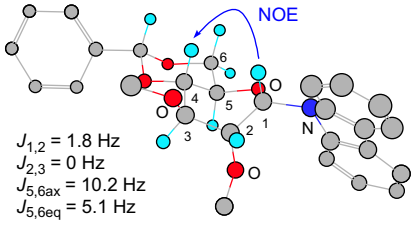

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Sinugibberosides A–E, new terpenoids with cyclic peroxyhemiketal from the soft coral *Sinularia gibberosa*

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Abstract—The organic extract of the soft coral *Sinularia gibberosa*, collected from the northern Taiwan, has been investigated and resulted in the isolation of five new xeniaphyllane-type diterpenoids with a rare cyclic peroxyhemiketal (3,6-dihydro-1,2-dioxin-3-ol) moiety, sinugibberosides A–E (**1**–**5**). The structures of the new terpenoids, including their stereochemistries, were established on the basis of extensive spectroscopic analysis, including 1D and 2D NMR (^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY), and by comparison of their NMR data with those of related compounds. Metabolites **1**–**5** represent the first example of marine terpenoids possessing a cyclic peroxyhemiketal moiety.

1. Introduction

Soft corals belonging to genus *Sinularia* (Alcyoniidae), have been well recognized as a rich source of structurally unique and biologically active diterpenoids[1] and norditerpenoids.[1–3] During the course of our screening of bioactive metabolites from marine organisms,[3–8] we have reported the isolation of xeniaphyllane-based diterpenoids and β-caryophyllane-type sesquiterpenoids from genus *Sinularia*.[3] Moreover, a chemical investigation on a sample of *Sinularia gibberosa* Tixier-Durivault, collected from the southern Taiwanese waters near Kenting, had furnished a variety of cytotoxic steroids[9] along with guaiane- and germacrane-derived sesquiterpenoids.[10] Recently, we reinvestigated the same organism, collected from the northern east coast of Taiwan, and found that the xeniaphyllane-type diterpenoids[3,11,12] is also the terpenoidal constituents of this organism. Five new xeniaphyllane-derived diterpenoids with the rare 3,6-dihydro-1,2-dioxin-3-ol[13] (cyclic peroxyhemiketal) subunit, sinugibberosides A–E (**1**–**5**), were isolated from the EtOAc extract of *S. gibberosa*. Their structures, including their stereochemistries, were elucidated on the basis of extensive spectroscopic (including 1D and 2D NMR) analysis and by comparison of their spectral data with those of the related compounds. Although polyketide-derived esters with cyclic peroxyketal moiety have been isolated from sponges of the genera *Chondrilla*[14] and *Xestospongia*,[15] and a sesquiterpenoid with cyclic peroxyhemiketal subunit was afforded from two asteraceous herbs,[16,17] Sinugibberosides A–E are reported herein as the first example of marine terpenoids possessing the rare 3,6-dihydro-1,2-dioxin-3-ol group (Fig. 1).

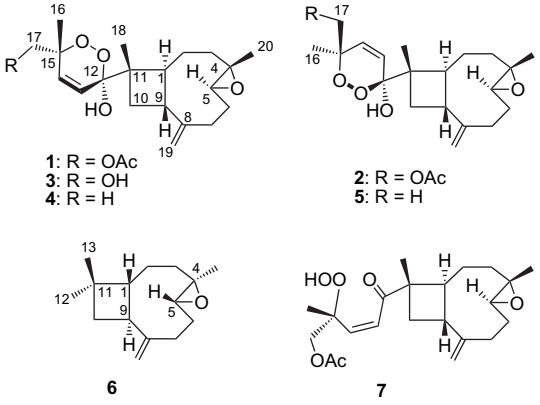

Figure 1. Structures of new metabolites **1**–**5**, a known compound **6**, and a hypothetical precursor **7**.

Keywords: Sinugibberosides A–E; Cyclic peroxyhemiketal; Xeniaphyllane; *Sinularia gibberosa*.
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2. Results and discussion

The minced bodies of *S. gibberosa* were exhaustively extracted with EtOAc and the concentrated extract was fractionated by column chromatography on silica gel using *n*-hexane and *n*-hexane–EtOAc mixture of increasing polarity. The eluted fractions were further purified by various chromatographic techniques including gel permeation and HPLC to afford **1**–**5** (see Section 3). All new metabolites were obtained as colorless oils and have been shown to exhibit a positive sign for optical rotations.

Sinugibberoside A (**1**) was found to possess a molecular formula $C_{22}H_{32}O_6$ as established from its HRESIMS (*m/z* 415.2093, $[M+Na]^+$), implying seven degrees of unsaturation. The IR spectrum indicated the presence of hydroxy (ν_{max} 3400 cm^{-1}) and ester (ν_{max} 1746 cm^{-1}) groups in the molecule. The ^1H NMR data (Table 1) revealed the presence of two upper field shifted methyls (δ 1.13 and 1.19, each 3H, s), one trisubstituted epoxide (δ 2.95, 1H, dd, J=10.5, 4.5 Hz), 1,1-disubstituted double bond (δ 4.88 and 5.04, each 1H, s), and two ^1H–^1H COSY correlated ring-juncture methines (δ 2.80, 1H, dd, J=10.0, 9.5 Hz and 2.63, 1H, q, J=9.5 Hz), characteristic of an 4,5-epoxycaryophyllene (**6**) moiety[3,11,12,18] in **1**. This was further supported by the similar ^{13}C NMR data (Table 2) of C-2 to C-11 and C-18 to C-20 with those of related metabolites.[3,11,12] Therefore, the substituent at C-11 of the molecule should require three degrees of unsaturation to comply with the MS data. This was satisfied by the presence of one acetoxyl (δ_H 2.08, 3H, s; δ_C 170.5, qC and 20.7, CH_3), one 1,2-disubstituted double bond (δ_H 5.83 and 5.88, each 1H, d, J= 10.5 Hz; δ_C 127.0 and 130.5, each CH), and one additional ring. The latter was suggested to be a 3,6-dihydro-1,2-dioxin-3-ol moiety on the basis of the chemical shifts of the two above-mentioned olefinic carbons and the oxycarbon signals appearing at δ 99.6 and 78.9 (each 1C, qC).[13,16] Also, the HMBC correlations found from H_3-18 (δ 1.13, 3H, s) and a hydroxy proton (δ 3.29, 1H, br s) at the quaternary oxycarbon (δ 99.6, qC, C-12) could assign the latter as the hemiketal carbon of the 3,6-dihydro-1,2-dioxin-3-ol group in **1**. Moreover, the detailed analyses of the ^1H–^1H COSY and HMBC correlations (Fig. 2) established **1** as one of the 4,5-epoxyxeniaphyllane diterpenoids.[3,11,12] The above correlations also revealed that the C-11 linked side chain is 12,15-epidioxidized to form a cyclic hemiketal group with hydroxy and methyl (δ_H 1.44, 3H, s; δ_C 19.6, CH_3) and acetoxymethyl (δ_H 3.98 and 4.18, each 1H, d, J=12.0 Hz; δ_C 66.4, CH_2) substituents at C-12 and C-15, respectively. Furthermore, the ion peak appeared in the MS spectrum at *m/z* 205 $[M-C_8H_{11}O_5]^+$ should be due to the cleavage of **1**, between C-11 and C-12 to afford the trisubstituted cyclic hemiketal moiety at *m/z* 187 $[C_8H_{11}O_5]^+$.

The NOE interactions (Fig. 3) displayed by the two *trans* ring-fused methine protons H-1 (δ 2.80, dd, J=10.0, 9.5 Hz) and H-9 (δ 2.63, q, J=9.5 Hz) with H-5 (δ 2.95, dd, J=10.5, 4.5 Hz) and H_3-20 (δ 1.19, s), respectively, revealed the 1*S**,4*S**,5*S**,9*R** configuration in **1**, as found in the related 4,5-epoxyxeniapyllenes previously isolated from *Sinularia* species by our group.[3] The NOE correlations observed between H-9 and H_3-18 (δ 1.13, s) positioned the methyl group at C-11 on the β-face of the molecule. This was further supported by the NOE correlations found between H-10β (δ 1.56, m) and both H-9 and H_3-18. Thus, C-11 is *S** configured. Moreover, the olefinic H-13 showed NOE interactions with both H_3-18 and H_2-10 but not with H_2-2, revealing that the 13,14-double bond of the cyclic peroxyhemiketal moiety at C-11 is placed in the position close to C-10 and the peroxide is located near C-1 of the β-caryophyllene moiety of **1**, respectively. Therefore, the significant NOE interaction displayed between the β-oriented H_3-18 and H_3-16 established the β-orientation of the methyl at C-15 and hence, the α-orientation of the hydroxyl at C-12.

Table 1. ^1H NMR chemical shifts of compounds **1**–**5**

	1[a]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
1	2.80 dd (10.0, 9.5)[d]	2.60 dd (9.5, 9.5)	2.78 dd (10.0, 9.6)	2.78 dd (10.0, 9.5)	2.58 dd (9.0, 9.0)
2α	1.77 ddd (15.0, 4.0, 4.0)	1.75 ddd (15.0, 4.8, 3.3)	1.77 ddd (14.6, 4.0, 3.6)	1.77 ddd (14.4, 3.6, 3.6)	1.78 ddd (14.6, 4.2, 2.8)
2β	1.45 m	1.52 m	1.45 m	1.45 m	1.49 m
3α	1.05 ddd (12.5, 12.5, 3.0)	1.02 ddd (12.9, 12.9, 4.8)	1.05 ddd (13.0, 13.0, 5.2)	1.06 m	1.02 ddd (12.6, 12.6, 4.2)
3β	2.06 m	2.07 m	2.04 ddd (13.0, 4.4, 4.4)	2.03 ddd (12.0, 3.6, 3.6)	2.06 ddd (12.6, 4.2, 2.8)
5	2.95 dd (10.5, 4.5)	2.93 dd (10.5, 4.0)	2.96 dd (10.5, 4.0)	2.95 dd (10.5, 4.0)	2.92 dd (10.5, 4.0)
6α	2.28 ddd (16.0, 8.0, 4.5)	2.27 m	2.28 ddd (16.4, 7.2, 4.0)	2.27 m	2.28 m
6β	1.33 m	1.31 m	1.31 m	1.32 m	1.31 m
7α	2.40 ddd (13.0, 9.0, 4.0)	2.38 ddd (12.6, 8.0, 4.0)	2.40 ddd (12.8, 8.8, 4.0)	2.40 ddd (12.8, 9.2, 4.0)	2.38 ddd (12.8, 8.4, 4.0)
7β	2.16 m	2.17 m	2.17 ddd (12.8, 7.2, 4.0)	2.17 m	2.16 ddd (12.8, 6.0, 4.0)
9	2.63 q (9.5)	2.62 q (9.5)	2.63 q (9.6)	2.62 q (9.5)	2.63 q (9.0)
10α	2.20 dd (10.5, 9.5)	2.27 dd (10.0, 9.5)	2.22 dd (10.4, 9.6)	2.22 dd (10.0, 9.5)	2.25 dd (10.0, 9.0)
10β	1.56 m	1.52 m	1.54 m	1.55 m	1.54 m
13	5.83 d (10.5)	5.86 d (10.5)	5.85 d (10.0)	5.65 d (10.0)	5.72 d (10.0)
14	5.88 d (10.5)	5.93 d (10.5)	5.95 d (10.0)	5.93 d (10.0)	5.95 d (10.0)
16	1.44 s, 3H	1.26 s, 3H	1.35 s, 3H	1.40 s, 3H	1.39 s, 3H
17	3.98 d (12.0), 4.18 d (12.0)	4.12 d (11.7), 4.27 d (11.7)	3.58 d (12.0), 3.63 d (12.0)	1.23 s, 3H	1.23 s, 3H
18	1.13 s, 3H	1.15 s, 3H	1.14 s, 3H	1.13 s, 3H	1.17 s, 3H
19	4.88 s, 5.04 s	4.88 s, 5.04 s	4.88 s, 5.04 s	4.87 s, 5.04 s	4.87 s, 5.04 s
20	1.19 s, 3H	1.19 s, 3H	1.19 s, 3H	1.18 s, 3H	1.19 s, 3H
Ac	2.08 s, 3H	2.11 s, 3H			
12-OH	3.29 br s	3.23 s	3.50 br s	3.15 s	3.12 s

[a] Spectra recorded at 500 MHz in $CDCl_3$.
[b] Spectra recorded at 300 MHz in $CDCl_3$.
[c] Spectra recorded at 400 MHz in $CDCl_3$.
[d] The J values are given in hertz in parentheses.

Table 2. ^{13}C NMR chemical shifts of compounds **1–5**

No.	**1**[a]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
1	—[d]	43.2 (CH)[e]	—	—	43.0 (CH)
2	28.3 (CH_2)	28.6 (CH_2)	28.5 (CH_2)	28.7 (CH_2)	28.8 (CH_2)
3	38.6 (CH_2)	38.6 (CH_2)	38.6 (CH_2)	38.9 (CH_2)	38.9 (CH_2)
4	59.8 (qC)	59.6 (qC)	59.8 (qC)	60.0 (qC)	59.9 (qC)
5	63.9 (CH)	63.9 (CH)	63.9 (CH)	64.2 (CH)	64.1 (CH)
6	30.2 (CH_2)	30.1 (CH_2)	30.2 (CH_2)	30.5 (CH_2)	30.4 (CH_2)
7	29.5 (CH_2)	29.4 (CH_2)	30.2 (CH_2)	30.0 (CH_2)	29.7 (CH_2)
8	151.6 (qC)	151.3 (qC)	151.6 (qC)	151.9 (qC)	151.7 (qC)
9	47.6 (CH)	48.2 (CH)	47.7 (CH)	47.9 (CH)	48.4 (CH)
10	33.2 (CH_2)	31.9 (CH_2)	33.2 (CH_2)	33.4 (CH_2)	32.4 (CH_2)
11	41.7 (qC)	41.5 (qC)	42.0 (qC)	42.1 (qC)	42.9 (qC)
12	99.6 (qC)	99.2 (qC)	99.6 (qC)	99.6 (qC)	99.5 (qC)
13	127.0 (CH)	127.2 (CH)	126.6 (CH)	124.3 (CH)	124.3 (CH)
14	130.5 (CH)	131.3 (CH)	131.7 (CH)	135.7 (CH)	136.2 (CH)
15	78.9 (qC)	78.0 (qC)	80.5 (qC)	77.4 (qC)	77.2 (qC)
16	19.6 (CH_3)	19.8 (CH_3)	19.3 (CH_3)	24.4 (CH_3)	24.3 (CH_3)
17	66.4 (CH_2)	64.6 (CH_2)	66.9 (CH_2)	24.8 (CH_3)	24.8 (CH_3)
18	15.7 (CH_3)	15.6 (CH_3)	15.8 (CH_3)	16.0 (CH_3)	15.6 (CH_3)
19	113.6 (CH_2)	113.7 (CH_2)	113.6 (CH_2)	113.7 (CH_2)	113.8 (CH_2)
20	17.1 (CH_3)	17.1 (CH_3)	17.1 (CH_3)	17.4 (CH_3)	17.3 (CH_3)
Ac	170.5 (qC), 20.7 (CH_3)	170.7 (CH_3), 20.8 (CH_3)			

[a] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C.
[c] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[d] Methine carbon signal was not observable and no corresponding HMQC and HMBC correlations were found.
[e] Attached protons were determined by DEPT experiments. The values are given in parts per million lower field from TMS.

Furthermore, the molecular mechanics calculations was performed to study the conformational behavior of **1**. The conformational analysis suggested the most stable conformation at 228.4 Kcal/mol as shown in Figure 4. It was found that the calculated distances between protons having key NOE correlations around C-12 of the cyclic peroxyhemiketal moiety were ranging from 2.8 to 3.2 Å as shown in Table 3. Based on the above findings, the structure of sinugibberoside A was fully established as (1*S**,4*S**,5*S**,9*R**,11*S**,12*R**,15*R**)-4,5-epoxy-12,15-epidioxy-17-acetoxy-xeniaphylla-8(19),13-dien-12-ol.

Sinugibberoside B (**2**) was found to possess the same molecular formula $C_{22}H_{32}O_6$ as that of **1** from the HRESIMS (*m*/*z* 415.2093 $[M]^+$) and NMR spectral data (Tables 1 and 2). The ^{13}C NMR data of **2** (Table 2) were found to be quite similar to those of **1**. Therefore, **2** was suggested to be an isomer of **1**. However, we observed the upper field shifts for C-10, C-15, and C-17 ($\Delta\delta$ −1.3, −0.9, and −1.8, respectively) and a lower field shift for C-9 ($\Delta\delta$ +0.6) of **2** relative to those of **1**. Also, although the ^1H NMR data of **2** (Table 1) showed high similarity with those of **1**, substantial differences for chemical shifts of H-1, H_3-16, and H-17 ($\Delta\delta$ −0.20, −0.18, and +0.14, respectively) relative to those of **1** were

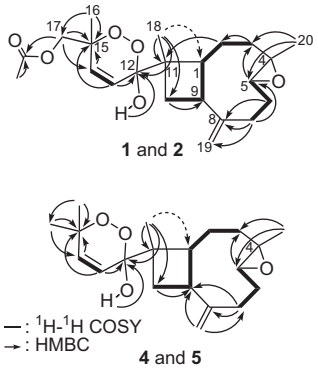

Figure 2. ^1H–^1H COSY and HMBC correlations for **1**, **2**, **4**, and **5**. Other HMBC correlation (dashed arrow) found for **2** and **5**.

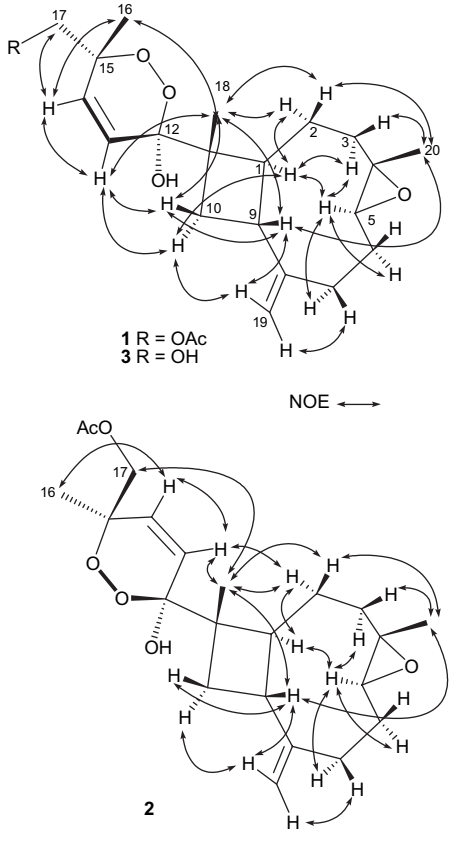

Figure 3. Observed NOESY correlations for **1–3**.

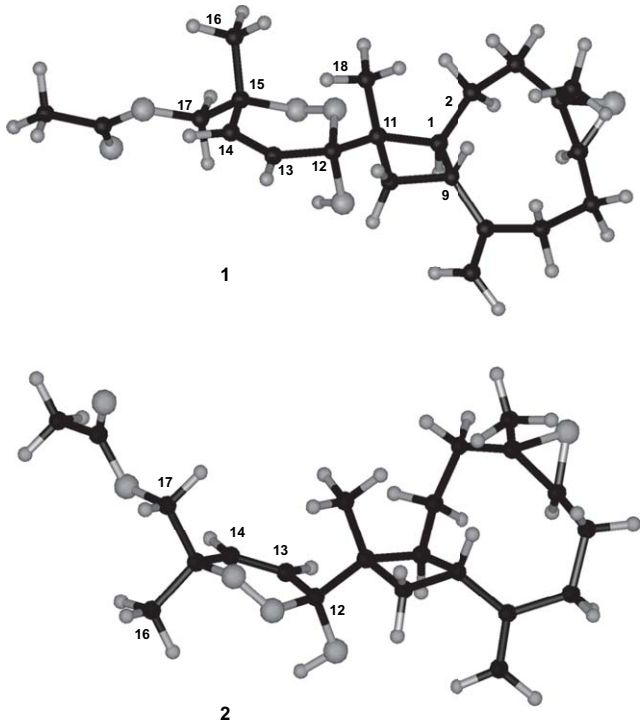

Figure 4. Stereo-view of **1** and **2** generated from computer modeling.

found. After the establishment of the planar structure of **2** by analysis of ^{1}H–^{1}H COSY and HMBC correlations (Fig. 2), we then carefully examined the NOESY correlations of **2** by comparison with those of **1** (Fig. 3). The NOESY spectrum of **2** exhibited similar NOE correlations as those found in **1**, and led to the determination of the *S**, *S**, *S**, *R**, and *S** configurations at C-1, C-4, C-5, C-9, and C-11, respectively. The NOE interactions found for the olefinic proton at C-13 with both H_3-18 (δ 1.15, s) and H-2α (δ 1.75, ddd, J=15.0, 4.8, 3.3 Hz), and H_3-18 with H-9 (δ 2.62, q, J=9.5 Hz) and H-17 (δ 4.12, d, J=11.7 Hz) further established the relative stereochemistry of **2** as shown in Figure 3. On the basis of the above findings, compound **2** was identified as the 12-epimer of **1**. Moreover, the molecular mechanics calculations suggested the most stable conformation at 232.3 Kcal/mol as shown in Figure 4. From this conformation, the calculated distances between those protons having key NOE correlations around C-12 were ranged from 2.9 to 3.3 Å as shown in Table 3. Therefore, sinugibberoside **2** was established as (1*S**,4*S**,5*S**,9*R**,11*S**,12*S**,15*R**)-4,5-epoxy-12,15-epidioxy-17-acetoxy-xeniaphylla-8(19),13-dien-12-ol.

Table 3. Calculated distances between selective protons having key NOE correlations of **1** and **2**

H/H	Distance (Å)	
	1[a]	**2**[b]
H_3-16/H_3-18	2.8	>4.0
H_2-17/H_3-18	>4.0	3.1
H-13/H_3-18	2.9	3.3
H-13/H-10α	2.8	>4.0
H-13/H-10β	3.2	>4.0
H-13/H-2α	>4.0	2.9

[a] Minimum energy conformers at 228.4 Kcal/mol.
[b] Minimum energy conformers at 232.3 Kcal/mol.

The most polar metabolite, sinugibberoside C (**3**), has a molecular formula $C_{20}H_{30}O_5$ as indicated from its HRESIMS (*m*/*z* 373.1989, $[M+Na]^+$) and NMR data (Tables 1 and 2). It differs with **1** and **2** in the absence of an ester moiety as revealed from its IR spectrum and by the lack of the acetate signals from the NMR spectra of **3**. Comparison of the NMR data of **3** with those of **1** and **2** designated **3** as a related 4,5-epoxyxeniaphyllane derivative with the same 3,6-dihydro-1,2-dioxin-3-ol moiety. The substantial upper field shift observed for H_2-17 (δ 3.58 and 3.63) in **3** than those of **1** (δ 3.98 and 4.18) and **2** (δ 4.12 and 4.27) indicated the presence of a hydroxymethyl at C-15 in **3** instead of the acetoxymethyl. However, we have also found that H-1 (δ 2.78) of **3** is resonating in a field quite close to that of **1** (δ 2.80), but in a lower field relative to that of **2** (δ 2.60). This suggested the close vicinity of the peroxide bridge to H-1 in **3** as in the case of **1**, revealing the same *R** configuration at C-12 for **3**. Analysis of NOE correlations displayed in the NOESY spectrum of **3** (Fig. 3) has led to similar results as that obtained from the NOESY spectrum of **1**, including the NOE responses for the olefinic H-13 with both H_2-10 and H_3-18 and for H_3-18 with H_3-16. Therefore, the structure of **3** was determined as (1*S**,4*S**,5*S**,9*R**,11*S**,12*R**,15*R**)-4,5-epoxy-12,15-epidioxy-xeniaphylla-8(19),13-dien-12,17-diol.

The HRESIMS (*m*/*z* 357.2040, $[M+Na]^+$) of sinugibberoside D (**4**) assigned a molecular formula $C_{20}H_{30}O_4$, with one oxygen atom less than that found for **3**. The IR (ν_{max} 3422 cm^{-1}) and ESIMS (*m*/*z* 317, $[M-H_2O+H]^+$) spectra revealed the presence of one hydroxy group in **4**. Moreover, the NMR data of **4** were found to be almost identical with those of **3** except for the replacement of the hydroxymethyl at C-15 (δ_H 3.58 and 3.63; δ_C 66.9) in **3** by a methyl (δ_H 1.23; δ_C 24.8) in **4**. Also, the identical chemical shift of H-1 in **4** and **3** disclosed a similar stereochemistry for the hemiketal carbon at C-12. After the establishment of the gross structure of **4** by the analysis of ^{1}H–^{1}H COSY and HMBC correlations (Fig. 2), we have found that the NOE interactions revealed by H-13 with both H_2-10 and H_3-18, and H_3-16 with H_3-18 also confirmed the 12*R** configuration of **4**, the same as that of **3**. Therefore, sinugibberoside D (**4**) was established as (1*S**,4*S**,5*S**,9*R**,11*S**,12*R**)-4,5-epoxy-12,15-epidioxy-xeniaphylla-8(19),13-dien-12-ol.

The HRESIMS (*m*/*z* 357.2041, $[M+Na]^+$) of sinugibberoside E (**5**) combined with NMR data suggested **5** to be an isomer of **4**. Also, a hydroxy group was revealed from the IR (ν_{max} 3422 cm^{-1}) and ESIMS (*m*/*z* 317, $[M-H_2O+H]^+$). Metabolite **5** was found to possess a cyclic peroxyhemiketal moiety from the chemical shift of C-12 (δ 99.5, qC), as those found in **1**–**4**. The significant lower field shift observed for C-9 ($\Delta\delta$ +0.5) and the upper field shift for C-10 ($\Delta\delta$ −1.0) relative to those in **4**, a case which is similar by comparison of the corresponding data of **2** with those of **1**, suggested that **5** might be the 12-epimer of **4**. This was supported by the upper field shift observed for H-1 (δ 2.58) of **5** relative to that of **4** (δ 2.78). Finally, the NOE interactions observed for H-13 with both H-2α and H_3-18, as those found in **2** established sinugibberoside E (**5**) as (1*S**,4*S**,5*S**,9*R**,11*S**,12*S**)-4,5-epoxy-12,15-epidioxy-xeniaphylla-8(19),13-dien-12-ol.

It is noteworthy to mention that sinugibberosides reported here are the first example of marine natural products possessing the rarely found tetrasubstituted peroxyhemiketal moiety,[16,17] although polyketide-derived metabolites with a trisubstituted cyclic peroxyketal moiety have been isolated from some sponges.[14,15] The biosynthesis of both **1** and **2** can be achieved by the nucleophilic attack of 15-OOH at the different sides of the carbonyl group at C-12 of a precursor **7**, respectively. Metabolites **3**–**5** were considered to be biosynthesized by the same approach from the similar precursors with structures related to **1**.

3. Experimental

3.1. General

Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Mercury Plus 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as an internal standard. LRMS data were obtained by EI on a VG Quattro GC–MS spectrometer or by ESI on a BRUKER APEX II mass spectrometer. HRMS were recorded on EI or ESI on a BRUKER APEX II mass spectrometer. Si gel (Merck, 230–400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography was performed on a Hitachi L-7100 apparatus with Merck Hibar Si-60 column (250×21 mm, 7 μm).

3.2. Organism

S. gibberosa was collected by hand via scuba from the northern east coast of Taiwan, in June 2004, at a depth of 15–20 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Resources, National Sun Yat-Sen University.

3.3. Extraction and separation

The bodies of *S. gibberosa* (1.3 Kg fresh weight) were minced and extracted exhaustively with EtOAc, and the extract was concentrated under reduced pressure to give a dark brown viscous residue (15.4 g). The residue was fractionated by open column chromatography on silica gel using *n*-hexane and *n*-hexane–EtOAc mixture of increasing polarity to yield 32 fractions. Fraction 15 eluted with *n*-hexane–EtOAc (5:1) was permeated through a Sephadex LH-20 column (2×90 cm) in acetone and the lipid-free fraction was subsequently isolated by normal phase HPLC using hexane–acetone (7:1 to 6:1) to give compounds **5** (0.7 mg) and **4** (0.5 mg). Fraction 20 eluted with *n*-hexane–EtOAc (4:1 to 2:1) was further separated by normal phase HPLC, using *n*-hexane–acetone (4:1 to 3:1) to yield **1** (0.5 mg), **2** (0.5 mg), and **3** (1.5 mg), respectively.

3.3.1. Sinugibberoside A (1). Colorless oil, $[\alpha]_D^{25}$ +18.3 (*c* 0.6, $CHCl_3$); IR (neat) ν_{max} 3400, 2956, 2926, 2876, 1746, 1647, 1456, 1385, 1242, 1046 cm^{-1}; for ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively; EIMS *m/z* 360 (0.1, $[M-O_2]^+$), 344 (0.1, $[M-3O]^+$), 205 (0.2, $[M-C_8H_{11}O_5]^+$), 187 (0.3, $[C_8H_{11}O_5]^+$), 175 (0.3), 171 (0.4), 163 (0.5), 159 (0.7), 147 (1.2), 135 (0.9), 121 (1.7), 111 (1.5), 105 (2.9), 97 (4.0), 93 (2.3), 91 (4.1), 79 (5.1); HRESIMS *m/z* 415.2093 (calcd for $C_{22}H_{32}O_6Na$, 415.2097).

3.3.2. Sinugibberoside B (2). Colorless oil, $[\alpha]_D^{25}$ +8.9 (*c* 1.7, $CHCl_3$); IR (neat) ν_{max} 3400, 2957, 2937, 2877, 1736, 1640, 1456, 1383, 1242, 1043 cm^{-1}; for ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; FABMS *m/z* 393 (16.6, $[M+H]^+$); HRESIMS *m/z* 415.2093 (calcd for $C_{22}H_{32}O_6Na$, 415.2097).

3.3.3. Sinugibberoside C (3). Colorless oil, $[\alpha]_D^{25}$ +33.3 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3400, 2930, 2880, 1647, 1541, 1456, 1387, 1049 cm^{-1}; for ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2, respectively; ESIMS *m/z* 373 (100, $[M+Na]^+$), 333 (3, $[M-H_2O+H]^+$), 285 (12, $[M-H_2O-3O+H]^+$), 267 (2, $[M-2H_2O-3O+H]^+$), 205 (6, $[M-C_6H_9O_4]^+$); ESIMS *m/z* 350 (0.1, $[M]^+$), 318 (0.5, $[M-2O]^+$), 316 (0.1, $[M-H_2O-O]^+$), 302 (0.2, $[M-3O]^+$), 205 (1.8, $[M-C_6H_9O_4]^+$), 187 (1.4), 175 (1.2), 164 (2.3), 163 (3.4), 159 (2.9), 149 (3.5), 148 (6.0), 135 (5.1), 121 (10.6), 111 (5.9), 105 (18.0), 97 (29.4), 93 (14.3), 91 (25.7), 79 (30.5); HRESIMS *m/z* 373.1989 (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

3.3.4. Sinugibberoside D (4). Colorless oil, $[\alpha]_D^{25}$ +47.2 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3422, 2924, 2874, 1647, 1512, 1456, 1396, 1051 cm^{-1}; for ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2, respectively; ESIMS *m/z* 357 (100, $[M+Na]^+$), 341 (30, $[M-O+Na]^+$), 335 (9, $[M+H]^+$), 317 (57, $[M-H_2O+H]^+$), 301 (13, $[M-H_2O-O+H]^+$), 285 (10, $[M-H_2O-2O+H]^+$); EIMS *m/z* 302 (0.2, $[M-2O]^+$), 205 (2.3, $[M-C_6H_9O_3]^+$), 187 (6.1), 173 (5.2), 164 (6.2), 161 (10.9), 149 (23.1), 148 (14.8), 138 (25.9), 122 (38.1), 105 (38.3), 95 (61.4), 91 (68.5), 79 (66.5); HRESIMS *m/z* 357.2040 (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

3.3.5. Sinugibberoside E (5). Colorless oil, $[\alpha]_D^{25}$ +36.1 (*c* 0.4, $CHCl_3$); IR (neat) ν_{max} 3422, 2924, 2874, 1647, 1541, 1456, 1397, 1049 cm^{-1}; for ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2, respectively; ESIMS *m/z* 357 (100, $[M+Na]^+$), 341 (74, $[M-O+Na]^+$), 317 (23, $[M-H_2O+H]^+$); EIMS *m/z* 302 (1.5, $[M-2O]^+$), 205 (3.3, $[M-C_6H_9O_3]^+$), 187 (8.2), 173 (5.3), 164 (8.4), 163 (12.0), 159 (17.4), 149 (16.9), 148 (13.2), 138 (24.9), 137 (26.2), 123 (58.4), 109 (37.2), 105 (49.2), 95 (54.9), 91 (62.3), 79 (77.7); HRESIMS *m/z* 357.2041 (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

3.3.6. Molecular mechanics' calculations. The minimum energy conformation of **1** and **2** was determined using the MSI Insight II/DISCOVER version 95 molecular modeling package incorporating an empirical force field, the consistent valence force field (CVFF),[19] on a Silicon Graphics IRIS Indigo XS24/R4000 workstation. Molecular mechanics was utilized to investigate the minimization,

and minimum energy was calculated by a conjugate gradient method until the maximum derivative was less than 0.001 Kcal/mol Å. The conformers shown in Figure 4 are the lowest energy conformation for **1** and **2**.

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High guest inclusion in 3β-amino-7α,12α-dihydroxycholan-24-oic acid enabled by charge-assisted hydrogen bonds

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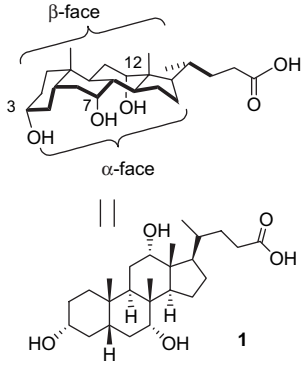
Abstract—3β-Amino-7α,12α-dihydroxycholan-24-oic acid (**2**) forms inclusion compounds with high ratio (host/guest=1/4) of guest methanol. Both hydrogen bonds and hydrophobic interactions are important to the solid structure. The cholates assemble in a head-to-tail fashion to form infinite hydrogen-bonded chains. The chains are interconnected between cholates and also through the guests. Large channels are formed along the crystallographic *a* axis where most of the methanol molecules are located. Presence of a dominant hydrogen-bonding motif (i.e., ammonium-carboxylate ion pairing) is probably responsible for high guest incorporation.

1. Introduction

Cholic acid (**1**) has an unusual distribution of functional groups: the α face is hydrophilic with three hydroxyl groups and the β face is hydrophobic consisting of only hydrocarbons. Because of its unique structure and commercial availability, cholic acid is a popular building block in supramolecular chemistry.[1] In recent years, it has been used to construct environmentally responsive molecules.[2–4] Taking advantage of the facial amphiphilicity of cholates,[5] we prepared molecular baskets that undergo transitions between micelle-like and reversed-micelle-like conformations induced by solvent changes[6] and cholate foldamers with nanometer-sized hydrophilic cavities.[7]



Another interesting feature of cholic acid (and bile acids in general) is their ability to form inclusion compounds with various organic compounds.[8] This is an attractive application because bile acids are chiral and can be used for enantiomeric and diastereomeric separation of guest molecules.[9,10] The number and the orientation of hydrogen bonds greatly influence the solid state structures of the bile acids as well as the inclusion compounds that can be formed. For example, deoxycholic acid, only different from cholic acid (**1**) by missing one hydroxyl group at C-7, is known for over a hundred years to form inclusion compounds with a wide variety of organic molecules including hydrocarbons, alcohols, ethers, ketones, acids, esters, and nitriles.[8,11] The ability of cholic acid to form inclusion compounds, however, was discovered much later, but received increased attention in recent years.[8] Its crystal lattice is quite stable and can survive reversible incorporation and removal of guest molecules in some cases,[12,13] making it potentially useful as 'organic zeolite' for separation and chemical reactions.

In our recent study of cholate derivatives, we synthesized 3β-amino-7α,12α-dihydroxycholan-24-oic acid (**2**) and found it could include guest molecule such as methanol. Most interestingly, large void volumes can be formed in the solid structure so that four solvent molecules can be incorporated per host molecule. In contrast, the number of guest molecules in preciously reported bile acid inclusion compounds almost never goes above two.

2. Results and discussion

Synthesis of **2** was adopted from literature procedures (Scheme 1).[14] Cholic acid was treated with catalytic amount

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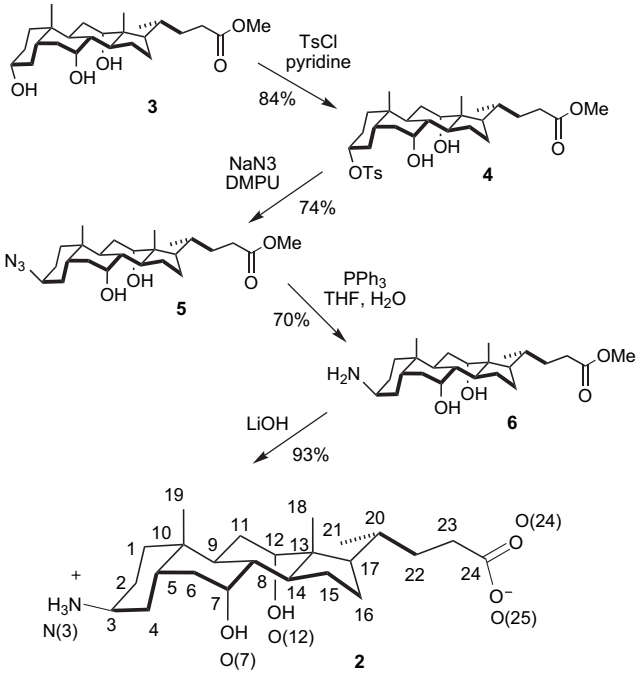
of sulfuric acid in refluxing methanol to give methyl ester **3**. Among the three hydroxyl groups, the one at 3α position is most reactive and was selectively tosylated in 84% yield. Tosylate **4** was replaced by azide through nucleophilic substitution with sodium azide in 74% yield. The azide intermediate **5** was then reduced by triphenylphosphine in aqueous THF and was hydrolyzed to give the final product **2** in good yield.



Scheme 1. Synthesis of compound **2**.

3β-Amino-7α,12α-dihydroxycholan-24-oic acid (**2**) has low solubility in many organic solvents including chloroform, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), and even dimethyl sulfoxide (DMSO)—the latter two typically dissolve cholate derivatives very easily. Apparently, charges from the ammonium and carboxylate interact more strongly than neutral hydrogen-bonding donors and acceptors in most bile acids and give exceptionally high stability to the solid. It is insoluble in water at neutral pH but is soluble under both acidic and basic conditions, presumably due to formation of micellar aggregates. The compound is soluble in hot methanol and easily forms large transparent needle-like crystals upon cooling.

According to single crystal X-ray structure determination one independent molecule of **2** and four methanol solvent molecules were found in asymmetric unit of orthorhombic cell (space group $P2_12_12_1$). The molecule assembles in a head-to-tail fashion with the amine and the carboxyl group hydrogen bond to each other (Fig. 1). The α faces of the cholates tilt up and down alternately along the chain. In fact, every other molecule along the chain is equivalent and can be converted to one other by translational operation. Similar to other bile acids, each repeating unit propagates along the crystallographic *c* axis in a helical fashion,[15] possibly as a result of the bent backbone caused by the cis-fused A/B rings. Along the *a* axis, the chains are completely parallel. These chains are bridged by methanols to give pleated sheets in this direction. The chains are zigzagged and antiparallel between neighboring layers. Along the crystallographic *b* axis, the chains are connected by hydrogen bonds between the carbonyl oxygen O(24) of one cholate and the hydroxyl group O(7) of another.

Amphiphilicity is important in the structure as both hydrophilic and hydrophobic portions of the molecules are clearly segregated (Fig. 1). Hydrophobic contact is maintained by closely packed methyl groups on the β faces of cholates between neighboring chains. Unlike most bile acids,[8] however, the hydrophobic layers are discontinuous along the *c* axis. This is the direct result of alternating α and β faces along the chains (which is likely caused by strong interactions between the amine and the carboxyl group and the β orientation of the amine). The hydrophobic contact is continuous along the *a* axis, forming multiple hydrophobic 'belts' in this direction. Hydrophilic region is located around the amine/carboxyl pair and the two hydroxyl groups O(7) and O(12) of another cholate molecule.

There are four cholates and 16 methanol molecules in one unit cell. This guest/host ratio (4/1) is unusually high for bile acid inclusion compounds. For example, cholic acid (**1**) only incorporates one or two methanol in its crystal.[16–18] Deoxycholic acid does not form inclusion compounds with simple alcohols. In fact, the guest/host ratio in the majority of bile acid inclusion compounds is 1:1 or lower.[8] Figure 2 shows the hydrogen-bonding network formed by the cholates and methanol. Not surprisingly, all the polar atoms (i.e., oxygen and nitrogen) from both the hosts and the guests are involved in hydrogen bonding. Each cholate is hydrogen bonded to six methanol molecules. The carboxylate of cholate A is bonded to the amine of cholate B and to hydroxyl

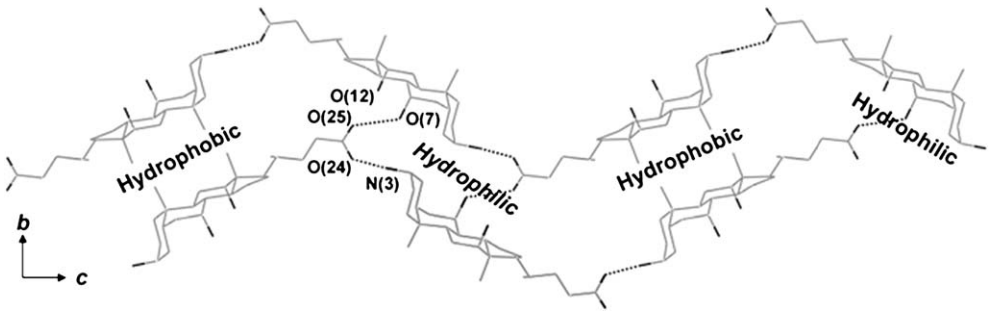


Figure 1. Two hydrogen-bonded chains of **2** viewed along the crystallographic *a* axis. Hydrogen bonds are shown in dotted lines. Hydrogen atoms are omitted for clarity.

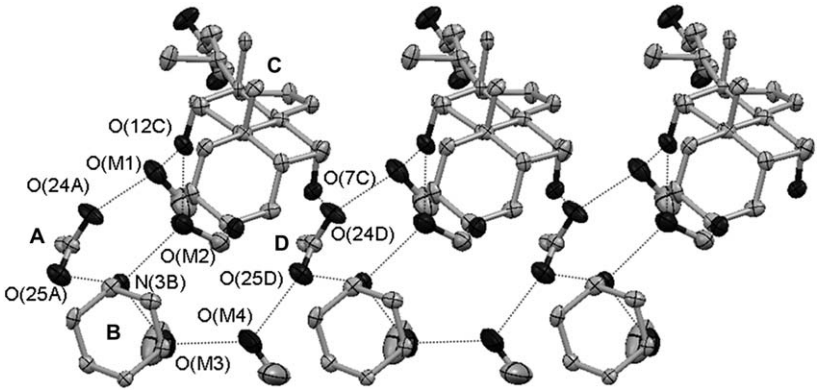


Figure 2. Hydrogen-bonding network within the crystal lattice of **2**. Hydrogen atoms and parts of cholates A, B, and D are omitted for clarity. O(M1), O(M2), O(M3), and O(M4) are the oxygen atoms on the four methanol molecules.

O(12) of cholate C through methanol M1. The hydroxyl O(12) of cholate C is then reconnected back to the amine of cholate B through methanol M2. Interestingly, two additional methanol molecules (M3 and M4) sit between closely bonded amine/carboxylate pairs from cholates A and B.

Typical hydrogen-bonded O···O distances range from 2.36 to 3.69 Å, with the latter being the van der Waals cutoff value.[19] Table 1 summarizes the hydrogen-bond distances and bond angles in the crystal structure. The O···O distance in our structure ranges from 2.65 to 2.79 Å, representing medium-strengthened (2.65–2.80 Å) hydrogen bonds according to literature classification.[19,20] Strong hydrogen bonds tend to have linear geometry. Many of the D–H–A bond angles, however, are smaller than 160°, possibly because the shape of **2** prevents optimal alignment of the donor and the acceptor atoms. Among all the polar atoms, O(7) is the only one that hydrogen bonds strongly to just one other polar atom—the next closest distance between O(7) and another polar atom is 3.30 Å. The O···N distance ranges from 2.70 to 2.86 Å (entries 7–9), similar to the values (2.66–3.12 Å with an average of 2.84 Å) found in amino acids and peptides.[21]

As in most supramolecular systems, the final product formed (crystal structure in this case) represents a minimum in either the global or local energy landscape (corresponding to the thermodynamically controlled or kinetically trapped structures). Multiple intermolecular forces have to work together and balance among themselves to reach the best compromise (i.e., to obtain at least a local energy minimum) in a crystal structure. In typical bile acid inclusion compounds, the most important interactions are hydrogen bonds and hydrophobic interactions.[8] Since all hydrogen bonds (O–H···O) are of similar nature, no one can dominate in a bile acid that is functionalized only with hydroxyl and carboxylic acid groups. Under such a circumstance, the molecules have many ways of optimization and can form tightly packed structures fairly easily. This probably explains why bile acid inclusion compounds rarely incorporate more than one or two guest per host even for small guests like methanol.

Table 1. Hydrogen-bond distances (with H···A distances <2.5 Å) and angles in the solid structure of **2**

Entry	Hydrogen bond[a]	D–H (Å)	H···A (Å)	D···A (Å)	D–H–A bond angle (°)
1	O(7C)–H···O(24D)	0.84	2.14	2.785(6)	133.4
2	O(12C)–H···O(M1)	0.84	1.99	2.755(7)	150.9
3	O(M3)–H···O(M4)	0.84	1.88	2.655(11)	153.6
4	O(M1)–H···O(24A)	0.84	1.86	2.660(8)	158.5
5	O(M2)–H···O(12C)	0.84	1.82	2.653(8)	171.0
6	O(M4)–H···O(25D)	0.84	1.83	2.660(10)	168.2
7	N(3B)–H···O(M2)	0.91	1.82	2.700(9)	162.5
8	N(3B)–H···O(25A)	0.91	2.02	2.831(8)	147.6
9	N(3B)–H···O(M3)	0.91	2.06	2.855(10)	145.5

[a] See Figure 2 for atom numbering. A, B, C, and D are the four labeled cholates. M1, M2, M3, and M4 are the four labeled methanol molecules.

In the current structure, however, the ammonium-carboxylate is the dominant force. In fact, charge-assisted hydrogen bonds are well known to be stronger than neutral ones[20,22,23] and are, therefore, generally maintained in the solid state. Görbitz surveyed 749 amino acids and peptides and found that ammonium carboxyl is always maintained despite the presence of many other hydrogen-bond donors and acceptors in the structures.[21] Aakeröy and co-workers had the same observation in a series of substituted benzylammonium benzoate derivatives.[24] Presence of a dominant force puts a severe constraint on the number of possible ways to optimize the structures. The price of maintaining a particular interaction is to sacrifice other hydrogen bonds and/or close packing of the molecules. Therefore, it should be much easier to incorporate a larger number of guests in such a system.

The crystal structure has channels along the *a* axis (Fig. 3). These channels are fairly hydrophobic except at the corners where the polar atoms are clustered. They are nearly triangular in shape and are fairly large in size: the shorter edge is about 5 Å and the longer ones roughly 7 Å in length. Three (M1, M3, and M4) of the methanol molecules are located within the channels and are connected to the 'wall' through hydrogen bonds. All three of them have their methyl groups pointing to the hydrophobic side of the wall. M1 has lower mobility because it is bonded to the wall via two connections (see also Fig. 2). M3 and M4, on the other hand, are interconnected to each other and are hydrogen bonded to the wall only through a one-point contact. As a result, they have the largest thermal motions among all the atoms, presumably because they can move up and down easily without significantly changing the hydrogen-bonding network. The fourth

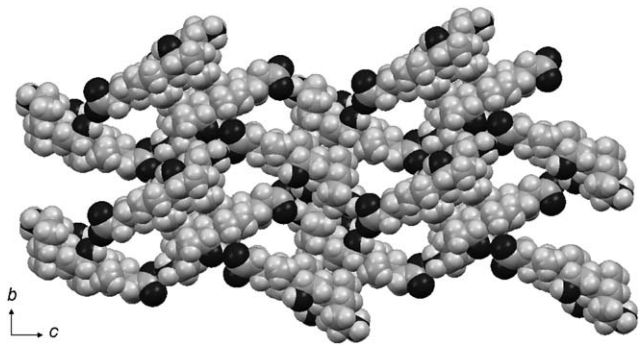


Figure 3. Space filling models of crystal structure of **2** viewed along the crystallographic *a* axis (carbon and hydrogen shown in light gray; oxygen and nitrogen shown in black). Methanol molecules are omitted to show the channels.

methanol (M2) is located in the hydrophilic region in between the carboxyl, amine, and hydroxyl O(7). It is tightly held in a narrow space, which explains the smallest thermal motion observed for this methanol among all the solvents.

3. Conclusions

Ammonium-carboxylate interaction is maintained in the crystal structure of 3β-amino-7α,12α-dihydroxycholan-24-oic acid (**2**). Combination of a dominant hydrogen-bonding interaction with shape awkwardness of the steroid backbone is probably responsible for incorporation of an unusually large number of guest molecules in the inclusion compound. Such a feature can be very useful in preparing inclusion compounds with high loading capacities. Another potentially beneficial feature of **2** as a supramolecular host is its low solubility in a range of polar and nonpolar solvents. This could be useful in reversible incorporation and release of guest molecules for separation and chemical reactions.[9,10]

Bile acid inclusion compounds occupy a unique position in the field of crystal engineering. They have multiple polar groups to stabilize the crystal lattice, facial amphiphilicity allowing incorporation of both hydrophilic and hydrophobic guests, chirality for enantiomeric and/or diastereomeric selectivity, and awkward shapes to avoid close packing. Many systematic modifications on the basic structures have been performed including variation on the number and the orientation of hydroxyl groups, on the type of functionality (e.g., acid, ester, amide, alcohol) at the C24 carbon, and the length of the carboxy tail.[8] In contrast, amino-derived cholates have received little or no attention in their inclusion abilities. Since charge-assisted hydrogen bonds are commonly used to rationally design molecular solids,[22,23] amino-derived bile acids as a group may become highly valuable host compounds for crystal engineering.

4. Experimental

4.1. General

Anhydrous tetrahydrofuran (THF) was dried by passage through a column of activated alumina under compressed nitrogen. All reagents and solvents were of A.C.S. certified grade or higher, and were used as received from commercial suppliers. All glassware and syringes were dried in an oven at least overnight prior to use. All Routine ^{1}H spectra were recorded on a Varian VXR-300 and VXR-400 spectrometer.

4.2. Synthesis

4.2.1. 3β-(4-Methylphenyl)sulfonyloxy-7α,12α-dihydroxycholan-24-oic acid methyl ester (4). This compound was prepared according to adopted literature procedures.[14,25] Methyl cholate **3** (3.03 g, 7.17 mmol) was dissolved in anhydrous pyridine (20 mL). Toluenesulfonyl chloride (1.95 g, 10.79 mmol) was added under N_2. The reaction mixture was stirred for 4 h at 50 °C. Solvent was removed in vacuo. The residue was dissolved in ethyl acetate (50 mL), washed with 2 N HCl (50 mL) and water (2×50 mL), dried with $MgSO_4$, and concentrated in vacuo to give a white powder (3.58 g, 6.21 mmol, 87% yield). This material was generally used in the next step without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz, δ) 7.74 (d, 2H, J=8.4 Hz), 7.42 (d, 2H, J=8.4 Hz), 4.21 (m, 1H), 3.71 (s, 1H), 3.52 (s, 3H), 2.58–0.78 (m, 33H), 0.54 (s, 3H).

4.2.2. 3β-Azido-7α,12α-dihydroxycholan-24-oic acid methyl ester (5). This compound was prepared according to literature procedures.[14] Tosylate **4** (3.58 g, 6.21 mmol) and NaN_3 (2.16 g, 33.22 mmol) were dissolved in *N*,*N*′-dimethylpropyleneurea (DMPU, 20 mL). The reaction mixture was stirred for 12 h at 60 °C. Water (100 mL) was added. The precipitate was collected by filtration and washed with water (2×50 mL). The residue was purified with column chromatography over silica gel using ethyl acetate/hexane (1/4) as the eluent to give a white powder (2.05 g, 4.59 mmol, 74% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, δ) 3.95 (br s, 1H), 3.73 (br s, 1H), 3.57 (br s, 1H), 3.53 (s, 3H), 2.58 (m, 1H), 2.32–0.73 (m, 29H), 0.54 (s, 3H).

4.2.3. 3β-Amino-7α,12α-dihydroxycholan-24-oic acid methyl ester (6). This compound was prepared according to literature procedures.[14] Azide ester **5** (203 mg, 0.459 mmol) and PPh_3 (168 mg, 0.641 mmol) were dissolved in THF (5 mL) and water (0.3 mL). The reaction mixture was heated to reflux for 12 h. Solvent was removed in vacuo. The residue was purified by column chromatography over silica gel using first ethyl acetate/hexane (4/1) and then methanol/triethylamine (50/1) as the eluents to give a white solid (135 mg, 0.321 mmol, 70% yield). Mp 225–230 °C dec. ^{1}H NMR (CD_3OD, 400 MHz, δ) 3.94 (br s, 1H), 3.80 (m, 1H), 3.64 (s, 3H), 3.09 (s, 1H), 2.57 (m, 1H), 2.42–2.11 (m, 3H), 1.96–0.91 (m, 26H), 0.71 (s, 3H).

4.2.4. 3β-Amino-7α,12α-dihydroxycholan-24-oic acid (2). This compound was prepared according to adopted literature procedures.[26] LiOH (2 M, 5 mL) was added to the solution of compound **5** (135 mg, 0.321 mmol) in methanol (10 mL). The mixture was stirred at room temperature for 21 h. HCl (2 N) was added until pH=7–8. Solvent was removed in vacuo. Residue was purified by column chromatography using MeOH/triethylamine (50/1) as eluent to give white solid (121 mg, 0.298 mmol, 93% yield). Mp 240–245 °C dec. ^{1}H NMR (CD_3OD/D_2O=1/1, 400 MHz,

δ) 3.59 (s, 1H), 3.26 (s, 1H), 2.00–0.75 (m, 30H), 0.52 (s, 3H).

4.3. X-ray crystallography

A colorless small solvent dependent crystal ($0.25\times 0.18\times 0.13$ mm^3) was covered with epoxy glue and immediately mounted and centered in the stream of cold nitrogen. The crystal evaluation and data collection were performed on a Bruker CCD-1000 diffractometer at 193 K, Mo Kα (λ=0.71073 Å) radiation, detector to crystal distance of 5.03 cm. The data were collected using the full sphere routine (0.3° scans in ω, 30 s per frame). This dataset was corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements[27] using SADABS software.[28] The structure was solved using direct methods and was refined in full-matrix anisotropic approximation for all nonhydrogen atoms. All hydrogen atoms were placed in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients.

The crystals of **2** are orthorhombic, $C_{24}H_{41}NO_4\times 4(CH_4O)$, space group $P2_12_12_1$; at 193(2) K, a=7.606(2), b=13.516(4), c=29.156(8) Å, V=2997.2(14) Å^3, Z=4, M=535.75, D_{calcd}=1.187 Mg m^{-3}, μ=0.085 mm^{-1}, $F(000)$=1184, $R1$=0.0814, $wR2$=0.2189 (data/parameters=2819/348), GOF=1.085.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 600390 copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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Diarylethenes with intramolecular donor–acceptor structures for photo-induced electrochemical change

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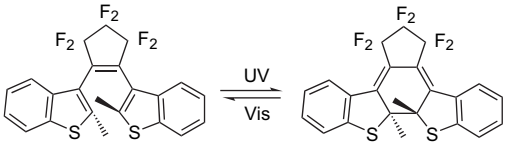
Abstract—Diarylethenes with donor–acceptor groups were synthesized to induce electrochemical switching by light. Photoisomerization was induced by 1,2-bis(2-methyl-1-benzo[*b*]thiophen-3-yl)perfluorocyclopentene (BTF, **1**) unit, while the 3,4-ethylenedioxythiophene (T) and nitro (N) groups were directly connected to BTF, to extend π-electron delocalization. Spectral change to a longer wavelength through photochromism was significant in the donor–acceptor structures (**6**), accompanied by an increase in the molar absorption coefficients, than those of the unsubstituted BTF (**1**) or the BTF substituted with only acceptor group (**2c** and **3c**). A significant peak shift toward lower redox energy was observed when the molecules were converted from an open isomer to a closed isomer. The plot of the reduction potentials ($(E^{red}_{1/2}$, V vs Ag/AgCl) vs LUMO energy eV) for the diarylethenes indicates that the reduction potential is strongly dependent on the nature of the substituents around the diarylethene unit. When **6** was applied to a photocell of Au/PC/ITO glass, in which PC is the polystyrene containing **6**, it became possible to switch the conductivity of the cell through the film by UV–vis irradiation, as estimated by the I–V plot on a photocell. The conductivity of the cell exposed to UV light was three times larger than that of the cell exposed to visible light, and 10 times larger than that of the cell containing **3**, indicating the importance of the push–pull structure for π-electron connectivity through the donor–BTF–acceptor.

1. Introduction

Light-induced reversible transformations of physical properties of two isomers are attracting strong interest in molecular switching.[1] In particular, the photochromic conversion of diarylethene, stimulated by irradiation with light at an appropriate wavelength,[2–8] makes it possible to switch the properties of molecules not only in electronic absorption but also for various physical and chemical properties, such as geometrical structure, refractive index, dielectric constant, and oxidation–reduction potential.[8–12] The most remarkable difference between diarylethene isomers is that the π-system of two aryl rings are separated in the open-ring isomer, whereas they are connected throughout the molecule in the closed-ring isomer. Therefore, any π-electron perturbation on the two aryl rings can modify the electrical switching properties arising from the interaction of the aryl rings through the conjugated pathway.

The diarylethenes with heterocyclic aryl rings have high application potentials due to additional characteristics, namely, the fatigue-resistant property and thermal irreversibility.[13–17] The coloration–decoloration cycle of benzothiophene derivatives such as BTF (**1**, Eq. 1) could be repeated more than 10^4 times while the thermally irreversible photochromic performance is kept in a solution.[13,17] Because of these advantages, researches on the optoelectronic application of diarylethene based on **1** are attracting strong interest.[8–18]



1o **1c** (1)

Substitution of the 6,6′-position on the benzothiophene ring of **1** with an electron acceptor and donor group could change the π-electron density and thus, the electronic properties and electrical conductivity of diarylethene through the conjugated pathway. In particular, introduction of the electron-donating and electron-accepting groups to the diarylethene units could stimulate intramolecular charge transfer along the closed isomer. Although many symmetrical diarylethene compounds have been reported, reports on nonsymmetrical derivatives and donor–acceptor structures are rare.[19–23]

Keywords: Photochromism; Diarylethene; Ring cyclization; Ring opening; Donor–acceptor; Photo-electrical switching.
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This paper reports on the synthesis and photo-induced electrical property change of the 6,6′-substituted nonsymmetrical diarylethenes. The nitro (N) and 3,4-ethylene-dioxythiophene (T) groups were substituted at the 6- and 6′-positions of **1** as the electron acceptor and donor group, respectively.

2. Results and discussion

2.1. Synthesis

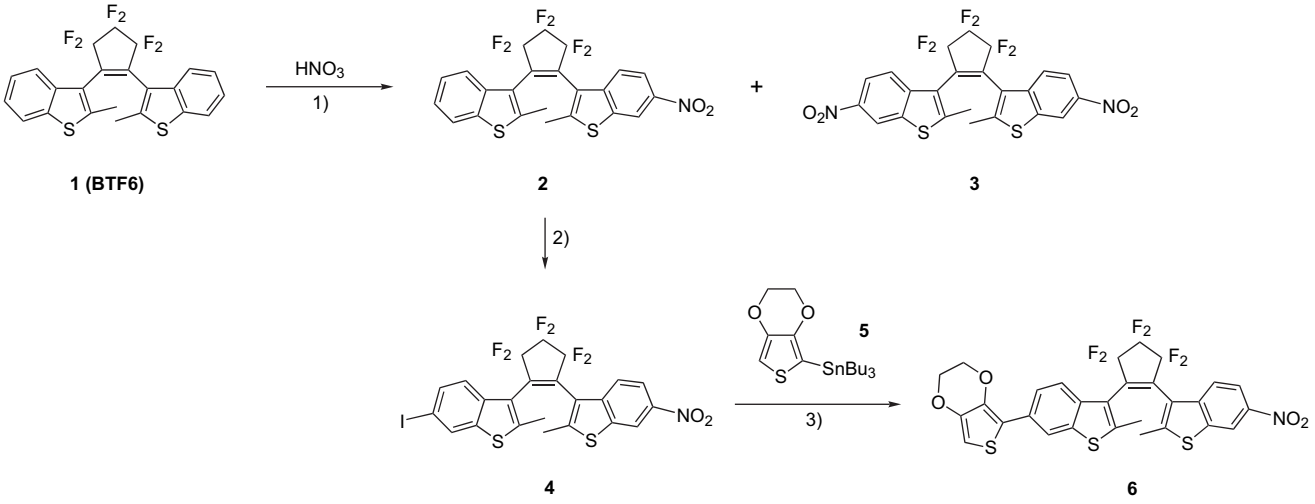
The known compound, 1,2-bis(2-methyl-1-benzo[*b*]thiophen-3-yl)perfluorocyclopentene (BTF, **1**),[24] was prepared from the reactions of perfluorocycloalkene with the organolithium compound.[8a,17] Nitration of **1** with fuming nitric acid yielded **2**. Dinitro compound **3** was synthesized using the same procedure used for **2**, but with excess nitric acid.[12,23] Then compound **2** was subjected to iodination with iodine and periodic acid in an acetic acid solution to yield **4**. Compound **6** was synthesized as a pale green powder through palladium-catalyzed Stille coupling of **4** with **5**[25] in the presence of a $Pd(PPh_3)_2Cl_2$ catalyst (Scheme 1).

The products were confirmed using the spectroscopic method and elementary analysis. The ^1H NMR spectrum of the unsymmetrical compounds **2**, **4**, and **6** showed four different methyl protons at δ 2.20–2.60, which arose from the two unsymmetric aryl groups with *anti*-parallel and parallel structures. The ratio of the *anti*-parallel structure to the parallel structure was about 65:35, as determined from the peak integration, in a solution (Supplementary data). The FTIR spectra of the nitrated compound (**2**, **3**, and **6**) showed characteristic asymmetric stretching vibration frequencies for the nitro group at 1500 and 1340 cm^{-1}.

2.2. Photochromic properties

Figure 1 shows the UV–vis spectral change upon the photochromic conversion of the diarylethenes in chloroform (1.0×10^{-5} M) through their exposure to a 365-nm light. A colorless solution that contained the open-ring isomer **2o** showed an absorption tail reaching 400 nm. Upon irradiation with UV light, new absorption bands appeared at longer wavelengths, which are ascribed to the closed-ring isomers. Most of the diarylethenes showed very large spectral shifts upon their photoisomerization from the open- to the closed-ring isomers (>6500 cm^{-1}).

In the closed-ring isomers, π-electrons were delocalized throughout the two benzothiophene rings and further



Scheme 1. Synthesis of **6**. (1) Acetic anhydride/acetic acid. (2) I_2, H_5IO_6/acetic acid, H_2SO_4, 70 °C. (3) $PdCl_2(PPh_3)_2$/toluene, reflux, 24 h.

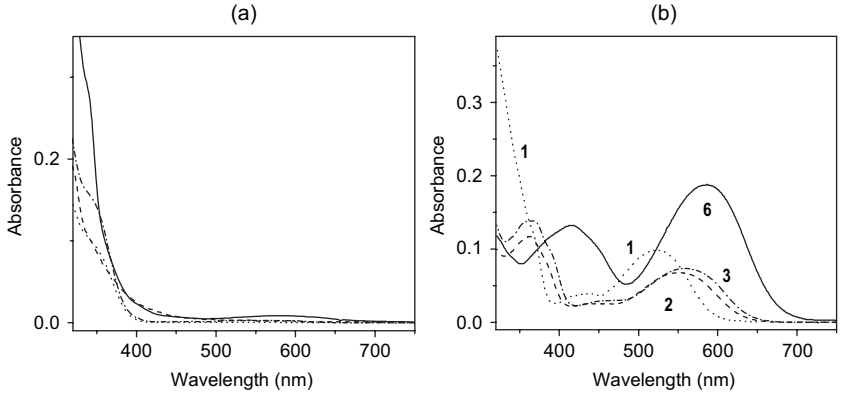


Figure 1. UV–vis spectra of compounds (1.0×10^{-5} M) in chloroform: (a) before and (b) after irradiation with 365 nm light for 2 min for compound **1** (dotted line), **2** (dashed line), **3** (dash–dotted line), and **6** (solid line).

extended to the donor and acceptor substituents. The absorption spectra of the closed-ring isomers thus depended on the substituents of the benzothiophene rings. The closed-form **2c** showed new bands at 362 nm (3.42 eV, ε=1.7×10^4 cm^{-1} M^{-1}) and 552 nm (2.24 eV, ε=7×10^3 cm^{-1} M^{-1}). Compared to the unsubstituted diarylethene (**1**), the absorption bands of **2** were red-shifted in both the open and closed isomers, indicating that the 6-nitro group affected the electronic transition of the diarylethenes. In particular, the red shift in the closed isomer (**2c**) was more significant than in the open isomer. Thus, compared to the unsubstituted diarylethene, **1c** (λ_{max}=534 nm, 2.32 eV, ε=1×10^4 cm^{-1} M^{-1}), the electronic transition of **2** was lowered to 0.08 eV in the closed form, in which the π-electrons were delocalized throughout the entire molecule. On the other hand, the ε value was decreased to 70% of **1c**. The di-nitrated **3c** showed a visible band at a similar position (559 nm) as that of **2c** with a similar ε value, indicating that the electronic transition of diarylethene was hardly modified by the second nitro group.

When the T group (3,4-ethylenedioxythiophene) was substituted at the 6′-position of the diarylethene, the spectral shift became more significant in the closed form. Thus, **6c** showed an absorption band at 587 nm (2.11 eV, ε=1.9×10^4 cm^{-1} M^{-1}), which corresponds to the lowering of the first electronic transition energy of 0.21 and 0.13 eV, compared to that of **2c** and **1c**, respectively. The π-electrons could be delocalized through the electron-donating T group to the electron-accepting nitro group in **6c** with a push–pull structure, which led to significant photo-induced polarization. These results correlate well with the previous observation of dithienyl derivatives.[26,27] UV–vis spectral data for diarylethenes in this study are summarized in Table 1.

Table 1. UV–vis spectral data for diarylethenes **1**, **2**, **3**, and **6** in chloroform

Compound	λ_{max} [nm] (ε×10^3 [cm^{-1} M^{-1}])	Energy (eV)
1o	300 (sh)[a]	4.14
1c	534 (10)	2.32
2o	300 (18)	4.14
2c	289 (18), 362 (17), 552 (7)	4.29, 3.43, 2.25
3o	304 (28)	4.08
3c	288 (23), 367 (14), 559 (7)	4.31, 3.38, 2.22
6o	322 (32)	3.85
6c	279 (27), 419 (13), 587 (19)	4.48, 2.96, 2.11

[a] Shoulder.

A diarylethene with five-membered heterocyclic rings have two conformations with the two rings in mirror symmetry (parallel conformation) and in C_2 symmetry (*anti*-parallel conformation).[7,13,28] The population ratio of the parallel to the *anti*-parallel conformations in **2** and **6** was 35:65, from the ^{1}H NMR study described above. The quantum yields of **2**, **3**, and **6** were determined as 0.34, 0.36, and 0.52, respectively, from the plot of the absorbance against the irradiation time.[29] The quantum yields of **6** were close to the maximum value, i.e., almost all the photo-excited *anti*-parallel conformations underwent the cyclization reaction. In other words, the actual quantum yields were close to 1.

On the other hand, the quantum yields of the cycloreversion of **6** dramatically decreased to 0.01 compared to those of **2** (0.07). The cycloreversion quantum yields of the dithienylethenes were reported to be dependent on the π-conjugation length of the aryl groups.[30] When the electron conjugative groups were attached to the aryl groups, the quantum yield of the cycloreversion reaction dramatically decreased due to the significant extension of the π-conjugation throughout the diarylethene molecule. Such a long extension of the π-conjugation in the donor–diarylethene–acceptor push–pull structure of **6** could enhance the photo-induced electrical switching between the open and closed isomers, as described below.

2.3. Photochromic electrochemical change

The open form of the nitro-substituted compounds (**2o** and **6o**) showed reduction peaks at −1.0 V in the cyclic voltammogram (CV), as shown in Figure 2. The nitro-thiophene compounds[31–33] and compound **3**[12] showed reduction potentials at about −1.5 to −1.0 V. Thus, the redox potential at −1 V (vs Ag/AgCl) could be assigned as the redox potential of the nitrated diarylethenes, **2o** and **6o**. The reduction potential of **6o** is almost the same as that of **2o**, indicating that the electron-donating T group does not affect the reduction process of **6o** because it is separated in the open form. In the anodic region, **2o** showed only an irreversible peak at >1.5 V, which can be ascribed to the oxidation of the diarylethene unit.[34] In general, there were no remarkable oxidation or reduction waves in the region from −2.2 to +1.6 V for *o*-BTF6 (unmodified) in acetonitrile (0.1 M TBAP) on a platinum electrode (vs Ag/Ag+) using cyclic voltammetry.[35] Similarly, no clear oxidation waves for **2o** and **3o** below 1.5 V were observed.

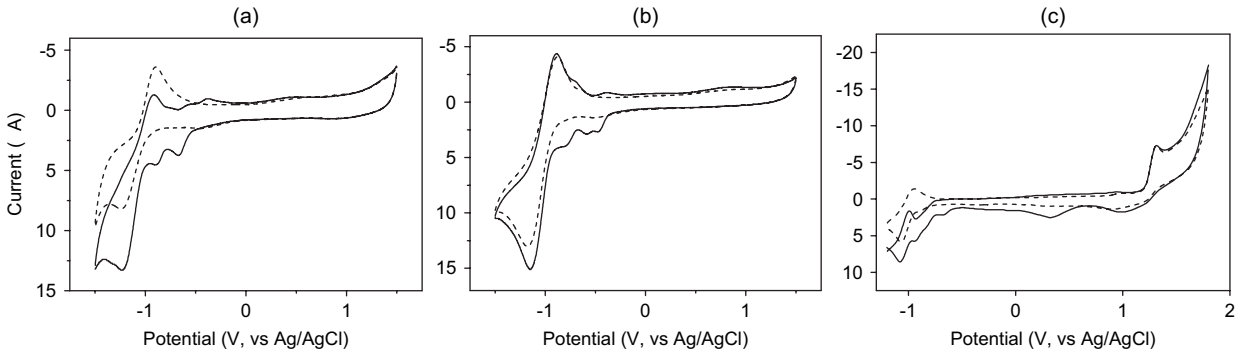


Figure 2. Cyclic voltammogram of diarylethenes (1×10^{-3} M) in dichloromethane containing 0.1 M of *n*-Bu_4NClO_4 before (dashed line) and after (solid line) irradiation with 365 nm light for 2 min for (a) **2**, (b) **3**,[12] and (c) **6**.

On the other hand, the CV of **6o** showed a reversible peak at 1.1 V and an irreversible peak at >1.5 V. The oxidation potentials of thiophene-containing molecules are reported as 0.8–1.6 V.[31–33,36] Thus, the CV waves of **6o** at 1.1 and >1.5 V can be considered as one-electron and two-electron oxidation of diarylethene, respectively. Substituted with the electron-donating T unit, **6o** can be oxidized at a lower potential than the unsubstituted analogues.

Interestingly, the CV of the solution that contained **2** after UV irradiation showed a significant current increase plus new redox peaks at −0.82, −0.61, and −0.4 V. It has been reported that the open-ring isomer of BTF does not show redox peaks, although the closed-ring isomer shows quasi-reversible redox potentials at −1.33 and +1.05 V.[35] Thus, the new peaks in CV could be ascribed to the reduction process of diarylethene containing the nitro group. The reduction peaks of **3c** shifted toward a positive potential,[12] thus lowering the energy for the reduction processes, possibly due to the additional nitro groups in **3c**, which facilitated the reduction process by the electron-withdrawing N group.

In the CV of **6c**, the reduction peak was observed at −0.84 V and shifted to a more negative potential than did the peaks of **2c** and **3c**, due to the presence of the electron-donating T group. The reduction current for the closed isomers (**2c**, **3c**, and **6c**, formed upon UV exposure) is higher than that of the open isomer because the π-electron conjugation is extended in the closed isomer to facilitate the electron transport in the closed form.[12,35]

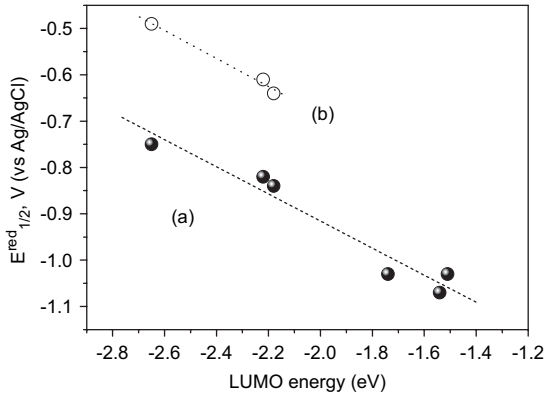


Figure 3. Plot of reduction potentials ($E^{red}_{1/2}$, V vs Ag/AgCl) versus LUMO energy (calcd, eV) for the diarylethenes, calculated using AM1 semi-empirical methods. (a) The correlations of the first reduction potential at the −1 V region for both closed and open isomers. (b) The second reduction potential from −0.7 to +0.4 V region of the closed isomers in Figure 2 against the LUMO energy of the corresponding molecules.

The addition of electron-donating groups has been reported to lower the oxidation potential, whereas the addition of electron-withdrawing groups has been reported to increase the oxidation potential.[37] Analogously, the addition of electron-donating groups will increase the reduction potential of diarylethene. The plot of the reduction potentials ($E^{red}_{1/2}$, V vs Ag/AgCl) versus LUMO energy of the diarylethenes, calculated using AM1 semi-empirical methods, indicates that the reduction potential is indeed strongly dependent on the nature of the substituents around the diarylethene molecular backbone (Fig. 3). The slopes of the plot for the first (Fig. 3a) and second reduction potentials (Fig. 3b) against the LUMO energy of diarylethene were almost identical (0.29 and 0.30, respectively) and indicate the sensitivity of these relationships, which decreases proportionally with decrease in the LUMO energy.

Compared to the reduction, the oxidation potential change through the ring closure was rather small in **2** and **3**. The CV of **6c** showed a new peak at 0.89 V (quasi-reversible) with an irreversible peak at 0.36 V, which arose from the one-electron oxidation of the BTF unit and the reduction of the diarylethene dication generated from the two-electron oxidation of the closed form according to the ECE mechanism, respectively.[34a] Furthermore, there was a significant current increase in the potential range above 1.5 V from the ring closure reaction upon UV exposure. This indicates that the electron transport becomes facile in the closed form as observed for the reduction current increase described above.

The potential shifts and the decrease in the potential difference between the oxidation and reduction peaks were more significant in **6** compared to those in **2** and **3**, indicating the importance of the push–pull structure for charge transfer. From the calculation using AM1 semi-empirical methods, the HOMO–LUMO energy difference was smallest in **6c** (Table 2), implying that the charge transfer must be faster in **6c** compared to that in others.

2.4. Photo-induced electrochemical switching of a photocell containing diarylethene

The diarylethene compounds in this study were homogeneously dispersed in polystyrene without phase separation to produce transparent polymer composite films. A photocell

Table 2. Electrochemical data for open- and closed-form diarylethenes obtained before and after UV irradiation, respectively

Sample	E_{red} (ΔE)[a] (V)	E_{ox} (V)	HOMO (eV)[b]	LUMO (eV)[b]	Δ (LUMO−HOMO) (eV)
2o	−1.07 (0.33)	>1.5[c]	−8.92	−1.54	7.38
2c	−0.82 (0.15), −0.61 (0.07), −0.4 (0.08)	>1.5	−8.72	−2.22	6.5
3o	−1.03 (0.28)	>1.5[c]	−9.508	−1.74	7.77
3c	−0.75 (0.11), −0.49 (0.18), −0.37 (0.21)	>1.5	−9.12	−2.65	6.47
6o	−1.03 (0.12)	0.92 (0.12), 1.1,[d] >1.5[c]	−8.64	−1.51	7.13
6c	−0.84 (0.17), −0.64 (0.07), −0.36[c]	0.89 (0.1), 1.1,[d] >1.5[c]	−8.58	−2.18	6.4

[a] Anodic–cathodic peak separation.
[b] Calculated using the AM1 semi-empirical method. Open and closed isomers are represented as **o** and **c**, formed before (dark) and after UV exposure, respectively.
[c] Irreversible process.
[d] Quasi-reversible process.

was fabricated with three layers of Au/PC/ITO glass, in which 'PC' represents the photochromic layer of **6** dispersed in a polystyrene binder. The PC layer was prepared from the solution of **6** (10 or 30 wt %) and polystyrene in a mixture of chloroform and trichloroethene (3:1, w/w). The cathode layer (Au) was in turn deposited on the photochromic film using the thermal evaporation method under the pressure of 3×10^{-6} Torr. The thickness of the Au layer and the photochromic layer were 40 and 220 nm, respectively, with an active area of 0.02 cm^2 (Fig. 4), and the prepared cell showed a color change from the UV and visible light sources (Fig. 5). Compared to the solution spectra, the band centered at 400 nm slightly changed due to the overlapping of the absorption tail with the binder.

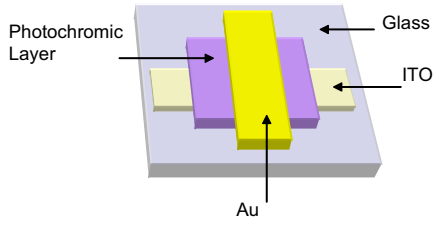
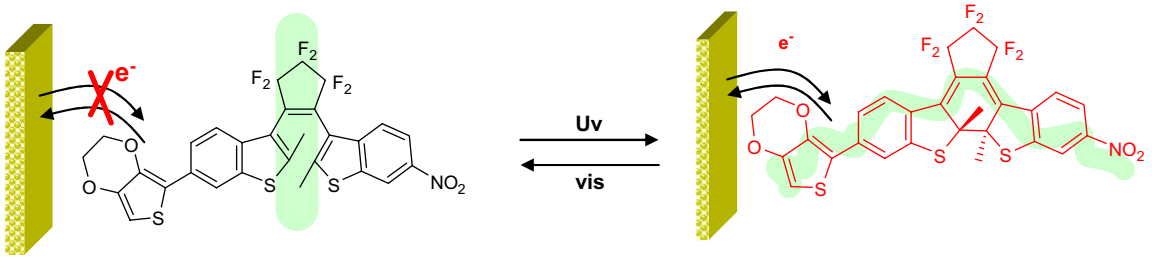


Figure 4. Structure of the thin-film photocell consisting of the Au/photochromic layer (diarylethene in polystyrene)/ITO.

The applied voltage dependence of the electric current is shown in Figure 6. It is important to note that the slope of the current–voltage (I–V) curve for the cell containing **6** significantly increased when the cell was irradiated with UV light. The current at 2 V for the colored cell, which contained closed form of **6**, was three times larger than that of the bleached cell. This demonstrates that the π-electron conjugation between the donor T and the acceptor N group in **6c** was extended, resulting in a higher current response than in the open form, in which such π-conjugation was limited. In particular, the donor–acceptor substituents could allow the electron push–pull mechanism in the closed form, in which the π-electron conjugation from the donor group through the diarylethene and then to the acceptor unit can be extended in the closed form, as schematically shown below.



(2)

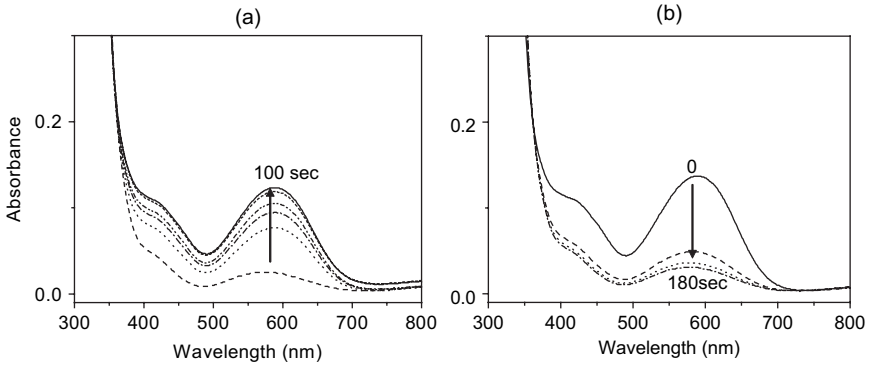


Figure 5. Spectral change in the photocell containing **6** (structured as in Fig. 4) through UV–vis irradiation. (a) UV exposure for 3, 20, 40, 60, 80, and 100 s. (b) Visible light (532 nm laser) exposure for 0, 60, 120, and 180 s.

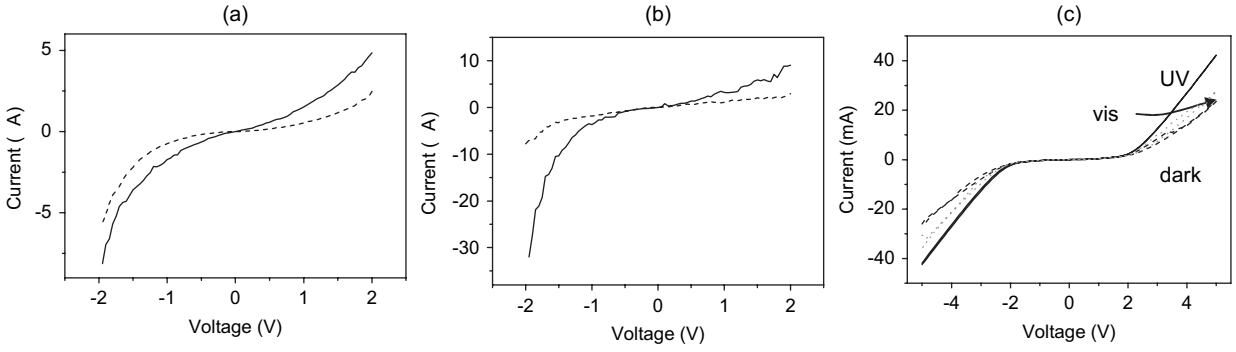


Figure 6. I–V plot for the cell containing (a) PS (90)/**6** (10) and (b) PS (70)/**6** (30) in the potentials between −2 and +2 V, before (dashed) and after (solid line) and (c) PS (70)/**6** (30) in the potentials between −5 and +5 V irradiation with 365 nm light, in dark (dashed) and after UV (solid), and then after visible light excitation (dotted line).

The conductivity was determined from the linear region of the I–V plot as 0.54×10^{-9} and 1.60×10^{-9} S cm^{-1} for the cell containing 10 wt % of **6**, before and after UV irradiation, respectively. The I–V curve was reversibly returned to the dark state upon irradiation with a visible light, allowing conductivity modulation through alternative irradiation with UV and visible lights. The switching efficiency, as defined by the ratio of the conductivities of the cells irradiated with UV and visible lights, was 2.9.

The conductivities of the cells increased to 1.51×10^{-9} and 3.52×10^{-9} S cm^{-1} before and after UV irradiation, respectively, when the content of **6** increased from 10 to 30 wt % in the PC layer. This shows that the conductivity of the photocell originated from the diarylethene molecules, which can act as active charge carriers. Similar I–V experiments were carried out on a photocell containing **3** (10 wt % in PS). The conductivity of the colored cell of **3** was lower than 10^{-10} S cm^{-1}. Moreover, the conductivity of the colored cell of 1 was 10^{-11} S cm^{-1}.[38] The conductivity of the PS binder without diarylethene was lower than 10^{-12} S cm^{-1}.[37] This implies that the charge transport must be facilitated in a donor–acceptor structure such as **6** that can push–pull the carrier during the transport between the molecules. In a doped system, in which a nonconductive binder (PS) is surrounded by diarylethene compounds, the content of the diarylethene and the donor–acceptor structure are important in carrier transport. Typically, carrier transport occurs through hopping of the charge carriers between active sites, and thus, is facilitated when the distance between the donor and the acceptor becomes shorter with the increased contents of the active carrier.

Although the I–V curve was reversible in the potentials' cycle between −2 and +2 V, it became less stable when the potential range was wider (from −5 to +5). There is a large current increase (40 mA at 5 V) by the UV excitation in the high potential range, however, the current drop by the visible light excitation was not completely overlapped to that of the dark and the I–V curve (visible in Fig. 6c) showed some hysteresis, possibly due to electrochemical decomposition at high voltage. Thus, working voltage for a stable switching effect of the photocell should be low (−2 to +2 V).

Such a reversible light-induced control of the electroactivity by means of the photoisomerization in a polymeric film allows the application of the functionalized electrode as an active interface to photostimulate selective electrochemical transformations: photon-mode actuation, electrochemical, and photochemical storage of information,[40] and in photoelectrode patterning for biological and nonbiological systems.[38,41] Furthermore, conductivity switching from an organic photochromic film could be utilized for injection control at the metal–organic interface of the photon mode.

3. Conclusions

A new diarylethene, with T and N as the electron donor and acceptor group, respectively, was synthesized to achieve photochromic and electrochemical switching with UV and visible light irradiation. The donor–acceptor structure **6** showed a significant shift in electronic transition, which resulted in spectral and redox potential changes. The closed form of **6**, generated through UV exposure, showed a higher current-to-voltage response, which resulted in a higher conductivity than that of the open form. Thus, conductivity switching was possible with UV and visible light switching. Such conductivity switching from an organic photochromic film opens up a new application potential in injection control at the metal–organic interface of the photon mode.

4. Experimental

4.1. General methodology

Methylene chloride and toluene were distilled from phosphorus pentoxide prior to use, and tetra-*n*-butylammonium perchlorate (TBAP) was purchased from TCI. The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in a $CDCl_3$ solution that contained Me_4Si as an internal standard with Bruker. IR spectra were recorded on a Shimadzu 540 spectrophotometer as KBr or film on KBr plates. An elemental analysis was performed by the Korea Basic Science Center. The FAB mass spectra were determined by the National Center for Inter-University Research Facilities. Column chromatography was performed using silica gel (200–400 mesh, Merck). All reactions were monitored for completion using thin-layer chromatography (TLC), which was performed using a precoated silica gel plate (Merck 60 F_{245}), and detection was performed with the aid of UV light. Melting points were measured on the Fisher–Jones melting point apparatus and uncorrected. The UV–vis spectra were recorded for chloroform solutions with a Jasco V-530 UV–vis spectrophotometer. Molar absorption coefficients were experimentally determined from the absorbance change at different concentrations using Lambert–Beer's law. The electrochemical properties were studied using cyclic voltammetry (CV) on a BAS 100B electrochemical analyzer (BAS, Inc.). The measurement was carried out in 10 mL methylene chloride solution that contained tetra-*n*-butylammonium perchlorate (TBAP, 0.1 M, 0.341 g) as a supporting electrolyte. Each of the photochromic compounds was dissolved under an argon atmosphere. The condition was composed of a three-electrode assembly equipped with a platinum working electrode, a platinum coil as the counter electrode, and an Ag/AgCl electrode as the reference electrode. Measurements were examined in a glass cell at room temperature. The cell was measured before and after it was irradiated with UV light and after it was blenched with visible light. The scan rate was 200 mV sec^{-1} and the voltage range was −1.5 to +1.5 V. The current–voltage (I–V) properties were measured using Agilent E5272A 2-Channel (High-power and Medium-power) Source/Monitor Units of Agilent Technologies. The measurement range was −2.0 to +2.0 V. For the study of I–V character photochromic compound (10, 30 wt %) and polystyrene (90, 70 wt %, Polysciences, Inc., 50,000 Mw) in chloroform, and the 1,1,2,2-tetrachloroethane solution was spin-coated on the ITO glass. Then the coated glass film was dried in an oven at 70 °C for more than 12 h, and gold was evaporated at a higher temperature. The thickness of the photochromic material was 220 nm and of gold was 40 nm. For coloration, the film

was exposed to UV light (365 nm, 0.5 mW) for 10 min, after which its properties (I–V curve, UV) were examined. After the film was bleached with light (532 nm, 2.0 mW), its properties were again investigated. The HOMO and LUMO energy of each compound were calculated using the AM1 semi-empirical method, as implemented in HyperChem ver. 7.0.[39]

4.1.1. 1-[6-Nitro-2-methyl-1-benzothiophen-3-yl]-2-(2′-methyl-1′-benzothiophen-3′-yl)hexafluorocyclopentene (2). To a solution of 30 mL acetic acid, 3 mL acetic anhydride was added with **1** (1 g, 2.13 mmol) at 10 °C. Fuming nitrate (1 mL) was slowly added to the solution, while the temperature was kept below 10 °C. The mixture was stirred overnight at room temperature, after which cold water was added to it. The solution was neutralized and extracted with ethyl acetate (3×30 mL). The organic layer was washed with 30 mL water, dried over $MgSO_4$, filtered, and evaporated. The residue was purified using column chromatography on a silica gel using hexane–ethyl acetate (5:1) as the eluent to produce **2** [yield: 75%; parallel (p):*anti*-parallel (ap)=40:60; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.21 (s, apMe, 2H), 2.31 (s, apMe, 2H), 2.49 (s, pMe, 1H), 2.58 (s, pMe, 1H), 7.20–7.76 (m, ArH, 5H), 8.10 (d, J=7 Hz, pArH, 0.4H), 8.26 (d, J=7 Hz, apArH, 0.6H), 8.54 (s, pArH, 0.4H), 8.64 (s, apArH, 0.6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.2, 15.8, 118.4, 118.6, 119.8, 120.0, 122.0, 122.3, 124.7, 124.9, 138.0, 142.6, 144.7]. HRMS (FAB) *m/z* (MH+) calcd for $C_{23}H_{14}F_6NO_2S_2$: 514.0370; obsd: 514.0369.

4.1.2. 1,2-Bis(2-methyl-6-nitro-1-benzothiophen-3-yl)-perfluorocyclopentene (3).[12,23] To a solution of acetic acid (30 mL), acetic anhydride (3 mL) was added with **1** (1 g, 2.13 mmol) at 10 °C. Fuming nitrate (3 mL) was slowly added to the solution, while the temperature was kept below 10 °C. The mixture was stirred overnight at room temperature, after which cold water was added to it. The solution was neutralized and extracted with ethyl acetate (3×30 mL). The organic layer was washed with water (30 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified using column chromatography on a silica gel with hexane–ethyl acetate (5:1) as the eluent to produce **3** [yield=75%; parallel (p):*anti*-parallel (ap) ratio=40:60; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.53 (s, apMe, 3.6H), 2.59 (s, pMe, 2.4H), 7.62 (d, J=9 Hz, pArH, 0.8H), 7.74 (d, J=9 Hz, pArH, 1.2H), 8.08 (d, J=9 Hz, apArH, 1.2H), 8.11 (d, J=9 Hz, apArH, 0.8H), 8.58 (d, J=2 Hz, pArH, 0.8H), 8.67 (d, J=2 Hz, apArH, 1.2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.8, 15.2, 113.0, 113.3, 114.8, 117.1, 133.0, 137.0, 140.0, 144.3, 144.7].

4.1.3. 1-(6-Nitro-2-methyl-1-benzothiophen-3-yl)-2-(6′-iodo-2′-methyl-1′-benzothiophen-3′-yl)hexafluorocyclopentene (4). Iodine (37 mg, 0.15 mmol) and H_5IO_6 (16 g, 0.07 mmol) were added to a stirred solution of **2** (100 mg, 0.19 mmol) in acetic acid (20 mL), sulfuric acid (1 mL), and water (1.5 mL). The mixture was stirred for 3 h at 70 °C in open air. The reaction mixture was poured into 500 mL of ice water. The organic layer was extracted with methylene chloride (3×30 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified using column chromatography on a silica gel with hexane–ethyl acetate (5:1) as the eluent to produce **4** [yield=85%; parallel (p):*anti*-parallel (ap) ratio=40:60; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.20 (s, apMe, 2H), 2.30 (s, apMe, 2H), 2.47 (s, pMe, 1H), 2.56 (s, pMe, 1H), 7.20–8.26 (m, ArH, 5H), 8.57 (s, pArH, 0.4H), 8.64 (s, apArH, 0.6H)].

4.1.4. 2-Tri-butylstannyl-3,4-ethylenedioxythiophene (5). To a solution of 3,4-ethylenedioxythiophene (4.50 g, 320 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) (4.25 g, 370 mmol) in anhydrous diethyl ether (100 mL), a solution of *n*-butyllithium (12.7 mL, 320 mmol, 2.5 M in hexane) was slowly added using a syringe under an argon atmosphere at room temperature. The mixture was stirred at room temperature for 10 min, and then refluxed for 30 min. A pink, milk-like mixture was formed. The mixture was cooled to −25 °C, and tributyltin chloride (10.33 g, 320 mmol) was added slowly to it over 1 h. The reaction mixture was warmed up to room temperature and stirred for 3 h. After it was quenched with saturated sodium chloride solution (50 mL), its organic layer was extracted with diethyl ether (3×30 mL), dried over $MgSO_4$, and filtered. Triethylamine (10 mL) was added to the filtrate, and the solvents were evaporated. The residue was purified using column chromatography with hexane on a pre-treated silica gel (silica gel was washed with neat triethylamine, and then with hexane). The solvent was removed in a vacuum and the residue was further purified through vacuum distillation to produce a colorless liquid [yield=68%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, J=7 Hz, Me, 9H), 1.10 (m, –CH_2, 6H), 1.34 (m, –CH_2–, 6H), 1.56 (m, –$SnCH_2$–, 6H), 4.15 (m, –OCH_2, 4H), 6.57 (s, –CH–, 1H)].

4.1.5. 1-[6-(3′,4′-Ethylenedioxy)thienyl]-2-methyl-1-benzothiophen-3-yl-2-[6′-nitro-2′-methyl-1′-benzothiophen-3′-yl]hexafluorocyclopentene (6). Under an argon atmosphere, 2-tributylstannyl-3,4-ethylenedioxythiophene (27 mg, 0.079 mmol) and **4** (50 mg, 0.079 mmol) were dissolved in toluene (20 mL). To this was added a catalytic amount of dichlorobis(triphenylphosphine)palladium(II) ($PdCl_2(PPh_3)_2$) (5 mg, 0.028 mmol) and the mixture was refluxed for 24 h. During the reaction, the color changed from yellow to black as Pd^0 was formed. After the reaction mixture was cooled, it was poured into a saturated sodium chloride solution (50 mL). Then benzene (20 mL) was added to it. The organic layer was extracted with benzene (3×30 mL), dried over $MgSO_4$, filtered, and evaporated. The residue was purified using column chromatography on a silica gel with hexane–ethyl acetate (5:1) as the eluent to produce **6** [yield=87%; parallel (p):*anti*-parallel (ap) ratio=35:65; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.20 (s, apMe, 2H), 2.32 (s, apMe, 2H), 2.48 (s, pMe, 1H), 2.57 (s, pMe, 1H), 4.26 (m, –OCH_2, 2H), 4.32 (m, –OCH_2, 2H), 6.30 (s, –CH–, 1H), 7.68–8.55 (m, ArH, 6H), 8.64 (s, apArH, 0.4H), 8.65 (s, pArH, 0.6H). HRMS (FAB) *m/z* (MH+) calcd for $C_{29}H_{18}F_6NO_4S_3$: 654.0302; obsd: 653.0301].

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Supplementary data

The conductivities determined from the I–V plot in Figure 6 (Table S1), and spectra for the characterization of compounds are available upon request. The supplementary data associated with this article can be found in the online version at, doi:10.1016/j.tet.2006.04.089.

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Leishmanicidal activity of withajardins and acnistins. An experimental and computational study

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Abstract—Several acnistins and withajardins were studied in their action against *Leishmania (V) panamensis* amastigotes; leishmanicidal activity and high toxicity were found. The 3D-QSAR analysis reveals certain correlation between bioactivity and some steric and electrostatic characteristics around the A and B rings of the steroidal skeleton; however, the models predict the same effects for both leishmanicidal and toxicity activities.

1. Introduction

Leishmaniasis is a protozoal disease with endemic distribution in 88 countries around the world, in particular in America and Asia;[1] about 1,500,000 new cases are reported each year. During the last 50 years the best treatment has been the application of compounds based in pentavalent antimonials; however, protozoal resistance is very frequent and additionally long treatment is required.[2] Due to the restricted number of available drugs, new compounds from synthetic or natural sources are essential to control this disease. Several classes of natural products have been assayed against *Leishmania* parasites, especially steroids[3] (agonists and antagonists of ecdysones), cardiac glycosides, and triterpenoid derivatives.[4] Recently, leishmanicidal activity was reported in steroidal alkaloids from *Holarrhena curtisii*[5] and *Dunalia brachycantha*.[6] In this paper, we report the leishmanicidal effect of another type of steroids, withajardins and acnistins. We analyze several structural and electronic properties of these compounds with molecular modeling programs to establish any relationship between the structure and the antiprotozoal activity. Because the activity of these substances is strongly structurally dependent, we have carried out a theoretical study to determine the structural features that may influence the activity and the interacting sites with a potential target molecule. These theoretical approaches represent a good alternative to improve the pharmacological profile of several types of substances or to develop new molecules based on the information obtained from classical QSAR analyses on the influence that different descriptors of the molecular structure have on the bioactivity.

2. Results and discussion

2.1. Leishmanicidal activity

The following differences can be established between the structures of acnistins and withajardins (Fig. 1):

- Acnistins have a bicyclic system (five and six members in each one) attached at C17, whereas withajardins have a similar but with six membered rings.
- The stereochemistry of C17-OH is β in acnistins and α in withajardins.
- Withajardins posses an additional C14α hydroxylation.

However, some compounds have similar substitution patterns in A and B rings so, several relationships between structure–activity can be derived:

(a) Acnistin E, **4**, and withajardin B, **9**, exhibit high leishmanicidal activity against *Leishmania (V) panamensis*

Keywords: Withanolides; Leishmanicidal activity; 3D-QSAR; Cytotoxicity; Lactone.
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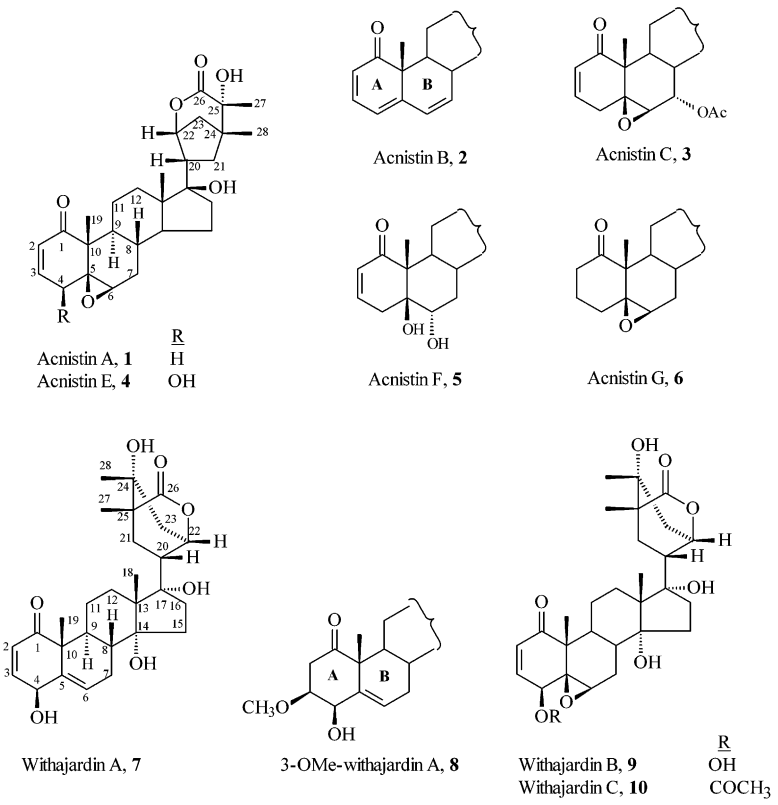

Figure 1. Structure of withajardins and acnistins.

amastigote (EC_{50}=1.0 and 1.1 μg/mL, respectively, Table 1); these compounds possesse a 2-en-1-one-system, a 5β,6β-epoxy, and one hydroxyl group in C4. Results are in disagreement to the activity previously reported in withanolides from *D. brachycantha* since 5β,6β-epoxy was less active than 4-deoxy compounds; however, strains used in that experiment besides *L. (L) amazonensis* were *L. (V) braziliensi* and *L. (L) donovani*.[4] Moreover, 4-deoxy-type compounds were also active (acnistins A, **1**, and C, **3**) but the acetylation of C4 hydroxy group causes an important reduction in the activity. Therefore, withajardin C, **10**, exhibits an EC_{50} of 12.8 μg/mL versus 1.1 μg/mL in withajardin B, **9**.

(b) Changes in 2-en-1-one system decrease or fully eliminate the activity, e.g., 3-methoxywithajardin A, **8** (EC_{50}>50 μg/mL). On the other hand, an epoxy compound without C2, C3-double bond (acnistin G, **6**) reduces its activity three times compared to the structural analog, acnistin A, **1**.

(c) Additionally, the 5β,6β-epoxide is essential for activity, because withajardin A, **7**, is a weak leishmanicidal compound. Besides, this group seems to be more important than unsaturated ketone system, since acnistin G, **6**, displays an EC_{50}=7.9 μg/mL. Opening of this group as in acnistin F, **5** (a 4-deoxy-5,6-dihydroxy molecule) causes a total loss of the activity (EC_{50}=76.0 μg/mL).

(d) The presence of a double bond instead of an epoxide ring in C5 and C6 (withajardin A, **7**, EC_{50}=30.8 μg/mL) decreases the activity as well as the formation of an Michael adduct in C3 (3-OMe-withajardin A, **8**). Moreover, a conjugated system without epoxide, such as acnistin B, **2**, is lacking activity.

Table 1. Leishmanicidal activity of withajardins and acnistins

Compound	LC_{50}, μg/mL (μM)	EC_{50}, μg/mL (μM)	SI (LC_{50}/EC_{50})
Acnistin A, **1**	0.27 (0.6)	3.0 (6.4)	0.1
Acnistin B, **2**	35.5 (78.4)	54.8 (121.2)	0.7
Acnistin C, **3**	1.7 (3.2)	2.3 (4.9)	0.7
Acnistin E, **4**	1.0 (2.1)	1.0 (2.1)	1.0
Acnistin F, **5**	158.5 (324.4)	76.0 (155.5)	2.1
Acnistin G, **6**	8.5 (18.0)	7.9 (16.7)	1.1
Withajardin A, **7**	18.4 (37.8)	30.8 (63.3)	0.6
3-OMe-withajardin A, **8**	248.0 (495.4)	>50.0	>5.0
Withajardin B, **9**	3.8 (7.6)	1.1 (2.2)	3.5
Withajardin C, **10**	9.5 (17.4)	12.8 (23.5)	0.8
Glucantime	400.0	6.7	59.7

EC_{50}: concentration of a compound, which caused a 50% reduction in survival or viability in comparison to identical cultures without this compound. SI=selectivity index, LC_{50}/EC_{50}; an acceptable value is SI>10.0.

Nevertheless, changes in the structure modify the cytotoxicity more than the activity. So, epoxide opening reduces it 600 times, while the activity decreases 25 times only (acnistin F, **5** vs acnistin A, **1**). Additionally, the α,β-unsaturated carbonyl group is less important than epoxide (acnistin G, **6** vs acnistin A, **1**) because the activity was reduced 2.6 times and cytotoxicity was reduced 31 times. In the withajardin series, the presence of a double bond instead of an epoxide reduces toxicity five times (withajardin A, **7** vs withajardin B, **9**), but some leishmanicidal activity is still retained.

Because of these results, it can be supposed that side chain substructure system is not necessary to explain this leishmanicidal activity.

To understand the electronic and topological facts involved in leishmanicidal activity, we have carried out a computational analysis as is described below.

2.2. Molecular modeling

From the activity data, a similar leishmanicidal activity in some acnistins and withajardins, specifically acnistins A, **1**, E, **4**, and C, **3** and withajardin B, **9**, can be observed. It can be assumed that the A and B rings in the steroidal substructure should be responsible for the bioactivity. To simplify the treatment in the following QSAR studies, bicyclic part of the molecule attached to C17 was not considered. The 3D-QSAR has been selected as the suitable tool to perform such analysis due to the relative structural rigidity of the steroidal system, which can partially avoid the alignment problem. Currently, this type of analysis is used to improve the pharmacological properties of bioactive compounds and to develop new molecules according to 3D descriptors in QSAR. The structural descriptors used in 3D-QSAR[7] correspond to three-dimensional fields in the molecular environment, related to a hypothetical receptor–ligand interaction. This type of methodology was selected because it gives an intuitive view on the structure–activity relationship. Also, the information that can be extracted from that relationship is localized in the three-dimensional space occupied by the molecule, facilitating its interpretation. Another positive aspect of 3D-QSAR methodology is the high flexibility in the treatment of the conformational variation in the set of molecules to be studied. The relative rigidity of the steroidal skeleton allows an easy alignment of the structures. Following the standard CoMFA of 3D-QSAR analysis, we initially considered the steric and electrostatic fields as responsible for the differences observed in the bioactivity. The different phases of the 3D-QSAR analysis performed in this work are described in the following sections.

2.3. Conformational analysis

As it was established above, the structures of the acnistins and withajardins were considered similar and both were lacking bicyclic side chain in a putative model. The steroidal skeleton constituted by four fused rings seems to be fairly rigid. The most remarkable difference among the set of compounds assayed can be localized in the A ring. This ring is pulled far from the pseudoplane formed by the steroidal structure due to the presence of a 5β,6β-epoxi group. The angle ϕ is defined between the planes formed on one hand by the atoms 1–4 in A ring and by atoms 5–17 in rings B, C, and D on the other (Fig. 2).

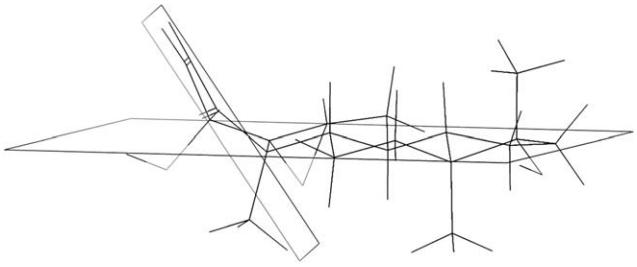

Figure 2. Angle ϕ between steroidal system and A ring plane.

Experimental data of leishmanicidal activity and toxicity versus ϕ angle are shown in Figure 3b. A quasi-linear increase in the bioactivity with the opening of the angle ϕ can be observed for both the sets of experimental data, specifically in the case of the leishmanicidal activity values. For acnistin A, **1**, a very active and toxic compound, which contains a 5,6β-epoxi, the measured angle is 47°, but it increases to 61° for acnistin E, **4**, the most active compound in the set. The angle between planes is more opened for this molecule by the presence of a hydroxyl group close to the epoxy group.

The molecular geometries optimized at the B3LYP/6-31G(d) level of theory are in agreement with similar crystallographic structures found in the Cambridge Crystallographic Database[8] giving a root mean squared deviation of less than 0.01 Å for the set of heavy atoms in the steroidal substructure. It should be mentioned however that the theoretical calculation finds a more stable conformer in the case of the acnistin E, **4**, compared to the experimental geometry. This is due to the extra stabilization coming from the intramolecular hydrogen bond between the hydroxyl group and the epoxy-oxygen atom. This interaction has not been observed in the experimental structures probably due to the crystal packing, which favors intermolecular interactions. The experimental conformation was chosen for the QSAR analysis according to the following approaches: (1) to keep the congruency with the crystallographic data, and (2) to assume that the intermolecular interaction would be favored in the case of binding with a putative receptor.

2.4. Alignment rule

Due to the rigidity of the steroidal skeleton, all molecules were aligned using atoms 5, 8, 9, 10, 13, and 14 as numbered in Figure 1. The result of such alignment for the set of molecules is shown in Figure 4, in addition to the cubic three-dimensional region where the molecular stereo electronic descriptors have been calculated and will be compared with experimental bioactivity.

2.5. PLS models[9]

Besides searching molecular models to explain the bioactivity of these compounds, the aim of the present study was the formulation of reliable models to explain separately the leishmanicidal and toxicity activities. This information would serve to propose new compounds or modifications on some of the tested set of molecules, to improve the bioactivity, and to reduce the toxicity. Thus, PLS models have been calculated separately for the antileishmanial activity and toxicity data. The standard CoMFA fields (steric and electrostatic) were selected firstly to describe the structural features of the acnistins and withajardins (Table 2).

The experimental values of activity and toxicity were transformed to a logarithmic scale to improve the data distribution as it is recommended in the usual QSAR practice:[10] the transformations used were Log ($1/LC_{50}$) and Log ($1/EC_{50}$). The experimental data display a high correlation between leishmanicidal and cytotoxicity values as can be seen in Figure 3a; the correlation coefficient was 0.76. Accordingly, it will be very difficult to separate activity and toxicity in the proposed models. The regression statistics

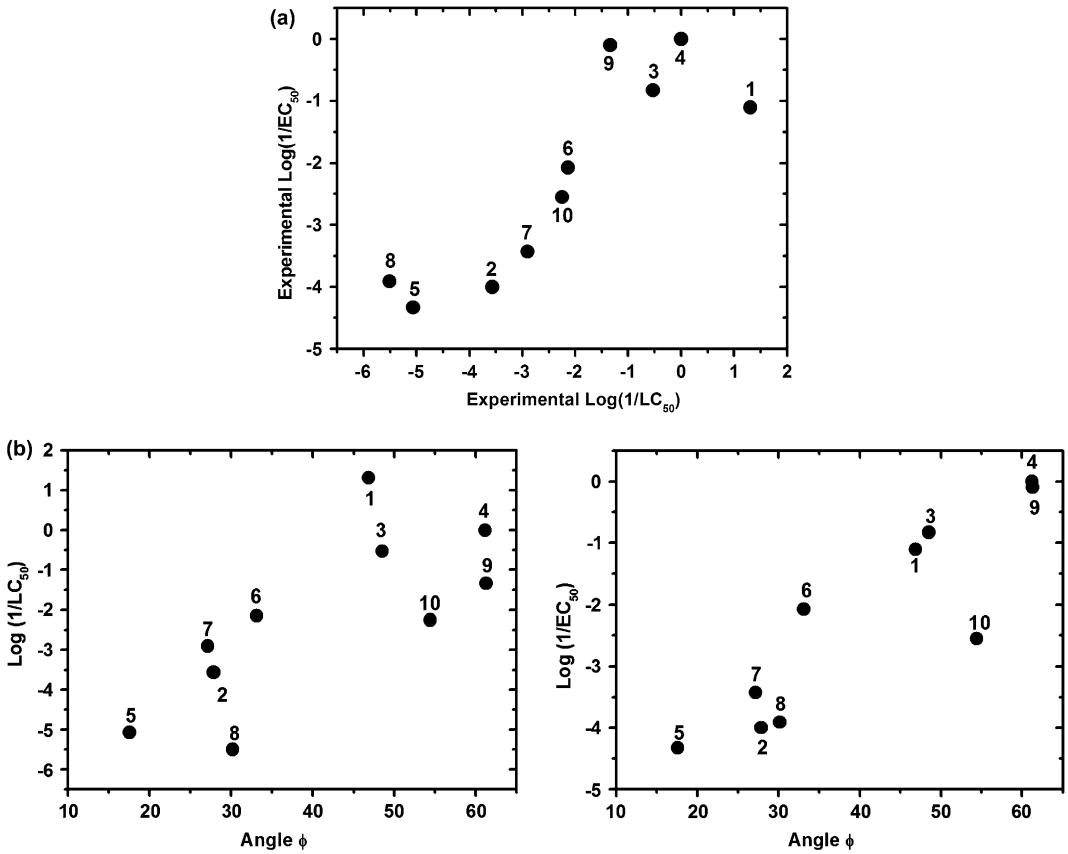

Figure 3. (a) Correlation between experimental values Log ($1/EC_{50}$) and Log ($1/LC_{50}$). (b) Correlation between Log ($1/LC_{50}$) and Log ($1/EC_{50}$) versus angle ϕ.

obtained through 'Leave One Out (LOO)' cross-validation for different combinations of molecular field, cutoff energies, and grid dimensions are shown in Table 2. The models that take into account only steric or electrostatic fields yield worse correlations and they were not considered further in this work. As it can be seen in Table 2 the regression models of the leishmanicidal activity present a good predictive ability with cross-validated correlation coefficients (q^2) greater than 0.5. The models for the toxicity have a worse predictive performance with q^2 values below 0.3. Finally, models with two components, optimal number after cross-validation, were obtained by taking into account all the compounds in the set. The corresponding fields were calculated with a grid spacing of 1.0 Å and cutoff values of 10.0 and 5.0 kcal/mol for both activities, respectively.

The statistics obtained for these models considering two components in each case were:

(a) For the antileishmanial activity: r^2=0.988, standard error of estimation=0.231 log units. The relative contribution of the steric field to the model is 36.4%.

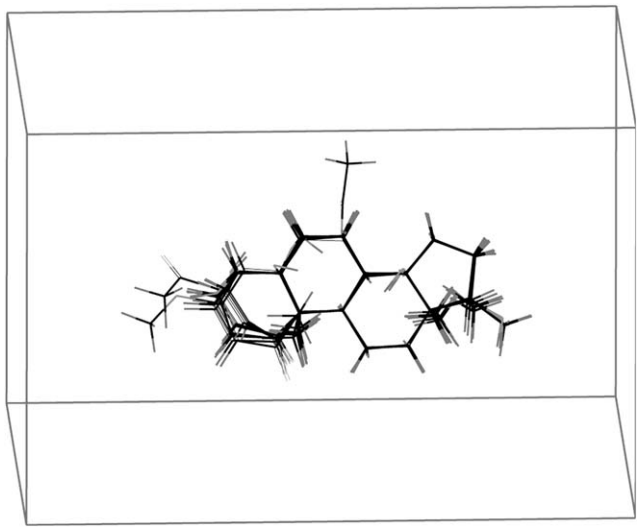

Figure 4. Alignment of the molecules and region used to calculate molecular fields.

Table 2. CoMFA parameters and 'cross-validated' statistics

Leishmanicidal activity						
Cutoff energy (kcal/mol)	30	30	30	90	10	5
Grid spacing (Å)	1	2	0.5	1	1	1
Q^2 coefficient[a]	0.555	0.441	0.545	0.544	0.562	0.552
Standard error of prediction (log units)	1.237	1.386	1.251	1.251	1.227	1.240
Cytotoxicity						
Cutoff energy (kcal/mol)	30	30	30	90	10	5
Grid spacing (Å)	1	2	0.5	1	1	1
Q^2 coefficient[a]	0.157	0.049	0.149	0.186	0.171	0.245
Standard error of prediction (log units)	2.264	2.404	2.274	2.224	2.245	2.145

[a] Two components model.

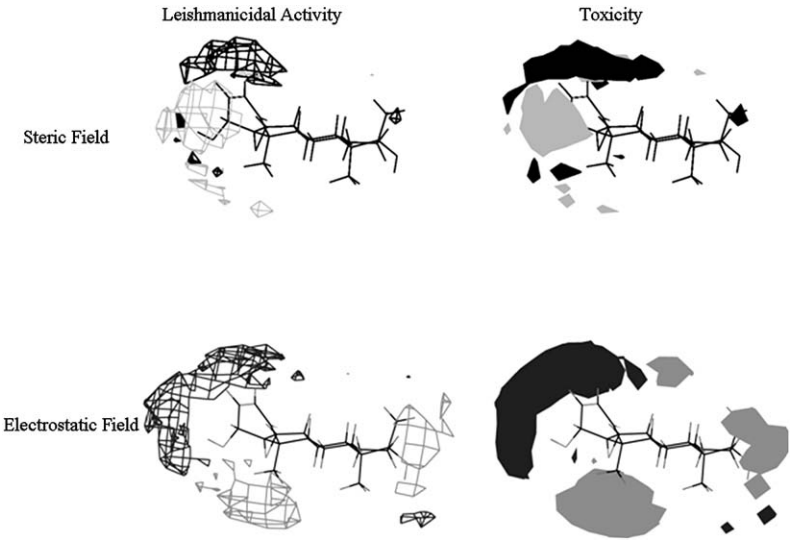

Figure 5. CoMFA fields. Equatorial view.

(b) For the toxicity: r^2=0.891, standard error of estimation=0.852 log units. The relative contribution of the steric field is 40.9% in this case. So, the CoMFA models marginally explain better the leishmanicidal activity than the toxicity.

The standard deviation (stdev)×QSAR coefficients (β) field gives a rough location where structure–activity relationship statements can be inferred discriminating areas where the local descriptor is important than those have no significance. The (stdev×β) field mapped on the simplified acnistin E substructure, **5**, is shown in Figures 5 and 6, in equatorial and axial perspectives, respectively. The isosurfaces correspond to the 80% level for the most positive contributions (dark gray) and to the 20% level for the most negative ones (light gray). The result obtained with the leishmanicidal activity data is shown on the left side of the figures with mesh representation of the isosurface. The models obtained for the cytotoxicity are graphed on the right side with a solid representation of the isosurfaces.

The contributions of the steric and electrostatic fields are plotted separately. In each case the most positive values are represented in black and the negative ones in light gray. The effect of the steric field is shown on the top of the figures. The areas of black mesh are indicative of regions where an increase of the steric field would be beneficial for the leishmanicidal activity. The opposite effect would be expected in the light gray areas. These regions are confined mainly around the A ring. The incorporation of bulk substituents in the area beyond the C2–C3 double bond would increase both the leishmanicidal activity and toxicity. On the other hand, steric bulk added in the region that would occupy the A ring (if this were coplanar with the rest of the steroidal skeleton) would not only be detrimental to leishmanicidal activity but also to toxicity. This observation agrees with the correlation found between the angle between planes ϕ and the experimental activity data (Fig. 3). The opening of the ϕ angle allows the access of the A ring to those steric regions of positive effect on both activities.

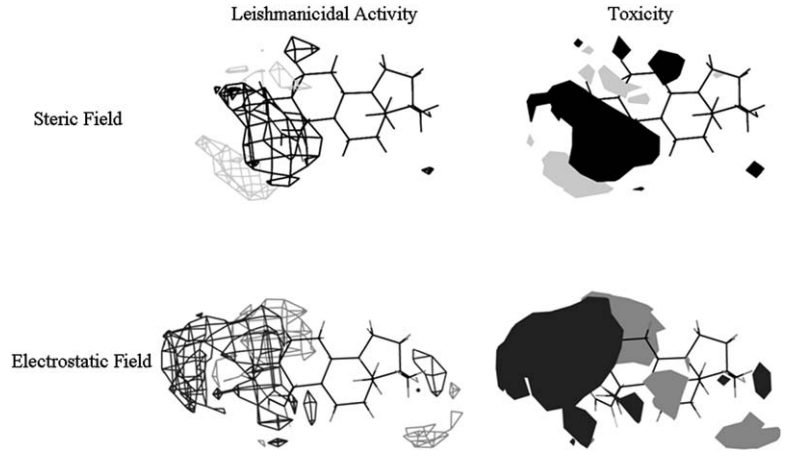

Figure 6. CoMFA fields. Axial view.

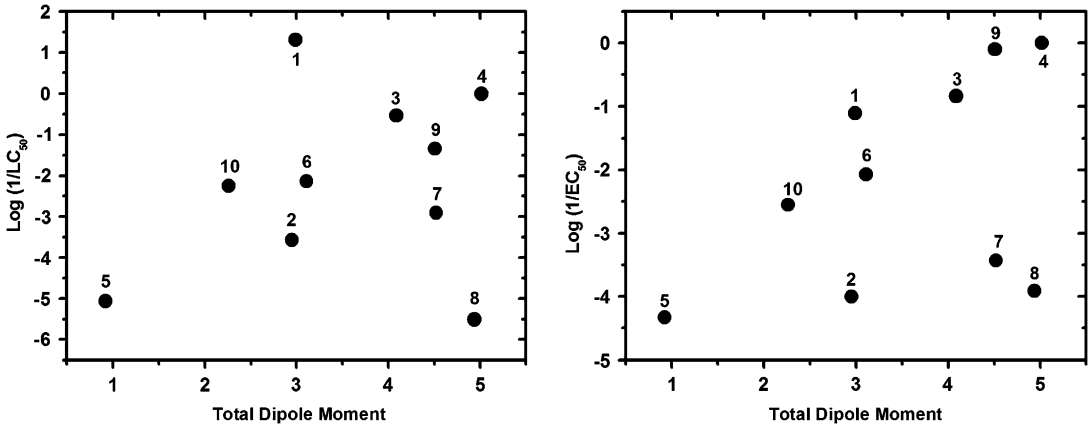

Figure 7. Correlations between Log ($1/LC_{50}$) and Log ($1/EC_{50}$) versus the total dipole moment.

The contribution of the electrostatic field to the models is shown in the bottom half of Figures 5 and 6. The significant isosurfaces for the standard deviation coefficients field are located mainly around the A and B rings. The dark area surrounding the A ring indicates that an increase in positive charge would enhance both the leishmanicidal activity and toxicity. On the other hand the light gray region around the epoxy-oxygen atom suggests that an increase in the negative charge in this position would improve the activity as well as the toxicity of this molecule. The influence of the electrostatic environment as an intramolecular dipole over the bioactivity can also be observed when we plot the experimental data against the molecular dipole (Fig. 7). From this figure it can be inferred that the larger values of dipole moment correlate to a higher activity, which is in agreement with the CoMFA result. However, the 3D-QSAR model can be used to identify the orientation of the dipole, which enhances the bioactivity.

Another source of lateral validation for the CoMFA model can be the calculation of the lipophilicity parameter *C* log *P*.[11] It can be seen in Figure 8 that there is a good correlation between experimental data and *C* log *P* values. However, the lipophilicity descriptor separates the acnistin compounds from the withajardin ones. For these two subsets it is clear that either leishmanicidal activity or toxicity increases as the *C* log *P* value decreases.

Withajardins present lower *C* log *P* values than acnistins because they contain extra polar groups in their structure, such as C14-OH. It can be observed that acnistin F, **5**, lies outwith the acnistin set and near the withajardin set because it contains two hydroxyl groups, which decrease its contribution to the *C* log *P* values. These observations suggest that bioactivity is enhanced by the hydrophilicity of the molecule. This in turn can be described by some types of polar interaction with a hypothetical receptor, in agreement with the CoMFA model.

Figure 9 shows the actual versus predicted activity plots obtained with the final non-validated models. As can be seen the correlation between experimental and theoretical values is better for the leishmanicidal activity than for toxicity, as was commented above.

Models taking into account the complete withanolide molecules including the bicyclic substructure have also been tried. Such models yielded poor statistics (cross-validated correlation coefficients, q^2, between 0.1 and 0.2) due probably to the extra noise introduced by the addition of the bicyclic side chain. In spite of the low statistical significance, these models showed steric and electrostatic contributions to explain bioactivity focused around the A and B steroidal rings, in a similar fashion to those described for the simplified structures.

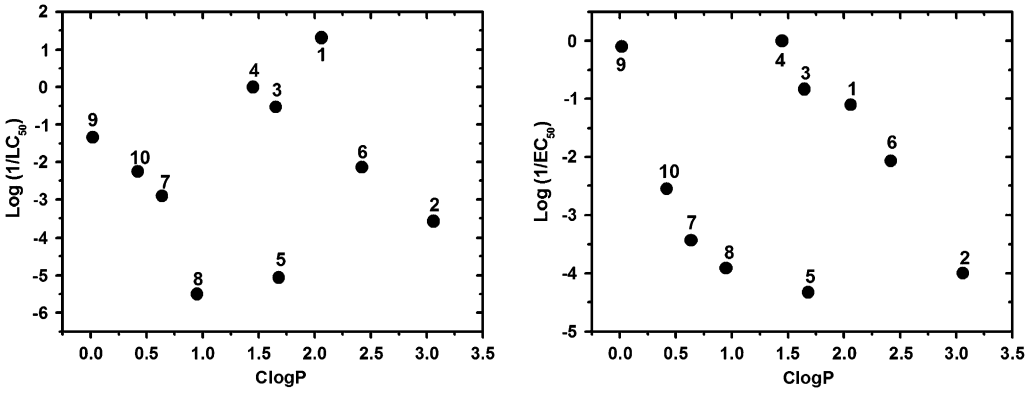

Figure 8. Correlations between Log ($1/LC_{50}$) and Log ($1/EC_{50}$) versus *C* log *P* values.

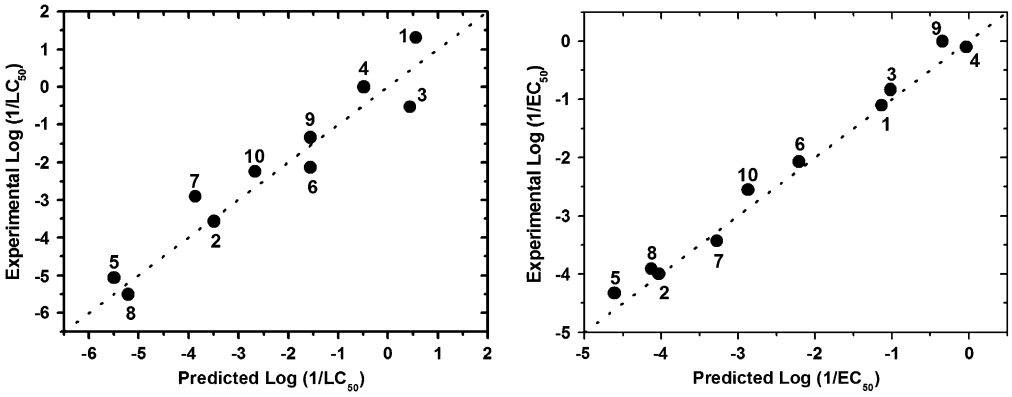

Figure 9. Experimental versus predicted Log ($1/LC_{50}$) and Log ($1/EC_{50}$) values.

3. Conclusions

Some withajardins and acnistins display high activity against leishmania amastigotes in dose dependent manner, but a high toxicity was also observed. A computational analysis was carried out toward design of structural analogs maintaining the activity and decreasing the toxicity, but retaining some functional characteristics of both A and B steroidal rings. A preliminary study has been done looking for a correlation between experimental bioactivity data and molecular structural descriptors for a series of withajardins and acnistins. The observed differences in bioactivity can be satisfactorily explained by the influence of steric and electrostatic fields in the surroundings of A ring. Mainly, a determined spatial disposition of A ring and of bulky substituents in 2, 3 positions increases the bioactivity. The model also shows that an increase of the positive charge near the 2, 3, 4 positions in A ring and of the negative charge near the epoxy group implies an increase of the bioactivity.

This spatial disposition of charge with different sign suggests a favorable interaction of a dipole of analog size to the skeletal bicycle with the hypothetical receptor. Also, changes in toxicity when the unsaturated carbonylic system and/or the epoxy group are modified show that it is possible to decrease the high reactivity of these groups, possibly with the addition of bulky or electron-withdrawing groups. Other three-dimensional fields could be considered in order to study their effect on the leishmanicidal activity and toxicity, among them, hydrogen bonds, frontier molecular orbitals, or Fukui functions. However, because of the high correlation observed between activity and toxicity it is very possible that the models do not discriminate between them. The high contribution of A and B rings to the activity seems to indicate that molecules smaller than natural withanolides could be synthesized and tested against *Leishmania* parasite.

On the other hand, the wide range of biological activities reported in withanolides should be derived from the reactivity of unsaturated 2-en-1-one system. Some bioactive molecules with this chemical system and a side lactone attached in C17 are reported as immunosuppressive, anti-inflammatory, antitumor, cytotoxic, antioxidant, inhibitors of cyclooxygenase-2, human IL-2, and cholinesterase, and antifeedant. In addition to compounds from *D. brachycantha*[6] some physalins with these chemical groups are reported as leishmanicidal too.[12]

4. Materials and methods

4.1. Biological assays

All compounds were obtained and identified as was reported elsewhere.[13–15] Cytotoxic activity of the synthesized compounds was evaluated according to previously described protocols.[16] For each of the concentrations of the different compounds, the net absorbance was registered. The percentage of dead cells was determined after calculating the viability percentage using the following formula: Viability %=(O.D. cells exposed to a compound/O.D. control cells without compound)×100. Mortality %=100−Viability %. The results were expressed as LC_{50}, which was calculated using the statistical package Probit.

The efficacy of the different compounds was evaluated against intracellular amastigotes following a modified methodology.[17] Cells cultivated in absence of the compounds but maintained under the same conditions were used as control; Glucantime® was used as control drug for leishmanicidal activity. Each compound concentration, and the controls without the drug were evaluated in triplicate. A diminishing of the parasite load evidenced the effect of the drug or compound. Due to the fact that the number of infected cells in absence of the compound is 100% of infection, the percentage of infected cells for each concentration was calculated using the following formula: % of infected cells=(# of cells infected in presence of the compound/# of control cells infected in absence of the compound)×100.

4.2. Computational methods

The molecular geometries were optimized at the B3LYP/6-31G(d) level using the Gaussian 03 package.[18] The 3D-QSAR analysis was carried out using the CoMFA[19] protocol implemented in the Sybyl[20] software. The Tripos force field was considered for the description of the steric field. Atomic charges derived from electrostatic potential were calculated following the CHELPG[21] methodology implemented in Gaussian 03. Different grid spacing and cutoff values for the interaction energies were considered as described in

Section 2. The probe atom used in the CoMFA calculations was the C sp^3 one with +1 charge.

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Radical dearomatization of benzene leading to phenanthridine and phenanthridinone derivatives related to (±)-pancratistatin

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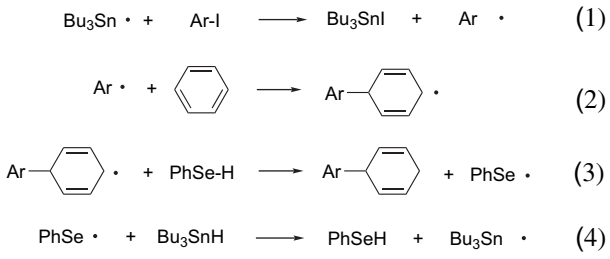
Abstract—The synthesis of the phenanthridinone nucleus common to the *Amaryllidaceae* series of natural products is achieved by a sequence involving tributylstannane-mediated, benzeneselenol-catalyzed addition of *ortho*-nitrogen functionalized aryl radicals to benzene, yielding aryl-substituted cyclohexadienes. These cyclohexadienes may be manipulated by oxidative ring closure sequences to generate functionalized phenanthridines. Beginning from 2-hydroxy-6-iodopiperonic acid a key intermediate in the Danishefsky synthesis of (±)-pancratistatin is achieved in two steps.

1. Introduction

Aryl radicals add rapidly to arenes to give cyclohexadienyl radicals substituted with aryl groups at the 6-position (Scheme 1).[1] Under typical preparative radical chain conditions the cyclohexadienyl radical is insufficiently reactive to propagate the radical chain by hydrogen atom abstraction from stannane or silane hydrogen atom donors and the eventual outcome is the formation of rearomatized biaryls.[2] Similarly the intramolecular version of this reaction, cyclization of an aryl radical onto an arene, is marked by the formation of fully re-oxidized products.[3] This breakdown in propagation typically results in poor conversion of the substrate and/or the need for excessive quantities of radical 'initiator'. Indeed, the azo-type initiators are now seen to serve the important function of oxidant for the cyclohexadienyl radical in addition to their more obvious planned function.[4] On the other hand, we have shown how the inclusion of a catalytic quantity of benzeneselenol in the stannane-mediated radical addition of aryl iodides to benzene, and other heterocycles, enables smooth trapping of the cyclohexadienyl radical by the selenol, leading to the isolation of aryl-substituted cyclohexadienes.[5] Although the Sn–H and Se–H bond dissociation energies are very similar,[6] the selenol traps alkyl radicals some 500 times faster than the stannane,[7] because of the operation of a polarity effect.[8]

The four-propagation step chain sequence is completed by regeneration of the selenol by reaction of the selenyl radical with the stannane (Scheme 1).[9]



Scheme 1. Mechanism of dearomatizing aryl radical addition to arenes.

The chemistry is rendered practical by the rapid in situ reduction of diphenyl diselenide to benzeneselenol by the stannane, which enables the direct handling of the air sensitive selenol to be avoided (Scheme 2).[9]

Scheme 2. In situ selenol generation.

The chemistry is particularly attractive when the aryl iodide is functionalized with a nucleophile at the *ortho*-position, thereby permitting the ring closing desymmetrization of the product cyclohexadiene.[10,11] We have employed this chemistry in syntheses of carbazomycin B (Scheme 3),[12] and of Kelly's β-sheet initiator (Scheme 4).[13]

Keywords: Radical; Arylation; Cyclohexadienyl; Dearomatization; Phenanthridinone.
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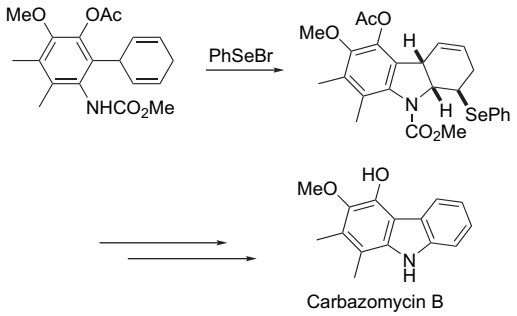
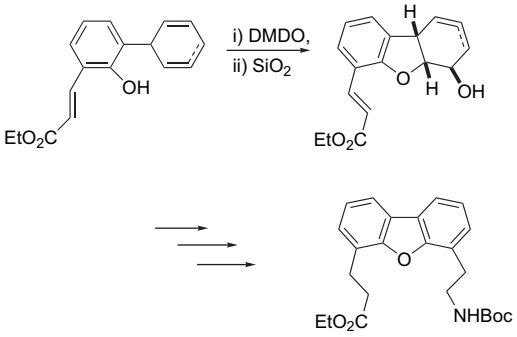
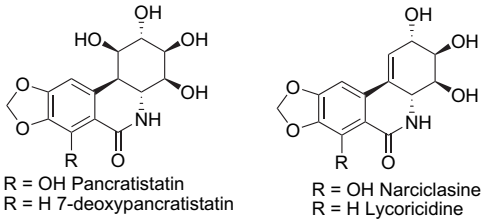


Scheme 3. Synthesis of carbazomycin B.



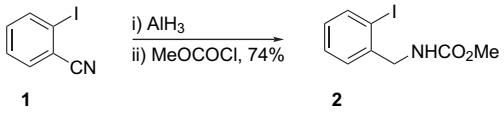
Scheme 4. Synthesis of a β-sheet initiator.

With a view to probing further the scope of the reductive radical arylation reaction and to exploiting more fully the potential of the aryl cyclohexadiene products we turned our attention to the preparation of phenanthridinone derivatives as found in the antineoplastic *Amaryllidaceae* natural products pancratistatin and lycoricidine and their analogs. The biological activity and densely arrayed functionality of these molecules have combined to make them the targets of numerous, successful synthetic endeavors since their discovery.[14–18] Moreover, it is especially noteworthy in the context of the present work that cyclohexadienes featured prominently in the original synthesis of (±)-pancratistatin by Danishefsky[15a] and in the very extensive work by Hudlicky group when they were generated, moreover, by dearomatization of arenes.[19] We report here on our work in this area, including an improved preparation of an intermediate in the Danishefsky synthesis of (±)-pancratistatin.



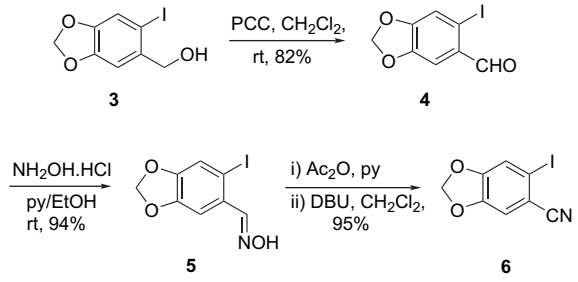
2. Results and discussion

We began our investigation by accessing the suitability of a series of *ortho*-functionalized aryl iodides for the key radical step, whose *ortho*-substituent should be convertible under mild conditions to a nucleophilic nitrogen species suitable for cyclization onto the cyclohexadiene formed in the dearomatization step. Thus, reduction of commercial *o*-iodobenzonitrile **1** with aluminum hydride,[20] followed by protection of the resulting amine with methyl chloroformate gave the iodo carbamate **2** (Scheme 5).[21]



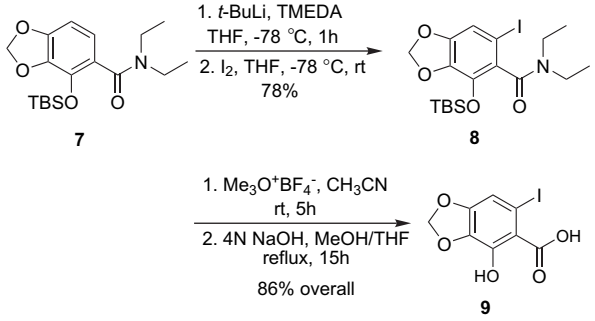
Scheme 5. Preparation of iodide **2**.

Iodide **3**, obtained from piperonyl alcohol with iodine and silver trifluoroacetate according to a literature procedure,[22] was converted to iodopiperonal **4** with PCC, and to the corresponding nitrile **6** by dehydration of oxime **5** (Scheme 6).



Scheme 6. Preparation of iodide **6**.

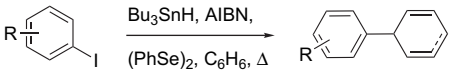
Finally, *ortho*-metallation of amide **7**, prepared by the Danishefsky route from resorcinol,[15a] and quenching with iodine gave the *o*-iodobenzamide **8**,[23] which could be hydrolyzed to the corresponding *des*-silyl acid **9** following conversion to the intermediate ester with trimethyloxonium tetrafluoroborate, and, then, heating with methanolic sodium hydroxide (Scheme 7).[24]

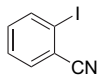
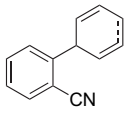
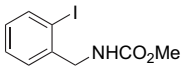
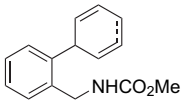
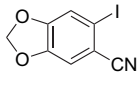
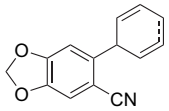
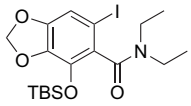
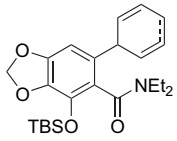
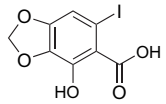
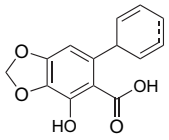
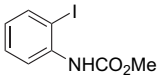
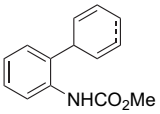
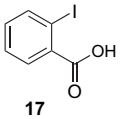
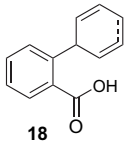


Scheme 7. Preparation of iodides **8** and **9**.

With these iodides in hand, the dearomatizing radical addition to benzene was investigated. These reactions were carried out according to a standard protocol involving the dropwise addition of tributyltin hydride and the initiator AIBN to a mixture of diphenyl diselenide and the substrate in benzene at reflux under argon, leading to the results outlined in Table 1, entries 1–5. As is typical for this type of addition[5a,10,12,13] the products were obtained as mixtures of 1,3- and 1,4-dienes in which the latter predominated, reflecting the known propensity of cyclohexadienyl radicals for kinetic trapping at the internal position.[25] The most

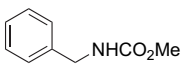
Table 1. Aryl radical addition to benzene

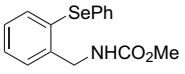


	Substrate	Product	% Yield ratio (1,4-/1,3)
1	 **1**	 **10**	44% (6/1)
2[b]	 **2**	 **11**	0%
3	 **6**	 **12**	44% (3/1)
4	 **8**	 **13**	Traces
5	 **9**	 **14**	30% (>10/1)
6	 **15**	 **16**	41% (3/2)[a]
7	 **17**	 **18**	54% (10/1)[a]

[a] Reproduced from Ref. 10.
[b] Compounds **19** and **20** were also isolated from this reaction in 30 and 32% yield, respectively.

satisfactory results were obtained with the two nitriles **1** and **6** both of which gave 44% yields of the benzene adducts (Table 1, entries 1 and 3). These yields while only moderate are typical for additions of this kind[5a,10,12,13] and, given the significant increase in complexity obtained and the simplicity of the starting materials, are acceptable for our purposes. The mass balance is typically made up of the deiodinated substrate and of the biaryl formally derived by oxidation of the cyclohexadiene: despite our best efforts over a number of years we have been unable to suppress formation of by-products of this type.[5a,10,12,13] The failure of the radical derived from the iodobenzyl carbamate **2** (Table 1, entry 2) was surprising, with carbamates having been previously shown to be compatible with the method (Table 1, entry 6)[10] Two major products **19** and **20** were isolated from this reaction in 30 and 32% yield, respectively, in addition to 35% of the recovered substrate. The formation of the *des*-iodo product **19** by intramolecular hydrogen atom transfer from the NH group to the aryl radical was excluded as a major pathway through an experiment employing *N*-deuterio **2** as substrate, when only 5% incorporation of deuterium into the *ortho*-position of **19** was observed. It is clear that most of the benzeneselenol is removed from this reaction mixture through the formation of selenide **20**. This results in a breakdown of the propagation cycle (Scheme 1), allowing an increase in stannane concentration as the addition proceeds, and ultimately results in the formation of the reduction product **19**. It is not clear why selenide **20** is formed in such high yield from substrate **2**, but it may be the result of hydrogen bonding of benzeneselenol to the carbamate, which facilitates a nucleophilic aromatic substitution reaction. Understandably in view of the obvious steric hindrance and the potential for intramolecular 1,5-hydrogen atom transfer from the amide group, only trace amounts of the adduct from the reaction between iodide **8** and benzene were obtained (Table 1, entry 4), with the major product **7** (64%) being that of simple reduction. This problem was remedied by use of the corresponding acid **9**, which reproducibly gave yields of 30% of the adduct **14** (Table 1, entry 5). Higher yields had been previously obtained with *o*-iodobenzoic acid (Table 1, entry 7),[10] but we were unable to improve on this yield despite repeated attempts. Nevertheless, it is interesting to note that the formation of **14** (Table 1, entry 5) took place with high regioselectivity in the hydrogen atom transfer step and afforded a product almost free of the minor, conjugated, isomeric diene. This unusually high regioselectivity in the quenching step was previously seen in the formation of **18** from *o*-iodobenzoic acid (Table 1, entry 7).[10]

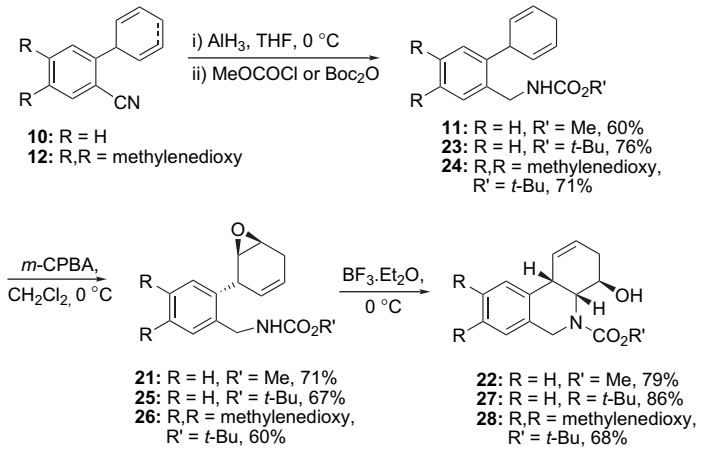

19


20

Turning to the desymmetrization step adduct **10** was converted to carbamate **11** by reduction with aluminum hydride,[20] and subsequent reaction with methyl chloroformate (Scheme 8).[26] Treatment with a controlled amount of *m*-CPBA subsequently afforded the mono epoxide **21** in 71%, which underwent the desired cyclization on exposure to boron trifluoride etherate[27] giving the phenanthridine derivative **22** in 79% yield (Scheme 8). The relative stereochemistry of **22**, with its *cis*-fused ring junction, was confirmed by X-ray crystallographic analysis.[†] An analogous series of experiments was also conducted on the corresponding *t*-butyl carbamates, and in the methylenedioxy series beginning from adduct **12**, with parallel results (Scheme 8).

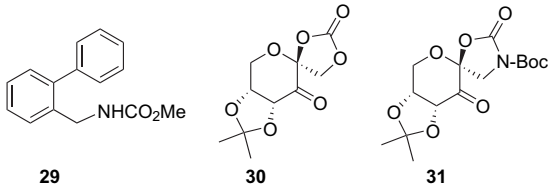
The asymmetric epoxidation of cyclohexadiene **11** with Jacobsen's catalyst[28] was attempted, but the only product

[†] CCDC 298768 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.



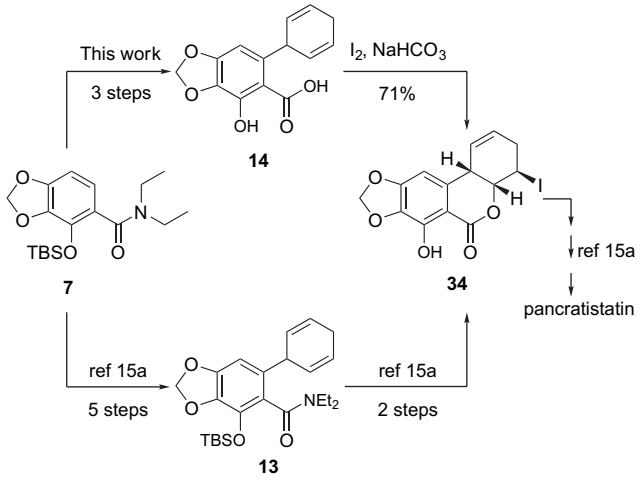
Scheme 8. Cyclization by epoxidation.

obtained was the biaryl system **29**.[29] A range of stoichiometric oxidants and additives was assayed in this catalytic protocol, including *N*-methyl morpholine *N*-oxide, sodium hypochlorite, iodosobenzene, and 4-phenylpyridine *N*-oxide, but we were unable to suppress the aromatization, which is, perhaps, not too surprising in view of the presence of the doubly allylic benzylic C–H bond in the substrate. Epoxidation of **11** with Shi catalyst **30** and dimethyl dioxirane did afford **21** in 48% yield, but in racemic form, consistent with the known substrate range of this system.[30] On the other hand, the more recent catalyst **31** developed by Shi group for *cis*-olefins gave **21** in 40% yield and 30% ee as determined by chiral HPLC methods.[31] In view of the poor enantioselectivity obtained with this system, no further investigations into enantioselective epoxidations were conducted although it is possible that other systems reported to bring about the enantioselective epoxidation of *cis*-alkenes, and which have appeared in the literature since this work was completed, may achieve the desired result.[32]

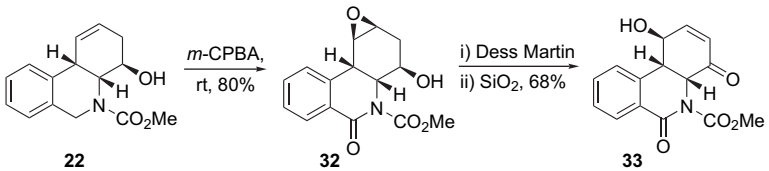


In exploring the further functionalization of the phenanthridine derivative **22**, an unexpected benzylic oxidation was encountered. Thus, treatment of **22** with excess *m*-CPBA in dichloromethane at room temperature gave not the expected simple epoxidation product but that of concomitant benzylic oxidation, the amide **32**, in 80% yield (Scheme 9). This interesting reaction has precedent in the work of Ma and co-workers who achieved the analogous oxidation of a series of cyclic and acyclic alkylarenes, including the transformation of ethylbenzene to acetophenone, with *m*-CPBA, for which they postulated a mechanism involving hydrogen atom abstraction and trapping of the benzylic radical by air.[33] Dess Martin oxidation[34] of **32** gave the corresponding ketone, which on passage over silica gel, afforded the anticipated hydroxyenone **33** in 68% yield, in which the enone ring derives its carbon skeleton from a benzene ring, of which every carbon has been modified.

Iodolactonization[35] of adduct **14** provided lactone **34**, a key intermediate in Danishefsky's synthesis of (±)-pancratistatin,[15a] in 71% yield (Scheme 10). In his synthesis Danishefsky constructed cyclohexadiene **13** from amide **7**,



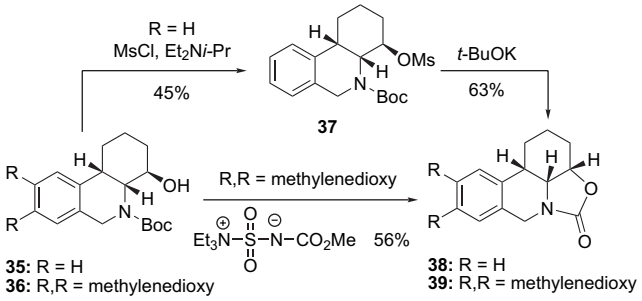
Scheme 10. Improved synthesis of a key intermediate in the Danishefsky (±)-pancratistatin synthesis.



Scheme 9. Functionalization of the 'benzene' ring.

and following removal of the silyl ether subjected it to iodolactonization to arrive at **34**. In that pioneering synthesis a sequence of five steps, including a tributyltin hydride mediated radical elimination reaction, were required to obtain **13** from **7**, and the complete sequence from **7** to iodolactone **34** was achieved in seven steps and 12.7% overall yield. The sequence of reactions that we describe here (Scheme 7, Table 1, and Scheme 10) proceeds from **7** to **34** in four steps and 14.3% overall yield, provides a convenient short cut in the original synthesis, and serves to highlight the advantages of the dearomatizing aryl radical addition to benzene as a means of aryl cyclohexadiene formation.

Finally, we note an interesting cyclization giving rise to the 7-oxa-γ-lycorane skeleton. Thus, catalytic hydrogenation of both **27** and **28** provided the dihydro analogs **35** and **36**, respectively, in excellent yield. The desmethylenedioxy system **35** was converted to the corresponding mesylate **37** by standard methods (Scheme 11). Interestingly enough this compound resisted all our attempts to bring about elimination of the mesyl group, with the major product being the oxazolidinone **38** resulting from displacement of the mesylate with inversion by the carbamate group. An analogous result was obtained on attempted elimination of water from **36** with the Burgess reagent,[36] when **39** was formed in 56% yield (Scheme 11).



Scheme 11. Formation of the 7-oxa-γ-lycorane skeleton.

3. Conclusion

The efficiency of the benzeneselenol-catalyzed, tributylstannane-mediated addition of aryl iodides to benzene depends strongly on the nature of the *ortho*-substituent. For reasons that are not yet clear, little or no addition takes place with an *o*-(methoxycarbonylaminomethyl) substituent, as in **2**, whereas the closely analogous *o*-(methoxycarbonylamino) group, as in **15**, is satisfactory. An *o*-cyano group functions well (**1** and **6**) and may be subsequently converted to the desired *o*-(methoxycarbonylaminomethyl) substituent by reduction with aluminum hydride followed by methyl chloroformate, without detriment to the 1,4-cyclohexadiene functionality. Further manipulations then lead rapidly to a variety of phenanthridine and phenanthridinone derivatives.

4. Experimental

4.1. General

All solvents were dried and distilled by standard techniques. All experiments were carried out in an atmosphere of dry nitrogen or argon. Extracts were dried over sodium sulfate and concentrated under reduced pressure at room temperature. Unless otherwise stated ^{1}H and ^{13}C NMR spectra were carried out at 400 and 100 MHz, respectively, in $CDCl_3$ solution. Chemical shifts are given in parts per million downfield from tetramethylsilane. Microanalyses were carried out by Midwest Microlabs, Indianapolis, IN. Mass spectra were recorded in the Research Resources Laboratory at UIC.

4.1.1. Methyl (2-iodobenzyl)carbamate (2). $LiAlH_4$ (173 mg, 4.6 mmol) was suspended in THF (8 mL), cooled to 0 °C, stirred vigorously, and treated with concentrated sulfuric acid (127 μL, 2.3 mmol). This mixture was stirred for 1 h at 0 °C, before a solution of 2-iodobenzonitrile (500 mg, 2.2 mmol) in THF (8 mL) was added dropwise. Stirring was continued for 1 h before the reaction was stopped by the addition of ethanol (10 mL) at 0 °C, followed by few drops of 2 N NaOH. The suspension was diluted with EtOAc (50 mL), filtered, and concentrated to provide a brown residue, which was taken directly to the next step. This residue was dissolved in CH_2Cl_2 (8 mL) and cooled to 0 °C, then treated with Et_3N (750 μL, 5 mmol) and methyl chloroformate (394 μL, 5 mmol). The resultant solution was stirred for 6 h, diluted with CH_2Cl_2 (50 mL), and washed with saturated aqueous $NaHCO_3$ (25 mL), and brine (25 mL). The dichloromethane layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 20% EtOAc in hexanes) to afford carbamate **2** (456 mg, 74%) as a white solid. Mp 77 °C (lit.[21] mp 74 °C); ^{1}H NMR: δ 3.68 (s, 3H), 4.37 (d, J=6.2 Hz, 2H), 5.25 (br s, 1H), 6.97 (t, J=8.0 Hz, 1H), 7.30–7.39 (m, 2H), 7.81 (d, J=7.7 Hz, 1H); ^{13}C NMR: δ 49.7, 52.3, 98.8, 128.5, 129.3, 129.5, 139.4, 140.5, 156.9.

4.1.2. 6-Iodo-1,3-benzodioxole-5-methanol (3). To a solution of piperonyl alcohol (5.15 g, 34 mmol), CF_3CO_2Ag (9.7 g, 44 mmol), and dry $CHCl_3$ (90 mL) at −5 °C was added I_2 (11.1 g, 44 mmol) in one portion. The resulting yellow mixture was maintained at −5 °C for 10 min, whereupon it was filtered. The filtrate was washed with 20% aqueous sodium thiosulfate (3x50 mL), dried, and concentrated. Recrystallization from chloroform afforded iodide **3** (6.1 g, 66%) as white needles. Mp 110–111 °C (lit.[22a] mp 106–107 °C); ^{1}H NMR (500 MHz): δ 2.06 (t, J=6.0 Hz, 1H), 4.57 (d, J=6.0 Hz, 2H), 6.0 (s, 2H), 6.98 (s, 1H), 7.26 (s, 1H); ^{13}C NMR (125 MHz): δ 69.2, 85.4, 101.7, 109.0, 118.5, 136.2, 147.9, 148.6.

4.1.3. 6-Iodo-1,3-benzodioxole-5-carbaldehyde (4). To a solution containing iodide **3** (6.1 g, 22.2 mmol) and dry CH_2Cl_2 (300 mL) at 0 °C was added PCC (9.6 g, 44 mmol). The mixture was allowed to warm to room temperature and was stirred for 5 h at 25 °C. The reaction mixture was concentrated to one third of its original volume and filtered through silica gel column (eluent: 30% EtOAc in hexanes) to give **4** as a pale yellow solid (5.7 g, 94%). Mp 110–112 °C; ^{1}H NMR (500 MHz): δ 6.08 (s, 2H), 7.32 (s, 1H), 7.35 (s, 1H), 9.86 (s, 1H); ^{13}C NMR (125 MHz): δ 93.3, 102.7, 108.8, 119.4, 129.5, 149.2, 153.5, 194.5. Anal. Calcd for $C_8H_5O_3I$: C, 34.81; H, 1.83. Found: C, 34.56; H, 1.70.

4.1.4. 6-Iodo-1,3-benzodioxole-5-carbaldoxime (5). A solution of aldehyde **4** (5.7 g, 20.7 mmol) in 1:1 pyridine (20 mL) and ethanol (20 mL) was treated with hydroxylamine hydrochloride (2.2 g, 31.1 mmol) and stirred at room temperature. After 8 h, the resulting solution was diluted with EtOAc (80 mL) and washed with saturated aqueous NH_4Cl solution (50 mL), followed by brine (50 mL). The organic layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 30% EtOAc in hexanes) to afford oxime **5** (5.9 g, 94%) as a white solid. Mp 148 °C; ^{1}H NMR (500 MHz, CD_3OD): δ 6.0 (s, 2H), 7.24 (s, 1H), 7.26 (s, 1H), 8.20 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ 87.2, 102.2, 105.6, 118.08, 128.0, 148.7, 149.7, 151.8. Anal. Calcd for $C_8H_6NO_3I$: C, 33.01; H, 2.08. Found: C, 33.12; H, 2.15.

4.1.5. 6-Iodo-1,3-benzodioxole-5-carbonitrile (6). A solution of oxime **5** (5.9 g, 19.4 mmol) and DMAP (236 mg, 1.9 mmol) in pyridine (50 mL) was treated with Ac_2O (2.7 mL, 29 mmol), and stirred at room temperature for 10 h. After complete consumption of **5**, DBU (3.5 mL, 23 mmol) was added to the reaction mixture, and stirring continued for 6 h during the course of which further DBU (2×1.7 mL) was added. The mixture was diluted with dichloromethane (70 mL), and washed with saturated NH_4Cl (2×40 mL) and brine. The dichloromethane layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 20% EtOAc in hexanes) to afford nitrile **6** (5.03 g, 95%) as pale yellow solid. Mp 127–29 °C; ^{1}H NMR (500 MHz): δ 6.09 (s, 2H), 7.01 (s, 1H), 7.28 (s, 1H); ^{13}C NMR (125 MHz): δ 89.8, 102.9, 113.0, 113.1, 119.2, 119.5, 148.3, 152.0. Anal. Calcd for $C_8H_4NO_2I$: C, 35.19; H, 1.48; N, 5.13; I, 46.48. Found: C, 35.10; H, 1.46; N, 4.96; I, 46.19.

4.1.6. *N*,*N*-Diethyl-4-(*tert*-butyldimethylsiloxy)-6-iodo-1,3-benzodioxole-5-carboxamide (8). In a dry flask, amide **7**[15a] (3.0 g, 8.54 mmol) was dissolved in THF (80 mL), and cooled to −78 °C. To this solution was added TMEDA (1.94 mL, 12.8 mmol), and *n*-BuLi (6.82 mL, 17.1 mmol, 2.0 M in hexanes). The resulting deep brown mixture was stirred at −78 °C for 1 h before I_2 (4.34 g, 17.1 mmol) in THF (20 mL) was added, and the reaction mixture allowed warm to room temperature overnight with stirring. The reaction mixture was quenched with saturated NH_4Cl, and the THF was removed in vacuo. The resulting residue was dissolved in EtOAc (50 mL) and H_2O (50 mL), and the aqueous layer was extracted with EtOAc (30 mL). The combined organic layers were washed with brine (30 mL) and dried. Purification by chromatography over silica gel (eluent: 12% EtOAc in hexanes) afforded **8** as yellow oil (3.2 g, 78%). ^{1}H NMR: δ 0.17 (s, 3H), 0.22 (s, 3H), 0.92 (s, 9H), 1.1 (t, J=7.2 Hz, 3H), 1.25 (t, J=7.2 Hz, 3H), 3.1–3.21 (m, 3H), 3.79–3.88 (m, 1H), 5.91 (s, 1H), 5.94 (s, 1H), 6.9 (s, 1H); ^{13}C NMR: δ −4.6, −4.1, 12.6, 13.8, 18.3, 25.7 (3C), 39.3, 43.1, 82.3, 101.4, 112.7, 130.5, 136.9, 137.6, 149.3, 167.5; ESIHRMS Calcd for $C_{18}H_{28}INO_4Si$ $[M+H]^+$: 478.0911, found: 478.0917.

4.1.7. 4-Hydroxy-6-iodo-1,3-benzodioxole-5-carboxylic acid (9). To a stirred solution of benzamide **8** (220 mg, 0.46 mmol) in CH_3CN (5 mL) was added Na_2HPO_4 (98 mg, 0.69 mmol), followed by trimethyloxonium tetrafluoroborate (205 mg, 1.4 mmol). The reaction was stirred at room temperature for 5 h after which the reaction was quenched by slow addition of saturated aqueous sodium bicarbonate solution (5 mL). The resulting mixture was stirred for 7 h at room temperature, and then diluted with EtOAc (25 mL), followed by water (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2×15 mL). The combined organic layer was dried and concentrated. The residue was dissolved in MeOH/THF (5 mL, 1/1) mixture, treated with 4 N NaOH (3 mL) and heated to reflux for 15 h. The reaction mixture was neutralized with 6 N HCl followed by removal of the methanol in vacuo. The resulting residue was dissolved in EtOAc (25 mL) and water (15 mL), and the aqueous layer was extracted with EtOAc (3×20 mL). The combined organic layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 70% EtOAc in hexanes) to afford **9** (122 mg, 86%) as white solid. Mp 147–148 °C; ^{1}H NMR: δ 6.0 (s, 2H), 7.14 (s, 1H); ^{13}C NMR: δ 85.4, 102.8, 112.8, 115.2, 136.4, 146.5, 152.3, 170.1; ESIHRMS Calcd for $C_8H_5IO_5$ $[M-H]^-$: 306.9103, found: 306.9105.

4.2. General procedure for radical addition to benzene

A dry flask was charged with aryl iodide (6.1 mmol) and diphenyl diselenide (380 mg, 1.22 mmol), fitted with a reflux condenser, and flushed with argon. Dry, degassed benzene (122 mL, 0.05 M) was added; the resulting solution was heated to reflux. A solution of AIBN (100 mg, 0.61 mmol) and tributyltin hydride (7.3 mmol, 1.96 mL) in dry degassed benzene (18 mL) was added via syringe pump at rate of 1.5 mL h^{-1}. On completion of the addition, the reaction mixture was refluxed for 1 h, then cooled to room temperature and the solvent removed in vacuo. The residue was taken up in acetonitrile (200 mL) and washed with (4×25 mL) hexane. The acetonitrile phase was concentrated and purified by silica gel chromatrography (eluent: EtOAc in hexanes) to yield the adducts.

4.2.1. 2-(2,5-Cyclohexadienyl)benzonitrile (10). Yield 44%; yellow oil; IR (neat): 2221, 2360, 2817, 2867, 3029 cm^{-1}; ^{1}H NMR (500 MHz): δ 2.78 (m, 2H), 4.43 (br t, J=7.5 Hz, 1H), 5.69–5.72 (br d, J=9.0 Hz, 2H), 5.89–5.92 (br d, J=10.5 Hz, 2H), 7.30 (t, J=7.3 Hz, 1H), 7.38 (d, J=7.9 Hz, 1H), 7.53 (t, J=7.9 Hz, 1H), 7.62 (d, J=7.7 Hz, 1H); ^{13}C NMR (125 MHz): δ 25.7, 40.2, 125.1, 125.3, 126.2, 126.8, 126.9, 129.4, 132.9, 132.91, 133.0, 133.1, 148.6; EIHRMS Calcd for $C_{13}H_{11}N$ $[M-H]^+$: 180.0813, found: 180.0747. The product was contaminated with an inseparable minor isomer (1,3-cyclohexadien-5-yl)-benzonitrile, which accounted for 14% of the mass as determined by NMR. This isomer was characterized by ^{1}H NMR (500 MHz): δ 2.34 (dddd, J=17.6 Hz, J=11.8 Hz, J=4.1 Hz, J=2.2 Hz, 1H), 2.67 (dddd, J=17.6 Hz, J=9.5 Hz, J=4.8 Hz, J=1.8 Hz, 1H), 4.05–4.10 (br t, J=10.0 Hz, 1H), 5.76–5.79 (m, 2H), 5.9–6.1 (m, 1H), 6.13–6.17 (m, 1H), 7.30 (t, J=7.3 Hz, 1H), 7.38 (d, J=7.9 Hz, 1H), 7.53 (t, J=7.9 Hz, 1H), 7.62 (d, J=7.7 Hz, 1H); ^{13}C NMR (125 MHz): δ 30.8, 37.7, 111.6, 117.9, 123.9, 125.1, 125.9, 126.8, 127.5, 128.4, 128.7, 133.0, 148.9.

4.2.2. 6-(2,5-Cyclohexadienyl)-1,3-benzodioxole-5-carbonitrile (12). Yield 44%; white solid; mp 101 °C; ^{1}H

NMR (500 MHz): δ 2.73–2.76 (m, 2H), 4.36–4.40 (m, 1H), 5.66 (ddt, *J*=10.0 Hz, *J*=5.5 Hz, *J*=1.5 Hz, 2H), 5.89 (ddt, *J*=10.5 Hz, *J*=5.5 Hz, *J*=1.5 Hz, 2H), 6.02 (s, 2H), 6.79 (s, 1H), 6.97 (s, 1H); ^{13}C NMR (125 MHz): δ 25.7, 39.8, 102.2, 109.5, 111.0, 118.1, 123.8, 125.2, 125.7, 126.5, 127.4, 145.5, 146.3, 152.0. Anal. Calcd for $C_{14}H_{11}NO_2$: C, 74.65; H, 4.92. Found: C, 74.46; H, 4.99. The product was contaminated with an inseparable minor isomer, 6-(1,3-cyclohexadien-1-yl)-1,3-benzodioxole-5-carbonitrile, which accounted for 25% of the mass as determined by NMR and which was characterized by the following resonances: ^{1}H NMR (500 MHz): δ 2.29 (dddd, *J*=17.6 Hz, *J*=11.1 Hz, *J*=4.4 Hz, *J*=2.3 Hz, 1H), 2.65 (dddd, *J*=17.6 Hz, *J*=9.5 Hz, *J*=4.7 Hz, *J*=1.8 Hz, 1H), 4.01 (br t, *J*=9.5 Hz, 1H), 5.71 (dd, *J*=9.0 Hz, *J*=4.0 Hz, 1H), 5.76 (dt, *J*=9.5 Hz, *J*=4.5 Hz, 1H), 5.96–5.99 (m, 1H), 6.03 (s, 2H), 6.06–6.14 (m, 1H), 6.98 (s, 1H), 6.99 (s, 1H); ^{13}C NMR (125 MHz): δ 30.8, 37.3, 103.3, 108.8, 113.3, 118.1, 125.0, 125.1, 126.3, 128.6, 128.7, 145.6, 145.3, 151.7.

4.2.3. 4-Hydroxy-6-(2,5-cyclohexadien-1-yl)-1,3-benzodioxole-5-carboxylic acid (14). Yield 30%; white solid; mp 152 °C; ^{1}H NMR: δ 2.71–2.76 (m, 2H), 4.96–5.0 (m, 1H), 5.69–5.74 (m, 2H), 5.82–5.87 (m, 2H), 6.05 (s, 2H), 6.61 (s, 1H); ^{13}C NMR: δ 25.7, 36.7, 102.5, 103.8, 106.9, 124.2, 128.3, 128.5, 130.3, 133.1, 145.8, 146.8, 153.4, 173.6; ESIHRMS Calcd for $C_{14}H_{12}O_5$ $[M-H]^-$: 259.0606, found: 259.0599.

4.2.4. Methyl benzylcarbamate (19). This compound was isolated in 30% yield from the attempted addition of **2** to benzene. White solid; mp 57–58 °C (lit.[37] mp 59–61 °C); ^{1}H NMR: δ 3.70 (s, 3H), 4.38 (d, *J*=5.9 Hz, 2H), 5.03 (br s, 1H), 7.26–7.35 (m, 5H); ^{13}C NMR: δ 45.0, 52.2, 127.2, 127.4, 127.5, 128.4, 128.6, 138.6, 157.2.

4.2.5. Methyl 2-phenylselenobenzylcarbamate (20). This compound was isolated in 32% yield from the attempted addition of **2** to benzene. Colorless oil; ^{1}H NMR: δ 3.65 (s, 3H), 4.46 (d, *J*=6.1 Hz, 2H), 5.05 (br s, 1H), 7.18 (t, *J*=7.5 Hz, 1H), 7.23–7.51 (m, 8H); ^{13}C NMR: δ 45.4, 52.2, 127.2, 127.8, 128.4, 128.5, 128.6, 128.9, 129.5 (2C), 130.3, 132.0, 135.6, 140.5, 156.9; EIHRMS Calcd for $C_{15}H_{15}NO_2Se$ $[M]^+$: 321.0268, found: 321.0257.

4.2.6. Methyl 2-(2,5-cyclohexadien-1-yl)benzylcarbamate (11). $LiAlH_4$ (250 mg, 6.6 mmol) was suspended in THF (8 mL), cooled to 0 °C, stirred vigorously, and treated with concentrated sulfuric acid (182 μL, 3.3 mmol). This mixture was stirred for 1 h at 0 °C, before a solution of diene **10** (570 mg, 3.14 mmol) in THF (7 mL) was added dropwise. Stirring was continued for 1 h before the reaction was stopped by the addition of ethanol (10 mL) at 0 °C, followed by few drops of 2 N NaOH. The suspension was diluted with EtOAc (50 mL), filtered, and concentrated to provide a brown residue, which was taken directly to the next step. This residue was dissolved in CH_2Cl_2 (8 mL) and cooled to 0 °C, and then treated with Et_3N (750 μL, 5 mmol) and methyl chloroformate (386 μL, 5 mmol). The resultant solution was stirred for 6 h, diluted with CH_2Cl_2 (50 mL), and washed with saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The dichloromethane layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 20% EtOAc in hexanes) to afford carbamate **11** (459 mg, 60%) as a white solid. Mp 81–83 °C; ^{1}H NMR (500 MHz): δ 2.75–2.79 (m, 2H), 3.68 (s, 3H), 4.21 (m, 1H), 4.47 (d, *J*=5.5 Hz, 2H), 4.92 (br s, 1H), 5.69 (ddt, *J*=10.0 Hz, *J*=5.0 Hz, *J*=1.0 Hz, 2H), 5.83–5.85 (m, 2H), 7.19–7.29 (m, 4H); ^{13}C NMR (125 MHz): δ 25.7, 38.1, 42.5, 52.2, 123.8, 124.8, 127.7, 127.9, 128.2, 128.8, 129.1, 129.8, 135.3, 143.0, 156.7; ESIHRMS Calcd for $C_{15}H_{17}NO_2$ $[M+Na]^+$: 266.1157, found: 266.1161.

4.2.7. *tert*-Butyl 2-(2,5-cyclohexadien-1-yl)benzylcarbamate (23). This carbamate was prepared from **10** analogously to **11**, except that the methyl chloroformate was replaced by Boc_2O. It was obtained as a colorless oil in 76% yield. IR (neat): 1168, 1511, 1694, 2976, 3340 cm^{-1}; ^{1}H NMR: δ 1.46 (s, 9H), 2.74–2.78 (m, 2H), 4.23 (br t, *J*=8.5 Hz, 1H), 4.42 (d, *J*=5.3 Hz, 2H), 4.72 (br s, 1H), 5.68 (br d, *J*=10.0 Hz, 2H), 5.82–5.85 (m, 2H), 7.17–7.27 (m, 4H); ^{13}C NMR: δ 25.7, 28.4 (3C), 37.8, 42.1, 79.5, 123.9, 126.6, 127.9, 128.1, 128.5, 128.9, 129.6, 130.0, 135.6, 143.1, 155.5; ESIHRMS Calcd for $C_{18}H_{23}NO_2$ $[M+Na]^+$: 308.1627, found: 308.1625.

4.2.8. *tert*-Butyl 6-(2,5-cyclohexadien-1-yl)-1,3-benzodioxole-5-ylmethylcarbamate (24). This carbamate was prepared from **12** in the same manner as **23** was obtained from **10**. It was obtained in 71% yield as a white solid. Mp 106 °C; ^{1}H NMR: δ 1.44 (s, 9H), 2.71–2.73 (m, 2H), 4.13 (m, 1H), 4.23 (d, *J*=5.1 Hz, 2H), 4.74 (br s, 1H), 5.59 (br d, *J*=10.1 Hz, 2H), 5.79 (br d, *J*=10.1 Hz, 2H), 5.89 (s, 2H), 6.72 (s, 1H), 6.73 (s, 1H); ^{13}C NMR: δ 25.7, 28.4 (3C), 37.4, 41.9, 79.4, 100.9, 108.6, 109.6, 123.7, 125.2, 128.2, 128.7, 129.9, 136.9, 146.0, 147.3, 155.5. Anal. Calcd for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04. Found: C, 69.35; H, 6.99.

4.3. General procedure for the epoxidation of dienes

3-Chloroperoxybenzoic acid (230 mg, 1.03 mmol) was added portionwise to a stirred solution of the diene (0.94 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C. After 2 h, the reaction mixture was diluted with dichloromethane (40 mL) and washed with saturated aqueous sodium bicarbonate (3×25 mL). The dichloromethane layer was dried, concentrated, and purified by chromatography on silica gel (eluent: EtOAC in hexanes) to afford the epoxide.

4.3.1. Methyl 2-(2-cyclohexen-5,6-epoxy-1-yl)benzylcarbamate (21). Yield 71%; white solid; mp 87–88 °C; ^{1}H NMR (500 MHz): δ 2.61–2.70 (m, 2H), 3.15 (m, 1H), 3.36 (m, 1H), 3.70 (s, 3H), 4.09 (m, 1H), 4.48 (d, *J*=6.5 Hz, 2H), 4.96 (br s, 1H), 5.53–5.60 (m, 2H), 7.22–7.35 (m, 4H); ^{13}C NMR (125 MHz): δ 24.9, 37.5, 42.7, 51.1, 52.3, 54.8, 121.5, 125.6, 127.2, 128.2, 128.8, 129.2, 136.0, 138.8, 156.7; ESIHRMS Calcd for $C_{15}H_{17}NO_3$ $[M+H]^+$: 260.1287, found: 260.1288.

4.3.2. *tert*-Butyl 2-(2-cyclohexen-5,6-epoxy-1-yl)benzylcarbamate (25). Yield 67%; white solid; mp 88 °C; IR (neat): 1167, 1248, 1519, 1699, 2359, 2977, 3354 cm^{-1}; ^{1}H NMR: δ 1.45 (s, 9H), 2.60–2.69 (m, 2H), 3.15 (d, *J*=4 Hz, 1H), 3.35 (m, 1H), 4.10 (m, 1H), 4.44 (d, *J*=5 Hz, 2H), 4.79 (br s, 1H), 5.54–5.58 (m, 2H), 7.21–7.22

(m, 4H); ^{13}C NMR: δ 25.1, 28.4 (3C), 37.5, 42.3, 51.9, 54.9, 79.7, 121.5, 125.7, 127.4, 128.1, 128.8, 129.2, 136.4, 138.8, 155.6; ESIHRMS Calcd for $C_{18}H_{23}NO_3$ [M+Na]$^+$: 324.1576, found: 324.1574.

4.3.3. *tert*-Butyl 6-(2-cyclohexen-5,6-epoxy)-[1,3]benzodioxole-5-ylmethyl)carbamate (26). Yield 60%; white solid; mp 143 °C; ^{1}H NMR: δ 1.44 (s, 9H), 2.60–2.69 (m, 2H), 3.08 (m, 1H), 3.31 (m, 1H), 4.01 (m, 1H), 4.30 (d, J=5.2 Hz, 2H), 4.80 (br s, 1H), 5.46–5.50 (m, 2H), 5.90 (s, 2H), 6.66 (s, 1H), 6.83 (s, 1H); ^{13}C NMR: δ 25.0, 28.3 (3C), 37.2, 42.4, 51.8, 54.9, 79.7, 101.2, 108.7, 109.3, 121.5, 125.9, 130.0, 132.1, 146.7, 147.3, 155.5. Anal. Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71. Found: C, 66.24; H, 6.70.

4.4. General procedure for cyclization to the tricyclic skeleton

A solution of epoxide (1.0 mmol) in CH_2Cl_2 (4 mL) was treated with $BF_3\cdot Et_2O$ (0.4 mmol, 118 μL) at −10 °C and stirred for 20 min, before the addition of saturated aqueous sodium bicarbonate (10 mL) and dichloromethane (40 mL). The dichloromethane layer was dried, concentrated, and purified by chromatography over silica gel (eluent: EtOAc in hexanes).

4.4.1. [(±)-4*S*,4a*S*,10b*R*] Methyl 4-hydroxy-4,4a,6,10b-tetrahydro-3*H*-phenanthridine-5-carboxylate (22). Yield 79%; colorless oil; IR (neat): 1242, 1456, 1698, 2360, 3479 cm^{-1}; ^{1}H NMR: δ 1.95 (br s, 1H), 2.17–2.23 (m, 1H), 2.52–2.58 (m, 1H), 3.63 (m, 1H), 3.78 (s, 3H), 4.31–4.50 (m, 3H), 4.75–5.08 (m, 1H), 5.74 (m, 1H), 6.14 (m, 1H), 7.09–7.32 (m, 4H); ^{13}C NMR: δ 35.7, 38.2, 43.5, 53.1, 56.1, 66.5, 125.3, 126.2, 126.3, 127.1, 127.3, 127.6, 131.4, 136.8, 158.4; EIHRMS Calcd for $C_{15}H_{17}NO_3$ [M]$^+$: 259.1208, found: 259.1204.

4.4.2. [(±)-4*S*,4a*S*,10b*R*] *tert*-Butyl 4-hydroxy-4,4a,6,10b-tetrahydro-3*H*-phenanthridine-5-carboxylate (27). Yield 86%; colorless oil; IR (neat): 1419, 1683, 2977, 3426 cm^{-1}; ^{1}H NMR: δ 1.45 (s, 9H), 2.15–2.21 (m, 1H), 2.52–2.58 (m, 1H), 3.55 (m, 1H), 3.74 (m, 1H), 4.43–4.98 (m, 3H), 5.76 (m, 1H), 6.14 (m, 1H), 7.11–7.26 (m, 4H); ^{13}C NMR: δ 28.4 (3C), 36.3, 38.5, 44.0, 55.7, 66.9, 80.7, 125.5, 126.1, 126.3, 127.0, 127.1, 127.7, 131.7, 137.0, 157.6; ESIHRMS Calcd for $C_{18}H_{23}NO_3$ [M+Na]$^+$: 324.1576, found: 324.1566.

4.4.3. [(±)-4*S*,4a*S*,10b*R*] *tert*-Butyl 4-hydroxy-4,4a,6,10b-tetrahydro-3*H*-[1,3]dioxolo[4,5-*j*]phenanthridine-5-carboxylate (28). Yield 68%; colorless oil; ^{1}H NMR: δ 1.45 (s, 9H), 2.03 (m, 1H), 2.50–2.55 (m, 1H), 2.67 (br s, 1H), 3.56 (m, 1H), 3.61 (m, 1H), 4.25–4.84 (m, 3H), 5.73 (m, 1H), 5.90 (s, 1H), 5.93 (s, 1H), 6.02 (m, 1H), 6.56 (s, 1H), 6.69 (s, 1H); ^{13}C NMR: δ 28.4 (3C), 34.5, 36.2, 42.3, 55.7, 66.7, 80.7, 100.9, 106.3, 107.3, 124.2, 126.5, 126.8, 130.3, 145.9, 146.7, 157.4; ESIHRMS Calcd for $C_{19}H_{23}NO_5$ [M+Na]$^+$: 368.1468, found: 368.1475.

4.4.4. Methyl 2-phenylbenzylcarbamate (29). To a solution of diene **11** (70 mg, 0.27 mmol) and (*R*,*R*)-(−)-*N*,*N*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III) chloride (7 mg, 0.01 mmol) in CH_2Cl_2 (0.6 mL) was added NaOCl (1 mL) buffered to pH 11.3. The reaction mixture was stirred at room temperature for 4 h, then was diluted with CH_2Cl_2 (20 mL) and washed with H_2O (2×20 mL). The aqueous layer was back extracted with CH_2Cl_2 (10 mL). The combined organic layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 25% EtOAc in hexanes) to yield **29** (40 mg, 58%) as a colorless oil. IR (neat): 1251, 1527, 1708, 3023, 3332 cm^{-1}; ^{1}H NMR: δ 3.6 (s, 3H), 4.33 (d, J=6.0 Hz, 2H), 4.76 (br s, 1H), 7.23–7.46 (m, 9H); ^{13}C NMR: δ 42.9, 52.1, 127.3, 127.4, 127.7, 128.4, 128.9, 129.0, 130.2, 135.7, 140.7, 141.6, 156.9; ESIHRMS Calcd for $C_{15}H_{15}NO_2$ [M+H]$^+$: 242.1175, found: 242.1170.

4.4.5. [(±)-1*R*,2*S*,4S,4a*S*,10b*R*] Methyl 1,2-epoxy-4-hydroxy-1,2,3,4,4a,10b-hexahydro-phenanthridin-6-one-5-carboxylate (32). A solution of 3-chloroperoxybenzoic acid (255 mg, 1.2 mmol) and alcohol **22** (100 mg, 0.38 mmol) in dry CH_2Cl_2 (5 mL), was stirred at room temperature for 24 h, then diluted with dichloromethane (30 mL), washed with saturated aqueous sodium bicarbonate (3×25 mL), and brine (25 mL). The dichloromethane layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 70% EtOAc in hexanes) to afford epoxide **32** as a white foam (89 mg, 80%). ^{1}H NMR: δ 2.09 (dd, J=15.6 Hz, J=9.3 Hz, 1H), 2.37–2.44 (m, 1H), 3.25 (br s, 1H), 3.29 (t, J=4.2 Hz, 1H), 3.63–3.68 (m, 1H), 3.91 (s, 3H), 3.91 (m, 1H), 4.10–4.14 (m, 1H), 4.70 (dd, J= 10.8 Hz, 5.1 Hz, 1H), 7.40 (t, J=7.5 Hz, 1H), 7.51 (d, J=7.8 Hz, 1H), 7.59 (t, J=6.5 Hz, 1H), 8.1 (d, J=8.6 Hz, 1H); ^{13}C NMR: δ 33.1, 36.2, 51.4, 54.5, 54.6, 56.8, 64.2, 124.3, 127.4, 128.6, 130.4, 133.8, 136.8, 156.3, 162.5; ESIHRMS Calcd for $C_{15}H_{15}NO_5$ [M+Na]$^+$: 312.0842, found: 312.0847.

4.4.6. [(±)-1*R*,4*S*,4a*S*,10b*R*] Methyl 1-hydroxy-4,6-dioxo-4,4a,6,10b-tetrahydro-1*H*-phenanthridine-5-carboxylate (33). Dess–Martin periodinane (88 mg, 0.21 mmol) was added to a solution of epoxide **32** (50 mg, 0.17 mmol) in dry CH_2Cl_2 (3 mL) and stirred for 4 h. When the reaction was complete, saturated aqueous sodium thiosulfate (15 mL) was added, and the reaction mixture stirred for 0.5 h, before it was poured into saturated aqueous sodium bicarbonate. The dichloromethane layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 3% MeOH in CH_2Cl_2) to afford hydroxyenone **33** (33 mg, 68%) as a white solid. Mp 98 °C; IR (neat): 1376, 1598, 1689, 1762, 3413 cm^{-1}; ^{1}H NMR (CD_3OD): δ 4.02 (s, 3H), 4.1 (m, 1H), 5.28 (dd, J=5.5, J=2.6 Hz, 1H), 5.82 (d, J=4.9 Hz, 1H), 5.92 (d, J=9.9 Hz, 1H), 7.01 (dd, J=10.0 Hz, 5.3 Hz, 1H), 7.41 (t, J=7.6 Hz, 1H), 7.44 (d, J=8.0 Hz, 1H), 7.55 (t, J=7.5 Hz, 1H), 8.06 (d, J=7.9 Hz, 1H); ^{13}C NMR (CD_3OD): δ 44.8, 53.3, 59.1, 63.3, 124.4, 127.6, 128.8, 128.9, 129.6, 133.7, 136.8, 146.3, 154.1, 163.1, 193.4; ESIHRMS Calcd for $C_{15}H_{13}NO_5$ [M+Na]$^+$: 310.0686, found: 310.0690.

4.4.7. [(±)-(4*S*,4a*S*,10b*R*)] 3,4,4a,10b-Tetrahydro-4-iodo-7-hydroxy-6*H*-[1,3]benzodioxolo[5,6-*c*]benzopyran-6-one (34). A solution of **14** (20 mg, 0.076 mmol) in THF (3 mL) was treated with $NaHCO_3$ (13 mg, 0.15 mmol), then I_2 (23 mg, 0.09 mmol), and stirred at room temperature

for 15 h. Saturated $Na_2S_2O_3$ solution (10 mL) was then added and the mixture was extracted with EtOAc (3×10 mL). The aqueous layer was washed with EtOAc (15 mL), and the combined organic layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 30% EtOAc in hexanes) to provide iodolactone **34** (30 mg, 71%) as a white solid. Mp 179 °C (lit.[26] mp 177 °C); ^{1}H NMR: δ 2.63–2.72 (m, 1H), 3.30–3.37 (m, 1H), 4.06–4.10 (m, 1H), 4.57–4.60 (m, 1H), 4.90–4.92 (m, 1H), 5.46–5.50 (m, 1H), 5.69–5.73 (m, 1H), 6.09 (s, 2H), 6.46 (s, 1H), 10.86 (s, 1H); ^{13}C NMR: δ 19.6, 30.8, 35.6, 77.7, 100.4, 102.7, 103.0, 124.2, 124.3, 133.5, 137.9, 145.8, 154.5, 168.0; EIHRMS Calcd for $C_{14}H_{11}IO_5$ $[M]^+$: 385.9651, found: 385.9663.

4.4.8. [(±)-4*R*,4a*R*,10b*R*] *tert*-Butyl 4-hydroxy-2,3,4,4a,6,10b-hexahydro-1*H*-phenanthridine-5-carboxylate (35). A solution of alcohol **27** (237 mg, 0.78 mmol) in methanol (4 mL) was stirred with 10% Pd–C (80 mg) under 1 atm of H_2 for 3 h, then was filtered through Celite® and purified by chromatography over silica gel (eluent: 30% EtOAc in hexanes) to yield **35** (77 mg, 95%) as a colorless oil. ^{1}H NMR: δ 1.25–1.27 (m, 2H), 1.45 (s, 9H), 1.56–1.59 (m, 1H), 1.67–1.76 (m, 1H), 1.96–1.99 (m, 1H), 2.30 (br s, 1H), 2.47 (dd, J=13.5 Hz, J=2.8 Hz, 1H), 3.30 (m, 2H), 4.25 (m, 1H), 4.45 (d, J=16.8 Hz, 1H), 4.70 (d, J=16.9 Hz, 1H), 7.11–7.29 (m, 4H); ^{13}C NMR: δ 19.6, 26.7, 28.4 (3C), 35.4, 37.4, 44.3, 58.3, 69.3, 80.5, 125.4, 126.1, 126.5, 126.8, 133.2, 135.2, 157.2; EIHRMS Calcd for $C_{18}H_{25}NO_3$ $[M]^+$: 303.1834, found: 303.1848.

4.4.9. [(±)-4*R*,4a*R*,10b*R*] *tert*-Butyl 4-hydroxy-2,3,4,4a,6,10b-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]phenanthridine-5-carboxylate (36). Analogous to the conversion of **27** to **35**, hydrogenation of alcohol **28** gave **36** in 95% yield as colorless oil. IR (neat): 1251, 1689, 2933, 3428 cm^{-1}; ^{1}H NMR: δ 1.22–1.30 (m, 2H), 1.44 (s, 9H), 1.49–1.56 (m, 2H), 1.96–1.98 (m, 1H), 2.31–2.34 (d, J=11.2 Hz, 1H), 2.60 (br s, –OH, 1H), 3.18 (m, 1H), 3.30 (m, 1H), 4.19 (m, 1H), 4.32 (d, J=13.2 Hz, 1H), 4.59 (d, J=13.0 Hz, 1H), 5.90 (s, 1H), 5.91 (s, 1H), 6.57 (s, 1H), 6.74 (s, 1H); ^{13}C NMR: δ 19.5, 27.2, 28.5 (3C), 35.3, 37.2, 44.3, 58.2, 69.1, 80.5, 100.9, 105.6, 106.5, 126.3, 128.6, 145.9, 146.8, 157.1; EIHRMS Calcd for $C_{19}H_{25}NO_5$ $[M]^+$: 347.1733, found: 347.1723.

4.4.10. [(±)-4*R*,4a*R*,10b*R*] *tert*-Butyl 4-methanesulfonyloxy-2,3,4,4a,6,10b-hexahydro-1*H*-phenanthridine-5-carboxylate (37). Alcohol **35** (92 mg, 0.3 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and cooled to 0 °C. To this solution was added Hunig's base (79 μL, 0.45 mmol) and methanesulfonyl chloride (37 μL, 0.45 mmol). The reaction mixture was slowly warmed to room temperature and stirred overnight. The mixture was diluted with dichloromethane (30 mL) and washed with saturated NH_4Cl (20 mL) and brine (20 mL). The dichloromethane layer was extracted, dried, and purified by chromatography over silica gel (20% EtOAc in hexanes) to provide mesylate **37** (52 mg, 45%) as a colorless oil, which was used immediately in the next step. ^{1}H NMR (500 MHz): δ 1.24–1.27 (m, 1H), 1.50 (s, 9H), 1.60–1.73 (m, 3H), 2.03–2.2 (m, 1H), 2.47–2.50 (m, 1H), 2.97 (s, 3H), 3.41 (m, 1H), 4.38–4.41 (m, 1H), 4.51–4.59 (m, 2H), 4.82–4.86 (m, 1H), 7.15–7.28 (m, 4H).

4.4.11. [(±)-3a*S*,11b*R*,11c*R*] 4-Oxa-1,2,3,3a,4,5,11b,11c-octahydro-7*H*-pyrrolo[3,2,1-*de*]phenanthridin-5-one (38). Mesylate **37** (19 mg, 0.05 mmol) dissolved in DMF (0.5 mL) was treated with potassium *tert*-butoxide (6 mg, 0.055 mmol) and heated to 65 °C for 6 h. The reaction mixture was diluted with EtOAc (30 mL), washed with saturated aqueous NH_4Cl (20 mL) and brine (20 mL). The organic layer was dried, concentrated, and purified by chromatography over silica gel (20% EtOAc in hexanes) to provide **38** (7 mg, 63%) as a colorless oil. IR (neat): 1308, 1751, 1800, 2940 cm^{-1}; ^{1}H NMR: δ 1.37–1.43 (m, 1H), 1.44–1.50 (m, 1H), 1.60–1.72 (m, 1H), 1.78–1.86 (m, 2H), 2.10–2.13 (m, 1H), 2.90 (dt, J=12.4 Hz, J=4.8 Hz, 1H), 3.98 (dd, J=7.6 Hz, 4.0 Hz, 1H), 4.35 (d, J=13.2 Hz, 1H), 4.75 (d, J=13.3 Hz, 1H), 4.80 (q, J=7.6 Hz, 1H), 7.15–7.26 (m, 4H); ^{13}C NMR: δ 15.3, 26.5, 28.5, 37.7, 43.1, 53.3, 74.1, 126.8, 127.0, 127.1, 129.3, 130.3, 136.6, 158.4; ESIHRMS Calcd for $C_{14}H_{15}NO_2$ $[M+H]^+$: 230.1181, found: 230.1173.

4.4.12. [(±)-3a*S*,11b*R*,11c*R*] 4-Oxa-1,2,3,3a,4,5,11b,11c-octahydro-7*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-5-one (39). A solution of alcohol **36** (40 mg, 0.12 mmol) in benzene (3 mL) was treated with methyl *N*-(triethylammoniosulfonyl)carbamate[38] (30 mg, 0.17 mmol) and heated to reflux. After 12 h, the reaction was diluted with EtOAc (25 mL) and washed with saturated aqueous NH_4Cl solution, followed by brine. The organic layer was dried, concentrated, and purified by chromatography over silica gel (eluent: 40% EtOAc in hexanes) to provide the oxazolidinone **39** as a colorless oil (18 mg, 56%). IR (neat): 1201, 1484, 1751, 2936 cm^{-1}; ^{1}H NMR: δ 1.35–1.45 (m, 2H), 1.61–1.81 (m, 3H), 2.05–2.08 (m, 1H), 2.77 (dt, J=12.4 Hz, J=4.8 Hz, 1H), 3.93 (dd, J=7.5 Hz, J=4.3 Hz, 1H), 4.23 (d, J=16.2 Hz, 1H), 4.62 (d, J=16.3 Hz, 1H), 4.77 (q, J=7.6 Hz, 1H), 5.93 (s, 2H), 6.58 (s, 1H), 6.59 (s, 1H); ^{13}C NMR: δ 19.2, 26.4, 28.9, 37.7, 43.2, 53.3, 74.1, 101.2, 106.3, 108.7, 123.3, 129.8, 146.8, 146.9, 158.2; ESIHRMS Calcd for $C_{15}H_{15}NO_4$ $[M+H]^+$: 274.1079, found: 274.1077.

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Highly efficient and selective oxidation of secondary alcohols to ketones under organic solvent and transition metal free conditions

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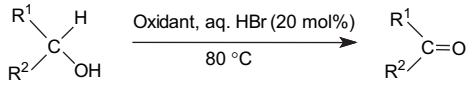
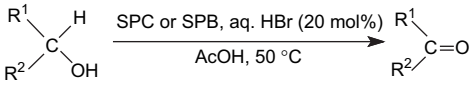
Abstract—The aqueous HBr/H_2O_2 was found to be highly efficient and green catalytic system for the selective oxidation of the secondary alcohols to ketones in excellent yields under organic solvent free conditions. The results of the oxidation of the secondary alcohols with solid alternatives of the aqueous hydrogen peroxide like SPC or SPB are also described.

1. Introduction

The development of catalytic synthetic methodologies using clean oxidants like molecular oxygen and hydrogen peroxide with a view to replace environmentally prohibitive stoichiometric oxidants is an area of current interest.[1] Hydrogen peroxide is an attractive, atom-economic, and environmentally benign oxidant as it is cheap, easily available, and produces only water as by-product. In the recent years, it has been extensively used in developing a variety of synthetically important oxidation methodologies like epoxidation, oxidation of alcohols, aldehydes, and sulfides using transition metal based catalysts both in homogeneous and heterogeneous phases.[2] The oxidation of secondary alcohols to carbonyl compounds is an important synthetic transformation[1a,3] and a variety of transition metal based catalysts, such as methyltrioxorhenium[4] dinuclear iron complexes,[5] vanadium phosphorus oxide,[6] cobalt(II) complexes,[7] Fe^{3+}/montmorillonite-K10 system,[8] and sodium tungstate[9] using hydrogen peroxide as oxidant, have been reported in the literature to accomplish it. However, most of these methods are associated with the limitations such as use of toxic, expensive metals, lower yields of the products, and oxidation of only activated such as benzylic and allylic alcohols. In the recent past, increasing emphasis is being placed toward the development of transition metal free ecofriendly synthetic methodologies to avoid the use of toxic and expensive metals and their complexes. In our preliminary communication,[10] we have reported a new and highly efficient methodology for the oxidation of secondary alcohols to ketones with aqueous H_2O_2 in the presence of catalytic amounts of HBr under very mild conditions. Our further observation that this system works more efficiently under organic solvent free conditions prompted us to describe the full details of this improved protocol along with the applications of solid oxidants in the place of aqueous hydrogen peroxide (Schemes 1 and 2).



Scheme 1.



Scheme 2.

2. Results and discussion

The oxidation of various secondary alcohols, both activated and non-activated, was carried out by heating the reaction mixture of substrate (1 mmol), aqueous 30 wt % hydrogen peroxide (2 mmol), and catalytic amount of aqueous HBr (20 mol %) at 80 °C under organic solvent free conditions. All the alcohols were selectively converted to the corresponding ketones in excellent yields and these results are presented in Table 1. Among the various alcohols studied, benzoins were found to be the most reactive and required shorter reaction times for their oxidation (Table 1, entries 17 and 18). Furthermore, aromatic substituted alcohols were found to be more reactive than aliphatic/alicyclic (Table 1, entries 1 and 2). Alcohols having both secondary

Keywords: Oxidation; Secondary alcohols; Ketones; Solid oxidant.
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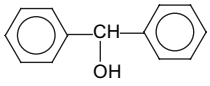
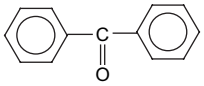
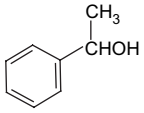
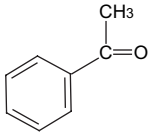
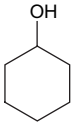
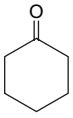
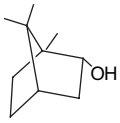
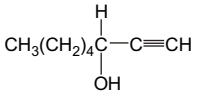
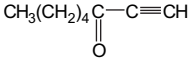
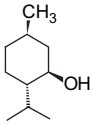
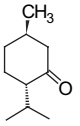
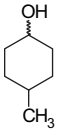
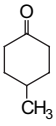
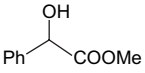
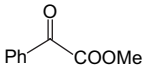
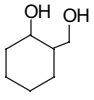
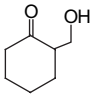
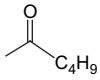
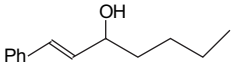
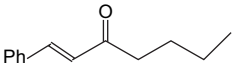
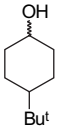
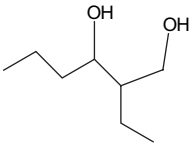
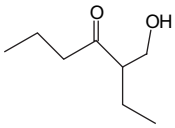
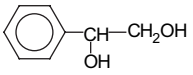
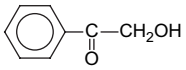
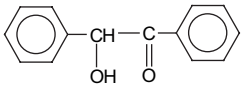
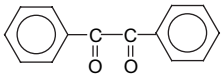
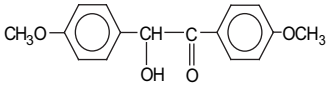
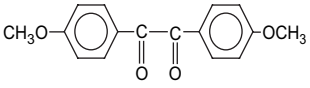
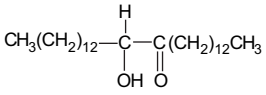
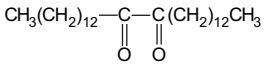
Table 1. Oxidation of secondary alcohols to ketones

Entry	Substrate	Product	Method A		Method B	
			Reaction time (h)	Yield[a]	Reaction time (h)	Yield[a]
1			0.50	98	1.0	96
2			0.75	94	1.0	90
3			2.0	89	2.0	80
4			3.5	75	3.0	72
5			5.00	82	5.5	75
6			4.50	76	4.0	70
7			2.5	85	3.5	82
8			1.75	82	2.0	80
9			2.0	89	2.5	86
10			2.0	87	2.75	84
11			1.5	82	2.5	80
12			3.5	75	4.0	70
13			2.75	85	3.5	82

(*continued*)

Table 1. (*continued*)

Entry	Substrate	Product	Method A		Method B	
			Reaction time (h)	Yield[a]	Reaction time (h)	Yield[a]
14			3.50	88	5.5	84
15			4.5	78	5.5	74
16			1.75	88	2.50	85
17			0.15	98	0.25	96
18			0.25	97	0.33	96
19			2.5	89	3.5	80

Method A—Reaction conditions: secondary alcohol (1 mmol), 30% H_2O_2 (2 mmol), and 48% aqueous HBr (20 mol %) at 80 °C under organic solvent free conditions.
Method B—Reaction conditions: secondary alcohol (1 mmol), 70% TBHP (2.5 mmol), and 48% aqueous HBr (20 mol %) at 80 °C under organic solvent free conditions.
[a] Isolated yields.

as well as primary hydroxyl groups such as 2-ethyl-1,3-hexanediol, 2-hydroxymethylcyclohexanol, and 1-phenyl-1,2-ethanediol were selectively converted into 2-ethyl-1-hydroxy-3-hexanone, 2-hydroxymethylcyclohexanone, and 2-hydroxy-1-phenylethanone, respectively, under these conditions, showing the usefulness of this method for the selective oxidation of secondary alcohols in the presence of primary alcohols (Table 1, entries 10, 15, and 16). Similarly, other functional groups such as double and triple bonds were found to be inert under these conditions (Table 1, entries 5 and 12). To evaluate the efficiency of this method, we also carried out the oxidation of benzhydrol to benzophenone in different organic solvents and these results are shown in Table 2. Although among the various organic solvents studied, acetonitrile was found to be more suitable, but in general, organic solvent free condition was found to be the best and required shorter reaction time. The effect of the reaction temperature was also evaluated and found that the oxidation of benzhydrol to benzophenone was slow at room temperature but could be conducted efficiently at 80 °C. Further increase in temperature affected the oxidation adversely in terms of yield of the benzophenone, probably due to fast decomposition of H_2O_2 at higher temperature.

Table 2. Effect of various solvents[a]

Entry	Substrate	Solvent	Method A		Method B	
			Reaction time (h)	Yield[b]	Reaction time (h)	Yield[b]
1	Benzhydrol	Acetonitrile	0.75	92	1.5	94
2	Benzhydrol	Methanol	2.0	40	3.0	35
3	Benzhydrol	Toluene	4.5	35	5.0	42
4	Benzhydrol	Dichloroethane	1.5	85	2.5	80
5	Benzhydrol	Neat	0.50	98[c]	1.0	96[c]

[a] Reaction conditions: substrate (1 mmol), 30% H_2O_2 (2 mmol), and 48% aqueous HBr (20 mol %), solvent (3 ml) under refluxing condition.
[b] Isolated yield.
[c] As mentioned in Table 1.

2.1. Effect of various bromine sources

To evaluate the effect of various bromine sources, the oxidation of benzhydrol was studied using catalytic amount of different bromine sources (20 mol %) in place of aqueous HBr with aqueous 30% hydrogen peroxide as an oxidant under similar reaction conditions and the results are presented in Table 3. Although the use of molecular bromine and

Table 3. Effect of various bromine sources under neat conditions[a]

Entry	Substrate	Bromine source	Reaction times (h)	Yields (%)[b]
1	Benzhydrol	Aqueous HBr	0.5	98
2	Benzhydrol	Br_2	0.5	94
3	Benzhydrol	NaBr	4.0	30
4	Benzhydrol	KBr	4.0	85
5	Benzhydrol	$KBr+V_2O_5$	1.5	85[c]
6	Benzhydrol	$PyHBr_3$	1.0	92

[a] Reaction conditions as mentioned in Table 1.
[b] Isolated yields.
[c] Using acetonitrile as solvent.

pyridiniumhydrobromide perbromide yielded comparable results, the use of salts such as NaBr and KBr gave very poor yield of the benzophenone. It was also observed that the addition of the catalytic amount of vanadium pentoxide in the reaction mixture of benzhydrol and aqueous H_2O_2 containing catalytic amount of KBr, accelerated the reaction rate and the oxidation was completed within 1.5 h (Table 3, entry 5).

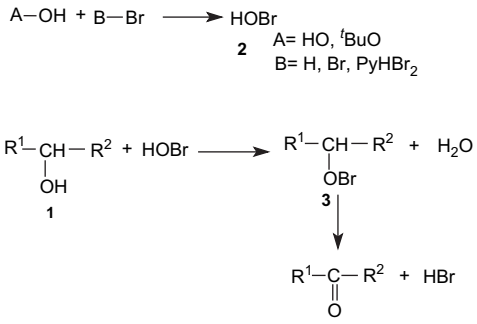
2.2. Effect of various oxidants

Further with a view to evaluate the efficiency of other oxidants, the oxidation of benzhydrol was carried out with molecular oxygen and aqueous 70% *tert*-butylhydroperoxide in the presence of catalytic amount of aqueous HBr under similar reaction conditions. While the reaction did not proceed with molecular oxygen, the use of TBHP as an oxidant yielded results comparable to aqueous H_2O_2. To evaluate the relative efficiencies of TBHP and aqueous H_2O_2 as oxidants, we studied the oxidation of various activated and non-activated alcohols with 70% TBHP in the presence of catalytic amount of aqueous HBr under organic solvent free conditions and the results obtained are presented in Table 1. All the alcohols were selectively converted to the corresponding ketones and reactivity order was found to be the same as obtained by using H_2O_2 as an oxidant. Benzoins in general were again found to be the most reactive while alcohols containing aromatic substituents were found to be more reactive than aliphatic/alicyclic alcohols. Oxidation of benzhydrol when carried out with TBHP in different solvents yielded results similar to hydrogen peroxide and again organic solvent free condition was found to be the most efficient. (Table 2).

The use of solid peroxy compounds, such as sodium perborate (SPB) and sodium percarbonate (SPC) due to their storage stability, crystalline nature, ease of handling, and higher hydrogen peroxide contents, has gained considerable attention in the recent years in various chemical transformations.[11] We therefore studied the oxidation of secondary alcohols to ketones with these solid oxidants using catalytic amount of aqueous HBr (Scheme 2). The protocol developed consists of oxidation of secondary alcohol (1 mmol) with solid oxidant (SPC/SPB) (2.5 mmol) in the presence of catalytic amount of aqueous HBr (20 mol %) in acetic acid (10 mmol) at 50 °C without using any organic solvent as reaction media (Table 4). All the alcohols were selectively and efficiently converted to the corresponding ketones in excellent yields. The presence of acetic acid was found to be essential in these reactions and in its absence the oxidation of benzhydrol in acetonitrile under similar reaction conditions was found to be very slow and gave poor yield of the benzophenone (Table 4, entry 1). This is probably due to the fast release of hydrogen peroxide from the solid oxidants in the presence of acetic acid.[12] To examine the effect of temperature, oxidation of benzhydrol was carried out at different reaction temperatures under similar reaction conditions, using SPC and SPB as solid oxidants. While the reaction was found to be slow at room temperature, at 50 °C the reaction rate was maximum and further increase in the reaction temperature decreases the reaction rate and afforded poor yield of the benzophenone probably due to faster decomposition of solid oxidants at higher temperature.[13]

It is worth mentioning that in contrast to aqueous H_2O_2 and SPC, sodium perborate could oxidize benzhydrol to benzophenone in the presence of catalytic amount of KBr (20 mol %) using acetic acid as a solvent (Table 4, entry 3).

The plausible mechanistic pathway for these reactions may involve the formation of hypobromous acid **2** by the reaction of peroxide oxidant with hydrobromic acid,[10] and its reaction with secondary alcohol **1** to afford hypobromite species **3** which on abstraction of hydrogen yielded corresponding ketone as shown in Scheme 3.



Scheme 3.

3. Conclusion

In summary, the present method describes an efficient, selective, and environmentally friendly synthetic methodology for the oxidation of various secondary alcohols to ketones under transition metal and organic solvent free conditions. The use of SPC and SPB as solid oxidants due to their ease of synthesis/handling, storage stability, and high hydrogen peroxide content makes it more advantageous than previously reported methods. Furthermore, the simplicity of the system, versatility toward a range of activated and non-activated alcohols, selective oxidation of secondary alcohols in the presence of primary alcoholic and other functional groups, simple reaction conditions, and excellent yields of the products make this method a facile, ideal, and attractive synthetic tool for the oxidation of secondary alcohols to ketones.

4. Experimental

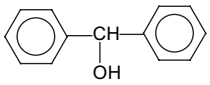
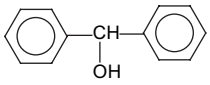
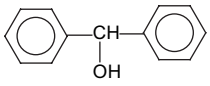
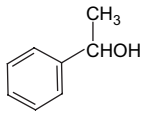
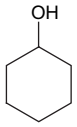
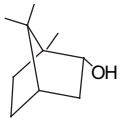
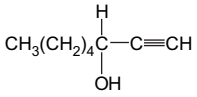
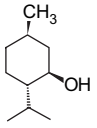
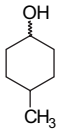
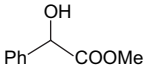
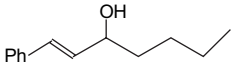
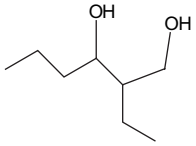
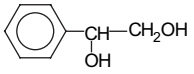
4.1. General

The melting points were determined in open capillaries on a Buchi apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker 300 MHz spectrometer and the chemical shifts (δ) are expressed in parts per million relative to tetramethylsilane (TMS) as an internal standard. The IR spectra were recorded on a Perkin–Elmer FTIR X 1760 instrument.

4.2. General experimental procedure for the oxidation of secondary alcohols using aqueous HBr/H_2O_2 system

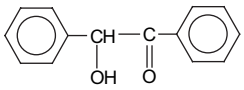
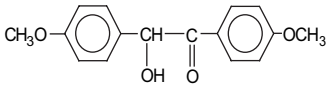
To a stirred solution of secondary alcohol (1 mmol) and aqueous 30% H_2O_2 (2 mmol) was added aqueous HBr (20 mol %) and the mixture was heated at 80 °C under organic solvent free conditions for the period given in

Table 4. Oxidation of secondary alcohols with solid oxidants SPC and SPB

Entry	Substrate	Method A		Method B	
		Reaction time (h)	Yield[a]	Reaction time (h)	Yield[a]
1		4.5	45	6.5	40[b]
2		1.0	96	1.5	94
3		—	—	1.25	90[c]
4		1.5	92	1.5	90
5		3.00	90	3.75	86
6		5.0	82	6.5	80
7		6.5	82	4.5	75
8		5.70	72	6.0	70
9		5.70	72	6.0	70
10		3.5	85	4.5	80
11		2.5	82	2.5	78
12		5.0	75	6.5	69
13		5.5	72	6.0	74

(*continued*)

Table 4. (*continued*)

Entry	Substrate	Method A		Method B	
		Reaction time (h)	Yield[a]	Reaction time (h)	Yield[a]
14		2.0	89	3.50	82
15		0.25	97	0.25	94

Method A—Reaction conditions: secondary alcohol (1 mmol), 48% aqueous HBr (20 mol %), SPC (2.5 mmol), and acetic acid (10 mmol) at 50 °C under organic solvent free conditions.
Method B—Experiments carried out with SPB.
[a] Isolated yields.
[b] Experiment carried out in acetonitrile without acetic acid.
[c] Experiment was carried out using KBr instead of aqueous HBr without any catalyst.

Table 1. Progress of the reaction was monitored by TLC (SiO_2). At the end of the reaction the excess hydrogen peroxide was destroyed by aqueous bisulfite followed by filtration through a Buckner funnel. After filtration, the reaction mixture was taken in dichloromethane and organic layer was washed with water (three times). The combined organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum to afford crude product, which was purified by column chromatography on silica gel using ethyl acetate/hexane (9:1) as an eluent. Evaporation of the solvent yielded corresponding ketones. Reaction times and yields of the products are given in Table 2.

4.3. Typical experimental procedure for oxidation of secondary alcohols using aqueous HBr/solid oxidant system

In a stirred mixture of benzhydrol (0.18 g, 1 mmol), SPB (0.39 g, 2.5 mmol), and acetic acid (0.6 ml, 10 mmol) was added dropwise aqueous HBr (20 mol %, 0.2 mmol, 0.03 ml) at 50 °C. The mixture was stirred for 2 h. After completion of the reaction, the solvent was evaporated under vacuum. The residue thus obtained was extracted with water and dichloromethane. The organic layer was removed and washed again with water (two times). Finally, the organic layer was removed and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to yield pure benzophenone (0.171 g, 94%). All the products were identified by comparing their physical and spectral data (IR and ^{1}H NMR) with the literature values. The physical and spectral data of the products are given below.

Benzophenone (Table 1, entry 1):[14] mp 47 °C (lit. 48–49 °C)[15] IR (KBr): 3028, 1661, 1489, 1204, 1151 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 7.30–7.55 (m, 6H, Ar H), 7.90–8.00 (m, 4H, Ar H).

Acetophenone (Table 2, entry 2):[16] oil, IR (KBr): 3086, 2923, 1685, 1430, 1267, 1160 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 2.50 (s, 3H, CH_3), 7.45–7.85 (m, 5H, Ar H).

Cyclohexanone (Table 1, entry 3):[14] bp 153 °C/760 mm (lit. 155 °C/760 mm)[15] IR (KBr): 2940, 1712, 1449, 1235 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 1.80–2.15 (m, 6H, CH_2), 2.30–2.38 (m, 4H, CH_2).

(±)-Camphor (Table 1, entry 4):[17] mp 172–173 °C (lit. 175–177 °C)[15] IR (KBr): 2935, 1715, 1440, 1369 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 0.97 (s, 6H, CH_3), 1.05–1.40 (m, 5H, CH_2, CH), 1.90 (s, 3H, CH_3), 2.10–2.20 (m, 2H, CH_2).

1-Octyne-3-one (Table 1, entry 5): oil, IR (KBr): 3278, 2950, 2105, 1680, 1180 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 0.89–0.95 (m, 3H CH_3), 1.10–1.40 (m, 6H, CH_2), 2.30–2.35 (m, 2H, CH_2), 3.10 (s, 1H, ≡CH).

(−)-Menthone (Table 1, entry 6):[14] bp 203–204 °C/760 mm (lit. 207–210 °C/760 mm)[15] IR (KBr) 2940, 1710, 1443, 1369 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 0.80–0.95 (m, 10H, CH_3, CH_2), 1.38–1.75 (m, 5H, CH_2, CH), 2.18–2.52 (m, 3H, CH_3).

4-Methylcyclohexanone (Table 1, entry 7):[18] colorless oil, IR (KBr): 2932, 2869, 1720, 1156 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 0.92 (d, J=6.8 Hz, 3H, CH_3), 1.10–1.25 (m, 5H, CH_2, CH), 1.24–1.32 (m, 4H, CH_2).

Methyl benzoylformate (Table 1, entry 8):[19] bp 247–248 °C (lit. 248–250 °C/760 mm),[15] IR (KBr): 3076, 2941, 1740, 1680, 1315, 1204. ^{1}H NMR ($CDCl_3$) δ ppm: 3.95 (s, 3H, CH_3), 7.40–8.20 (m, 5H, Ar H).

2-Methylcyclohexanone (Table 1, entry 9):[18] colorless oil, IR (KBr): 2935, 2863, 1718, 1370, 1156 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 1.00 (d, J=7.0 Hz, 3H, CH_3), 1.34–1.36 (m, 6H, CH_2), 1.62–1.80 (m, 3H).

2-Hydroxymethylcyclohexanone (Table 1, entry 10):[20] colorless oil, IR (KBr) 3421, 2960, 1710, 1169 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 1.36–2.61 (m, 9H, CH_2), 2.79 (br s, 1H, OH), 3.50–3.67 (m, 2H, CH_2).

Hexan-2-one (Table 1, entry 11):[16] oil, IR (KBr): 2961, 2937, 1718, 1368, 1165 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 0.90 (t, J=7.2 Hz, 3H, CH_3), 1.27–1.32 (m, 2H, CH_2), 1.52–1.58 (m, 2H), 2.14 (s, 3H, CH_3) 2.43 (t, J=7.2 Hz, 2H, CH_2).

1-Phenyl-1-hepten-3-one (Table 1, entry 12):[21] colorless oil, IR (KBr): 3010, 2961, 2937, 1720, 1364, 1162 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ ppm: 0.90 (t, J=7.0 Hz, 3H, CH_3), 1.35–1.55 (m, 4H, CH_2), 2.58 (t, J=6.2 Hz, 2H, CH_2),

6.74 (d, 1H, J=13.2 Hz, =CH), 7.28–7.60 (m, 6H, =CH and Ar H).

4-tert-Butyl cyclohexanone (Table 1, entry 13):[23a,24a] mp 45–47 °C (lit. 47–50 °C)[15] IR (KBr): 2955, 1718, 1468, 1366 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 0.90 (s, 9H, CH_3), 1.30–1.60 (m, 5H), 2.10–2.35 (m, 4H).

Cyclopentanone (Table 1, entry 14):[23b,24b] bp 129–130 °C/760 mm (130–131 °C/760 mm)[15] IR (KBr) 2942, 1446, 1236 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 1.82–210 (m, 4H, CH_2), 2.34–2.40 (m, 4H, CH_2).

3-Hydroxymethyl-4-heptanone (Table 1, entry 15):[20] colorless liquid. IR (KBr): 3422, 1705 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 0.90 (t, J=7.4 Hz, 3H, CH_3), 0.95 (t, J=7.5 Hz, 3H, CH_3), 1.39–1.70 (m, 4H, CH_2), 2.14 (br s, 1H, OH), 2.45 (t, J=7.3 Hz, 2H, CH_2), 2.60–2.68 (m, 1H, CH), 3.70–3.75 (m, 2H, CH_2).

2-Hydroxy-1-phenylethanone (Table 1, entry 16):[22] mp 83–84 °C (lit. 84–85 °C) IR (KBr): 3428, 1682 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 2.98 (s, 1H, OH), 4.85 (s, 2H, CH_2), 7.50–7.85 (m, 5H, Ar H).

Benzil (Table 1, entry 17):[14] mp 92–93 °C (lit. 94–95 °C)[15] IR (KBr): 3043, 1678, 1659, 1594, 1315, 1176 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 7.52–7.61 (m, 6H, Ar H), 7.80–7.89 (m, 4H, Ar H).

4,4′-Dimethoxybenzil (Table 1, entry 18):[25] mp 130–131 °C (lit. 132–134 °C)[15] IR (KBr): 3046, 2959, 1682, 1598, 1314, 1171 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 3.80 (s, 6H, CH_3), 6.94 (d, J=8.0 Hz, 4H, Ar H), 7.94 (d, J=8.0 Hz, 4H, Ar H).

14,15-Octacosanedione (Table 1, entry 19): mp 67–69 °C (lit. 70–72 °C)[26] IR (KBr) 2941, 2877, 1724, 1449, 1389, 1163 cm^{-1}. ^1H NMR ($CDCl_3$) δ ppm: 0.89–1.00 (m, 6H, CH_3), 1.10–1.72 (m, 44H, CH_2), 2.30 (m, 4H, CH_2).

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HSAB-driven chemoselective N1-alkylation of pyrimidine bases and their 4-methoxy- or 4-acetylamino-derivatives

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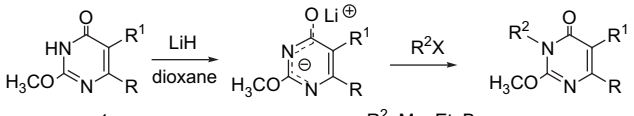
Abstract—The lithium salts of the conjugated bases of 4-methoxy- and 4-acetylamino-2(1*H*)-pyrimidinones **1–3** undergo highly chemoselective N^1-methylation or ethylation when treated with methyl- or ethylsulfate (hard electrophiles) in dry dioxane, while the use of DMF as solvent results in competitive O^2-alkylation. Potassium salts of the same bases in DMF undergo prevalent O^2-attack. Under the same conditions, a similar but less chemoselective behaviour is observed in alkylation of thymine and uracil, where some N^3-attack occurs. This can be rationalised in terms of the HSAB principle.

1. Introduction

N^1-Alkylated nucleic acid bases are widely used as nucleoside analogs to exert anti-viral[1] and anti-inflammatory activities,[2] to inhibit the activity of reverse transcriptase in the HIV virus,[3] as anti-cancer agents,[4] as agonists of AMPA and kainate receptors[5] and as GnRH receptor antagonists.[6] Although N^1-alkylated pyrimidinones can be obtained by intramolecular condensation[6] or by Pd(0)-catalysed reactions,[1a] the most general approach involves an S_N^2 displacement. In this process, the conjugate base of the substrate is usually reacted with electrophiles (alkyl phosphates,[7] halides[3b,5,7b,8] and diazoalkanes[9]) in polar solvents. However, due to the tetradentate nucleophilic nature of the intermediate conjugated base, these reactions suffer from low chemoselectivity and lead to mixtures of N^1-monoalkylated and N^1,N^3-dialkylated products[1a,3b,5,7a,8] along with some products resulting from O^2- and O^4-attack.[7a,9a] In order to avoid these problems, various methodologies using 2,4-dimethoxy-[10] or 2,4-disilyloxy-pyrimidines[11] as starting materials have been developed and are widely applied.[3b,c,e]

Formation of the N^1,N^3-dialkylated products can be easily explained in terms of deprotonation and subsequent realkylation of the N^1-monoalkylated products. However, the lack of N^3-monoalkylation derivatives is difficult to explain in terms of the higher acidity of N^1–H than N^3–H, as stated in the literature.[3e] In fact, Wittenburg[12] and Ganguly and Kundu[13] have shown that first deprotonation of thymine occurs at N^3 with a pK_a value of 9.9, but the N^{3-} species is in equilibrium with the N^{1-} species in a 1:1 ratio. The same behaviour is also operative in uracil, with a pK_a value of 9.5.[13,14] Therefore, the lack of N^3-attack should be related to an intrinsic higher reactivity of N^1.

While investigating the hypothesis that the HSAB principle[15] is responsible for the above regioselectivity, we first studied the chemoselectivity on simplified models: 2-methoxy-4(3*H*)-pyrimidinone derivatives (Scheme 1), where the intermediate tridentate conjugate base can undergo N^1-, N^3- or O^4-attack. We reported[16] that a very high N^3-chemoselectivity is observed when reactions are carried out in a low polarity solvent (dioxane) and both a hard base (LiH) and a hard electrophile (alkylsulfate or tosylate) are used. Under these conditions, tight ionic pairs



Scheme 1.

Keywords: Pyrimidinones; Alkylation; Regioselection; HSAB principle.
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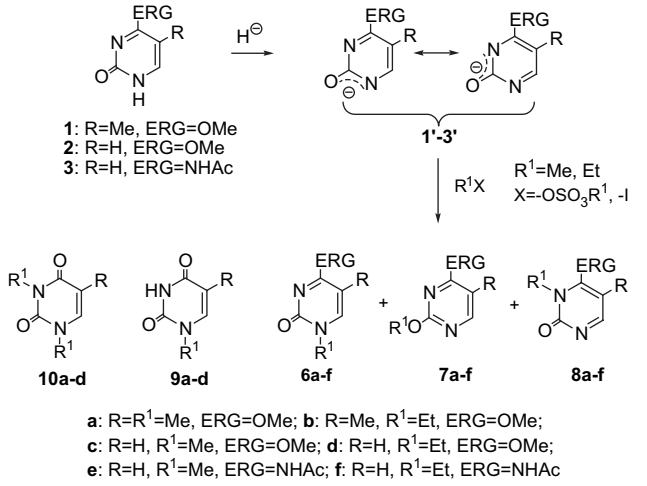
between the O^4-anion and the lithium cation are obtained, so preventing O^4-alkylation, in addition, the electron releasing methoxy group at C_2 enhances the hardness of the N^3-anion with respect to that of N^1, giving rise to a favourable hard–hard match with the hard alkylating agents. Accordingly, the use of either softer counterions (Na^+ or K^+) or a softer alkylating agent (BnBr) results in competitive O^4- or N^1-attack.[16]

The above findings prompted us to further investigate the applicability of the HSAB principle to the selective alkylation of pyrimidinone derivatives. We proposed that, moving the methoxy group from C_2 to C_4 would result in an inversion of the hardness of the two nitrogen in favour of the N^1 position and so give selective N^1-alkylations. Hence, in order to confirm our hypothesis and to take synthetic advantage, we carried out a model study to investigate the effects of different solvents and alkylating agents on the distribution of products arising from S_N^2 alkylation of the 4-methoxy- and the 4-acetylamino-2(1*H*)-pyrimidinones **1**–**3** (Scheme 2). In addition, to obtain information about the real effect of the electron releasing group (ERG) at O^4 (or O^2) on the chemoselectivity, the same studies were extended to the unprotected pyrimidine bases thymine **4** and uracil **5** (Scheme 4).

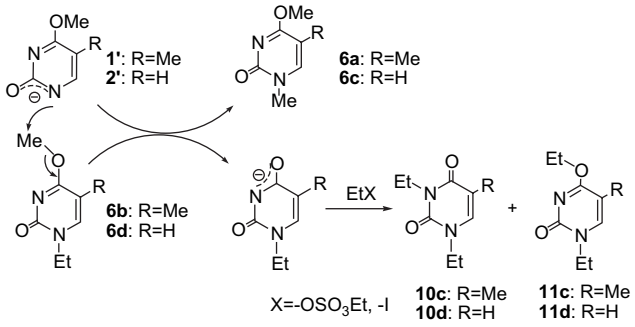


Scheme 2.

2. Results and discussion

Initial experiments were concerned with the chemoselection of the methylation or ethylation of **1**–**3** where, due to the tridentate nucleophilic character of the intermediate metal salts **1′**–**3′**, the monoalkylated compounds **6a–f**, **7a–f** and **8a–f** (Scheme 2) were expected as products, with their relative amounts depending on the metal counterion, the solvent and the alkylating agent. Substrate **3** is commercial, while compounds **1** and **2** were prepared as reported in the literature.[17] Surprisingly, we noticed that substrates **1**–**3** are remarkably less soluble in dioxane than the corresponding, previously studied,[16] 2-methoxy-derivatives (Scheme 1). This feature was found to be in agreement with the calculated logarithms of the partition coefficient for *n*-octanol/water,[18] which are −0.599, −1.098 and −1.680 for compounds **1**, **2** and **3**, respectively, versus 0.133 and −0.385 for the 2-methoxy-derivatives corresponding to **1** and **2**. This lower solubility necessitated the reactions in dioxane to occur in heterogeneous phase, even at 60 °C, and accounted for the low reaction rates observed.

Trial experiments allowed the isolation and characterisation (see Section 4 and Scheme 2) of pure standards of the unprecedented products of N^1- and O^2-alkylation **6b**, **6f**, **7b**, **7d** and **7f**, as well as the known compounds **6a**,**c**,**d**,[9a] **6e**,[19] **7a**,**c**[17] and **7e**,[20] while products **8a–f** were never found. In addition, variable amounts of the commercial compounds **9a**,**c**,**d** and **9b**,[21] resulting from hydrolysis of the methoxy group at C_4 during either workup or chromatographic separation, were isolated from the reaction mixtures of **1** and **2** together with the already known N^1,N^3-double alkylation products **10a–d**.[22–24] Finally, small amounts of the known N^1,O^4-diethyl derivatives **11b**[24] and **11d**[9a] (Scheme 3) were identified in some ethylation experiments.



Scheme 3.

The metal salts **1′**–**3′** were prepared using LiH or KH as hard or soft counterions, respectively, while dry dioxane (ε=2.2) or DMF (ε=38.2) were used as solvents in order to generate either tight or loose ionic couples. Dry dimethylsulfate (DMS) and diethylsulfate (DES) were chosen as hard electrophiles, while the corresponding iodides were used as soft alkylating agents. All reactions were monitored and analysed by HPLC, over 24 h, in the case of substrates **1** and **2**, and over 48 h for substrate **3**. Results are shown in Table 1, where the HPLC percentages of the obtained products **6** (including their hydrolysis products **9**) and **7** are reported in the N^1 and O^2 columns, respectively, together with the corresponding N^1/O^2 ratios. HPLC percentages of the unexpected (vide infra) products **10**, arising from N^1,N^3-double alkylation, are also reported, while minor products (less than 1%) are not shown.

The data in Table 1 support our hypothesis of HSAB-driven chemoselectivity. N^3-Alkylation is always absent so that only N^1 and O^2 compete for the electrophile. This can be explained as a result of the very hard character of N^1 versus N^3. When the LiH/dioxane/alkylsulfate system is used to favour both tight O^-Li^+ pairs and hard–hard nucleophile–electrophile matches, N^1-alkylation is always the main process with chemoselectivities ranging from >200:1 (methylations) or 36:1 (ethylations) for the 4-methoxypyrimidinones **1**–**2**, and up to >30:1 for the acetylcytosine **3**. Accordingly, the use of soft electrophiles, such as iodides, does not affect the chemoselectivity, but results only in a poorer conversion of the substrates.

Alkylation of the N^4-acetylcytosine **3** showed some differences with that of **1** and **2**. The reactivity was always lower

Table 1. HPLC % distribution of products in the S_N^2 alkylation of **1–3** under different conditions

Run	Base	R^1X	Solvent	N^1	O^2	N^1,N^3	Unreacted substrate	N^1/O^2 ratio
4-Methoxy-5-methyl-2(1*H*)-pyrimidinone, **1**[a]								
1	LiH	DMS	Dioxane	93.8	—	—	6.1	>200
2	LiH	MeI	Dioxane	74.0	—	—	26.0	>200
3	LiH	DES	Dioxane	76.2	2.1	—	21.0	36.3
4	LiH	EtI	Dioxane	21.6	Traces	—	75.5	>20
5	KH	DES	Dioxane	51.2	24.5	—	21.2	2.2
6	LiH	DMS	DMF	76.0	8.8	—	14.8	8.6
7	LiH	MeI	DMF	75.2	9.8	0.4	13.6	7.7
8	LiH	DES[b]	DMF	42.1	37.4	3.8	15.5	1.1
9	LiH	EtI[b]	DMF	29.9	24.0	8.0	36.5	1.2
10	KH	DES	DMF	9.8	64.2	—	25.0	0.1
4-Methoxy-2(1*H*)-pyrimidinone, **2**[a]								
11	LiH	DMS	Dioxane	90.0	—	5.0	4.5	>200
12	LiH	MeI	Dioxane	87.6	—	5.1	7.1	>200
13	LiH	DES[c]	Dioxane	89.7	2.5	4.5	3.0	35.9
14	LiH	EtI[c]	Dioxane	29.1	Traces	1.0	69.0	>20
15	KH	DES	Dioxane	46.7	33.7	Traces	18.0	1.4
16	LiH	DMS	DMF	80.1	8.7	4.0	6.0	9.2
17	LiH	MeI	DMF	65.5	8.5	5.0	20.7	7.7
18	LiH	DES[c]	DMF	55.3	37.6	3.3	3.0	1.5
19	LiH	EtI[c]	DMF	43.8	31.2	5.9	18.5	1.4
20	KH	DES	DMF	40.6	54.0	2.1	2.5	0.7
4-Acetylamino-2(1*H*)-pyrimidinone, **3**[d]								
21	LiH	DMS	Dioxane	77.9	2.1	—	17.8	37.1
22	LiH	MeI	Dioxane	17.0	0.5	—	81.9	34.0
23	LiH	DES	Dioxane	54.5	1.8	—	43.5	30.2
24	LiH	EtI	Dioxane	2.6	—	—	97.4	—
25	KH	DES	Dioxane	44.5	30.3	—	24.2	1.5
26	LiH	DMS	DMF	60.0	6.5	—	33.0	9.2
27	LiH	MeI	DMF	56.4	7.0	—	36.2	8.0
28	LiH	DES	DMF	65.8	8.8	—	25.0	7.5
29	LiH	EtI	DMF	55.0	7.9	—	36.8	7.0
30	KH	DES	DMF	37.5	39.6	—	21.0	0.9

[a] Reactions carried out at 60 °C for 24 h.
[b] Small amounts of **6a** and **11b** were detected.
[c] Small amounts of **6c** and **11d** were detected.
[d] Reactions carried out at 60 °C for 48 h.

and small amounts of O^2-alkylation products were always present, even in methylations. This is not unexpected[25] and could be related to the weaker electron releasing effect of the acetylamino group, resulting in a smaller difference in hardness between O^2 and N^1.

In contrast, the use of DMF, where loose O^-Li^+ pairs were expected, gave rise to a considerable amount of O^2-alkylation, which increased with the hardness of the electrophile (compare runs 6, 16 and 26 with runs 8, 18 and 28, respectively). This decrease of the N^1/O^2 chemoselectivity confirms the typical trend of reactions, which obey the HSAB principle. Accordingly, the use of the soft K^+ cation (runs 5, 10, 15, 20, 25 and 30) led to a sharp inversion of chemoselectivity in favour of O^2-alkylation as observed previously.[25]

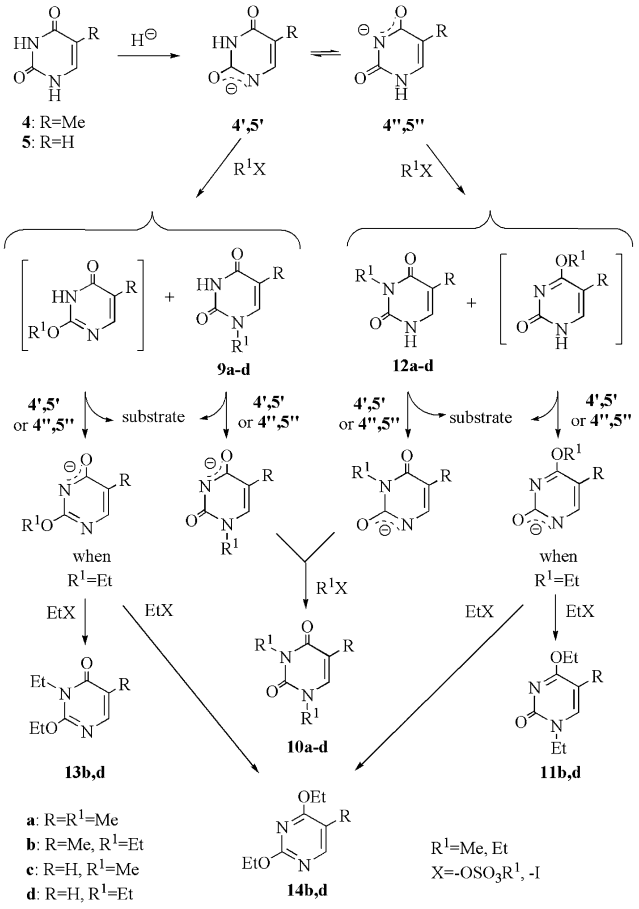
Formation of the unexpected N^1,N^3-double alkylation products **10**, usually reported as important by-products in the literature,[1a,3b,5,7b,8] is the result of a side reaction. N^1,N^3-Dialkylated products occur in very small amounts for the methoxy derivative **1** and increased amounts for substrate **2**, but are absent for the N^4-acetylcytosine **3**. This trend can be rationalised as a result of a competitive nucleophilic displacement of the O^4-methyl group by the suitable conjugated base **1′** (or **2′**), as shown in Scheme 3 for the ethylation reactions.

This side reaction is suppressed by the steric hindrance of the methyl group at C_5 in the thymine derivative **1** and is impossible for the acetylcytosine **3**.

The detection of small amounts of the N^1,O^4-dimethylated products **6a** (or **6c**) and the corresponding diethylated products **11b** (or **11d**), together with the N^1,N^3-derivatives **10b** (or **10d**), in some ethylation reactions (Table 1, runs 8, 9, 13, 14, 18 and 19), supports this hypothesis. In conclusion, N^1-attack was the most prevalent, if not the only, monoalkylation process observed with the LiH/alkylsulfate/dioxane system and this is in favour of a strong activation at N^1 due to a N^1-hardening effect operated by the ERG at C_4.

With the above results in hand, and in order to verify the real effect of the C_4-ERG (or C_2-ERG)[16] in driving chemoselection, we extended the same experiments to the C_4-unprotected pyrimidine bases thymine **4** and uracil **5**. Here, as outlined in Section 1, single proton extraction can give rise to the two different conjugated bases **4′**, **4″** and **5′**, **5″** (Scheme 4) in a ratio of 1:1,[12–14] so that each alkylation could afford, in theory, four monoalkylated compounds. Trial experiments showed that alkylation of **4** and **5** was always very slow giving the monoalkylation products **9a–d** and **12a–d**[16] together with the N^1,N^3-double alkylation derivatives **10a–d**, as major products. O^2- or O^4-Monoalkylation products were never found, but small amounts of the already

mentioned N^1,O^4-diethyl derivatives **11b**,**d** together with the corresponding unprecedented N^3,O^2-isomers **13b**,**d** were identified in some ethylation experiments. The latter compounds were not isolated, but detected by both GC–MS and by their conversion into **12b**,**d**, respectively, after acid hydrolysis of their mixtures.



Scheme 4.

As depicted in Scheme 4, compounds **11** and **13** are most likely derived from an easier re-alkylation of the more reactive and previously formed O^2- or O^4-monoalkylation products, respectively. Therefore, the relative amounts of N,O-bis-alkylation products account for the percentage of O^2- or O^4-attack. The same route is followed for the formation of **10** from **9** or **12**.

Product distributions under different conditions were measured as described above for substrates **1**–**3** and the results are reported in Table 2, where N^1, N^3 and N^1,N^3 columns report the relative amounts of compounds **9**, **12** and **10**, while columns O^2 and O^4 report the amounts of compounds **13** and **11**, respectively. Ratios N^1/N^3, N^1/O^2 and N^1/O^4 are also reported.

The data shown in Table 2 depict a trend similar to that observed for compounds **1**–**3**, but both chemoselection and reactivity are reduced. N^1-Alkylation was certainly the preferred process, but, in contrast to previous reports,[1a,3b,3e,5,7a,8] N^3-attack was always competitive, especially with soft electrophiles and/or in polar solvent. In addition, O^2- and O^4-attack also took place, even in dioxane, when a harder ethyl electrophile was used. Therefore, the N^1 versus N^3-chemoselectivity was still good for alkylations carried out with the LiH/dioxane/sulfate system, but dropped down if softer iodides and/or polar solvents were used.

Irrespective of the hard/soft features of the reagents, N^1,N^3-double alkylation products were always present, their relative amount increasing with polarity. This can be related to an easier proton transfer (Scheme 4) from the monoalkylated products **9** and **12** to the conjugated bases **4′**, **5′** (or **4″**, **5″**). As expected, a sharp increase of both N^3- and O-alkylation products, including the known O^2,O^4-bis-alkylation derivatives **14b**,**c**,[9,24] was observed when K^+ was used as counterion and DMF as solvent (runs 9 and 18 in Table 2). Therefore, the most used reaction conditions for preparative purposes[1a,3b,5,7–9] give rise to the worst chemoselection.

Table 2. HPLC % distribution of products in the S_N^2 alkylation[a] of either lithium or potassium salts of **4** and **5** in polar or apolar solvents

Run	Base	R^1X	Solvent	N^1	N^3	O^2	O^4	N^1,N^3	Unreacted substrate	N^1/N^3 ratio	N^1/O^2 ratio	N^1/O^4 ratio
Thymine, **4**												
1	LiH	DMS	Dioxane	68.7	2.2	—	—	3.6	25.0	31.2	—	—
2	LiH	MeI	Dioxane	47.4	2.2	—	—	1.1	48.5	21.5	—	—
3	LiH	DES	Dioxane	63.2	3.2	1.4	2.8	4.7	23.5	19.7	45.1	22.5
4	LiH	EtI	Dioxane	21.7	2.3	—	—	1.0	68.7	9.4	—	—
5	LiH	DMS	DMF	17.8	4.0	—	—	5.8	71.8	4.5	—	—
6	LiH	MeI	DMF	18.8	5.5	—	—	4.5	69.7	3.4	—	—
7	LiH	DES	DMF	32.1	3.6	1.7	2.0	9.0	49.7	8.9	18.9	16.0
8	LiH	EtI	DMF	30.6	3.9	1.9	2.1	11.5	48.5	7.8	16.1	14.6
9	KH	DES[b]	DMF	34.0	8.7	2.7	6.3	13.8	29.2	3.9	12.6	5.4
Uracil, **5**												
10	LiH	DMS	Dioxane	59.7	2.2	—	—	5.0	32.0	27.1	—	—
11	LiH	MeI	Dioxane	12.2	0.8	—	—	2.4	83.8	15.2	—	—
12	LiH	DES	Dioxane	22.9	1.4	0.6	1.0	2.4	70.8	16.3	37.9	22.9
13	LiH	EtI	Dioxane	16.5	2.3	—	—	7.1	73.2	7.1	—	—
14	LiH	DMS	DMF	29.7	4.7	—	—	10.5	54.6	6.3	—	—
15	LiH	MeI	DMF	41.0	8.2	—	—	2.2	47.5	5.0	—	—
16	LiH	DES	DMF	41.3	5.1	2.0	2.7	12.1	35.9	8.0	20.6	15.3
17	LiH	EtI	DMF	38.8	5.2	2.1	2.8	14.4	36.2	7.5	18.5	13.9
18	KH	DES[c]	DMF	34.4	8.5	2.6	5.8	18.6	25.3	4.0	13.2	5.9

[a] Methylations were carried out at 60 °C for 24 h, ethylations for 72 h.
[b] Compound **14b** (3.2%) was also present.
[c] Compound **14d** (4.0%) was also present.

Despite the already mentioned 1:1 ratio between the two conjugate bases of thymine and uracil,[12–14] the N^1 position was always preferentially alkylated and this behaviour was enhanced when hard–hard matches were set up by the use of the LiH/dioxane/sulfate system. This is true for an HSAB-driven chemoselection, if N^1 is assumed to be harder than N^3, and accounts for the widely reported preferential alkylation at that position.

3. Conclusion

The above results show that chemoselection in S_N^2 alkylation of pyrimidine bases **4**–**5** is driven by the HSAB principle, the N^1 position being intrinsically harder than the N^3. Therefore, the use of both hard alkylating agents, such as sulfates, and apolar solvents results in an increased N^1-attack. Moreover, the intrinsic higher hardness of N^1 is strongly enhanced by the introduction of ERG at C_4, as in compounds **1**–**3**, and this gives rise to both higher reactivity and almost quantitative N^1-chemoselectivity, under the above reaction conditions. In contrast, when the ERG is at C_2, as in the 2-methoxy-pyrimidinones (Scheme 1), the N^3 site becomes harder than that of the N^1 and regioselection is completely reversed to give quantitative N^3-alkylation.[16]

In conclusion, high yielding chemoselective alkylations at either N^1 or N^3 can be obtained if the HSAB principle is applied to pyrimidinone derivatives carrying ERGs at the C_4 or C_2 position, respectively.

4. Experimental

4.1. General

Chromatographic separations were carried out on silica gel (Fluka, 70–230 mesh) washed with 0.1 M HCl and rinsed with hot distilled water, while Fluka silica gel TLC plates (5–17 μm, 0.25 mm) were used for TLC analyses. HPLC analyses were performed on a TSP Spectra Series P200 apparatus equipped with a Thermo Hypersil BDS C18 column (250×4.6 mm, 5 μm) at λ=254 nm.

GC/MS analyses were obtained on a FISON GC 8000 gas-chromatograph equipped with a capillary column (MEGA SE52MS, 30 m-long, ID 0.25 mm, film thickness 0.25 μm) and coupled with a FISON MD800 mass detector. FTIR spectra were recorded in $CHCl_3$ on a Brucker Vector 22 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were performed in CD_3OD on Gemini 200 and VARIAN XL300 spectrometers. HRMS spectra were recorded with Micromass Q-TOF *micro* Mass Spectrometer (Waters).

Substrates **1** and **2** were prepared according to the literature,[17] while substrates **3**–**5** were purchased (Sigma–Aldrich). Alkylsulfates (DMS and DES) were distilled to neutrality over $NaHCO_3$, while alkyliodides (MeI and EtI) were distilled over P_2O_5.

4.2. Products from alkylation of 1–5

LiH (1.2 equiv) was added to a solution of the appropriate substrate (1.0 g) in dry DMF (80 ml) under a dry Ar atmosphere and the mixture was stirred at 60 °C for 0.5 h. Next, 1.2 equiv of the appropriate freshly distilled alkylsulfate (DMS or DES) were added, the solution was stirred at 60 °C and the reaction progress was monitored by TLC. After two days (three days in the case of uracil **5**), the solution was left to cool, neutralised while stirring with 1.2 equiv of solid NH_4Cl and evaporated under reduced pressure at 40 °C. The residue was suspended in *n*-butanol, dried over anhydrous Na_2SO_4, filtered and evaporated to leave a crude mixture, which was separated by chromatography (SiO_2, 1:100; eluent $CHCl_3$/MeOH=99.5:0.5).

All isolated new compounds were determined to be >95% pure by HPLC or GLC and are reported in elution order together with their spectroscopic properties. All isolated known products were identified by comparison with either authentic samples or by their literature described spectroscopic properties and are reported in elution order.

4.2.1. Products from 4-methoxy-5-methyl-2(1*H*)-pyrimidinone 1.

4.2.1.1. Methylation. 5-Methyl-2,4-dimethoxypyrimidine **7a** (110 mg, 10%);[17] 1,3,5-trimethyl-2,4-pyrimidinedione **10a** (12 mg, 1%);[24] 4-methoxy-1,5-dimethyl-2(1*H*)-pyrimidinone **6a** (0.65 g, 59%);[9a] 1,5-dimethyl-2,4(3*H*)-pyrimidindione **9a** (0.15 g, 15%).

4.2.1.2. Ethylation.

*4.2.1.2.1. 2-Ethoxy-4-methoxy-5-methylpyrimidine (**7b**).* Colourless oil (0.42 g, 35%); δ_H 8.05 (1H, q, *J* 1.0 Hz, C_6H), 4.23 (2H, q, *J* 7.0 Hz, O^2-CH_2), 3.95 (3H, s, O^4-CH_3), 2.07 (3H, d, *J* 1.0 Hz, C_5-CH_3), 1.33 (3H, t, *J* 7.0 Hz, CH_3); δ_C 170.8 (C_4), 168.8 (C_2), 161.3 (C_6), 110.2 (C_5), 63.4 (O^2-C), 55.0 (O^4-C), 13.6 (Me), 13.1 (C_5-Me); *m/z* (%) 168 (38), 153 (60), 140 (53), 125 (36), 124 (100), 82 (70), 55 (83); ν_{max} 1570, 1455, 1256, 1065 cm^{-1}; HRMS found, 168.0898. $C_8H_{12}N_2O_2$ requires 168.0899.

*4.2.1.2.2. 1-Ethyl-4-methoxy-5-methyl-2(1H)-pyrimidinone (**6b**).* White solid (0.46 g, 39%); δ_H 7.17 (1H, q, *J* 1.0 Hz, C_6H), 3.96 (3H, s, OCH_3), 3.86 (2H, q, *J* 7.0 Hz, N^1-CH_2), 1.92 (3H, d, *J* 1.0 Hz, C_5-CH_3), 1.31 (3H, t, *J* 7.0 Hz, CH_3); δ_C 170.66 (C_2), 156.60 (C_4), 143.60 (C_6), 104.61 (C_5), 54.53 (O-C), 45.16 (N^1-C), 14.55 (CH_3), 12.10 (C_5-CH_3); *m/z* (%) 168 (100), 167 (20), 140 (60), 125 (22), 110 (78), 82 (20), 55 (19); ν_{max} 1644, 1628, 1545, 1485 cm^{-1}; HRMS found, 168.0896. $C_8H_{12}N_2O_2$ requires 168.0899.

4-Ethoxy-1-ethyl-5-methyl-2(1*H*)-pyrimidinone **11b** (4 mg, 0.3%);[24] 1,3-diethyl-5-methyl-2,4-pyrimidindione **10b** (52 mg, 4%);[22] 1-ethyl-5-methyl-2,4(3*H*)-pyrimidindione **9b** (22 mg, 2%).[21] Traces of **6a** were also found by GC–MS.

4.2.2. Products from 4-methoxy-2(1*H*)-pyrimidinone 2.

4.2.2.1. Methylation. 2,4-Dimethoxypyrimidine **7c** (120 mg, 11%);[17] 1,3-dimethyl-2,4-pyrimidindione **10c** (45 mg, 4%);[24] 4-methoxy-1-methyl-2(1*H*)-pyrimidinone **6c** (0.77 g, 70%);[9a] 1-methyl-2,4(3*H*)-pyrimidindione **9c** (80 mg, 8%).

4.2.2.2. Ethylation.

*4.2.2.2.1. 2-Ethoxy-4-methoxypyrimidine (**7d**).* Colourless oil (0.41 g, 39%); δ_H 8.17 (1H, d, *J* 5.5 Hz, C_6H), 6.42 (1H, d,

J 5.5 Hz, C_5-H), 4.40 (2H, q, *J* 7.0 Hz, O^2-CH_2), 3.97 (3H, s, O^4-CH_3), 1.32 (3H, t, *J* 7.0 Hz, CH_3); δ_C 171.1 (C_4), 169.5 (C_2), 161.1 (C_6), 101.0 (C_5), 62.9 (O^2-C), 54.9 (O^4-C), 14.1 (Me); *m/z* (%) 154 (28), 139 (41), 126 (34), 110 (100), 96 (42), 68 (60); ν_{max} 1580, 1460, 1380, 1268, 1085 cm^{-1}; HRMS found, 154.0744. $C_7H_{10}N_2O_2$ requires 154.0742.

4-Ethoxy-1-ethyl-2(1*H*)-pyrimidinone **11d** (3 mg, 0.2%);[9a] 1,3-diethyl-2,4-pyrimidindione **10d** (46 mg, 3.5%);[23] 1-ethyl-4-methoxy-2(1*H*)-pyrimidinone **6d** (0.63 g, 52%);[9a] 1-ethyl-2,4(3*H*)-pyrimidindione **9d** (33 mg, 3%). Traces of **6c** were also found by GC–MS.

4.2.3. Products from 4-acetylamino-2(1*H*)-pyrimidinone 3.

4.2.3.1. Methylation. 4-Acetylamino-2-methoxypyrimidine **7e** (54 mg, 5%);[20] 4-acetylamino-1-methyl-2(1*H*)-pyrimidinone **6e** (0.52 g, 48%).[19]

4.2.3.2. Ethylation.

*4.2.3.2.1. 4-Acetylamino-2-ethoxypyrimidine (**7f**).* White solid (106 mg, 9%); δ_H 8.22 (1H, d, *J* 5.5 Hz, C_6H), 7.62 (1H, d, *J* 5.5 Hz, C_5H), 4.26 (2H, q, *J* 7.0 Hz, O^2-CH_2), 2.07 (3H, s, N^4-$COCH_3$), 1.27 (3H, t, *J* 7.0 Hz, CH_3); δ_C 172.85 (C_2), 165.99 (C=O), 161.26 (C_4), 161.14 (C_6), 104.63 (C_3), 64.42 (O^2-C), 24.29 (C(O)-CH_3), 14.78 (CH_3); *m/z* (%) 181 (M^+, 20), 166 (47), 137 (47), 111 (47), 95 (100), 68 (28), 43 (83); ν_{max} 3230, 1675, 1390, 1265 cm^{-1}; HRMS found, 181.0849. $C_8H_{11}N_3O_2$ requires 181.0851.

*4.2.3.2.2. 4-Acetylamino-1-ethyl-2(1H)-pyrimidinone (**6f**).* White solid (0.75 g, 63%); δ_H 7.91 (1H, d, *J* 7.0 Hz, C_6H), 7.28 (1H, d, *J* 7.0 Hz, C_5H), 3.84 (2H, q, *J* 7.0 Hz, N^1-CH_2), 2.07 (3H, s, N^4-$COCH_3$), 1.23 (3H, t, *J* 7.0 Hz, CH_3); δ_C 73.04 (C=O), 164.20 (C_4), 158.68 (C=O), 150.70 (C_6), 98.25 (C_5), 41.12 (N^1-C), 24.53 (C(O)-CH_3), 14.50 (CH_3); *m/z* (%) 81 (M^+, 70), 166 (100), 139 (41), 138 (64), 111 (62), 81 (49), 43 (97); ν_{max} 3240, 1700, 1674, 1645, 1495 cm^{-1}; HRMS found, 181.0853. $C_8H_{11}N_3O_2$ requires 181.0851.

4.2.4. Products from thymine 4.

4.2.4.1. Methylation. 1,3,5-Trimethyl-2,4-pyrimidindione **10a** (86 mg, 7%);[24] 3,5-dimethyl-2,4(1*H*)-pyrimidindione **12a** (56 mg, 5%);[16] 1,5-dimethyl-2,4(3*H*)-pyrimidindione **9a** (0.22 g, 20%).

4.2.4.2. Ethylation.

*4.2.4.2.1. 2-Ethoxy-3-ethyl-5-methyl-2(3H)-pyrimidinone (**13b**).* *m/z* (%) 182 (M^+, 40), 154 (55), 126 (100), 110 (83), 83 (55), 82 (58) in 1:5 mixture (0.13 g, 12%) via HPLC with **10b**;[22] the mixture **13b+10b** (50 mg) was dissolved in 2 M HCl (2.5 ml), warmed at 60 °C for 2 h, evaporated, redissolved in MeOH and analysed via HPLC to give a 1:5 mixture of **12b** and **10b**.

4-Ethoxy-1-ethyl-5-methyl-2(1*H*)-pyrimidinone **11b** (43 mg, 3%);[24] 3-ethyl-5-methyl-2,4(1*H*)-pyrimidindione **12b** (50 mg, 4%);[16] 1-ethyl-5-methyl-2,4(3*H*)-pyrimidindione **9b** (0.42 g, 34%).

When KH was used in place of LiH (see run 9 in Table 2) 2,4-diethoxy-5-methylpyrimidine **14b** (3.2%)[24] was detected by HPLC among the other products and recognised by GC–MS coupling; *m/z* (%) 182 (M^+, 27), 167 (18), 154 (100), 138 (20), 126 (64), 110 (54), 96 (50).

4.2.5. Products from uracil 5.

4.2.5.1. Methylation. 1,3-Dimethyl-2,4-pyrimidindione **10c** (0.15 g, 12%); 3-methyl-2,4(1*H*)-pyrimidindione **12c** (56 mg, 5%);[16] 1-methyl-2,4(3*H*)-pyrimidindione **9c** (0.33 g, 30%).[24]

4.2.5.2. Ethylation.

*4.2.5.2.1. 2-Ethoxy-3-ethyl-2(3H)-pyrimidinone (**13d**).* *m/z* (%) 168 (M^+, 57), 140 (56), 112 (53), 96 (40), 82 (100), 69 (30), 68 (31) in 1:6 mixture (0.22 g, 15%) via HPLC with **10d**;[23] the mixture **13d+10d** (50 mg) was dissolved in 2 M HCl (2.5 ml), warmed at 60 °C for 2 h, evaporated, redissolved in MeOH and analysed via HPLC to give a 1:6 mixture of **12d** and **10d**.

4-Ethoxy-1-ethyl-2(1*H*)-pyrimidinone **11d** (45 mg, 3%);[9a] 3-ethyl-2,4(1*H*)-pyrimidindione **12d** (62 mg, 5%);[16] 1-ethyl-2,4(3*H*)-pyrimidindione **9d** (0.52 g, 42%).

When KH was used in place of LiH (see run 18 in Table 2) 2,4-diethoxy-pyrimidine **14d** (4%)[9a] was detected by HPLC among the other products and recognised by GC–MS coupling; *m/z* (%) 168 (M^+, 29), 140 (53), 124 (17), 112 (68), 96 (100), 82 (25), 70 (51).

4.3. HPLC distribution of products

One equivalent of the appropriate metal hydride (LiH or KH) was added under stirring to 5.0 ml of a 0.07 M solution of the substrates **1**–**5** in dioxane or DMF, and the mixture was stirred for 30 min at 60 °C under a dry Ar atmosphere. Subsequently, 1.3 equiv of the appropriate alkylating agent (DMS, DES, MeI or EtI) were added and stirring at 60 °C was continued for the time reported in Tables 1 and 2. After this time, 1 equiv of solid NH_4Cl was added and the resulting suspension was filtered. DMF solutions were analysed directly by HPLC, while reactions in dioxane were evaporated under reduced pressure and redissolved in MeOH. Reaction mixtures from **2** and **5** were analysed through a gradient elution from 100% H_2O (1 min) to 100% MeCN in 15 min (flow=0.7 ml/min), while a gradient elution from H_2O/MeCN=90:10 to 100% MeCN in 20 min (flow=1 ml/min) was used for mixtures from **1**, **3** and **4**. Results are reported in Tables 1 and 2 as average of two independent runs.

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Stereochemistry of the intermediates in the synthesis of 1,4,7,10-tetraazacyclododecane from triethylenetetramine, glyoxal and diethyl oxalate

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Abstract—The equilibrium and rearrangement phenomena encountered in two steps for the synthesis of 1,4,7,10-tetraazacyclododecane from triethylenetetramine, glyoxal and diethyl oxalate were studied and elucidated after the development of two micellar electrokinetic chromatographic (MEKC) methods. The latter were able to separate: (i) the four bis-aminals (**2**–**5**) obtained from the condensation of triethylenetetramine with glyoxal; (ii) the four diones (**6**–**9**) derived from the reaction of the bis-aminals with diethyl oxalate, whose solid state structures were determined by single crystal X-ray diffraction. The three not yet reported diones (**6**, **7** and **9**) were synthesised by taking advantage of both the reaction conditions and the use of a particular catalyst (MeONa). A plausible reaction mechanism, as well as a discussion of the solid state structures, is presented.

1. Introduction

Within the past two decades, the importance of 1,4,7,10-tetraazacyclododecane (cyclen, **1**) has continuously grown since it became an intermediate for the synthesis of chelating agents, which found applications in diagnostics and therapeutics.[1] In particular, the complexes of such ligands with paramagnetic metal ions, like the gadolinium ion, are largely used as magnetic resonance imaging (MRI) contrast agents.[2] Accordingly, a series of synthetic routes to cyclen appeared in the literature[3–12] and we wish to report here some recent findings regarding one of those synthetic paths.[4–7,9]

2. Results and discussion

The reaction of triethylenetetramine with glyoxal affords a mixture of four bis-aminals, i.e., *cis*-octahydro-3*H*,6*H*-2*a*,5,6,8*a*-tetraazacenaphthylene (**2**), *trans*-octahydro-3*H*,6*H*-2*a*,5,6,8*a*-tetraazacenaphthylene (**3**), *cis*-decahydro-diimidazo-[1,2-a:2′,1′-*c*]pyrazine (**4**) and *trans*-decahydro-diimidazo-[1,2-a:2′,1′-*c*]pyrazine (**5**).[6,7,10] By amidation with diethyl oxalate (DEO), the related mixture containing *cis*-octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]acenaphthylene 1,2-dione (**6**), *trans*-octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]acenaphthylene 1,2-dione (**7**), *cis*-octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]acenaphthylene 3,4-dione (**8**) and *trans*-octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]acenaphthylene 3,4-dione (**9**) is obtained.[6] The subsequent reduction of the amide carbonyls, followed by removal of the central bridging moieties of compounds **10** and **11**, leads to **1** (see Scheme 1).[6,7]

The composition of the mixture containing **2**–**5** was at first determined by means of NMR studies, which were in accordance with those reported in the literature,[10,13] and showed that **2**, the thermodynamically favoured product, was predominant in reactions carried out in water at 5 °C in the presence of $Ca(OH)_2$ (Bracco procedure)[5,6] while **5**, the kinetically favoured product, was the major component working in EtOH at rt (Nycomed procedure).[7] By means of GC analysis,[5,6] we could detect the couple of compounds **2**+**3** and **4**+**5** but we did not make further efforts to find the conditions that are able to separate them. Indeed, we could not exclude that the harsh conditions used for the GC analysis might affect the composition of the mixture, as it is known[10,13] that isomerisation may occur. Accordingly, a micellar electrokinetic chromatographic (MEKC) method was developed to allow the separation of the four isomers and their quantification. Analytical results, obtained using

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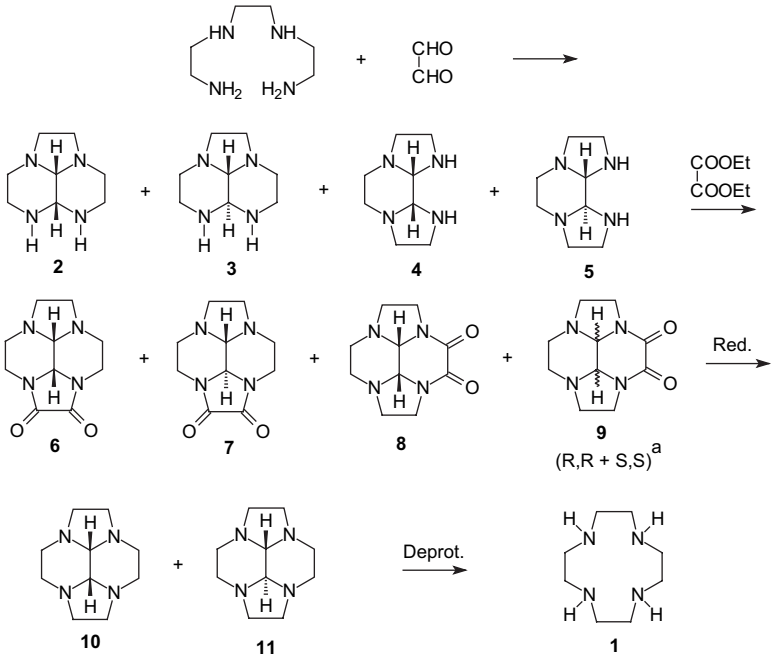


Scheme 1. [a]Both enantiomers of **9** are present in the solid state, as crystallised in an achiral space group.

both synthetic procedures for the preparation of **2–5** mixtures, are reported in Table 1.

As partly anticipated, the composition of the mixtures greatly depends on the reaction conditions. In particular: (i) in water at 5 °C in the presence of $Ca(OH)_2$,[5,6] **2** is formed as the main component, followed by **5**. Compound **3** is present in low percentage, while **4** only appears in a late reaction stage (entries 1–4); (ii) in EtOH at rt,[7] **5** is largely predominant along with **4** while **2** and **3**, which were derived from an isomerisation, are formed in small percentages only after the work up (entries 5 and 6). These results confirm those obtained from NMR studies and reported in the literature.[13]

We subsequently checked the stability of the mixtures obtained with the Bracco procedure,[5,6] especially under the reaction conditions used in the following step (reflux in EtOH, without or with a catalyst). The results are summarised in Table 2.

In particular: (i) the mixture stored at rt changed its composition over time: the content of the trans isomers increased whereas the content of the cis isomers decreased (entry 3 vs entry 1); (ii) heating at reflux a solution in EtOH led to an increase in the content of **3** and a decrease in the content of **2** while no significant change in the content of the other isomers was observed (entry 2 vs entry 1); (iii) heating at reflux a solution in EtOH, in the presence of MeONa, had almost no influence on the composition of the mixture (entry 4 vs entry 3).

Later on, we reacted mixtures having different compositions in each of the **2–5** isomers with DEO in EtOH under the conditions reported in Section 4. As we observed that compounds **4** and **5** were more reactive than **2** and **3**, we took advantage of this peculiarity for the preparation of pure **8** and **9**. Indeed, the reactions were performed, without or with MeONa, using about half of theoretical DEO. The latter preferentially reacted with **4** and **5** affording mixtures enriched with **8** and **9** from which the isolation of the products resulted easier. The work up of the different reaction mixtures allowed us to obtain pure **6**, **7**, **8** and **9** as crystals suitable for the determination of the solid state structure by single crystal X-ray diffraction (the structure of **8** was already reported in the literature[9]). After a MEKC method for the separation of each of the four isomers was devised, we were able to follow the amidation reaction, which was performed using the mixture reported in Table 1, entry 4, without or with a catalyst. Quite interestingly, even in the

Table 1. Percentages of the mixture containing **2–5** determined by MEKC

Entry[a]	2	3	4	5
1[b]	74.8	4.5	—[c]	20.7
2[d]	77.0	4.4	—[c]	18.6
3[e]	71.3	4.0	7.0	17.7
4[f]	70.2	5.9	6.0	17.9
5[b]	—[c]	—[c]	12.3	87.6
6[f]	6.6	5.7	11.9	75.8

[a] Entries 1–4: prepared according to Bracco procedure;[5,6] entries 5 and 6: prepared according to Nycomed procedure.[7]
[b] Reaction (0.5 h).
[c] Not detected.
[d] Reaction (2 h).
[e] Reaction (18 h).
[f] Isolated product.

Table 2. Percentages of the mixture containing **2–5** determined by MEKC

Entry	2	3	4	5
1[a]	70.2	5.9	6.0	17.9
2[b]	63.2	13.0	6.2	17.6
3[c]	68.8	9.6	2.6	19.0
4[d]	67.9	10.2	2.4	19.5

[a] Starting material.
[b] Mixture of entry 1 heated for 10 h in EtOH at reflux.
[c] Mixture of entry 1 reanalysed after one month at rt.
[d] Mixture of entry 3 heated for 10 h in EtOH at reflux in the presence of MeONa (1 mol equiv).

Table 3. Percentages of the mixture[a] containing **6–9** determined by MEKC

Entry	Time (h)[b]	Conversion (%)	6	7	8	9
1[c]	24	75	64.1	—[d]	35.9	—[d]
2[e]	6	91	76.3	—[d]	23.7	—[d]
3[f]	1	95	61.5	16.4	3.4	18.7

[a] Prepared starting from the mixture reported in Table 1, entry 4.
[b] Reflux time of a 12.5% ethanol solution containing DEO (3 mol equiv for the reaction of entry 1; 2 mol equiv for the reactions of entries 2 and 3).
[c] Without catalyst.
[d] Not detected.
[e] Catalysed by 2-pyridinol (0.5 mol equiv).
[f] Catalysed by MeONa (1 mol equiv).

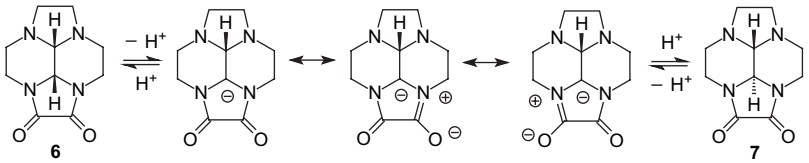
reactions performed with an excess of DEO (2–3 mol equiv) or in neat DEO, no oligomers were found. This clearly indicates that the monoamidated intermediate, which we could not detect, undergoes a rapid cyclisation reaction leading to compounds **6–9** instead of being attached by the amino groups of other molecules.

Some significant results, obtained from the same starting material, are reported in Table 3 while other un-tabulated data will be mentioned in the following discussion.

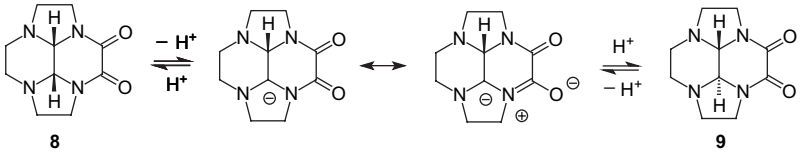
In the uncatalysed and in the 2-pyridinol catalysed reactions,[14] only the *cis* bis-aminals **6** and **8** were obtained (Table 3, entries 1 and 2). When a 95% pure *cis* bis-aminal **2** was reacted at rt, the related cis compound **6** was obtained. This is in disagreement with a previous report[9] asserting that **2** does not react at rt and that only polymers are formed upon heating. Indeed, in our experiment the conversion to **6** increased after heating at reflux. When pure, isolated *trans* bis-aminal **3** was reacted, the cis compound **6** was exclusively obtained. It was already reported in the literature[9] that *trans* bis-aminal **5** was quantitatively transformed into the *cis* diamide **8** as a consequence of an initial isomerisation of **5** into **4** before reacting with DEO. Our data confirmed that assumption and we think that the analogous isomerisation of **3** into **2** takes place, leading to **6**.

The results obtained in the presence of MeONa deserve some comments. The catalytic effect of MeONa could be explained by the in situ generation of dimethyl oxalate that might be more reactive than the corresponding diethyl ester. Such a mechanism was ruled out when we used dimethyl in place of diethyl oxalate and we obtained very similar results. Alternatively, generation of a more nucleophilic species through deprotonation assisted by the catalyst could occur. However, a complete transfer of a proton from the bis-aminals to the catalyst should be difficult because the bis-aminals are very weak acids. Therefore, we can suppose that MeONa assists the cleavage of hydrogen ion from the tetrahedral intermediate. Moreover, using NaOMe, along with **6** and **8**, the trans compounds **7** and **9** were also formed, while these compounds were absent in the reaction run in the absence of NaOMe (Table 3, entry 3 vs entries 1 and 2). As a preliminary assumption, MeONa could play a role in determining the stereochemical configuration of the acylation products in three different ways, as it could: (i) catalyse the interconversion of the mixtures containing **2–5**; (ii) catalyse the interconversion of the mixtures containing **6–9**; (iii) change the stereochemical course of the uncatalysed acylation. Quite interestingly, the percentage of **9** was well related to the content of trans isomer **5** in the starting material while the content of **7** resulted considerably higher than that of **3** (Table 3, entry 3 vs Table 1, entry 4). However, the reaction of pure **3** in the presence of MeONa initially afforded a 1:4 mixture of **6** and **7**, which upon heating, was transformed into a 4:1 mixture of **6** and **7**. Both **6** and **7** proved to be stable in refluxing EtOH but, when they were individually dissolved in EtOH containing MeONa, again a 4:1 mixture of **6** and **7** was obtained. Compound **8** proved to be stable in refluxing EtOH and the addition of MeONa afforded only 3% of **9**, along with residual **8** and degradation products. Considering that MeONa: (i) practically does not affect the composition of the **2–5** mixture (Table 2, entry 4); (ii) promotes the interconversion between **6** and **7**; (iii) has nearly no effect on the transformation of **8** into **9**, we propose that the interconversion between **6** and **7** occurs through an assisted deprotonation of the ethinic carbon atom (see Scheme 2). The intermediate carbanion is stabilised by: (i) the field effect[15] associated with the ylide-type system of the carbanion and the positively charged nitrogen of the dipolar form of the amide function; (ii) the resonance effect, between two equivalent structures, which share the carbanion.

Once applied to **8**, the same mechanism would involve the same field effect between the carbanion and the dipolar structure but, although in this case there are two equivalent ethinic hydrogen atoms, the absence of the resonance effect decreases the acidity of such hydrogens (see Scheme 3).



Scheme 2.



Scheme 3.

Accordingly, the stereochemical stability of **8** and **9** to MeONa is higher in comparison with that of **6** and **7**.

Therefore, in the noncatalysed reaction (Table 3, entry 1) only compounds **6** and **8** are formed because there is enough time for the isomerisation of **3** and **5** into **2** and **4**, respectively, to occur. In the presence of 2-pyridinol (Table 3, entry 2) the reaction, although accelerated, is still sufficiently slow to permit the isomerisation. When the amidation is performed in the presence of MeONa (Table 3, entry 3), **7** can be formed from **3** and from a partial inversion of configuration of **6** according to the mechanism depicted in Scheme 2, while **9** could be the consequence of the catalytic effect of MeONa, which makes the amidation of **5** faster than its stereoisomerisation into **4**. Indeed, the content of **9** (18.7%) is very similar to that (17.7%) of **5** in the starting material.

As previously mentioned, crystals of **6**, **7**, **8** and **9** were obtained and, by means of single crystal X-ray diffraction, their solid state structures were determined. The structure of compound **8** resulted isomorphous and isostructural with the already published structure,[9] thus our data will not be reported and those already known for **8**[9] will be taken into account for comparative purposes.

Bond distances and angles are those expected for this kind of molecules. Concerning the overall geometry of compounds **6–9**, both the trans isomers, **7** and **9**, show a more regular shape with respect to the cis ones (**6** and **8**). Indeed, in compound **7** (Fig. 1) a pseudo-symmetry plane passing through the C9–C10 bond and perpendicular to the mean plane defined by all the nonhydrogen atoms can be recognised, while in **9** a twofold pseudo-symmetry axis, bisecting bonds C1–C2, C5–C6 and C9–C10 is present (Fig. 2).

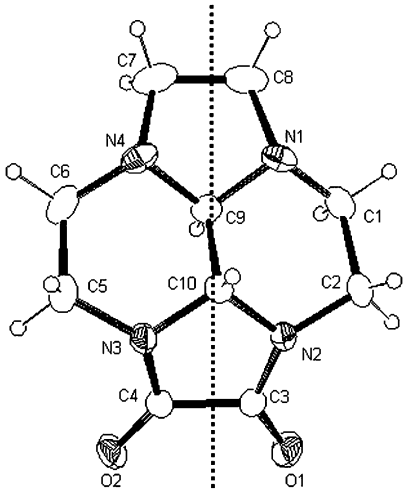


Figure 1. ORTEP3 view of compound **7**. The pseudo-symmetry plane is represented by the dotted line.

Obviously, the overall shape of molecules **6–9** results from the relative arrangement of the four condensed cycles A–D (see Scheme 4) and from their intrinsic symmetry, or better from their conformations. These latter results are summarised in Table 4.

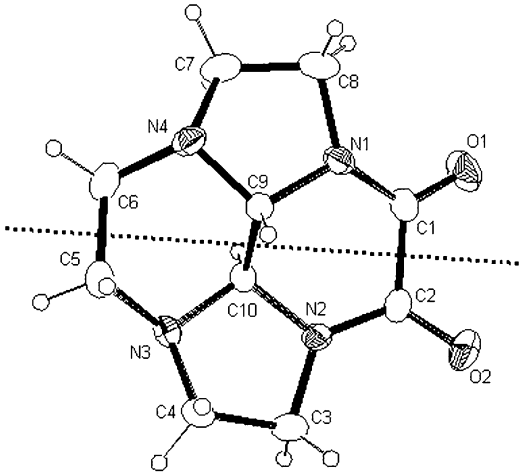
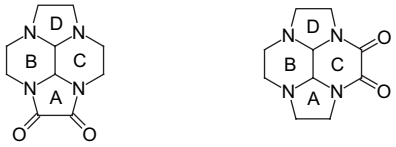


Figure 2. ORTEP3 view of compound **9**. The twofold pseudo-symmetry axis is represented by the dotted line.



Scheme 4.

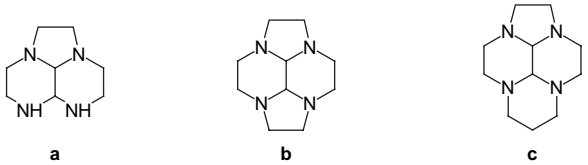
Table 4. Conformations of the four condensed rings in **6**, **7**, **8** and **9**

Ring	6	7	8[9]	9
A	Planar	Planar	Envelope	Envelope
B	Twist	Chair	Chair	Twist
C	Twist	Chair	Twist	Twist
D	Envelope	Envelope	Envelope	Envelope

Given the hybridisation of C3, C4, N2 and N3, the five-membered ring labelled 'A' is planar in compounds **6** and **7**, while in compounds **8** and **9** (where only N2 is sp^2) it show an envelope conformation. Ring D shows the same conformation (envelope) in all the four isomers.

The six-membered rings, B and C, have the same conformation in compounds **6** and **9** (twist), and in compound **7** (chair). In compound **8** the two six-membered rings show a chair (B) and a twist (C) conformation.

For comparative purposes a search for the solid state structures of fragments shown in Scheme 5 was performed in the Cambridge Structural Database (CSD, v. 5.26).[16] However, the very small number of entries prevents from any statistical analysis. In addition, because all the deposited molecules show a cis junction between the six-membered rings, retrieved data will be compared only with **6** and **8**.



Scheme 5.

Fragments **a** (one entry),[17] **b** (four entries)[18] and **c** (one entry)[19] show an almost identical 3D arrangement of the common rings as provided by the root mean square value (RMS, calculated using all the carbon and nitrogen atoms) that ranges between 0.054 and 0.152 Å. In all cases the five- and six-membered rings have envelope and chair conformations, respectively. Accordingly, the cis junction of three (**a** fragment) and four rings (**b** and **c** fragments) does not influence the conformational behaviour of these fully saturated rings. In the cis isomer **6** the conformation of both the six-membered cycles differs either from that (chair) observed in the retrieved solid state structures (**a**–**c** type) or from that (still of chair type) expected for six-membered rings having one sp^2 atom (N2 and N3). On the contrary, both the smaller rings (A and D) have the expected conformation.

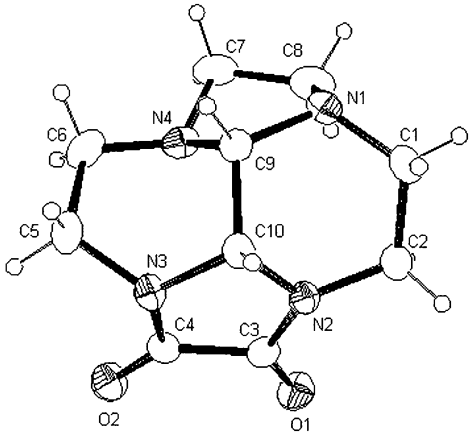


Figure 3. ORTEP3 view of compound **6**.

In compound **8** all the four fused rings show the expected conformations (twist for the C ring due to the presence of four sp^2 atoms). Thus, concerning the cis isomers, **6** and **8**, the introduction of sp^2 atoms in the smaller ring (A in **6** vs C in **8**) causes a more significant 3D rearrangement in the nearby rings (B and C in **6** vs A, B and D in **8**). However, this cannot be the unique reason for such distortion, as provided by the 'regular' (not distorted with respect to that expected) geometry of the four condensed rings in **7**. Accordingly, also the six-membered rings' junction plays an important role.

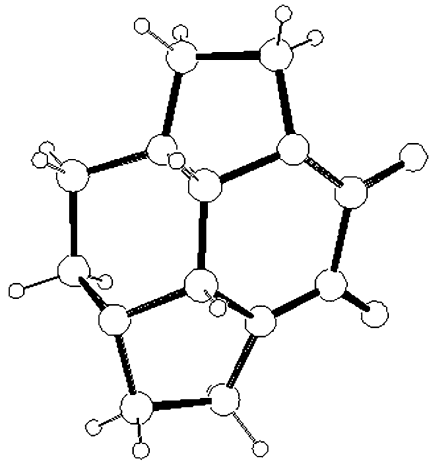


Figure 4. PLUTO view of compound **8** (the atomic coordinates are those reported in the literature.[9] The atom labelling is consistent with the other solid state structures reported here).

3. Conclusion

trans Bis-aminals **3** and **5** can isomerise into their corresponding cis isomers **2** and **4**, which in turn can be transformed, by reaction with DEO in EtOH without catalyst or in the presence of 2-pyridinol, into the related *cis* diamides **6** and **8**. When the amidation is performed in the presence of MeONa, the so far unknown *trans* diamides **7** and **9** are formed. Diamide **7** can be derived from both amidation of bis-aminal **3** and the partial inversion of configuration of the *cis* diamide **6** according to the mechanism described in Scheme 2, while **9** could be the consequence of the catalytic effect of MeONa, which makes the amidation faster than the stereoisomerisation of **5** into **4**.

The comparison of the solid state structures of compounds **6** and **8** with those of similar cis fragments deposited in the Cambridge Structural Database evidenced that the introduction of sp^2 atoms in a five-membered ring (**6**), instead of in the six-membered one (**8**), causes a significant 3D rearrangement in the nearby rings. However, also the six-membered rings' junction should play an important role in determining the rings' conformation, given the regular (i.e., not distorted with respect to the expected one) geometry of the four condensed rings in **7**.

4. Experimental

4.1. General

All reagents and solvents, obtained from commercial sources, were used without further purification. MeONa was used as a 1 M solution obtained by dissolution of sodium in MeOH. Melting points (°C, uncorrected) were measured with a Büchi 510 instrument. IR spectra were recorded on a Perkin–Elmer 882 spectrophotometer, using potassium bromide disks. MS spectra were acquired on a TSQ700 ThermoFinnigan Spectrometer using CH_3OH as the solvent. ^1H and ^{13}C NMR spectra were recorded at 298 K in $CDCl_3$ at 400.13 and 100.61 MHz, respectively, with a Bruker DRX 400 spectrometer. In order to have a complete assignment of the structures, 2D spectra were recorded using ^1H–^1H COSY45, HMQC and HMBC standard pulse sequences. The chemical shifts are given in δ units (ppm) relative to TMS (δ=0). For the assignment, see the numeration of the atoms in the related ORTEP figures. Elemental analyses were carried out at the Redox Laboratories (Monza, Milano, Italy).

4.2. Micellar electrokinetic chromatography (MEKC)

Analyses were performed on a Hewlett–Packard 3D Capillary Electrophoresis System equipped with an autosampler, column thermostat set at 15 °C and diode array detector set at 200 nm. A hydrodynamic injection of a 1–2 mg/mL solution into a fused silica capillary column (50 μm inner diameter) 80.5 cm long was used and a voltage of 30 kV was applied. For the analyses of the mixtures containing **2**–**5**

we used: (i) injection: 50 mbar, 5 s; (ii) electrolyte: 30 mM sodium borate buffer at pH 9.3, 0.3 mM EDTA, 20 mM sodium dodecyl sulfate (SDS), 1% MeOH. For the analyses of the mixtures containing **6–9** we used: (i) injection: 50 mbar, 3 s; (ii) electrolyte: 50 mM sodium borate buffer at pH 8.1, 0.3 mM EDTA, 180 mM sodium dodecyl sulfate (SDS), 1% MeOH.

4.3. Synthetic methods

4.3.1. *cis*-Octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]-acenaphthylene 1,2-dione (6). A solution of bis-aminals having the composition described in Table 1, entry 4 (21 g; 0.125 mol) in EtOH (160 mL) was treated with MeONa (6.75 g; 0.125 mol) and DEO (36.5 g; 0.25 mol). After 2 h at reflux under nitrogen atmosphere, the mixture was cooled to 30 °C and 37% HCl (12.3 g; 0.125 mol) was dropped in. The precipitated hydrochloride was filtered, washed with EtOH (3×20 mL), dissolved in water (80 mL), and then the solution was neutralized with Na_2CO_3 (6.9 g; 0.065 mol) and taken to dryness. The residue was boiled with MeOH (55 mL), the suspension was filtered and the clear solution was left at rt for 24 h. The crystalline precipitate was filtered, washed with MeOH (10 mL) and dried to afford **6** (7.5 g; 27%). Mp 158–160 °C. IR (KBr) ν 1429, 1719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.56 (ddd, 2H, J=2.2, 5.7, 10.3 Hz: 6, 1), 2.73 (m, 2H: 7, 8), 2.95 (m, 4H: 7, 8, 6, 1), 3.11 (ddd, 2H, J=5.7, 10.0, 13.9 Hz: 5, 2), 3.64 (d, 1H, J=4.6 Hz: 9), 4.20 (ddd, 2H, J=2.2, 6.4, 13.9 Hz: 5, 2), 5.17 (d, 1H, J=4.6 Hz: 10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.0 (5, 2), 48.0 (6, 1), 50.4 (7, 8), 65.4 (10), 74.8 (9), 160.0 (4, 3); MS *m*/*z* (ESI) 223 $(M+H)^+$, 245 $(M+Na)^+$; Anal. Calcd for $C_{10}H_{14}N_4O_2$: C, 54.04; H, 6.35; N, 25.21. Found: C, 54.02; H, 6.39; N, 25.18.

4.3.2. *trans*-Octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]-acenaphthylene 1,2-dione (7). The mother liquor obtained from the filtrate of the hydrochloride used for the preparation of **6** was evaporated to dryness and the residue was purified by silica gel chromatography (6:3:1 $CHCl_3$/CH_3OH/25% NH_4OH). The fractions enriched with the desired compound were combined and evaporated to dryness, then the residue was crystallised three times from methanol to afford **7** (1.5 g; 5.4%). Mp 258–260 °C. IR (KBr) ν 1442, 1737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.48 (d, 1H, J=6.2 Hz: 9), 2.33 (m, 2H: 6, 1), 2.46 (m, 2H: 7, 8), 3.22 (dd, 2H, J=4.7, 10. 9 Hz: 6, 1), 3.38 (m, 4H: 7, 8, 5, 2), 4.22 (d, 1H, J=6.2 Hz: 10), 4.28 (dd, 2H, J=3.1, 14.1 Hz: 5, 2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.2 (5, 2), 50.8 (7, 8), 52.3 (6, 1), 67.0 (10), 92.8 (9), 157.5 (4, 3); MS *m*/*z* (ESI) 223 $(M+H)^+$, 245 $(M+Na)^+$; Anal. Calcd for $C_{10}H_{14}N_4O_2$: C, 54.04; H, 6.35; N, 25.21. Found: C, 53.97; H, 6.41; N, 25.15.

4.3.3. *cis*-Octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent[*fg*]-acenaphthylene 3,4-dione (8). A solution of bis-aminals having the composition reported in Table 1, entry 6 (30 g; 0.178 mol) in EtOH (225 mL) was treated with DEO (13 g; 0.089 mol) and heated at reflux for 18 h under nitrogen atmosphere. After addition of further DEO (2.6 g; 0.018 mol) and heating for 4 h at reflux, the solid, which spontaneously precipitated was filtered after cooling the suspension to rt. The product was crystallised from MeOH (270 mL) to afford **8** (6.2 g; 26%). Mp 255–257 °C (lit.[9] 252 °C). IR (KBr) ν 1458, 1687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (see the atom numbers in the ORTEP view of the parent compound **9**) δ 2.60 (m, 2H: 5, 6), 2.76 (m, 4H: 4, 7, 5, 6), 3.12 (m, 2H: 4, 7), 3.45 (m, 2H: 3, 8), 3.89 (m, 2H: 3, 8), 4.13 (s, 2H: 9, 10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.5 (3, 8), 47.1 (5, 6), 49.7 (4, 7), 69.8 (9, 10), 157.6 (1, 2); MS *m*/*z* (ESI) 223 $(M+H)^+$, 245 $(M+Na)^+$; Anal. Calcd for $C_{10}H_{14}N_4O_2$: C, 54.04; H, 6.35; N, 25.21. Found: C, 53.95; H, 6.40; N, 25.12.

4.3.4. *trans*-Octahydro-2*a*,4*a*,6*a*,8*a*-tetraazacyclopent-[*fg*]acenaphthylene 3,4-dione (9). A solution of bis-aminals having the composition reported in Table 1, entry 6 (5.5 g; 0.033 mol) in EtOH (80 mL) was treated with MeONa (0.88 g; 0.0163 mol) and DEO (2.38 g; 0.0163 mol). After heating at reflux for 8 h under nitrogen atmosphere, the reaction mixture was concentrated to 20 g. The suspension thus obtained was filtered and the solid was crystallised from MeOH (10 mL) to afford **9** (0.5 g; 13.8%). Mp 255–256 °C. IR (KBr) ν 1433, 1682 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.71 (ddd, 2H, J=6.5, 8.7, 10.2 Hz: 4, 7), 2.90 (m, 2H: 5, 6), 3.38 (m, 4H: 4, 7, 5, 6), 3.49 (ddd, 2H, J=5.9, 10.2, 11.6 Hz: 3, 8), 4.20 (ddd, 2H, J=0.7, 6.5, 11.6 Hz: 3, 8), 4.42 (s, 2H: 9, 10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 44.6 (3, 8), 49.9 (5, 6), 53.3 (4, 7), 71.8 (9, 10), 156.9 (1, 2); MS *m*/*z* (ESI) 223 $(M+H)^+$, 245 $(M+Na)^+$; Anal. Calcd for $C_{10}H_{14}N_4O_2$: C, 54.04; H, 6.35; N, 25.21. Found: C, 53.88; H, 6.44; N, 24.98.

4.4. X-ray crystallographic study

Cell parameters and intensity data for compounds **6**, **7**, **8** and **9** were obtained on a Siemens P4 diffractometer, using graphite monochromated Cu Kα radiation (λ=1.54180 Å). Cell parameters were determined by least squares fitting of 25 centered reflections. The intensities of three standard reflections were measured every 60 min to check the stability of the diffractometer and the decay of the crystals. Intensity data were corrected for Lorentz and polarisation effects, an absorption correction was applied once the structures were solved by using the Walker and Stuart method.[20] Structures were solved using the SIR-97[21] program and subsequently refined by the full-matrix least squares program SHELX-97.[22] Given that the solid state structure of compound **8** was found to be isomorphous and isostructural with the already published structure,[9] our X-ray data will not be published. The hydrogen atoms of compounds **6**, **7** and **9** were introduced in calculated position and their coordinates refined in agreement with those of the linked atoms. All the nonhydrogen atoms were refined anisotropically. Atomic scattering factors and anomalous dispersion corrections for all the atoms were taken from the literature.[23] The molecular plots were produced by the ORTEP3 program[24] and the ORTEP views of compounds **6**, **7** and **9**, along with a PLUTO view of compound **8**, are detailed in Figures 1–4. Crystal parameters and structure refinement data for **6**, **7** and **9** are resumed in Table 5.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Structural Data Centre as supplementary publication numbers CCDC 292972, CCDC 292973 and CCDC 292974.

Table 5. Crystal data and refinement parameters of compounds **6**, **7** and **9**

	6	**7**	**9**
Empirical formula	$C_{10}H_{14}N_4O_2$	$C_{10}H_{14}N_4O_2$	$C_{10}H_{14}N_4O_2$
Formula weight	222.25	222.25	222.25
T (K)	293	293	293
λ (Å)	1.54180	1.54180	1.54180
Crystal system, space group	Orthorhombic, $P2_1cn$	Triclinic, P-1	Orthorhombic, $P2_1nb$
Unit cell dimensions (Å, °)	a=5.906(1)	a=7.868(5), α=111.190(5)	a=7.373(2)
	b=11.550(1)	b=8.735(5), β=95.210(5)	b=8.768(1)
	c=14.871(3)	c=9.543(5), γ=112.060(5)	c=15.934(3)
Volume (Å^3)	1014.4(3)	546.9(5)	1030.1(4)
Z, d_{calcd} (g/cm^3)	4, 1.455	2, 1.350	4, 1.433
μ (mm^{-1})	0.871	0.807	0.857
2θ range for data collection (°)	9.5–130.0	10.0–130.0	11.0–130.0
Reflections collected/unique	1274/902	1936/1574	1299/917
	[R(int)=0.0246]	[R(int)=0.0354]	[R(int)=0.0295]
Data/restraints/parameters	902/1/147	1574/0/155	917/1/147
Final R indices [$I>2\sigma(I)$]	$R1$=0.0467, $wR2$=0.1162	$R1$=0.0512, $wR2$=0.1255	$R1$=0.0424, $wR2$=0.1087
R indices (all data)	$R1$=0.0468, $wR2$=0.1163	$R1$=0.0521, $wR2$=0.1265	$R1$=0.0427, $wR2$=0.1089

4.5. Cambridge structural database

For comparative purposes crystal data of compounds containing fragments **a–c** (Scheme 5) were retrieved in the Cambridge Structural Database (v. 5.26).[16] Given the low number of entries featuring fragments **a–c**, no filters were turned on, except that only entries having 3D coordinates have been considered.

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Convenient syntheses of metabolically important quercetin glucuronides and sulfates

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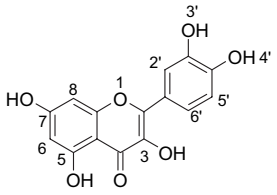
Abstract—Synthetic approaches to the major human plasma metabolites of quercetin, quercetin 3′-sulfate and the β-D-glucopyranosiduronic acid derivatives 3′-methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide), quercetin 3-glucuronide and quercetin 3′-glucuronide are described. This is the first report of the chemical synthesis of quercetin 3′-glucuronide. All procedures start from the same precursor, 4′,7-di-*O*-benzylquercetin, and all are more convenient than existing methods.

1. Introduction

The human diet includes several classes of plant flavonoids; many appear to be protective against coronary heart disease and/or a variety of carcinomas.[1] Quercetin **1** (Scheme 1) is the major flavonol found in plants (though usually in glycosylated forms)[2] and is thus a ubiquitous part of the human diet. In the past, the majority of evidence for the health benefits of quercetin came from in vitro experiments on free quercetin. Recent work has studied the human absorption and metabolism of quercetin derivatives and has shown that quercetin itself is not present in human plasma, but is found instead in various glucuronidated and sulfated forms.[3] In order to better determine the biological effects of quercetin, we required 100 mg quantities of the most abundant circulating forms—the β-D-glucopyranosiduronic acid derivatives quercetin 3-glucuronide **2**, quercetin 3′-glucuronide **3** and 3′-methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide) **4**, together with quercetin 3′-sulfate **5**. Although quercetin glucuronides have been synthesised using liver microsomal preparations,[4,5] this is not convenient if larger quantities of glucuronides are needed; and existing chemical syntheses of both the sulfate and the glucuronides, where available, are either involved and/or low yielding. We describe convenient syntheses from a common easily prepared precursor, 4′,7-di-*O*-benzylquercetin **6** (Scheme 2).



1

Scheme 1. Structure of quercetin **1** (showing ring numbering).

2. Results and discussion

2.1. 4′,7-Di-*O*-benzylquercetin, 6

4′,7-Di-*O*-benzylquercetin, **6**, was prepared as described by Jurd.[6] Although the yields were modest (17–29%), gram quantities of pure **6** were easily obtained without the need for chromatography.

2.2. Quercetin 3-glucuronide, 2

Wagner et al. first reported the synthesis of **2** in 1970.[7] Glucuronidation of **6** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide **7** in the presence of silver oxide (Ag_2O), gave **9** in 44% yield (Scheme 2); debenzylation and deacetylation afforded **2** in 24% overall yield. When we attempted this procedure, we failed to obtain any glucuronidated products, or to recover **6**. To ensure anhydrous conditions, we had stirred **6**, Ag_2O and a desiccant (either calcium sulfate, $CaSO_4$, or 3 Å molecular sieves) in pyridine for 2 h before adding **7**. Conversely, when we added **7** immediately, the reaction gave two mono-glucuronidated products, **8a** and **8b**, in 18 and 19% yields, respectively, after

Keywords: Quercetin glucuronide; Quercetin sulfate; Quercetin 3-glucuronide; Quercetin 3′-glucuronide; Isorhamnetin 3-glucuronide; Quercetin 3′-sulfate; Glucuronidation; Sulfation; Synthesis.
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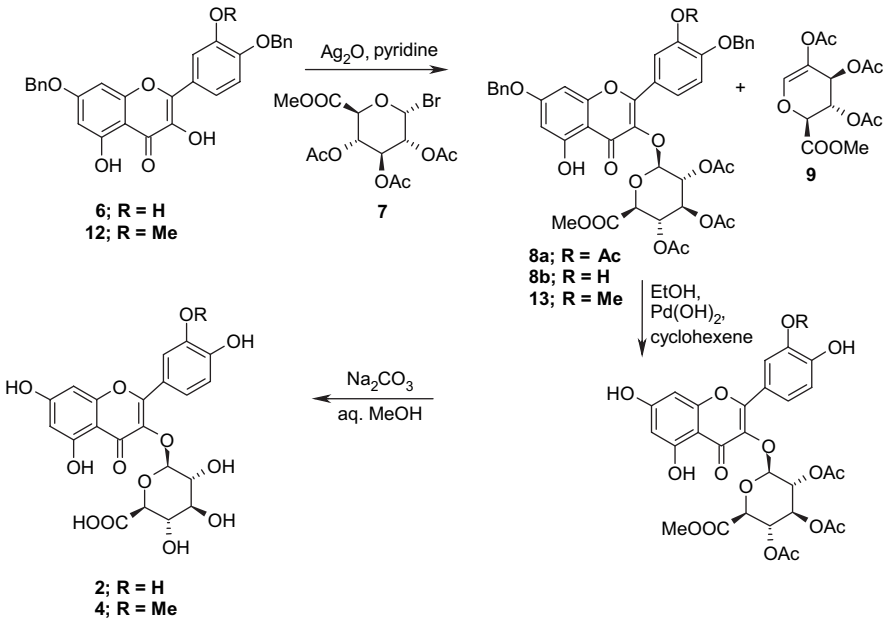


Scheme 2. Synthesis of quercetin 3-glucuronide **2** and isorhamnetin 3-glucuronide **4**.

chromatography. Compound **8a** was contaminated with a trace of the glycal **9** (Scheme 2), a known by-product of glucuronidation of phenols.[8] Both **8a** and **8b** gave only **2** after debenzylation[9] and ester hydrolysis[10] in a combined purified yield of 23% from **6**. Compound **8b** was the expected product; but **8a** was, in addition, 3′-*O*-acetylated. (In contrast, glucosylation of **6** with the corresponding tetraacetylglucosyl bromide gave only the expected 3-glucosylated product—data not shown. We noted the tendency of **7** to transfer an acetyl group to a less acidic, unprotected phenolic hydroxyl during the development of our synthesis of the isoflavone conjugate daizein 7-glucuronide.)[11] When the reaction was repeated at 0 °C, **8a** and **8b** were obtained in an improved combined yield of 52%. Debenzylation and hydrolysis gave, after purification, **2** in 40% overall yield in three steps from **6**. This represents a considerable improvement over both the original report, and a more recent procedure involving selective oxidation of a glucoside derivative.[12]

We briefly investigated why delayed addition of **7** was deleterious to product yield. A ^{1}H NMR spectrum after workup of a mixture of **6**, Ag_2O, pyridine and $CaSO_4$ after 2 h showed no signs of debenzylation. The flavonol signals were greatly broadened, suggesting complexation to silver had occurred, which might account for the failure to react with **7**. Thus the problem might be anticipated in other Ag_2O-catalysed glycosylations where the substrate has a suitable site for complexation, unrelated to the intended reactive site (such as vicinally positioned 5-hydroxy and 4-oxo groups here). No problem occurred in the synthesis of quercetin 7-glucuronide,[13] where all hydroxyls—except the 7—were protected.

2.3. Quercetin 3′-glucuronide, 3

Treatment of **6** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate **10** gave **11** and recovered **6** (Scheme 3), which was partially purified by chromatography. No 3-*O*-glucuronidation was observed. Debenzylation and de-esterification gave, after purification, **3** in 11% yield. Although this yield is low, this is the first reported chemical synthesis of this compound. The different regioselectivities of **7** and **10** are noteworthy. Characteristic downfield shifts of the 2′ and 6′-protons were evident in the ^{1}H NMR spectrum (see Table 1 for comparative data). We confirmed the structure of **3** by HMBC, when the expected long range ^{1}H–^{13}C connectivities were observed. Thus H″-1 showed ^3J correlation to C-3′, C-3′ showed ^3J correlation to H-5′ and ^2J correlation to H-2′.

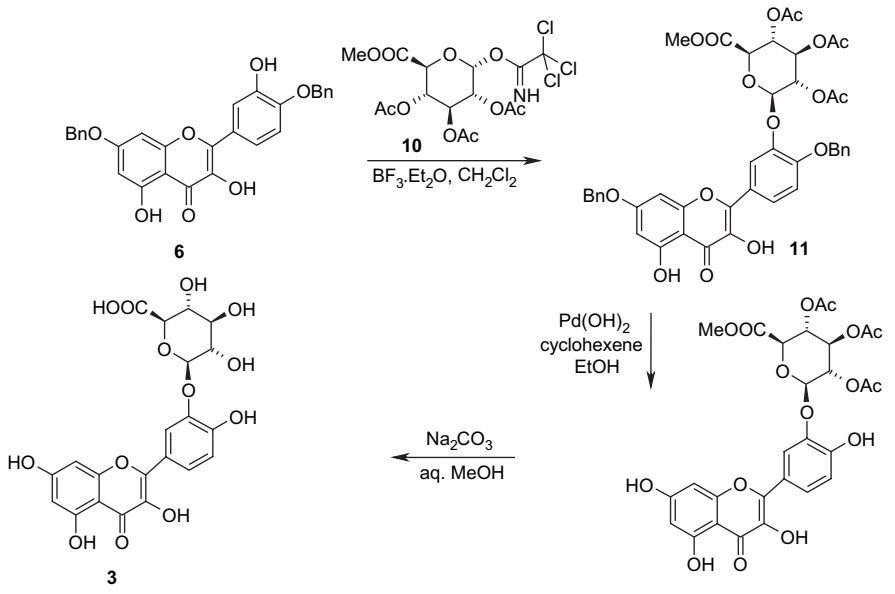
A synthesis of **3** using liver microsomes has been described.[4] Table 2 shows comparative NMR data for this material (the ionic form of the material was not specified) and chemically synthesised **3** (as both free acid and sodium salt).

2.4. 3′-Methylquercetin 3-glucuronide, 4 (isorhamnetin 3-glucuronide)

We reported the first synthesis of this compound, from **6**,[10] but the yield was very low (5%). The procedure was based on the treatment of 3′-*O*-methyl-4′,7-*O*-dibenzylquercetin **12** (derived from **6**) with **10**. Our synthesis of **3** suggested that reaction with **7** would be a better approach, and indeed treatment of **12** with **7** and Ag_2O in pyridine at 0 °C gave crude **13** in 51% yield (Scheme 2). Debenzylation, hydrolysis and purification of **13** gave **4** in 33% yield from **12** (see Table 1). We confirmed the structure of **4** by HMBC, when the expected long range ^{1}H–^{13}C connectivities were observed. Thus H″-1 showed ^3J correlation to C-3 and C-3′ showed ^3J correlation to the protons of the methyl group.

2.5. Quercetin 3′-sulfate 5

Although treatment of **1** with sulfamic acid gives **5**,[3] it is as part of a complex mixture, which is difficult to purify. We thus considered alternative approaches based on sulfation of 3,4′,7-tri-*O*-benzylquercetin **14**. Compound **14** was prepared in 66% yield by treatment of **6** with 1.6 equiv of



Scheme 3. Synthesis of quercetin 3′-glucuronide **3**.

Table 1. ^{1}H NMR shifts/δ (CD_3OD) for quercetin glucuronides (H^+ form)

Proton	Quercetin (**1**)	Quercetin 3-glucuronide (**2**)	Quercetin 3′-glucuronide (**3**)	Isorhamnetin 3-glucuronide (**4**)
2′	7.72	7.61	8.03	7.95
6′	7.62	7.64	7.89	7.54
5′	6.87	6.84	6.95	6.86
8	6.37	6.38	6.39	6.37
6	6.17	6.19	6.15	6.17

Table 2. ^{1}H NMR shifts/δ (DMSO-d_6) for quercetin 3′-glucuronide **3**

Proton	Quercetin (**1**)[a]	**3**		**3**[a,b]
		H^+ form	Na^+ form	
2′	7.67	7.85	7.93	7.98
6′	7.53	7.88	7.85	7.90
5′	6.87	6.99	6.97	6.96
8	6.40	6.48	6.50	6.59
6	6.18	6.20	6.19	6.16

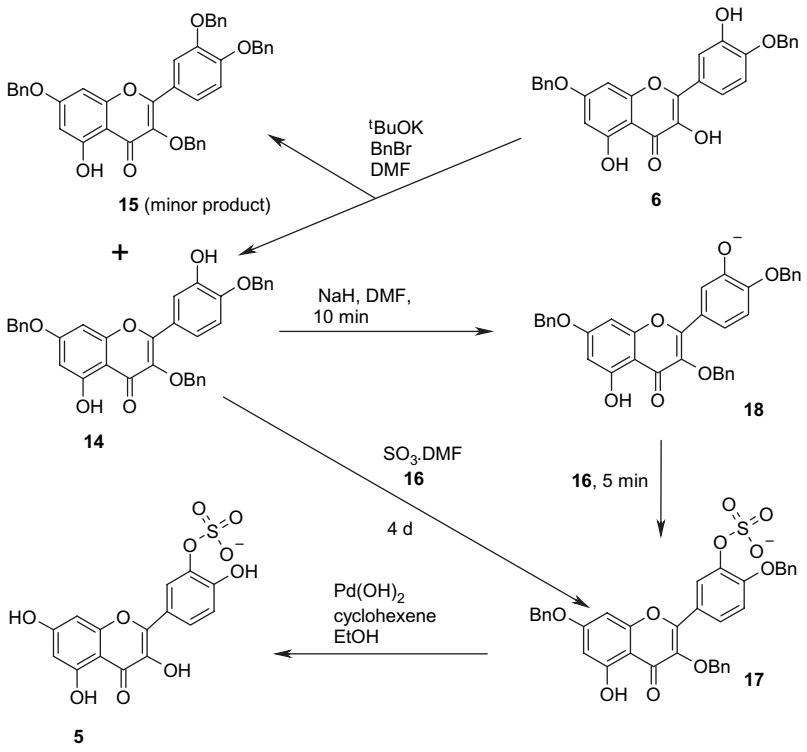
[a] Ref. 4.
[b] Cationic form unspecified.

potassium *tert*-butoxide and benzyl bromide in DMF (Scheme 4; 3,3′,4′,7-tetra-*O*-benzylquercetin **15** was also produced, but these conditions maximised the yield of **14**). Treatment of **14** with a chlorosulfonic acid/pyridine mixture at room temperature—conditions used to sulfate partially protected daidzein derivatives[14]—or overnight at 80 °C was unsuccessful. Treatment of **14** in DMF with sulfur trioxide-*N*,*N*-dimethylformamide complex **16**, a highly reactive sulfating reagent[15] for 4 d, gave **17**, which was debenzylated to give a mixture of **1** and **5** (Scheme 4). The latter were easily separated on a C-18 reverse phase solid phase extraction (SPE) cartridge; elution with water gave pure **5** in 68% yield from **14**. (Alternatively, treatment of **14** with a slight excess of sodium hydride for 10 min, to give the phenoxide anion **18**, followed by addition **16**, also gave, after debenzylation of **18**, a mixture of **1** and **5**, from which **5** was isolated in 45% yield. Though this approach had the advantage of a short reaction time, it was difficult to stoichiometrically control NaH addition on the small scale employed, which lowered the yield). Thus **5** was obtained directly as its sodium salt, avoiding the need for the elaborate purification procedures typically used in sulfate synthesis (see below); but only if the **14** used was pure. We found **14**, prepared by benzylation of **1** as described,[16] contained small amounts of two isomeric tribenzylquercetins, which we were unable to remove; and that these led, after sulfation and deprotection to a mixture of **5** and **1**, together with two other compounds, tentatively identified as quercetin 7-sulfate and quercetin 4′-sulfate (data not shown). Purification of **5** in this case required preparative HPLC (see below); complete separation of the closely running monosulfates was difficult, and the yield of **5** was lowered as a result.

2.6. Purification of quercetin glucuronides and sulfates by preparative HPLC

C-18 reverse phase columns, and gradients of 0.1% aq trifluoroacetic acid (TFA) and acetonitrile, were effective for preparative scale purification of glucuronides. It was necessary, however, to load the crude glucuronides in their acid forms in 50% methanol. Solubility in this solvent was limited; this restricted loading capacity, but this was the best compromise between solubility and solvent polarity. Though sodium salts could be loaded in concentrated aqueous solution, two peaks—one early peak corresponding to the sodium form, and a later one to the acid form—were obtained for each glucuronide, making the approach suited to simple mixtures only.

We also report a substantial improvement in the HPLC purification of sulfates. Although it was not ultimately required for **5** prepared as above, it was used to obtain pure sulfated products from several procedures that were ultimately less efficient and are therefore not reported here.



Scheme 4. Synthesis of quercetin 3′-sulfate **5**.

Aq TFA–acetonitrile gradients were found to be unsuitable. Retention times were highly variable (due presumably to the low pK_a of sulfates) and fractions containing sulfates were prone to acid catalysed sulfate cleavage during subsequent evaporation (data not shown). We found that substituting 50 mM aq ammonium acetate for aq TFA overcame this problem, and products were easily desalted by lyophilisation. Previously reported flavonoid sulfate purifications are generally involved, employ several steps, and use techniques such as size exclusion chromatography, normal phase HPLC, preparative TLC and separation on polyamide.[17–20] As far as we are aware, NH_4OAc–CH_3CN gradients and reverse phase HPLC have not been used before for the isolation of pure flavonoid sulfates.

2.7. Storage and handling of glucuronides and sulfates

We initially stored glucuronides in their acid forms, as protection against possible autooxidative reactions (to which **1** is increasingly susceptible as temperature, pH and/or salt concentrations are raised),[21] in solution in 50% aq methanol at −20 °C; they were indefinitely stable. Solutions were warmed to room temperature and briefly sonicated before use. Although the latter apparently redissolved any material that had precipitated during refrigeration, subsequent filtration through a 0.2 μm membrane led to losses. Methanolic solutions (100%) were filtered without loss, but under these conditions the glucuronic acid moieties were prone to self-catalysed methyl esterification. The addition of water successfully prevented esterification during storage at −20 °C, or, for example, rotary evaporation. Sulfates were stored as solid Na salts, or in solution in aq methanol or methanol, at −20 °C. More recently we have converted glucuronides to their sodium salts by titration, in 50% aq methanol, to pH 6.0 with aq sodium hydroxide, followed by immediate evaporation. The resultant solids were stored at −20 °C, and have again been stable. The salt forms of these materials are freely soluble in water, which is highly convenient for use in cell culture studies, etc. Frozen, millimolar solutions have also been stable. We are unable yet to fully comment on long-term stability; but we have found solutions to be stable for 48 h at 37 °C in culture media.[5]

3. Experimental

3.1. General methods

Solvents were dried over freshly activated 3 Å molecular sieves. Evaporations were performed in vacuo at 50 °C. Solids were dried overnight in vacuo over P_2O_5 before use. TLC was performed on Macherey-Nagel Silica Gel 60/UV254 plates using UV light, or 50% sulfuric acid and charring, for visualisation. MPLC used pre-packed silica cartridges (Isolute Flash Si, Argonaut Technologies) and UV detection. Analytical HPLC and HPLC–ESMS used a 5 μm Lunar column (250×4.4 mm) eluted at 1 mL min^{-1} at 30 °C. Eluant A 80% for 5 min (isocratic); to 10% A at 35 min (gradient). Eluate was monitored by UV detection at 205 and 280 nm. Preparative HPLC of glucuronides used a 5 μm Prodigy ODS3 column (Phenomenex Inc., 250×21.2 mm+60×21.2 mm guard) eluted at 5 mL min^{-1}. Eluant A—0.1% CF_3COOH (TFA); Eluant B—CH_3CN. 80% A for 15 min (isocratic); then to 10% A at 75 min (gradient). Preparative HPLC of sulfates used the same column and flow rate, but different elution conditions: Eluant A—50 mM aq NH_4OAc, pH 6.5; Eluant B—CH_3CN. 80% A for 15 min (isocratic); then to 40% A at 75 min (gradient).

Solid phase extraction (SPE) was performed on TechElut SPE C-18 2000/12 mL cartridges, preconditioned with MeOH and water. Melting points were determined with a Reichert 7905 hot stage microscope, and are uncorrected. ^{1}H and ^{13}C NMR spectra were run on a JEOL EX-270 spectrometer at 21 °C. HMBC NMR spectra were run on a Bruker Avance 600 spectrometer at 27 °C. NMR chemical shifts were referenced to residual solvent absorption. ESMS, APCIMS and HPLC–ESMS analyses were performed on a Micromass Quattro II mass spectrometer. HRMS data were obtained by syringe pump injection of solutions in aq CH_3CN, using either a Micromass LCT MS or a Bruker microTOF, equipped with electrospray sources. IR spectra were measured by Attenuated Total Reflectance (ATR), using a BioRad FTS175C Fourier transform infrared spectrometer with HgCdTe detector and Specac GoldenGate single reflection diamond Horizontal ATR system; 128 scans at 2 cm^{-1} resolution, background spectrum empty crystal. Optical rotation measurements were made on a Perkin Elmer 341 Polarimeter.

3.2. Synthesis of quercetin conjugates

3.2.1. 4′,7-Di-*O*-benzylquercetin, 6. 4′,7-Di-*O*-benzylquercetin, **6**, was prepared from quercetin pentaacetate (25 g) as described by Jurd,[6] except that the product was initially purified by extraction with toluene at 80 °C, rather than with refluxing benzene. Yield of recrystallised **6** (as yellow needles) 4.0–6.8 g, 17–29% (lit.[6] 13%), mp 181–182 °C (lit.[6] 181 °C); δ_H ($CDCl_3$) 11.70 (s, 1H, 5-OH), 7.77 (dd, 1H, *J* 9.6, 2.3 Hz, H-6′), 7.78 (d, 1H, *J* 2.3 Hz, H-2′), 7.29–7.46 (m, 10H, 2×Ph), 7.02 (d, 1H, *J* 9.6 Hz, H-5′), 6.61 (s, 1H, 3-OH), 6.55 (d, 1H, *J* 2.3 Hz, H-8), 6.44 (d, 1H, *J* 2.3 Hz, H-6), 5.77 (s, 1H, 3-OH), 5.18, 5.13 (2×s, 2×2H, 2×C*H*$_2$Ph).

3.2.2. Quercetin 3-glucuronide, 2. Ag_2O (0.27 g, 2.5 equiv), $CaSO_4$ (dried at 120 °C, 0.5 g) and dry pyridine (4 mL) were stirred, in the dark, under Ar at 0 °C for 5 min. Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide **7** (0.23 g, 580 μmol, 1.25 equiv) was added; and, after a further 5 min, 4′,7-di-*O*-benzylquercetin **6** (0.22 g, 460 μmol). Stirring was continued at 0 °C. After 16 h, aq KCl (10%, 20 mL) and aq AcOH (10%, 100 mL) were added and the mixture was filtered through Celite. The latter was washed with water (2×100 mL), and the crude product eluted with acetone (2×50 mL). The latter was evaporated to give a light brown solid (0.35 g), which was purified by MPLC (20 g silica, 4% acetone/96% toluene isocratic elution) to give two main products **8a** and **8b**.

Compound **8a**: δ_H ($CDCl_3$) 12.42 (s, 1H, 5-OH), 8.01 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.85 (d, 1H, *J* 2.3 Hz, H-2′), 7.31–7.42 (m, 10H, 2×Ph), 7.10 (d, 1H, *J* 8.9 Hz, H-5′), 6.49 (d, 1H, *J* 2.0 Hz, H-8), 6.42 (d, 1H, *J* 2.0 Hz, H-6), 5.75 (d, 1H, *J* 7.9 Hz, H-1″), 5.1–5.4 (m, 3H, H-2″, H-3″, H-4″), 5.26, 5.11 (2×s, 2×2H, 2×C*H*$_2$Ph), 3.94 (d, 1H, *J* 9.9 Hz, H-5″), 3.58 (s, 3H, OCH_3), 2.33 (s, 3H, 3′-$COCH_3$), 2.10, 2.03, 2.01 (3×s, 3×3H, 3×sugar $COCH_3$); *m*/*z* (APCI, +ve mode) 863 [$(M+Na)^+$].

Compound **8b**: δ_H ($CDCl_3$) 12.43 (s, 1H, 5-OH), 7.68 (dd, 1H, *J* 8.6, 2.3 Hz, H-6′), 7.63 (d, 1H, *J* 2.3 Hz, H-2′), 7.27–7.46 (m, 10H, 2×Ph), 7.02 (d, 1H, *J* 8.6 Hz, H-5′), 6.49 (d, 1H, *J* 2.0 Hz, H-8), 6.42 (d, 1H, *J* 2.0 Hz, H-6), 5.83 (s, 1H, 3′-OH), 5.73 (d, 1H, *J* 7.9 Hz, H-1″), 5.1–5.4 (m, 3H, H-2″, H-3″, H-4″), 5.21, 5.11 (2×s, 2×2H, 2×C*H*$_2$Ph), 4.00 (d, 1H, *J* 10.2 Hz, H-5″), 3.60 (s, 3H, OCH_3), 2.10, 2.02, 2.00 (3×s, 3×3H, 3×$COCH_3$); *m*/*z* (APCI, +ve mode) 821 [$(M+Na)^+$].

Compounds **8a** and **8b** were not further purified or characterised but were debenzylated, de-esterified and purified by HPLC as follows. The products were recombined and suspended in a mixture of EtOH (80 mL) and cyclohexene (20 mL). Twenty percent $Pd(OH)_2$ on charcoal (200 mg) was added. The mixture was refluxed under Ar for 30 min, cooled and filtered through a 0.5 μm filter. The filtrate was evaporated and the residue was suspended in 50% aq MeOH (200 mL). Aq Na_2CO_3 (0.5 M, 6 mL) was added and the mixture was stirred under Ar at room temperature for 2.5 h. After cooling Dowex 50W resin (H^+ form) was added with stirring until the pH fell to below 3.0. The mixture was filtered (0.5 μm), the resin was washed (50% aq MeOH, 1×50 mL; MeOH, 1×50 mL) and filtrate and resin washes were combined and evaporated. The residue was dissolved in 50% aq MeOH (8 mL). Compound **2** was isolated by preparative HPLC (2×4 mL injections) as an orange glass and was >95% pure by analytical HPLC. It was identical to authentic material.[22] Yield 89 mg (41% from **6**).

Compound **2** (H+ form): δ_H (CD_3OD) 7.64 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.61 (d, 1H, *J* 2.3 Hz, H-2′), 6.84 (d, 1H, *J* 8.9 Hz, H-5′), 6.38 (d, 1H, *J* 2.0 Hz, H-8), 6.19 (d, 1H, *J* 2.0 Hz, H-6), 5.32 (d, 1H, *J* 7.6 Hz, H-1″), 3.74 (d, 1H, *J* 9.6 Hz, H-5″), 3.42–3.62 (m, 3H, H-2″, H-3″, H-4″).

Compound **2** (lit.[22] H+ form): δ_H (CD_3OD) 7.63 (H-6′), 7.63 (H-2′), 6.84 (H-5′), 6.37 (H-8), 6.18 (H-6), 5.31 (H-1″), 3.75 (H-5″), 3.47–3.59 (H-2″, H-3″, H-4″).

3.2.3. Quercetin 3′-glucuronide, 3. Compound **6** (0.36 g, 0.75 mmol), powdered 3 Å molecular sieves (0.5 g) and dry CH_2Cl_2 (5 mL) were stirred at room temperature under Ar for 2 h. The trichloroacetimidate **10** (0.39 g, 1.1 equiv) was added and the mixture was cooled to −15 °C. $BF_3{\cdot}Et_2O$ (freshly distilled, 120 μL, 1.25 equiv) was added drop wise over 10 min. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was filtered through Celite and the latter was washed with CH_2Cl_2, acetone and methanol until the washings were colourless. Filtrate and washings were combined and evaporated to give a bright yellow glass. The crude product was purified by MPLC (20 g silica, 5% acetone/95% toluene isocratic elution) to give recovered **6** (0.21 g, 58%) and crude **11** (0.10 g).

Compound **11**: δ_H ($CDCl_3$) 11.65 (s, 1H, 5-OH), 8.05 (d, 1H, *J* 2.3 Hz, H-2′), 7.94 (dd, 1H, *J* 8.6, 2.3 Hz, H-6′), 7.28–7.46 (m, 10H, 2×Ph), 7.02 (d, 1H, *J* 8.6 Hz, H-5′), 6.69 (s, 1H, 3-OH), 6.58 (d, 1H, *J* 2.0 Hz, H-8), 6.41 (d, 1H, *J* 2.0 Hz, H-6), 5.76 (d, 1H, *J* 7.9 Hz, H-1″), 5.1–5.4 (m, 3H, H-2″, H-3″, H-4″), 5.36, 5.14 (2×s, 2×2H, 2×C*H*$_2$Ph), 4.16 (d, 1H, *J* 10.2 Hz, H-5″), 3.70 (s, 3H, OCH_3), 2.05 (s, 3×3H, 3×$COCH_3$).

Compound **11** was not further characterised or purified but was debenzylated, de-esterified and purified in the same

way as **8a** and **8b** (proportionately scaled down) to give **3** as an orange glass. Yield 39 mg (11% from **6**). The substitution position was confirmed by HMBC (see text); **3** ν_{max} (ATR of solid) 3208br d, 1675, 1652, 1616, 1595, 1570, 1502, 1412, 1357, 1268, 1189, 1161, 1136, 1003; $[\alpha]_D^{25}$ −44 (*c* 1.0, water).

Compound **3** (H^+ form): δ_H (CD_3OD) 8.03 (d, 1H, *J* 2.0 Hz, H-2′), 7.89 (dd, 1H, *J* 8.6, 2.0 Hz, H-6′), 6.95 (d, 1H, *J* 8.6 Hz, H-5′), 6.39 (d, 1H, *J* 2.0 Hz, H-8), 6.15 (d, 1H, *J* 2.0 Hz, H-6), 4.92 (d, 1H, *J* 6.9 Hz, H-1″), 4.04 (d, 1H, *J* 9.6 Hz, H-5″), 3.52–3.70 (m, 3H, H-2″, H-3″, H-4″).

Compound **3** (H^+ form): δ_H (DMSO-d_6) 12.43 (s, 1H, 5-OH), 9.45 (br d s, 1H, COOH), 7.88 (dd, 1H, *J* 8.2, 2.0 Hz, H-6′), 7.85 (d, 1H, *J* 2.0 Hz, H-2′), 6.99 (d, 1H, *J* 8.2 Hz, H-5′), 6.48 (d, 1H, *J* 2.0 Hz, H-8), 6.20 (d, 1H, *J* 2.0 Hz, H-6), 5.2 (br d s, 1H, 3-OH), 4.92 (d, 1H, *J* 6.9 Hz, H-1″), 3.79 (d, 1H, *J* 8.9 Hz, H-5″), 3.3–3.5 (m, 3H, H-2″, H-3″, H-4″).

Compound **3** (Na^+ form, obtained by titration in aq MeOH to pH 6.0 with aq NaOH, and evaporation): δ_H (DMSO-d_6) 12.46 (s, 1H, 5-OH), 7.93 (d, 1H, *J* 2.3 Hz, H-2′), 7.85 (dd, 1H, *J* 8.6, 2.3 Hz, H-6′), 6.97 (d, 1H, *J* 8.6 Hz, H-5′), 6.50 (d, 1H, *J* 1.6 Hz, H-8), 6.19 (d, 1H, *J* 1.6 Hz, H-6), 5.04 (br d s, 1H, 3-OH), 4.73 (d, 1H, *J* 6.9 Hz, H-1″), 3.44 (d, 1H, *J* 8.9 Hz, H-5″), 3.2–3.4 (m, 3H, H-2″, H-3″, H-4″); δ_C (DMSO-d_6) 175.9 (C-4), 171.5 (C-6″), 164.1 (C-7), 160.5 (C-5), 156.1 (C-9), 149.6 (C-4′), 146.3 (C-2), 145.3 (C-3′), 135.9 (C-3), 124.2 (C-6′), 121.6 (C-1′), 117.9 (C-2′), 116.5 (C-5′), 103.3 (C-1″), 102.9 (C-10), 98.2 (C-6), 93.7 (C-8), 74.2 (C-5″), 74.5, 76.0, 73.0 (C-2″, C-3″, C-4″).

HRMS (ESI, −ve mode): $(M-H)^-$, found 477.0686. $C_{21}H_{17}O_{13}$ requires 477.0669.

3.2.4. 3′-*O*-Methyl-4′,7-di-*O*-benzylquercetin, 12. 3′-*O*-Methyl-4′,7-di-*O*-benzylquercetin, **12**, was prepared from 3,3′,5,4′,7-di-*O*-benzylquercetin triacetate (1.66 g) as described by Jurd,[6] except that the crude product after filtration and acidification was purified by MPLC (silica, 2% acetone/toluene). Yield of **12** (as a yellow powder) 0.18 g, 15% (lit.[6] 12%); mp 172–174 °C (lit.[6] 175 °C); δ_H ($CDCl_3$) 11.71 (s, 1H, 5-OH), 7.79 (d, 1H, *J* 2.3 Hz, H-2′), 7.74 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.33–7.46 (m, 10H, 2×Ph), 7.00 (d, 1H, *J* 8.9 Hz, H-5′), 6.60 (s, 1H, 3-OH), 6.56 (d, 1H, *J* 2.0 Hz, H-8), 6.52 (d, 1H, *J* 2.0 Hz, H-6), 5.24, 5.14 (2×s, 2×2H, 2×CH_2Ph), 3.98 (s, 3H, OCH_3).

3.2.5. 3′-Methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide), 4. Ag_2O (0.71 g, 2.5 equiv), $CaSO_4$ (dried at 120 °C, 0.6 g) and dry pyridine (10 mL) were stirred, in the dark, under Ar at 0 °C. After 5 min, methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide **7** (0.61 g, 580 μmol, 1.25 equiv) was added; and, after a further 5 min, 3′-*O*-methyl-4′,7-di-*O*-benzylquercetin **12** (0.61 g, 1.23 mmol). Stirring was continued at 0 °C. After 16 h, the reaction was worked up as described for **8a** and **8b** (on 2.5×the scale) to give 0.82 g of a light brown solid. MPLC (50 g silica, 5% acetone/95% toluene, isocratic elution) gave 0.42 g (51%) of **13**; δ_H ($CDCl_3$) 12.40 (s, 1H, 5-OH), 7.77 (d, 1H, *J* 2.0 Hz, H-2′), 7.53 (dd, 1H, *J* 8.6, 2.0 Hz, H-6′), 7.29–7.46 (m, 10H, 2×Ph), 6.92 (d, 1H, *J* 8.6 Hz, H-5′), 6.41 (d, 1H, *J* 2.0 Hz, H-8), 6.35 (d, 1H, *J* 2.0 Hz, H-6), 5.80 (d, 1H, *J* 7.9 Hz, H-1″), 5.12–5.41 (m, 3H, H-2″, H-3″, H-4″), 5.21, 5.01 (2×s, 2×2H, 2×CH_2Ph), 4.05 (d, 1H, *J* 9.9 Hz, H-5″), 4.00 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 2.10, 2.02, 1.99 (3×s, 3×3H, 3×$COCH_3$).

Compound **13** was not further characterised or purified but was debenzylated, de-esterified and purified in the same way as **8a** and **8b** (proportionately scaled up) to give **4** as an orange glass, identical in all respects to authentic material,[10] yield 198 mg (33% from **6**). The substitution position was confirmed by HMBC (see text); ν_{max} (ATR of solid) 3223br d (OH), 1680, 1652, 1599, 1563, 1463, 1427, 1353, 1286, 1242, 1198, 1181, 1167, 1124; $[\alpha]_D^{25}$ −13 (*c* 1.0, water).

Compound **4** (H^+ form): δ_H (CD_3OD) 7.95 (d, 1H, *J* 2.4 Hz, H-2′), 7.54 (dd, 1H, *J* 8.2, 2.0 Hz, H-6′), 6.86 (d, 1H, *J* 8.2 Hz, H-5′), 6.37 (d, 1H, *J* 2.0 Hz, H-8), 6.17 (d, 1H, *J* 2.0 Hz, H-6), 5.48 (d, 1H, *J* 7.2 Hz, H-1″), 3.93 (s, 3H, OCH_3), 3.78 (d, 1H, *J* 9.2 Hz, H-5″), 3.46–3.62 (m, 3H, H-2″, H-3″, H-4″); δ_C (CD_3OD) 179.1 (C4), 171.6 (C6″), 166.0 (C7), 163.0 (C5), 158.7 (C2), 158.4 (C9), 150.9 (C3′), 148.4 (C4′), 135.1 (C3), 123.5 (C6′), 122.8 (C1′), 116.0 (C5′), 114.4 (C2′), 105.7 (C10), 104.0 (C1″), 99.9 (C6), 94.8 (C8), 77.5 (C3″), 77.2 (C5″), 75.6 (C2″), 73.0 (C4″), 55.7 (OCH_3).

Compound **4** (Na^+ form): δ_H (CD_3OD) 7.99 (d, 1H, *J* 2.0 Hz, H-2′), 7.56 (dd, 1H, *J* 8.3, 2.0 Hz, H-6′), 6.89 (d, 1H, *J* 8.3 Hz, H-5′), 6.38 (d, 1H, *J* 2.0 Hz, H-8), 6.17 (d, 1H, *J* 2.0 Hz, H-6), 5.38 (m, 1H, H-1″), 3.93 (s, 3H, OCH_3), 3.44–3.57 (m, 4H, H-2″, H-3″, H-4″, H-5″).

HRMS (ESI, +ve mode): $(M+H)^+$, found 537.0642. $C_{22}H_{19}Na_2O_{13}$ requires 537.0616.

3.2.6. 3,4′,7-Tri-*O*-benzylquercetin, 14. Compound **6** (2 g, 4.15 mmol) was dissolved with stirring in dry DMF (35 mL) under Ar at 21 °C. ^{t}BuOK (0.745 g, 1.6 equiv) was added, followed by BnBr (790 μL, 1.6 equiv). Stirring was continued overnight at 21 °C for 17 h. The mixture was poured into stirred 10% aq HCl (375 mL) and cooled to 5 °C for 2 h. The resultant solid was recovered by filtration, washed with water, dissolved in EtOAc (300 mL), washed twice with water (2×100 mL) and dried ($MgSO_4$). Evaporation gave a solid (2.38 g) that was purified by MPLC (50 g silica, 100% toluene for 10 min, then to 5% acetone/95% toluene after another 5 min) to give **14** (1.57 g, 66%) as yellow needles, mp 155 °C (lit.[16] mp not given).

Compound **14**: δ_H (DMSO-d_6) 12.67 (s, 1H, 5-OH), 9.44 (s, 1H, 3′-OH), 7.57 (d, 1H, *J* 2.3 Hz, H-2′), 7.53–7.29 (m, 16H, H-6′, 3×Ph), 7.13 (d, 1H, *J* 8.8 Hz, H-5′), 6.80 (d, 1H, *J* 2.0 Hz, H-8), 6.48 (d, 1H, *J* 2.0 Hz, H-6), 5.23–5.32 (br s, 4H, 2×CH_2Ph), 5.03 (s, 2H, CH_2Ph).

Compound **14** (lit.[16]): δ_H (DMSO-d_6) 12.67 (s, 1H, 5-OH), 9.47 (s, 1H, 3′-OH), 7.56 (d, 1H, *J* 1.5 Hz, H-2′), 7.52–7.29 (m, 16H, H-6′, 3×Ph), 7.13 (d, 1H, *J* 8.8 Hz, H-5′), 6.80 (d, 1H, *J* 1.5 Hz, H-8), 6.47 (d, 1H, *J* 1.5 Hz), 5.23–5.32 (br s, 4H, 2×CH_2Ph), 5.02 (s, 2H, CH_2Ph).

3.2.7. Quercetin 3′-sulfate, 5. Compound **14** (0.252 g, 440 μmol) was dissolved in dry DMF (5 mL) with stirring

under Ar. A solution of sulfur trioxide-*N*,*N*-dimethylformamide complex **16** (0.340 g, 5 equiv, weighed and kept under Ar) in DMF (5 mL) was added. After 4 d, the reaction mixture was evaporated, and satd $NaHCO_3$ (6 mL) was added with stirring (final pH 7.6). The mixture was again evaporated to dryness and the residue was dissolved in acetone (150 mL) and filtered (sinter). The residual solid was washed with acetone (50 mL). (Attempts to isolate more products from the residual undissolved material were unsuccessful.) The acetone washings and filtrate were combined and evaporated to a yellow glass (0.27 g). This material was not further purified but was dissolved in EtOH (50 mL); cyclohexene (25 mL) and $Pd(OH)_2$/C (250 mg) were added, and the mixture was heated at reflux (30 min) and cooled. The mixture was filtered (0.5 μm membrane) and evaporated to give a yellow solid (0.34 g). The latter was redissolved in water (10 mL), loaded onto an SPE cartridge and eluted with water (100 mL) to give **5** (121 mg, 68% from **14**) as a yellow solid, identical to authentic material.[23]

Compound **5** (Na^+ form): δ_H (DMSO-d_6) 12.45 (br s, 1H, 5-OH), 9.47 (br s, 2H, 2×OH), 8.03 (d, 1H, *J* 2.0 Hz, H-2′), 7.85 (dd, 1H, *J* 8.6, 2.0 Hz, H-6′), 6.98 (d, 1H, *J* 8.6 Hz, H-5′), 6.44 (d, 1H, *J* 1.9 Hz, H-8), 6.19 (d, 1H, *J* 1.9 Hz, H-6).

Compound **5** (lit.[23], Na^+ form): δ_H (DMSO-d_6) 7.99 (d, 1H, *J* 2.4 Hz, H-2′), 7.80 (dd, 1H, *J* 7.6, 2.4 Hz, H-6′), 6.93 (d, 1H, *J* 7.6 Hz, H-5′), 6.39 (d, 1H, *J* 1.5 Hz, H-8), 6.14 (d, 1H, *J* 1.5 Hz, H-6).

Compound **5** (Na^+ form): δ_H (CD_3OD) 8.20 (d, 1H, *J* 2.3 Hz, H-2′), 8.01 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.01 (d, 1H, *J* 8.9 Hz, H-5′), 6.42 (d, 1H, *J* 1.9 Hz, H-8), 6.16 (d, 1H, *J* 1.9 Hz, H-6). *m*/*z* (ESI, −ve mode) 381 ($(M-H)^-$).

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Synthesis of substituted 5-aminomethyl tetrahydro-isoquinolines and dihydro-isoindoles

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Abstract—The synthesis of ten substituted aminomethylene tetrahydro-isoquinolines is described, proceeding in eight steps from 5-hydroxyisoquinoline via reductive amination of *N*-Boc tetrahydro-isoquinoline 5-carboxaldehyde. Likewise, reductive amination was used to prepare four substituted dihydro-isoindoles from the corresponding aldehyde. The dihydro-isoindole ring system was conveniently accessed via a 2+2+2 cycloaddition reaction.

1. Introduction

The introduction of conformational restraint is a frequently employed tactic for improving the potency and selectivity of biologically active compounds.[1] Di- or multi-functional compounds are useful components for building compound libraries to investigate structure–activity relationships rapidly. Hence difunctional compounds that possess a well-defined spatial relationship between the functional groups are particularly sought after as templates.[2] In connection with a programme to discover novel, selective α_1-adrenergic antagonists, we needed to prepare a range of tetrahydro-isoquinolines (**1**) and dihydro-isoindoles (**2**) (Fig. 1), bearing a variable basic substituent (NR^1R^2) attached to the ring system via a methylene group.

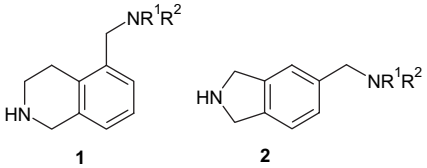

Figure 1.

The synthesis of **1** began with the reduction of commercially available 5-hydroxyisoquinoline (Scheme 1); hydrogenation over Adam's catalyst in acetic acid solvent[3] gave 5-hydroxy-tetrahydro-isoquinoline **3**. Treatment with an excess of Boc-anhydride afforded a mixture of *N*-Boc and *N*,*O*-diprotected products, which were not separated but exposed to sodium hydroxide, resulting in the selective removal of the *O*-Boc group. Compound **4** was isolated in 84% yield. The phenol group was converted into the nitrile **5** by reaction with *N*-phenyl triflamide[4] followed by palladium-catalysed displacement of the triflate by zinc cyanide.[5] Reduction of the nitrile to the aldehyde **6** was best achieved using an excess of DIBAL-H in toluene at −78 °C,[6] followed by a careful quenching of the excess reagent using methanol, and a brief exposure to hydrochloric acid to hydrolyse the intermediate imine. The aldehyde was obtained in 88% yield without loss of the Boc group.

Aldehyde **6** was reacted with a variety of amines (see Table 1) in the presence of sodium triacetoxyborohydride,[7] followed by treatment of the products (**7a–j**) with a saturated solution of hydrogen chloride in dichloromethane to afford the deprotected amines (**1a–j**) as their hydrochlorides. In some cases (i.e., **7f–j**), the amine was more conveniently employed as its hydrochloride or trifluoroacetate salt; thus 1 equiv of triethylamine or sodium acetate was also added to the reductive amination to buffer the reaction mixture.

The amine used to prepare **7j**, 3-methoxy-3-methylazetidine, was made using the four-step route shown in Scheme 2.

Keywords: Tetrahydro-isoquinoline; Dihydro-isoindole; Diamine; Reductive amination.
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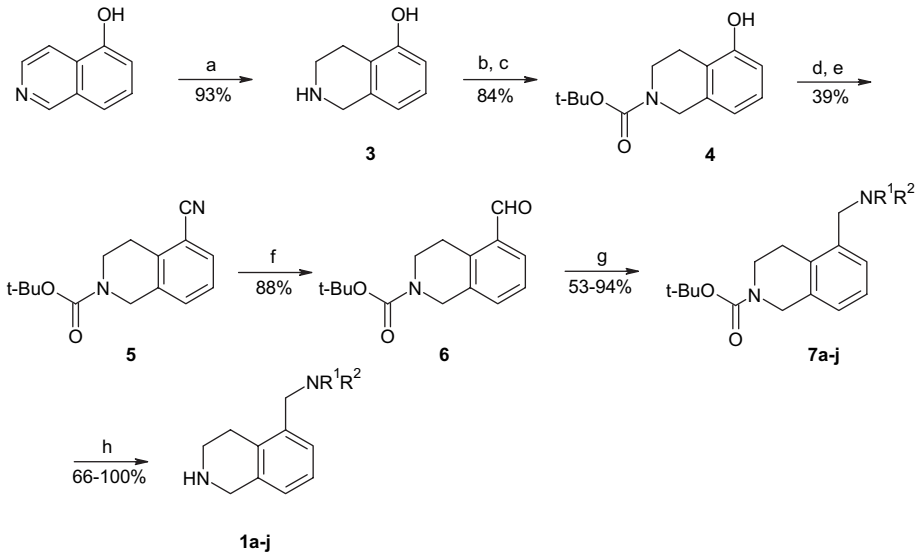
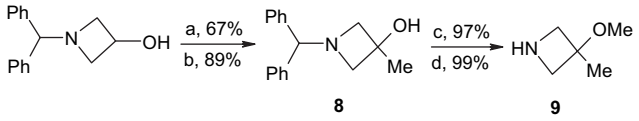


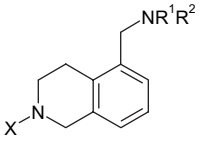
Scheme 1. Reagents and conditions: (a) H_2 (3 bar), PtO_2, HOAc, 16 h; (b) $(Boc)_2O$, NaOH, dioxane/water, 20 °C, 16 h; (c) 2 M NaOH (aq), MeOH/dioxane, 20 °C, 3 h; (d) $(Tf)_2NPh$, Et_3N, CH_2Cl_2, 0–20 °C, 16 h; (e) $Zn(CN)_2$ (1 equiv), 4 mol % $Pd(PPh_3)_4$, LiCl (1 equiv), DMF, 80 °C; (f) DIBAL-H (2.3 equiv), toluene, −78 °C, 2 h, then 1 M aq HCl, 0 °C, 20 min; (g) R^1R^2NH (1–1.5 equiv), MeCN, THF or CH_2Cl_2, 20 °C, 1–3 h, then $NaBH(OAc)_3$ (2.5 equiv), 18 h; (h) $HCl_{(g)}$, CH_2Cl_2, 20 °C, 1–2 h.

Thus, commercially available *N*-benzhydrylazetidin-3-ol was oxidised to the ketone using DMSO and pyridine–sulfur trioxide complex[12] and then treated with methylmagnesium bromide to afford **8**. Alkylation of the alcohol and removal of the benzhydryl group using chloroethyl chloroformate[13] gave **9** as the hydrochloride salt.

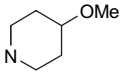
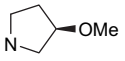
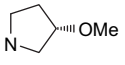


Scheme 2. (a) DMSO, Py–SO_3, 20 °C, 1.5 h; (b) MeMgBr (3 M in ether), THF, 0 °C, 2 h; (c) NaH, DMF, 0–20 °C, 2 h, then MeI, 0–20 °C, 2 h; (d) chloroethyl chloroformate, MeCN, reflux, 1 h, then MeOH, reflux, 2 h.

Table 1. Structures and yields of diamine products **7a–j** and **1a–j**



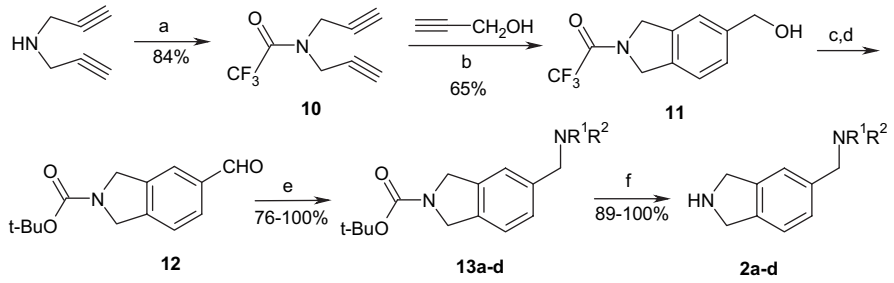
1: X = H; **7**: X = Boc

NR^1R^2	Compd (%yield)	Compd (%yield)	
NMe_2	**7a** (83)	**1a** (100)	
$NHCH_2CH_2OMe$	**7b** (81)	**1b** (100)	
$NMeCH_2CH_2OMe$	**7c** (64)	**1c** (90)	
	**7d** (77)	**1d** (99)	
	**7e** (66)	**1e** (66)	Ref. 8
	**7f** (94)	**1f** (99)	Ref. 9
	**7g** (89)	**1g** (99)	Ref. 10
	**7h** (53)	**1h** (84)	Ref. 11
	**7i** (65)	**1i** (100)	Ref. 10
	**7j** (74)	**1j** (100)	

For the synthesis of **2**, we utilised 2+2+2 cycloaddition chemistry to build up the isoindoline ring, as shown in Scheme 3.

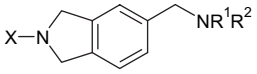
Thus, *N*,*N*-dipropynylamine was protected as its trifluoroacetyl derivative **10**, followed by reaction with 4 equiv of propynol in the presence of Wilkinson's catalyst to give hydroxymethylisoindoline **11**.[14] The trifluoroacetyl protecting group was chosen as it is easily removed under mild, basic conditions, however, we found that it was sometimes labile under reductive amination conditions. We therefore switched to the more robust Boc protection prior to oxidation using the modified Swern oxidation. The aldehyde **12** was obtained in good yield. Four amines were used in the reductive amination, and removal of the Boc group, as before, afforded the compounds shown in Table 2 (yields in parentheses).

In summary, we have developed efficient routes to a range of substituted tetrahydro-isoquinolines (**1**) and dihydro-isoindoles (**2**). These compounds were elaborated further to explore structure–activity relationships of α_1-adrenergic antagonists. Details of the synthesis of the target compounds, and their biological screening data, will be reported elsewhere.

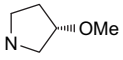


Scheme 3. (a) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0–20 °C; (b) 3 mol % $(PPh_3)_3RhCl$, EtOH, 0–20 °C, 17 h; (c) K_2CO_3, H_2O, MeOH, 20 °C, 30 min then $(Boc)_2O$, 20 °C, 6 h, 89%; (d) $(CF_3CO)_2O$, DMSO, CH_2Cl_2, −78 °C, then *i*-Pr_2NEt, warmed to 20 °C, 78%; (e) R^1R^2NH, HOAc, $NaBH(OAc)_3$, THF, 20 °C, 19 h; (f) $HCl_{(g)}$, CH_2Cl_2, 20 °C, 1 h.

Table 2. Structures and yields of diamine products **13a–d** and **2a–d**



2: X = H; **13**: X = Boc

NR^1R^2	Compd (%yield)	Compd (%yield)
	**13a** (99)	**2a** (99)
$NMeCH_2CH_2OMe$	**13b** (76)	**2b** (100)
	**13c** (100)	**2c** (99)
	**13d** (85)	**2d** (89)

2. Experimental

2.1. General

Melting points were determined using open glass capillary tubes and a Gallenkamp melting point apparatus and are uncorrected. Spectroscopic data were recorded on a Perkin–Elmer 983 (IR), Finnigan Mat. Navigator (LRMS, either positive (ES^+) or negative (ES^-) electrospray mode) and Varian Unity Inova (^1H NMR 300 or 400 MHz) instruments and are consistent with the assigned structures. Combustion analyses were performed by Exeter Analytical (UK) Limited, Uxbridge, Middlesex. Accurate mass determinations for molecular ions were obtained using a commercially available Apex II Fourier Transform Mass Spectrometer (Bruker Daltonics Inc., Billerica, MA, USA) equipped with a 4.7 T, passively shielded, superconducting magnet and an electrospray ionisation source (ESI), used in positive ion mode (Analytica of Branford, Branford, CT, USA) and calibrated using sodium trifluoroacetate. Reactions were performed under an atmosphere of dry nitrogen unless otherwise noted. Flash chromatography refers to column chromatography on silica gel (Kieselgel 60, 230–400 mesh, from E. Merck, Darmstadt). Kieselgel 60 F_{254} plates from E. Merck were used for TLC and compounds were visualised using UV light or 0.5% aqueous potassium permanganate solution.

2.1.1. 1,2,3,4-Tetrahydro-5-hydroxy-isoquinolinium acetate, 3.[3] A suspension of 5-isoquinolinol (24 g, 165 mmol) in acetic acid (250 mL) was hydrogenated over platinium dioxide (8 g), with overhead stirring, at 3 bar for 16 h at room temperature. The reaction mixture was decanted from the catalyst and the catalyst was thoroughly rinsed with methanol (1 L). The combined solutions were evaporated to dryness to give an oil (65 g). The residual oil was dissolved in methanol (200 mL) and diethyl ether was added. The solid, which precipitated, was collected by filtration, washed with diethyl ether and dried under vacuum to give **3** as an off-white solid (32.1 g, 93%). δ_H ($CDCl_3$, 300 MHz) 2.60 (m, 2H), 3.10 (m, 2H), 3.90 (m, 2H), 6.4 (d, *J* 8 Hz, 1H), 6.60 (d, *J* 8 Hz, 1H), 6.9 (t, *J* 8 Hz, 1H); LRMS: *m*/*z* (ES^+) 150 [MH^+].

2.1.2. *tert*-Butyl 3,4-dihydro-5-hydroxy-2(1*H*)-isoquinolinecarboxylate, 4. A solution of di-*tert*-butyl dicarbonate (66.75 g, 0.31 mol) in 1,4-dioxane (300 mL) was added to a mixture of **3** (32.0 g, 153 mmol) and 1 M aqueous sodium hydroxide (200 mL) and the resulting suspension stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the residue partitioned between 1 M hydrochloric acid (300 mL) and dichloromethane (500 mL). The aqueous phase was re-extracted with dichloromethane (200 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give an orange oil. The crude product was dissolved in 1,4-dioxane (200 mL) and methanol (100 mL) followed by the addition of 2 N aqueous sodium hydroxide (150 mL) and the resulting cloudy mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure and the residue partitioned between ethyl acetate (600 mL) and water (200 mL). The organic phase was separated, washed with 2 N hydrochloric acid (200 mL), brine (250 mL) then dried ($MgSO_4$) and concentrated under reduced pressure to give a tan solid. The solid was suspended in dichloromethane (150 mL), then pentane (800 mL) was added and filtered to give **4** as a white solid (32.04 g, 84%). δ_H ($CDCl_3$, 300 MHz) 1.49 (s, 9H), 2.76 (t, *J* 6 Hz, 2H), 3.66 (t, *J* 6 Hz, 2H), 4.56 (s, 2H), 5.29 (br s, 1H), 6.63 (d, *J* 8 Hz, 1H), 6.71 (d, *J* 8 Hz, 1H), 7.05 (t, *J* 8 Hz, 1H); LRMS: *m*/*z* (ES^+) 272 [MNa^+]. Found: C, 67.30; H, 7.68; N, 5.61, $C_{14}H_{19}NO_3$ requires C, 67.45; H, 7.68; N, 5.62%.

2.1.3. *tert*-Butyl 5-cyano-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate, 5. Triethylamine (20.1 mL, 144 mmol) was added to a suspension of **4** (32.65 g, 131 mmol) in dichloromethane (400 mL). The mixture was cooled to 0 °C and *N*-phenyl-bis-(trifluoromethane)sulfonimide (51.46 g, 144 mmol) was added portionwise. The resulting brown solution

was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was washed consecutively with water (200 mL), 0.5 M hydrochloric acid (200 mL), brine (250 mL) and then dried ($MgSO_4$) and concentrated under reduced pressure to give a brown oil. The crude product was purified by column chromatography on silica gel, eluting with a solvent gradient of *n*-pentane/diethyl ether (100:0–70:30). The product was co-evaporated with dichloromethane (2×100 mL) to give *tert*-butyl 5-[(trifluoromethanesulfonyl)oxy]-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate as a colourless gum (40.1 g, 80%). δ_H ($CDCl_3$, 300 MHz) 1.49 (s, 9H), 2.89 (t, *J* 5 Hz, 2H), 3.65 (t, *J* 5 Hz, 2H), 4.59 (s, 2H), 7.13 (m, 2H), 7.24 (m, 1H); LRMS: *m*/*z* (ES^+) 404 [MNa^+]. The triflate (20.0 g, 52 mmol) was dissolved in anhydrous DMF (120 mL) under nitrogen. Zinc cyanide (6.15 g, 52 mmol), lithium chloride (2.22 g, 52 mmol) and tetrakis(triphenylphosphine)palladium (0) (2.42 g, 2.1 mmol) were added and the mixture was heated at 110 °C for 8 h. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between dichloromethane (500 mL) and saturated sodium bicarbonate solution (250 mL). The aqueous phase was re-extracted with dichloromethane (300 mL). The combined organic solutions were dried ($MgSO_4$) and concentrated under reduced pressure to give a golden oil. The crude product was purified by column chromatography on silica gel using *n*-pentane/ethyl acetate (90:10) as eluant. The product was co-evaporated with dichloromethane (2×100 mL) to give **5**, as a colourless oil (13.32 g, 49%). δ_H ($CDCl_3$, 300 MHz) 1.48 (s, 9H), 3.02 (t, *J* 6 Hz, 2H), 3.70 (t, *J* 6 Hz, 2H), 4.58 (s, 2H), 7.20 (t, *J* 7 Hz, 1H), 7.44 (d, *J* 7 Hz, 1H), 7.55 (d, *J* 7 Hz, 1H); LRMS: *m*/*z* (ES^+) 281 [MNa^+].

2.1.4. *tert*-Butyl 5-formyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate, 6. A solution of compound **5** (9.1 g, 35 mmol) in anhydrous toluene (100 mL) was cooled to −78 °C. Over 1 h, diisobutylaluminium hydride (80 mL of a 1 M solution in toluene, 80 mmol) was added dropwise keeping the internal temperature below −60 °C and the resulting mixture was stirred for 2 h at −78 °C. Methanol (20 mL) was pre-cooled to −78 °C and added dropwise to the reaction mixture keeping the internal temperature below −60 °C. Over 20 min, the reaction mixture was poured into 1 N hydrochloric acid (200 mL) that had been pre-cooled to 0 °C. The reaction mixture was extracted with ethyl acetate (3×400 mL) and the combined organic extracts were washed with brine (200 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The product was co-evaporated with dichloromethane (2×50 mL) to give **6**, as a yellow oil (8.14 g, 88%). δ_H (DMSO-d_6, 400 MHz) 1.40 (s, 9H), 3.19 (t, *J* 6 Hz, 2H), 3.55 (t, *J* 6 Hz, 2H), 4.55 (s, 2H), 7.40 (t, *J* 8 Hz, 2H), 7.47 (d, *J* 8 Hz, 1H), 7.70 (d, *J* 8 Hz, 1H), 10.16 (s, 1H); LRMS: *m*/*z* (ES^+) 284 [MNa^+].

2.1.5. 4-Methoxypiperidine hydrochloride. Sodium hydride (1.19 g, 60% in mineral oil, 29.7 mmol) was added portionwise to a cooled (10 °C) solution of *tert*-butyl 4-hydroxy-1-piperidinecarboxylate in anhydrous THF (80 mL) and the suspension was stirred at room temperature for 1 h. Iodomethane (1.85 mL, 29.7 mmol) was added and the reaction was stirred at 50 °C for 20 h. The mixture was diluted with water (50 mL), extracted with ethyl acetate (2×150 mL) and the combined organic extracts were washed with saturated sodium bicarbonate solution (50 mL), dried ($MgSO_4$) and evaporated under reduced pressure to afford *tert*-butyl 4-methoxy-1-piperidinecarboxylate as a golden oil, 5.24 g. ^1H NMR δ_H ($CDCl_3$, 400 MHz) 1.47 (s, 9H), 1.50 (m, 2H), 1.80 (m, 2H), 3.08 (m, 2H), 3.34 (m, 4H), 3.75 (m, 2H); LRMS: *m*/*z* (ES^+) 238 [MNa^+]. This material was dissolved in dichloromethane (100 mL), cooled to 0 °C and hydrogen chloride was bubbled through the solution. After being stirred for 1.5 h, the solution was purged with nitrogen and evaporated under reduced pressure to afford 4-methoxypiperidine hydrochloride as an off-white solid, 3.67 g, (82% over 2 steps).

δ_H (DMSO-d_6, 400 MHz) 1.87 (m, 2H), 1.99 (m, 2H), 3.10 (m, 2H), 3.28 (m, 2H), 3.36 (s, 3H), 3.54 (m, 1H); LRMS: *m*/*z* (ES^+) 231 [$2MH^+$].

2.1.6. *N*-Benzhydryl-3-methylazetidin-3-ol, 8. Triethylamine (49.4 mL, 0.36 mol) was added to a solution of *N*-benzhydrylazetidin-3-ol (10 g, 36 mmol) in DMSO (50 mL). A solution of sulfur trioxide–pyridine complex (36 g, 0.22mol) in DMSO (110 mL) was added dropwise and the resulting yellow solution was stirred at room temperature for 1.5 h. The reaction was poured onto ice-water (300 mL) and extracted with ethyl acetate (2×300 mL). The combined organic extracts were washed with brine (3×200 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using cyclohexane/ethyl acetate (8:2) as eluant to give *N*-benzhydrylazetidin-3-one as a white solid (5.77 g, 67%).

δ_H ($CDCl_3$, 300 MHz) 3.99 (s, 4H), 4.58 (s, 1H), 7.2 (m, 2H), 7.25 (t, *J* 8 Hz, 4H), 7.47 (d, *J* 8 Hz, 4H); LRMS: *m*/*z* (ES^+) 238 [MH^+], 260 [MNa^+]. The azetidinone (5.77 g, 24.3 mmol) was dissolved in THF (60 mL) and cooled to 0 °C. Methylmagnesium bromide (16.2 mL, 48.7 mmol) was added dropwise and stirring continued at 0 °C for 2 h. The reaction was carefully quenched with saturated ammonium chloride (100 mL) and extracted with ethyl acetate (150 mL). The organic solution was washed with brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using cyclohexane/ethyl acetate (8:2) as eluant to give *N*-benzhydryl-3-methylazetidin-3-ol[15] as a golden oil (5.48 g, 89%). δ_H ($CDCl_3$, 300 MHz) 1.52 (s, 3H), 2.97 (d, *J* 8 Hz, 2H), 3.18 (d, *J* 8 Hz, 2H), 4.35 (s, 1H), 7.17 (t, *J* 6 Hz, 2H), 7.26 (t, *J* 6 Hz, 4H), 7.40 (d, *J* 6 Hz, 4H); LRMS: *m*/*z* (ES^+) 254 [MH^+], 276 [MNa^+].

2.1.7. 3-Methoxy-3-methylazetidine hydrochloride, 9. Azetidinol **8** (3.46 g, 13 mmol) was dissolved in DMF (50 mL) and cooled to 0 °C. Sodium hydride (820 mg, 60% in mineral oil, 20.5 mmol) was added portionwise and the suspension was stirred for 2 h. The mixture was re-cooled to 0 °C and a solution of iodomethane (1.06 mL, 17 mmol) in DMF (10 mL) was added dropwise. After being stirred for 3 h, the mixture was diluted with ethyl acetate (125 mL) and washed with water (2×80 mL). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using cyclohexane/ethyl acetate (8:2) as eluant to give *N*-benzhydryl-3-methoxy-3-methylazetidine as a

golden oil (3.55 g, 97%). δ_H ($CDCl_3$, 300 MHz) 1.49 (s, 3H), 3.01 (d, *J* 9 Hz, 2H), 3.10 (d, *J* 9 Hz, 2H), 3.18 (s, 3H), 4.38 (s, 1H), 7.17 (t, *J* 9 Hz, 2H), 7.25 (t, *J* 9 Hz, 4H), 7.41 (d, *J* 9 Hz, 4H); LRMS: *m/z* (ES^+) 268 [MH^+], 290 [MNa^+]. The benzhydryl-protected compound (500 mg, 1.87 mmol) was dissolved in acetonitrile (10 mL) and cooled to 0 °C. 1-Chloroethyl chloroformate (0.26 mL, 2.43 mmol) was added and the reaction was refluxed for 1 h. The mixture was concentrated under reduced pressure, re-dissolved in methanol (10 mL) and refluxed for 2 h. The mixture was concentrated under reduced pressure and the residue was dissolved in water (10 mL), washed with cyclohexane (2×20 mL), the aqueous was concentrated under reduced pressure and co-evaporated with ethanol (50 mL) then dichloromethane (50 mL) to give **9** as a white solid (256 mg, 100%). δ_H (DMSO-d_6, 400 MHz) 1.42 (s, 3H), 3.15 (s, 3H), 3.70 (m, 2H), 3.85 (m, 2H), 9.20 (br s, 1H), 9.60 (br s, 1H).

2.1.8. (*N*,*N*-Dipropynyl)trifluoroacetamide, 10. A solution of trifluoroacetic anhydride (8.40 g, 40 mmol) in anhydrous dichloromethane (15 mL) was added dropwise to a stirred solution of *N*,*N*-dipropargylamine (3.162 g, 34 mmol) and triethylamine (4.24 g, 42 mmol) in anhydrous dichloromethane (55 mL) at 4 °C. The resulting solution was allowed to warm to room temperature and stirred for 17 h. The mixture was poured into saturated aqueous sodium bicarbonate (100 mL) and extracted with dichloromethane (75 mL and 50 mL). The combined organic solutions were washed with water (75 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography, (gradient elution with hexane/ethyl acetate) to give **10** (5.4 g, 84%). δ_H ($CDCl_3$, 300 MHz) signals doubled due to rotational isomerism 2.14, 2.18 (t, *J* 3 Hz, 1H), 4.17, 4.20 (d, *J* 3 Hz, 2H); IR ν_{max} (film) 3300, 2130, 1700 cm^{-1}. $C_8H_6F_3NO$ requires C, 50.80; H, 3.20; N, 7.41%, found C, 50.78; H, 3.21; N, 7.24%.

2.1.9. 2,3-Dihydro-5-hydroxymethyl-2-trifluoroacetyl(1*H*)isoindole, 11. Propargyl alcohol (4.28 g, 76 mmol) was added dropwise to a solution of **10** (3.61 g, 19.1 mmol) in ethanol (80 mL) at 0 °C. Tris(triphenylphosphine)rhodium (I) chloride (535 mg, 0.579 mmol) was added in one portion and the reaction was stirred at room temperature for 17 h. The mixture was concentrated under reduced pressure and the crude product was purified by flash chromatography using pentane/ethyl acetate (2:1) as eluant to give **11** as an off-white solid (2.73 g, 58%), mp 83–86 °C. δ_H ($CDCl_3$, 300 MHz) 1.80 (br s, 1H, exchanges with D_2O), 4.70 (s, 2H), 4.90 (s, 2H), 5.05 (s, 2H), 7.2–7.4 (m, 3H); LRMS (TSP): *m/z* 262 [MNH_4^+]; IR ν_{max} (film) 3350, 3250, 1700 cm^{-1}. Found: C, 53.76; H, 4.07; N, 5.63, $C_{11}H_{10}F_3NO_2$ requires C, 53.88; H, 4.11; N, 5.71%

2.1.10. 2,3-Dihydro-5-formyl-2-*tert*-butoxycarbonyl(1*H*)isoindole, 12. 2,3-Dihydro-5-hydroxymethyl-2-trifluoroacetyl(1*H*)isoindole (2.73 g, 11.1 mmol) was dissolved in methanol (80 mL) and a solution of potassium carbonate (12.27 g, 88.8 mmol) in water (32 mL) was added. The cloudy mixture was stirred at room temperature for 30 min before adding di-*tert*-butyldicarbonate (4.85 g, 22.2 mmol) and stirring for 6 h. The reaction mixture was diluted with ethyl acetate (200 mL), washed with water (2×100 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give 2,3-dihydro-5-hydroxymethyl-2-*tert*-butoxycarbonyl(1*H*)isoindole as an off-white solid (2.49 g, 89%). δ_H ($CDCl_3$, 300 MHz) 1.51 (s, 9H), 4.6–4.70 (m, 6H), 7.15–7.3 (m, 3H); APCI: *m/z* 250 (MH^+).

Dimethylsulfoxide (2.28 mL, 32.2 mmol) in dichloromethane (6 mL) was added dropwise to a suspension of trifluoroacetic anhydride (3.42 mL, 24.2 mmol) in dichloromethane (60 mL) under nitrogen at −78 °C. After 10 min, a solution of 2,3-dihydro-5-hydroxymethyl-2-*tert*-butoxycarbonyl(1*H*)isoindole (4.0 g, 16.1 mmol) in dichloromethane (20 mL) was added dropwise to the mixture for over 25 min. After a further 20 min at −78 °C, *N*,*N*-diisopropylethylamine (14.02 mL, 80.5 mmol) was added dropwise for over 15 min. The mixture was stirred at −78 °C for 20 min before allowing to warm up to room temperature. The light brown solution was diluted with diethyl ether (200 mL), washed sequentially with water (50 mL) and dilute citric acid (2×50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give a light brown solid. The crude product was purified by flash chromatography using cyclohexane/ethyl acetate (3:1) as eluant to give **12** as a white solid (3.117 g, 78%), mp 118–119 °C. δ_H ($CDCl_3$, 300 MHz) 1.52 (s, 9H), 4.70–4.80 (m, 4H), 7.35–7.46 (m, 1H), 7.70–7.85 (m, 2H), 10.0 (s, 1H). Found: C, 67.91; H, 6.92; N, 5.70, $C_{14}H_{17}NO_3$ requires C, 68.00; H, 6.93; N, 5.66%.

2.2. Reductive aminations: general method

A mixture of aldehyde (**6** or **12**) (1.05 mmol), the required amine (1.0 equiv) and acetic acid (1.15 equiv) in THF (10 mL) was stirred at room temperature for 0.5 h. In some cases the amine was used as its hydrochloride salt; thus anhydrous sodium acetate (1.0 mmol) was also added to buffer the mixture. Sodium triacetoxyborohydride (2.5 equiv) was added and the mixture stirred at room temperature for 18 h. The mixture was diluted with ethyl acetate and basified to pH 11 using concentrated aqueous ammonia. The layers were separated, the aqueous phase extracted with ethyl acetate and the combined organic solutions dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane/methanol/concentrated aqueous ammonia (typically between 97:3:0 and 90:10:1) as eluant to afford **7a–j** and **13a–d** as oils. Yields are shown in Tables 1 and 2.

Spectroscopic data for the amines **7a–j** and **13a–d** are given below.

Amine **7a**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.5 (s, 9H), 2.2 (s, 6H), 2.90 (t, *J* 6 Hz, 2H), 3.35 (s, 2H), 3.62 (t, *J* 6 Hz, 2H), 4.55 (s, 2H), 7.00 (m, 1H), 7.10 (m, 2H); LRMS: *m/z* (ES^+) 291 [MH^+], 313 [MNa^+].

Amine **7b**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.47 (s, 9H), 2.8–2.9 (m, 4H), 3.34 (s, 3H), 3.49 (t, *J* 6 Hz, 2H), 3.65 (t, *J* 6 Hz, 2H), 3.76 (s, 2H), 4.55 (s, 2H), 7.00 (d, *J* 6 Hz, 1H), 7.13 (t, *J* 6 Hz, 1H), 7.16 (d, *J* 6 Hz, 1H); LRMS: *m/z* (ES^+) 321 [MH^+], 343 [MNa^+].

Amine **7c**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 2.21 (s, 3H), 2.59 (t, *J* 6 Hz, 2H), 2.91 (t, *J* 6 Hz, 2H), 3.31 (s,

2H), 3.47 (m, 5H), 3.62 (t, *J* 6 Hz, 2H), 4.55 (s, 2H), 6.99 (m, 1H), 7.10 (m, 2H); LRMS: *m/z* (ES^+) 335 [MH^+], 357 [MNa^+]. Found: C, 66.74; H, 8.88; N, 8.20, $C_{19}H_{30}N_2O_3\cdot 0.4H_2O$ requires C, 66.79; H, 9.09; N, 8.20%.

Amine **7d**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.50 (s, 9H), 2.43 (m, 4H), 2.93 (t, *J* 5 Hz, 2H), 3.44 (s, 2H), 3.67 (m, 6H), 4.58 (s, 2H), 7.03 (m, 1H), 7.12 (m, 2H); LRMS: *m/z* (ES^+) 333 [MH^+], 355 [MNa^+].

Amine **7e**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.47 (s, 9H), 1.56 (m, 2H), 1.84 (br, 2H), 2.13 (m, 2H), 2.68 (br, 2H), 2.90 (t, *J* 6 Hz, 2H), 3.20 (m, 1H), 3.31 (s, 3H), 3.41 (s, 2H), 3.62 (m, 2H), 4.56 (s, 2H), 7.00 (m, 1H), 7.10 (m, 2H). Found: C, 69.64; H, 8.94; N, 7.72, $C_{21}H_{32}N_2O_3$ requires C, 66.97; H, 8.95; N, 7.77%.

Amine **7f**: pale pink oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 1.77 (m, 1H), 2.02 (m, 1H), 2.5 (m, 2H), 2.61 (m, 1H), 2.77 (m, 1H), 2.88 (t, *J* 6 Hz, 2H), 3.25 (s, 3H), 3.56 (s, 2H), 3.62 (t, *J* 6 Hz, 2H), 3.90 (m, 1H), 4.55 (s, 2H), 6.99 (d, *J* 8 Hz, 1H), 7.08–7.18 (m, 2H); LRMS: *m/z* (ES^+) 347 [MH^+]. Found: C, 68.68; H, 8.73; N, 8.04, $C_{20}H_{30}N_2O_3\cdot 0.16H_2O$ requires C, 68.76; H, 8.74; N, 8.02%.

Amine **7g**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 1.76 (m, 1H), 2.02 (m, 1H), 2.5 (m, 2H), 2.61 (m, 1H), 2.77 (m, 1H), 2.88 (t, *J* 6 Hz, 2H), 3.25 (s, 3H), 3.56 (s, 2H), 3.62 (t, *J* 6 Hz, 2H), 3.90 (m, 1H), 4.55 (s, 2H), 6.99 (d, 1H), 7.10 (t, *J* 8 Hz, 1H), 7.16 (d, *J* 8 Hz, 1H); LRMS: *m/z* (ES^+) 347 [MH^+], 369 [MNa^+]. Found: C, 68.71; H, 8.71; N, 7.86, $C_{20}H_{30}N_2O_3\cdot 0.1H_2O$ requires C, 68.97; H, 8.74; N, 8.04%.

Amine **7h**: oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 1.69 (d, *J* 10 Hz, 1H), 1.85 (d, *J* 10 Hz, 1H), 2.57 (d, *J* 10 Hz, 1H), 2.83 (d, *J* 10 Hz, 1H), 2.90 (t, *J* 6 Hz, 2H), 3.38 (s, 1H), 3.63 (t, *J* 6 Hz, 3H), 3.70 (d, *J* 2 Hz, 2H), 4.08 (d, *J* 6 Hz, 1H), 4.4 (s, 1H), 4.6 (s, 1H), 7.00 (d, *J* 6 Hz, 1H), 7.08–7.2 (m, 2H); LRMS: *m/z* (ES^+) 345 [MH^+], 367 [MNa^+].

Amine **7i**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.45 (s, 9H), 2.81 (t, *J* 6 Hz, 2H), 2.95 (t, *J* 6 Hz, 2H), 3.22 (s, 3H), 3.60 (m, 6H), 4.01 (m, 1H), 4.58 (s, 2H), 7.00 (m, 1H), 7.13 (m, 2H); LRMS: *m/z* (ES^+) 333.3 [MH^+], 355 [MNa^+].

Amine **7j**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.46 (s, 3H), 1.48 (s, 9H), 2.82 (t, *J* 6 Hz, 2H), 3.03 (d, *J* 6 Hz, 2H), 3.18 (s, 3H), 3.20 (m, 2H), 3.59 (s, 2H), 3.63 (t, *J* 6 Hz, 2H), 4.56 (s, 2H), 7.00 (br, 1H), 7.14 (m, 2H); LRMS: *m/z* (ES^+) 347 [MH^+], 369 [MNa^+].

Amine **13a**: white waxy solid; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 2.42 (m, 4H), 3.48 (s, 2H), 3.68 (m, 4H), 4.65 (br, 4H), 7.20 (br m, 2H); LRMS: *m/z* (ES^+) 319 [MH^+], 341 [MNa^+].

Amine **13b**: yellow oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 2.24 (s, 3H), 2.39 (t, *J* 6 Hz, 2H), 3.31 (s, 2H), 3.49 (t, *J* 6 Hz, 2H), 3.53 (s, 3H), 4.55–4.7 (br s, 4H), 7.06–7.35 (br m, 3H); LRMS: *m/z* (ES^+) 321 [MH^+], 343 [MNa^+].

Amine **13c**: colourless oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 2.95 (t, *J* 6 Hz, 2H), 3.23 (s, 3H), 3.57 (t, *J* 6 Hz, 2H), 3.61 (s, 2H), 4.02 (m, 1H), 4.6–4.7 (br s, 4H), 7.1–7.2 (br, 3H); LRMS: *m/z* (ES^+) 319 [MH^+], 341 [MNa^+].

Amine **13d**: yellow oil; δ_H ($CDCl_3$, 400 MHz) 1.48 (s, 9H), 1.8 (br, 1H), 2.05 (m, 1H), 2.52 (br, 2H), 2.64 (m, 1H), 2.75 (br, 1H), 3.25 (s, 3H), 3.61 (s, 2H), 3.91 (br, 1H), 4.6–4.7 (br s, 4H), 7.1–7.25 (br, 3H); LRMS: *m/z* (ES^+) 333 [MH^+], 355 [MNa^+].

2.3. Deprotection of *N*-Boc derivatives (7a–j and 13a–d): general methods

Hydrogen chloride was bubbled through an ice-cooled solution of the Boc-protected amine (**7a–j** or **13a–d**) in dichloromethane (10–12 mL/g) for 20 min. The solution was then stirred for a further 30 min at room temperature and evaporated under reduced pressure to afford **1** or **2** (hydrochloride). Alternatively, a solution of the Boc-protected amine in dichloromethane (10–12 mL/g) was treated with an equal volume of trifluoroacetic acid and the solution stirred at room temperature for 2 h. Removal of the solvents under reduced pressure gave **1** or **2** (trifluoroacetate). Spectroscopic data for the amines **1a–j** and **2a–d** are given below.

Amine **1a**·HCl: colourless foam; δ_H (DMSO-d_6, 400 MHz) 2.50 (s, 6H), 3.15 (t, *J* 6 Hz, 2H), 3.35 (br, 2H), 4.26 (m, 4H), 7.30 (m, 2H), 7.55 (d, *J* 8 Hz, 1H), 9.55 (br, 2H); LRMS: *m/z* (ES^+) 191 [MH^+], 213 [MNa^+]. Found: C, 47.24; H, 7.14; N, 8.49, $C_{12}H_{18}N_2\cdot HCl\cdot H_2O\cdot CH_2Cl_2$ requires C, 47.36; H, 7.03; N, 8.50%.

Amine **1b**·2HCl: colourless foam; δ_H (DMSO-d_6, 400 MHz) 3.06 (t, *J* 6 Hz, 2H), 3.14 (t, *J* 6 Hz, 2H), 3.30 (s, 3H), 3.35 (t, *J* 6 Hz, 2H), 3.67 (t, *J* 6 Hz, 2H), 4.13 (s, 2H), 4.25 (s, 2H), 7.25 (d, *J* 6 Hz, 1H), 7.32 (t, *J* 6 Hz, 1H), 7.50 (d, *J* 6 Hz, 1H); LRMS: *m/z* (ES^+) 221 [MH^+], 243 [MNa^+]. Found: C, 50.1; H, 7.68; N, 8.78, $C_{13}H_{20}N_2O\cdot 2HCl\cdot H_2O$ requires C, 50.17; H, 7.77; N, 9.00%.

Amine **1c**: free base, pale yellow gum; δ_H ($CDCl_3$, 400 MHz) 2.23 (s, 3H), 2.6 (t, *J* 5 Hz, 2H), 2.82 (t, *J* 5 Hz, 2H), 3.16 (t, *J* 5 Hz, 2H), 3.32 (s, 3H), 3.47 (s, 2H), 3.50 (t, *J* 5 Hz, 2H), 4.01 (s, 2H), 6.9 (d, *J* 8 Hz, 1H), 7.06 (t, *J* 8 Hz, 1H), 7.13 (d, *J* 8 Hz, 1H); LRMS: *m/z* (ES^+) 235 [MH^+]. Found: C, 70.6; H, 9.56; N, 11.76, $C_{14}H_{22}N_2O\cdot 0.2H_2O$ requires C, 70.67; H, 9.49; N, 11.77%.

Amine **1d**·2HCl, colourless solid; δ_H (DMSO-d_6, 400 MHz) 3.20 (m, 4H), 3.37 (m, 2H), 3.62 (m, 2H), 3.90 (m, 4H), 4.22 (m, 2H), 4.32 (s, 2H), 7.30 (m, 2H), 7.62 (m, 1H), 9.58 (br s, 2H), 11.40 (br s, 1H); LRMS: *m/z* (ES^+) 233 [MH^+].

Amine **1e**: free base, gum; δ_H ($CDCl_3$, 400 MHz) 1.56 (m, 2H), 1.86 (br, 2H), 2.16 (dt, *J* 10 and 3 Hz, 2H), 2.69 (br, 2H), 2.82 (t, *J* 6 Hz, 2H), 3.15 (t, *J* 6 Hz, 2H), 3.20 (m, 1H), 3.32 (s, 3H), 3.39 (s, 2H), 4.01 (s, 2H), 6.90 (d, *J* 7 Hz, 1H), 7.03 (t, *J* 7 Hz, 1H), 7.10 (d, *J* 7 Hz, 1H). Found C, 72.88; H, 9.29; N, 10.69, $C_{16}H_{24}N_2O\cdot 0.3H_2O$ requires C, 72.80; H, 9.32; N, 10.61%.

Amine **1f**·2HCl: colourless foam; δ_H ($CDCl_3$, 400 MHz) 2.23 (br, 2H), 3.17 (br, 2H), 3.35 (s, 3H), 3.40 (br, 2H), 3.50 (br, 2H), 3.75 (m, 2H), 4.13 (br, 1H), 4.32 (br, 2H),

7.25 (d, *J* 8 Hz, 1H), 7.33 (t, *J* 8 Hz, 1H), 7.58 (d, *J* 8 Hz, 1H); LRMS: *m*/*z* (ES^+) 247 [MH^+].

Amine **1g**·2HCl: colourless foam; δ_H ($CDCl_3$, 400 MHz) 2.23 (br, 2H), 3.18 (br, 2H), 3.35 (s, 3H), 3.4 (br, 2H), 3.5 (br, 2H), 3.78 (m, 2H), 4.13 (br, 1H), 4.35 (br, 2H), 7.23 (d, *J* 6 Hz, 1H), 7.32 (t, *J* 6 Hz, 1H), 7.58 (d, *J* 6 Hz, 1H); LRMS: *m*/*z* (ES^+) 247 [MH^+].

Amine **1h**·2HCl: pale pink solid; δ_H (DMSO-d_6, 400 MHz), 2:1 mix of diastereomers due to protonation, 2.00 (m, 1H, major+minor), 2.43 (m, 1H, major+minor), 3.05–3.25 (m, 3H, major+minor), 3.4–3.6 (m, 3H, major+minor), 3.61 (d, *J* 8 Hz, 1H, major), 3.68 (d, *J* 11 Hz, 1H, minor), 4.2–4.5 (m, 6H, major+minor), 4.62 (s, 1H, minor), 4.68 (s, 1H, major), 7.30 (m, 2H, major+minor), 7.60 (d, *J* 8 Hz, 1H, minor), 7.70 (d, *J* 8 Hz, 1H, major), 9.60 (m, 2H, major+minor), 11.22 (br s, 1H, major), 11.56 (br s, 1H, minor); LRMS: *m*/*z* (ES^+) 245 [MH^+].

Amine **1i**·2HCl: colourless foam; δ_H (DMSO-d_6, 400 MHz) 3.01–3.40 (m, 7H), 3.90 (br s, 1H), 4.01 (br s, 1H), 4.22 (m, 6H), 4.40 (s, 2H), 7.28 (m, 2H), 7.43 (m, 1H), 9.40–9.56 (m, 2H); APCI: *m*/*z* (ES^+) 233 [MH^+].

Amine **1j**·HCl: yellow solid; δ_H (MeOH-d_4, 400 MHz), some signals doubled and broadened due to protonation, 1.53 and 1.55 (each br s, 3H total), 3.20 (br, 2H), 3.30 (s, 3H), 3.55 (t, *J* 6 Hz, 2H), 4.1–4.22 (br, 4H), 4.40 (s, 2H), 4.48 and 4.53 (each br s, 2H total), 7.35 (d, 1H), 7.40 (m, 2H); LRMS: *m*/*z* (ES^+) 247 [MH^+].

Amine **2a**·2HCl: colourless powder; δ_H (MeOH-d_4, 400 MHz) 3.13–3.38 (br m, 4H), 3.70–4.10 (br m, 4H), 4.38 (s, 2H), 4.67 (d, *J* 5 Hz, 4H), 7.54 (d, *J* 9 Hz, 1H), 7.59 (d, *J* 9 Hz, 1H), 7.66 (s, 1H); LRMS: *m*/*z* (ES^+) 219 [MH^+]; HRMS: found: 219.1493 (MH^+), $C_{13}H_{18}N_2O$ requires 219.1492.

Amine **2b**·2HCl: purple solid; δ_H (MeOH-d_4, 400 MHz) 2.84 (s, 3H), 3.34 (m, 1H), 3.40 (s, 3H), 3.60 (m, 1H), 3.73 (m, 2H), 4.34 (d, *J* 13 Hz, 1H), 4.50 (d, *J* 13 Hz, 1H), 4.66 (m, 4H), 7.55 (s, 2H), 7.6 (s, 1H); LRMS: *m*/*z* (ES^+) 221 [MH^+].

Amine **2c**·2HCl; colourless sticky foam; δ_H (MeOH-d_4, 400 MHz) 3.29 (s, 3H), 4.02 (m, 2H), 4.35 (m, 3H), 4.45 (s, 2H), 4.66 (m, 4H), 7.51 (s, 2H), 7.55 (s, 1H); LRMS: *m*/*z* (ES^+) 219 [MH^+]; HRMS: found: 219.1493660, $C_{13}H_{19}N_2O$ requires 219.1491897.

Amine **2d**·2HCl: pale purple foam; δ_H (MeOH-d_4, 400 MHz) 2.13 (m, 1H), 2.33 (m, 1H), 3.27 (s, 3H), 3.34 (m, 2H), 3.58 (m, 2H), 4.17 (br s, 1H), 4.44 (m, 2H), 4.66 (m, 4H), 7.52 (d, *J* 6 Hz, 1H), 7.61 (s, 1H); LRMS: *m*/*z* (ES^+) 233 [MH^+].

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Facile solid-phase synthesis of biotinylated alkyl thiols

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Abstract—Biotinylated alkyl thiols with the capacity to graft avidin proteins are in increasing demand for the development of self-assembled monolayers on gold. Here we propose 2-Chlorotrityl Chloride solid-phase resin as a new platform to produce these functionalized alkyl thiols. Biotinylated alkyl thiols of non-obvious solution synthesis were obtained rapidly using this method and without previous purification steps.

1. Introduction

Self-assembled monolayers (SAMs) are a popular tool for tailoring the reactive properties of surfaces. In order to produce these monolayers, molecules may be physisorbed from solution or more tightly grafted by covalent bond formation with gold substrates and alkyl thiols. Because of the features of the functional group of alkyl thiols, surface properties can be easily modified by simply changing the chemical nature of the terminal groups. Alkyl thiols are widely used, mainly in biological applications.[1] Gold surfaces can be derivatized to bind proteins,[2] carbohydrates, peptides,[3] DNA,[4] haptenes[5] or to produce new surfaces for cell culture studies such as cell attachment, differentiation or proliferation.[6] For all these purposes, rapid and efficient access to a diversity of functional alkyl thiols is required. In an attempt to minimize the difficulty of access to these molecules, researchers introduce the desired functionalities (biotins, haptenes, polyglycols) by reaction over amino- or acid-terminating SAMs. Reactions over a previously formed SAM do not ensure perfect derivatization because the processes between surfaces and solution are not kinetically well afforded. This problem is exacerbated when the gold surface to be derivatized is a fragile gold AFM tip or a micro(nano)electrode because the manipulation and the solvent rinsing procedures required to remove the undesired reagents may cause damage. Consequently, a thiol with the desired functionality is required. Given that dip pen nanolithography, the AFM-based soft-lithography developed by Mirkin et al. in 1999,[7] involves the direct deposition of thiols in nanometer scale using an AFM probe, the development of functional alkyl thiols is crucial for this technique.

Here we propose a novel solid-phase strategy for the development of biotin alkyl thiol (BAT) derivates. BAT structures are formed by the thiol, the aliphatic chain (n=11, 16), a PEG linker, and the biotin group (Fig. 1).

BATs are useful for the development of biosensors as they allow the production of well-defined biotinylated surfaces (Fig 2a). Biotin surfaces are one of the most used tools to immobilize antibodies (i.e., antigens, enzymes or DNA) onto surfaces through the biotin-streptavidin (i.e., neutravidin, avidin) pair (K_d~10^{-15} M) as building block. Streptavidin has four equivalent sites for biotin (two on one side and two on the opposite). Streptavidin vacancies for biotin can be used to link the protein almost irreversibly to the surface and to create well-oriented free biotin sites that are exposed to the surface (Fig. 2b). These exposed sites allow the grafting of biotinylated biomolecules for the preparation of biosensors and have minimal impact on biological activity (Fig. 2c). BAT structures must include PEG groups in order to avoid the non-specific adsorption of streptavidin and other proteins onto surfaces, and to allow a good orientation of the streptavidin molecule.

Keywords: Solid-phase; Biotinylated alkyl thiols; Self-assembled monolayers.
Abbreviations: AFM, atomic force microscope; BAT, biotinylated alkyl thiols; DCM, dichloromethane; DIPEA, *N*,*N*′-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*′-dimethylformamide; HOAt, 1-hydroxy-7-azabenzotriazole (3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*] pyridine); MALDI-TOF, matrix assisted laser desorption/ionization time of flight (mass spectrometry); MeCN, acetonitrile; MeOH, methanol; MHDA, mercaptohexadecanoic acid; NMR, nuclear magnetic resonance; PDMS, polydimethylsiloxane; PEG, polyethylene glycol; SAM, self-assembled monolayers; TFA, trifluoroacetic acid; TES, triethylsilane.
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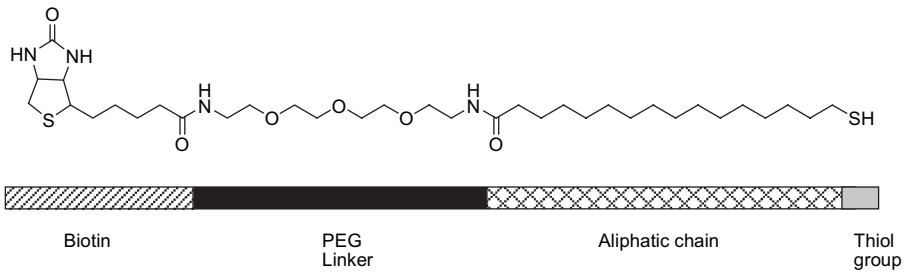


Figure 1. BAT structure.

To date, the synthesis of BATs has been described through solution-phase.[8] In the original report, BAT was attained in poor yield and no characterization data were given. In this case, the molecule was constructed by the reaction of the PEG linker with the biotin molecule, followed by attachment of the thiol-protected acid-activated alkyl chain. New syntheses in solution have recently been reported but the procedure is still long, and tedious silica sol–gel purification work-up steps are required in each step of synthesis.[9,10] In all solution-phase syntheses, the protection and deprotection of the thiol cannot be avoided during the procedure and consequently large amounts of starting products and reagents are needed to obtain sufficient amounts of the thiols of interest.

Here we describe a new solid-phase approach, based on the 2-Chlorotrityl Chloride® (CTC) resin, for the development of BATs. CTC resin is a polymer support functionalized with chlorotrityl groups that graft nucleophiles, such as thiols,[11] amines[12] or carboxylates,[13] thereby allowing cleavage of the final product under acidic conditions. One of the advantages of this resin is that it can be regenerated several times.[14] Moreover, it offers a new platform to obtain alkyl thiols of interest.[15] The solid-phase method reported here allows the synthesis of high purity BATs in only a few days. The number of carbons of the alkyl chain, the presence or number of glycols, in the case of BAT with PEG linkers, and the functional group can be chosen freely. In solution-phase synthesis, modifications in molecule design change the purification procedures, which must be optimized for any new molecule. We obtained two BATs, with and without PEG, using the same solid-phase synthetic procedure, which indicates the robustness of the new method. The development of this method allows the production of custom-designed BATs or other functionalized alkyl thiols.

2. Results and discussion

Solid-phase synthesis has been widely used for the synthesis of biopolymers such as peptides, oligonucleotides, and polysaccharides and has dramatically improved yield, purity, and length of synthesis over traditional solution-phase methodology. Solid-phase approaches have evolved exponentially in recent decades because they provide a means to develop small bioactive organic molecules. Moreover, these procedures allow the parallel synthesis of analog families with high purity.[16]

The structure of functionalized alkyl thiols as consecutive building blocks assembled by amide, ester, and ether bonds make this kind of molecule highly suitable for synthesis by solid-phase approaches. Here we focused on the development of BATs in compounds **1** and **2** (Fig. 3).

BAT structures have a long chain with a thiol group at one end and convenient functional group at the other. This strategy is based on chain growth from thiol to functional group. The functional group is added in the last step. This methodology facilitates the generation of compound families with a range of functional groups (Scheme 1). The cornerstone

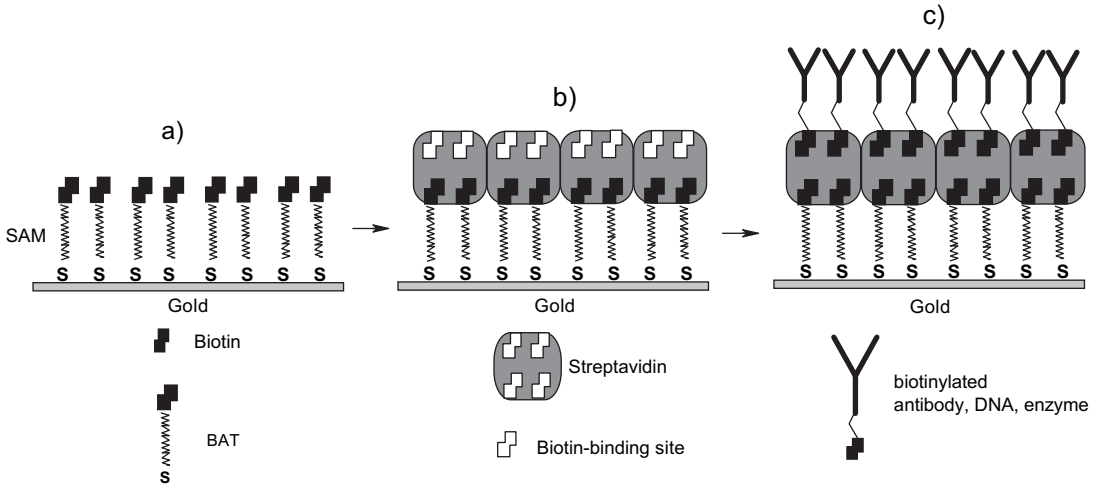


Figure 2. Streptavidin–biotin procedure to immobilize biomolecules: (a) biotinylated gold surface, (b) streptavidin-grafted surface through biotin groups and formation of biotin vacancies on the surface, (c) grafting of biotinylated biomolecules over the surface.

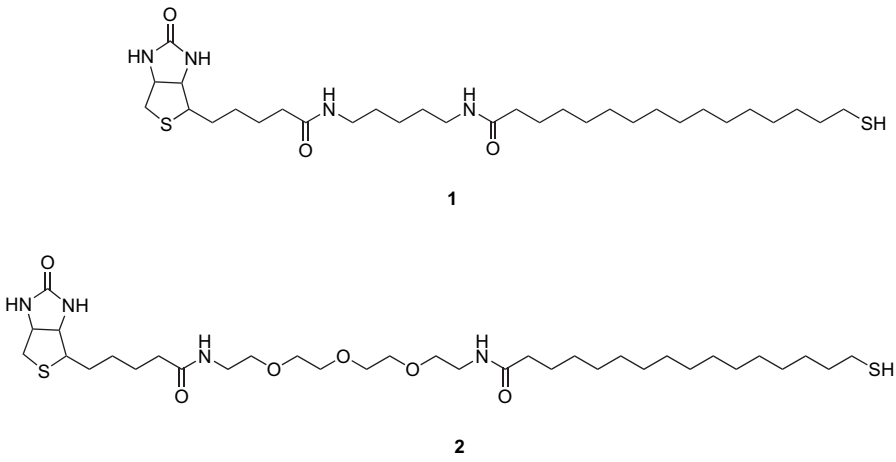
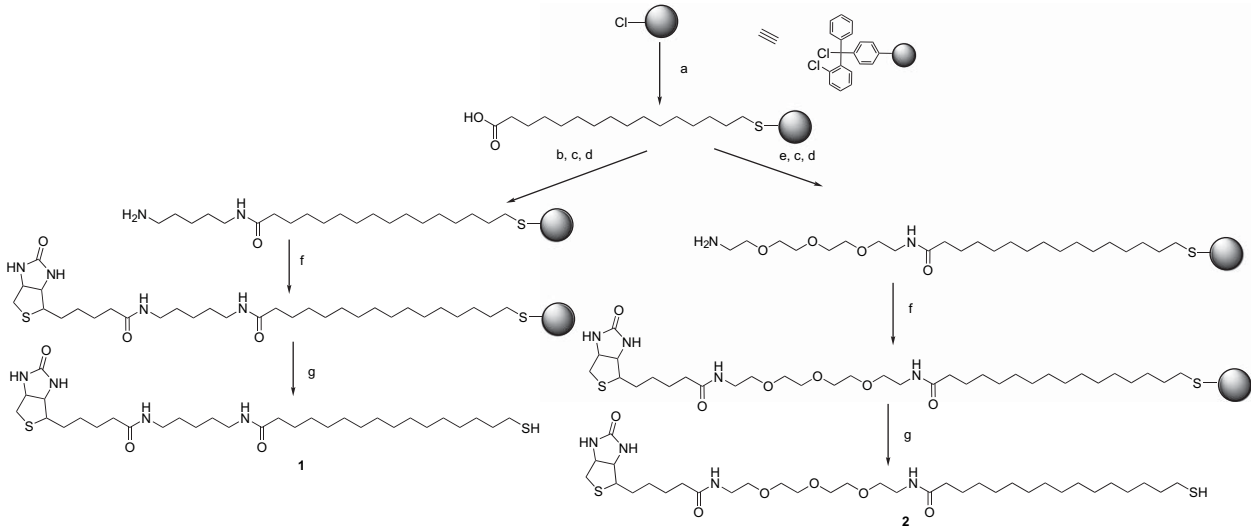


Figure 3. BAT structures.

of this strategy is the use of CTC resin as solid-support; (i) CTC resin is functionalized with a chlorotrityl group, which can be attached by nucleophile groups such as amines, carboxylates, and thiols; (ii) cleavage of final compound is under very mild acidic conditions, thereby recovering the initial functional group; (iii) the capacity of CTC resin for regeneration reduces the cost of synthesis.

Chain elongation is through available protected building blocks using the standard coupling reagents, as in peptide solid-phase synthesis. On solid-support, coupling reaction with quantitative yields greatly facilitates the removal of the excess of reagents and soluble side-products by simple filtration and washings.

The solid-phase resin protects the thiol group and prevents the formation of non-desired compounds via reaction with the thiol instead of acid group. Several attempts were made before achieving the attachment of the 16-mercaptohexadecanoic acid through its thiol group instead of the carboxylic group. We performed qualitative colorimetric tests,[17] such as Ellman's test for thiol groups and Malachite green for carboxylic groups, to identify the group that was not attached to the resin. Thus, a two-fold molar excess of 16-mercaptohexadecanoic acid and less than an equimolar amount of diisopropylethylamine (DIPEA) was left to react with the resin (156 mg) for 2 h in DCM–DMF (1:1, v/v).[18,13,19] However, in our case, under these conditions, the attachment of mercapto acid through the carboxylic group was partial. The same result was observed when the reaction time was extended overnight. The experiment was repeated without DIPEA. Attachment was not complete through the thiol group. A fourth attempt was performed with DMF only, with no positive results. Finally, the expected result was achieved using DCM without base overnight. The absence of base makes the thiol group more reactive than the carboxylic one and the DCM solvates the aliphatic long chain better than DMF. The carboxylic acid attached to the CTC resin was split into two in order to obtain two BAT-modifying linkers. One half was treated



Scheme 1. Solid-phase of **1** and **2**. (a) MHDA (2 equiv), DCM, overnight, rt; (b) Fmoc-diaminopentane hydrochloride (3 equiv), DIPCDI (3 equiv), HOAt (3 equiv), DIPEA (3 equiv), DMF, overnight, rt; (c) MeOH (2 mL/g resin), DIPEA (0.3 mL/g resin), DCM, 10 min, rt; (d) piperidine–DMF (1:4, v/v), 1×1 min, 2×10 min, rt; (e) Fmoc-1-amino-4,7,10-trioxa-13-tridecanamine hydrochloride (3 equiv), DIPCDI (3 equiv), HOAt (3 equiv), DIPEA (3 equiv), DMF, overnight, rt; (f) Biotin (5 equiv), DICPDI (5 equiv), HOAt (5 equiv), DMF, overnight, rt; (g) TFA 65% in DCM/TES (95:5, v/v).

with protected diamino compound Fmoc-diaminopentane hydrochloride and DIPEA to release the free amine, and DIPCDI–HOAt was used as coupling reagent. This inverse solid-phase amide formation (activation of the supported carboxylic groups, which react with excess of the solution of the amino component) requires a powerful coupling reagent such as DIPCDI–HOAt.[20,21] Coupling was performed by mixing the amino compound with all reagents prior to adding to the resin. The remaining active sites (free chloride groups of the resin) were capped by addition of MeOH (200 μL/100 mg resin) and DIPEA (29 μL) for 10 min. The Fmoc group was removed by treatments with piperidine–DMF (1:4, v/v) for 10 min, and absorbance (λ 290 nm, ε 5800 L/mol/cm) of washes was measured. A good resin loading (0.85 mmol/g resin) was achieved. The second fraction of 16-mercaptohexadecanoic acid–CTC resin was treated with PEG linker (Fmoc-1-amino-4,7,10-trioxa-13-tridecanamine hydrochloride) neutralized by DIPEA, and DIPCDI–HOAt was used as coupling reagents. Coupling and Fmoc removal were performed as described above. Biotin was attached to the two resin fractions in a parallel way. Coupling was made with a five molar excess of biotin and using DIPCDI (5 equiv), HOAt (5 equiv), and DIPEA (5 equiv) as coupling reagents. The poor solubility of the biotin derivative makes the solid-phase synthesis the strategy of choice for the incorporation of the biotin into the macromolecule.[22] Thus, excess of the derivative can be used to drive the reaction to completion and excesses can be removed by single filtration and washings. To afford compounds **1** and **2**, the two resin fractions were treated with TFA–TES–DCM (65:2:33, v/v/v, 3×10 min). Compounds **1** (41 mg, 80% yield) and **2** (61 mg, 96% yield) were obtained as white solids. A ^{1}H NMR spectrum revealed traces of TES in the final product (See Supplementary data). Modified cocktail cleavage should be addressed in further studies to avoid the use of TES.

To test the functionality of these compounds synthesized on solid-phase, micro-contact printing was used. As an example, the interaction of streptavidin with BAT with PEG linker (compound **2**, without purification) was examined on gold surfaces. BAT (4 mM) in acetonitrile was used to ink a 2.5 μm PDMS-positive stamp. Before thiol inking, the PDMS stamp was oxidized with O_2 plasma to ensure good coverage of the stamp surface with the thiol. After keeping the stamp in contact with gold surface, the samples were immersed in PEG-terminated alkyl thiol, 1 mM diluted ethanol solution for 24 h. This PEG thiol prevented the non-specific adsorption of proteins on the non-functionalized gold areas. Incubation of the samples with streptavidin labeled with a fluorescent dye (Texas Red) and inspection of the surface with fluorescent microscopy showed the patterned areas of proteins over the gold surface and indicated the functionality of the BAT (Fig. 4).

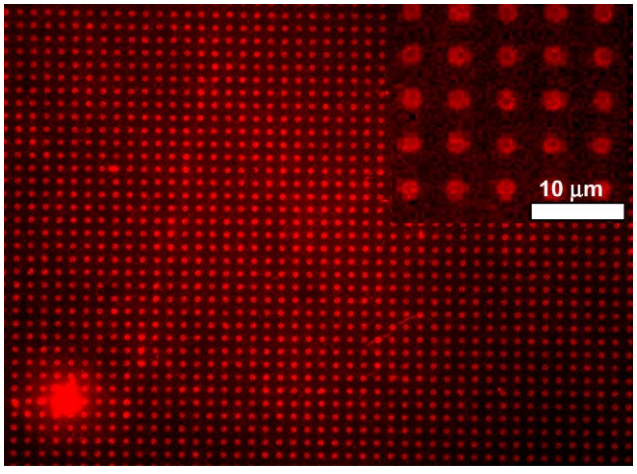


Figure 4. Fluorescent picture of a micropatterned gold surface with BAT showing the interaction between streptavidin labeled with a fluorescent dye and biotin.

3. Conclusions

CTC resin is a rapid, efficient, and versatile tool for the production of BATs. The solid-phase method described here improves the synthesis of BATs compared with solution-phase syntheses reported to date. This methodology contributes to the development of molecules that are increasingly required for nanobiotechnology applications.

4. Experimental

4.1. General procedures and instrumentation

CTC was a gift from Rohm and Haas, USA. 16-Mercaptohexadecanoic acid and Biotin was obtained from Aldrich (Milwaukee, WI, USA). Fmoc-diaminopentane hydrochloride was supplied by Bachem AG (Bubendorf, Switzerland). Fmoc-1-amino-4,7,10-trioxa-13-tridecanamine hydrochloride was from Neosystem (Strasbourg, France). DIPCDI and TES were obtained from Fluka Chemika (Buchs, Switzerland). HOAt was supplied by Applied Biosystems, DIEA was from Merck Schuchardt (Hohenbrunn, Germany). TFA was supplied by Scharlau (Barcelona, Spain). DCM, DMF, MeOH, MeCN, piperidine, and *tert*-butanol were obtained from SDS (Peypin, France). $(EG)_6$-terminated alkyl thiol was purchased from Prochimia (Gdansk, Poland).

Mass spectra were recorded on a MALDI-TOF Applied Biosystems 4700 Proteomics Analyzer.

^{1}H NMR spectroscopy was performed on a Nuclear Magnetic Resonance Varian: Mercury-400 (^{1}H de 400 MHz, $CDCl_3$) spectrophotometer.

UV detection was performed at 290 nm on a UV–vis Shimadzu UV mini 1240 spectrophotometer.

Elastomeric stamp fabrication for μCP has been described previously.[23] Here we use positive stamps, fabricated from polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning), where the structures protrude from the bulk PDMS surface. The PDMS stamps were replicated from silicon-based molds that had previously been structured using deep reactive ion etching (DRIE).

4.2. General synthesis

The solid-phase synthesis was performed in 5-mL polypropylene syringes fitted with polyethylene porous disks.

Solvents and soluble reagents were removed by suction. After a second coupling, a MeOH capping (200 μL MeOH–29 μL DIPEA–100 mg resin) in DCM was carried out (10 min). Next, Fmoc was removed with piperidine–DMF (1:4, v/v) (1×1 min, 2×10 min). Resins were washed before use and between deprotection, and coupling and subsequent deprotection steps were done with DMF (3×30 s) and DCM (3×30 s) using 10 mL of solvent/g of resin each time. Cleavage was performed using TFA 65% in DCM–TES (95:5, v/v) (3×10 min) followed by washing with DCM (3×1 min); to avoid the high concentration of TFA in the balloon, 200 μL of water was added before cleavage. Given the high insolubility of the compounds, once evaporated, lyophilization was performed under *tert*-butanol. To change the contra ion, a second lyophilization was done under *tert*-butanol and 100 μL of HCl 1 N.

4.3. Loading calculation

Deprotection with piperidine gave the fulvene–piperidine adduct, which was determined by quantitative spectrophotometry at λ 290 nm, ε 5800 L/mol/cm.

4.4. Synthesis of compound 1

A two-fold molar excess of 16-mercaptohexadecanoic acid (MHDA) (51 mg, 0.18 mmol) was left to react with CTC resin (78 mg, 0.086 mmol) in DCM overnight. Then the Fmoc-diamminopentane hydrochloride (93 mg, 0.26 mmol) coupling was performed using DIPCDI (40 μL, 0.26 mmol), HOAt (39 mg, 0.28 mmol) as coupling reagents, and addition of DIPEA (44 μL, 0.26 mmol) in DMF overnight at rt. The amino compound was mixed with all reagents prior to adding to the resin. A capping step with methanol was then performed before treatment with piperidine, with several washings between steps (the loading was 0.85 mmol/g of resin). The next coupling was done with biotin (108 mg, 0.44 mmol), DIPCDI (67 μL, 0.44 mmol), HOAt (66 mg, 0.48 mmol), and DIPEA (73 μL, 0.43 mmol), in DMF overnight as mentioned above. After cleavage, compound **1** was obtained as a white solid, the yield was 80% (41 mg). The product was characterized by ^{1}H NMR δ_H (400 MHz, $CDCl_3$–CD_3OD (1:1)) 4.34–4.31 (2H, m, C*H*CH_2S of biotin), 3.20–3.16 (4H, m, CH_2NHCO of biotin) and (1H, m, C*H*S of biotin) both overlapped, with correct integration, 2.94 (1H, dd, CH_2S of biotin), 2.74 (1H, d, CH_2S of biotin), 2.52 (2H, t, CH_2SH), 2.20–2.15 (4H, m, CH_2CON), 1.41–1.24 (36H, m, CH_2) and MALDI-TOF *m/z* calcd for $C_{31}H_{58}N_4O_3S_2$ 598.95, found 599.43 $[M+H]^+$, 621.42 $[M+Na]^+$, 637.39 $[M+K]^+$ (See Supplementary data).

4.5. Synthesis of compound 2

The first attachment of the 16-mercaptohexadecanoic acid (53 mg, 0.18 mmol) to the CTC resin (78 mg, 0.086 mmol) was done as explained above, then Fmoc-1-amino-4,7,10-trioxa-13-tridecanamine hydrochloride (13 mg, 0.27 mmol) coupling was performed using DIPCDI (40 μL, 0.26 mmol), HOAt (37 mg, 0.27 mmol) as coupling reagents and DIPEA (44 μL, 0.26 mmol) in DMF overnight. The amino compound was mixed with all reagents prior to adding to the resin. Next, a capping step with methanol was carried out before treatment with piperidine, with several washings between steps. The loading was 0.45 mmol/g of resin. The next coupling was done with Biotin (113 mg, 0.46 mmol), DIPCDI (66 μL, 0.43 mmol), HOAt (63 mg, 46 mmol), and DIPEA (72 μL, 0.42 mmol) in DMF overnight as mentioned above. After cleavage, compound **2** was obtained as a white solid, the yield was 96% (61 mg). The product was characterized by ^{1}H NMR[3,4] δ_H (400 MHz, CD_3OD) 4.49–4.47 (1H, m, C*H*CH_2S of biotin), 4.31–4.28 (1H, m, C*H*CH_2S of biotin), 3.63–3.52 (12H, m, CH_2O), 3.20 (4H, td, CH_2NHCO), 3.19–3.17 (1H, m, C*H*S of biotin), 2.92 (1H, dd, CH_2S of biotin), 2.69 (1H, d, CH_2S of biotin), 2.48 (2H, t, CH_2SH), 2.21–2.19 (4H, m, CH_2CON), 1.47–1.30 (34H, m, CH_2) and MALDI-TOF *m/z* calcd for $C_{36}H_{68}N_4O_6S_2$ 716.46, found 717.62 $[M+H]^+$, 739.59 $[M+Na]^+$, 755.56 $[M+K]^+$ (See Supplementary data).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.090.

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Synthesis and reactivity of *trans*-2-aryl-3-chloroazetidines

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Abstract—Several *trans*-2-aryl-3-chloroazetidines were synthesized in a stereoselective way by reduction of the corresponding β-lactams, which were formed by a Staudinger reaction using different benzaldimines and chloroketene. The resulting chloroazetidines proved to be excellent building blocks for the synthesis of different 3-substituted azetidines through nucleophilic substitution of the chlorine by different carbon, nitrogen, sulfur and oxygen nucleophiles in good to high yields. Since these substitution reactions took place with retention of stereochemistry, the intermediacy of bicyclic azonio[1.1.0]bicyclobutanes is proposed.

1. Introduction

Within azaheterocyclic chemistry, azetidines are an extraordinary class of strained compounds because of their wide range of known biological activities.[1–4] In the literature, several 3-substituted azetidines with known physiological effects have been reported. 1-Cyclohexyl-3-guanidinylazetidine has an antihypertensive activity,[5] 3-carbamoyl substituted azetidines exhibit a tranquilizing effect on the central nervous system[6] and azetidine-3-carboxylic acid is a known gametocide.[7] In various recent patents, 3,3-difluoroazetidines have been described as therapeutically active and selective inhibitors of the dipeptidyl peptidase-IV enzyme (DPP-IV)[8–12] making them potential drugs for the treatment of type 2 diabetes.[13] Indeed, 3-haloazetidines form an important subclass within the azaheterocycles because of their potential physiological activities and their use as substrates towards the synthesis of 3-substituted azetidine derivatives.

Various reports on the synthesis of 3-haloazetidines can be found in the literature. 2-Aryl-3-bromoazetidinyl-1-tosylates were prepared by electrophile-induced cyclization of *N*-cinnamyl tosylamine with bis(collidine)bromine(I) hexafluorophosphate as electrophile.[14] Furthermore, azetidinyl-3-tosylates and -mesylates are known precursors for 3-haloazetidine formation by substitution reactions,[15–20] and azetidin-3-ols also underwent substitution reactions at the C3 carbon atom upon treatment with triphenylphosphine dibromide or triphenylphosphine in tetrachloromethane towards 3-bromo- and 3-chloroazetidines.[21–23] Another straightforward synthetic method for the synthesis of 3-haloazetidines compromises ring opening of the peculiar and difficultly accessible 1-azabicyclo[1.1.0]butanes. Opening of the bridging 1,3-bond with *p*-toluenesulfonyl chloride,[24–26] hydrogen halides[25,27–29] or acid chlorides[25,30] resulted in a broad group of different 3-haloazetidines. 3-Haloazetidines, such as 1-*tert*-butyl-3-chloroazetidine, have already been used in different reactivity studies with various nucleophiles. Although moderate to good yields were reported, the reaction procedures required long reaction times (up to 11 days) and extreme reaction conditions (heating in steel bombs).[22,31]

In this report, a straightforward and efficient synthesis of *trans*-2-aryl-3-chloroazetidines is disclosed as versatile substrates in organic chemistry. The scope of these synthons for the preparation of a large variety of 3-substituted azetidines was demonstrated by means of different nucleophiles in an efficient and elegant way.

2. Results and discussion

trans-2-Aryl-3-chloroazetidines **4a–g** were prepared by condensation of different aromatic aldehydes **1** with a variety of amines in dichloromethane in the presence of magnesium sulfate as drying agent, and the corresponding aldimines **2** were isolated in excellent yield after reflux for 1 h. In the next step, the latter imines **2** underwent a Staudinger [2+2]-cycloaddition using chloroacetyl chloride as a ketene precursor and 2,6-lutidine as base, affording *trans*-4-aryl-3-chloro-β-lactams **3a–g** (Scheme 1).[32] The stereoselectivity of this reaction was confirmed by literature data regarding the coupling constants of *cis*- and *trans*-3-hydroxy-2-methyl-1-*tert*-butylazetidine between the protons at C3 and C4.[33,34]

Keywords: β-Lactams; Azetidines; Azonio[1.1.0]bicyclobutanes.
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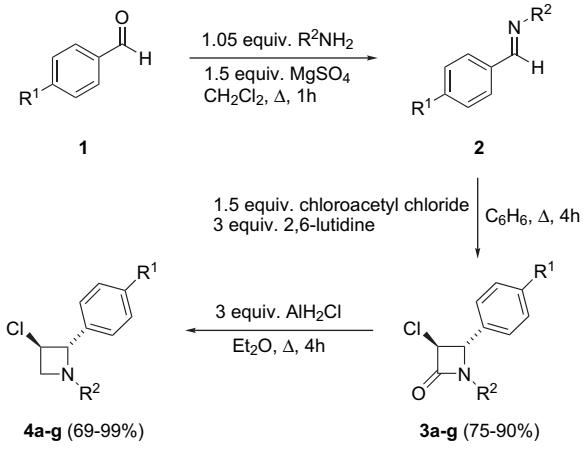
For a cis configuration, coupling constants of 5–6 Hz have been reported, and for a trans configuration a *J*-value of 0–2 Hz.[33,34] All the synthesized β-lactams **3a–g** had a coupling constant of 1.7 or 1.8 Hz between the protons at C3 and C4, indicative of a trans configuration of the chlorine atom with respect to the aromatic substituent (Scheme 1).



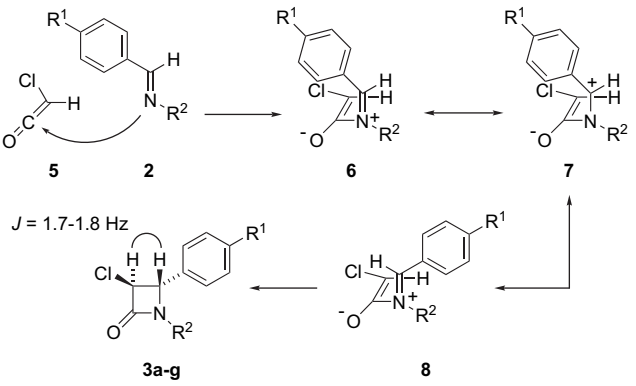
Scheme 1.

The use of monochloroalane has already been proven to be an efficient method for the reduction of β-lactams towards their corresponding azetidines.[35] Also in this case, the use of 3 equiv of monochloroalane in diethyl ether proved to be an efficient method for the stereoselective reduction of the 4-aryl-3-chloroazetidin-2-ones **3a–g**, affording *trans*-2-aryl-3-chloroazetidines **4a–g** in high yield after reflux for 4 h without the formation of undesired ring opening products (Scheme 1, Table 1).

The mechanism of the stereochemical outcome of this Staudinger reaction has been studied thoroughly in the past (Scheme 2).[36] The ketene **5**, formed by dehydrochlorination of the acid chloride with 2,6-lutidine as a base, is attacked by the nitrogen lone pair of the imine **2** at the less hindered side of the ketene with the plane of the imine perpendicular to the ketene. When using *N*-(alkylidene)amines, the formed zwitterionic intermediate **7** undergoes ring closure in the thermodynamic less stable cis isomer in a conrotatory way. In this case however, using aromatic aldimines **2**, isomerization of the imine bond is possible as the positive charge, which occurs during this isomerization, can be stabilized at the benzylic position, allowing rotation of the nitrogen–carbon double bound towards intermediate **8**. Hence, by conrotatory ring closure, the β-lactams **3a–g** are formed in the thermodynamic more stable trans-configuration.[36]

Table 1. Synthesis and yield of 4-aryl-3-chloroazetidin-2-ones **3a–g** and 2-aryl-3-chloroazetidines **4a–g**

Compound	R^1	R^2	Yield of **3** (%)	Yield of **4** (%)
a	H	Allyl	85	73
b	Cl	Allyl	82	80
c	H	Benzyl	75	87
d	Cl	Benzyl	90	69
e	H	*i*Pr	77	83
f	H	cHex	86	79
g	Me	*i*Pr	81	86



Scheme 2.

The retention of the trans configuration of the formed 2-aryl-3-chloroazetidines **4a–g** was confirmed by comparing the coupling constants of the protons at the C2, C3 and C4 carbon atoms with literature data.[37–39] According to the literature, *cis*-3-hydroxy-2-methyl-1-*tert*-butylazetidine has coupling constants of 6.6 and 1.8 Hz between the protons at C3 and C4, whereas *trans*-3-hydroxy-2-methyl-1-*tert*-butylazetidine has coupling constants of 6.7 and 6.4 Hz. The coupling constants between the C2 and C3 protons were about 6 Hz in both cases.[37–39] Upon analysis of the spectral data of azetidines **4a–g**, the coupling constants of the azetidine ring protons were observed to be larger than 5 Hz, indicating the trans-configuration. The proton at the C3 carbon atom was distinguished as a quadruplet-like multiplet in the ^{1}H NMR spectra, which exists of a d×d×d system with similar coupling constants. The protons at the C4 carbon have a triplet like appearance since both coupling constants are about the same size (6.6–7 Hz) (Fig. 1).

Insertion of new functional groups on azetidine rings is a challenging synthetic topic within organic chemistry because of the interesting biological and chemical properties of substituted azetidines. Since no systematic study of nucleophilic substitution reactions of 3-haloazetidines has been performed up to now and the known methods are very laborious, 2-aryl-3-chloroazetidines **4a–g** were treated with different nucleophiles in dimethylsulfoxide (DMSO) and methanol, resulting in several 3-substituted azetidines (Scheme 3). *trans*-2-Aryl-3-chloroazetidines **4a–g** reacted with 4 equiv of sodium azide, potassium cyanide, sodium acetate and potassium thiocyanate, respectively, in dimethylsulfoxide resulting in *trans*-2-aryl-3-azidoazetidines **9a–g**, *trans*-2-aryl-3-cyanoazetidines **9h–j**, *trans*-3-acetoxy-2-arylazetidines **9k–n** and *trans*-2-aryl-3-thiocyanoazetidines **9o–p**, respectively, as substitution products in good yields after overnight heating at 80–120 °C (Table 2).

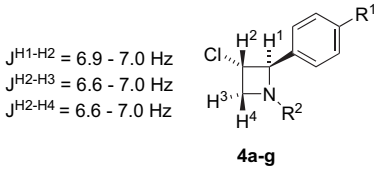
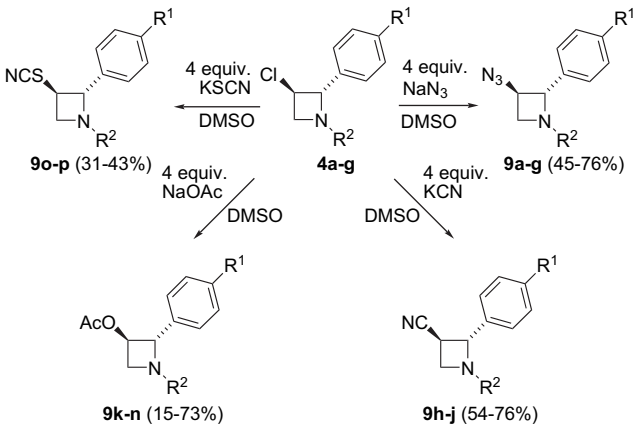


Figure 1.



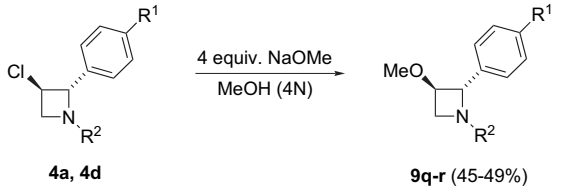
Scheme 3.

Table 2. Substitution reactions of *trans*-2-aryl-3-chloroazetidines **4a–g** towards 3-substituted azetidines **9a–p**

Starting compound **4**	Reagent MX	Temperature (°C)	Reaction time (h)	Isolated compound **9**	Yield (%)
4a	NaN_3	80	22	**9a**	47
4b	NaN_3	90	22	**9b**	62
4c	NaN_3	90	22	**9c**	45
4d	NaN_3	110	22	**9d**	63
4e	NaN_3	80	18	**9e**	62
4f	NaN_3	80	18	**9f**	70
4g	NaN_3	80	18	**9g**	76
4a	KCN	90	22	**9h**	54
4b	KCN	90	46	**9i**	62
4d	KCN	120	22	**9j**	65
4a	NaOAc	90	22	**9k**	52
4b	NaOAc	100	72	**9l**	73
4c	NaOAc	110	22	**9m**	52
4d	NaOAc	125	21	**9n**	15
4a	KSCN	90	22	**9o**	31
4b	KSCN	90	22	**9p**	43

DMSO has proven to be the most suitable solvent for this type of substitution reactions. Treatment of *trans*-2-aryl-3-chloroazetidines **4a–g** with potassium cyanide in acetonitrile or methanol at reflux did not result in substitution, and the starting material was recovered. The required reaction temperature had to be above 80 °C, since a reaction temperature below 80 °C did not result in substitution reactions.

Only for the synthesis of 3-methoxyazetidines **9q–r**, a different solvent had to be chosen. Treatment of *trans*-2-aryl-3-chloroazetidines **4a–d** with 4 equiv of sodium methoxide in methanol (4 N) afforded the corresponding *trans*-2-aryl-3-methoxyazetidines **9q–r** in moderate yields (Scheme 4, Table 3).



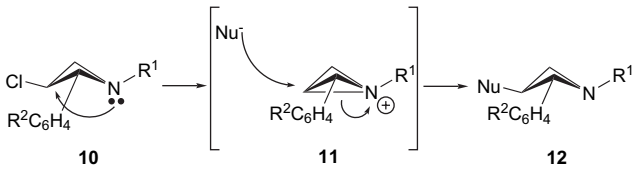
Scheme 4.

The method developed for the stereospecific substitution of *trans*-2-aryl-3-chloroazetidines **4a–d** can be used for a number of different carbon, sulfur, oxygen and nitrogen nucleophiles. In an efficient, straightforward and simple way, several potentially bioactive compounds can be synthesized, which broaden the already significant azetidine chemistry.

Table 3. Reaction of *trans*-2-aryl-3-chloroazetidines **4a** and **4d** with sodium methoxide towards *trans*-2-aryl-3-methoxyazetidines **9q–r**

Starting compound **4**	Temperature (°C)	Reaction time (h)	Isolated compound **9**	Yield (%)
4a	Reflux, Δ	22	**9q**	49
4d	Reflux, Δ	72	**9r**	45

It should be stressed that all substitution reactions took place with retention of stereochemistry, and only trans substituted azetidines were detected in the reaction mixtures. This behaviour can be explained by the formation of a bicyclic azonia[1.1.0]bicyclobutane intermediate **11**, followed by attack of the nucleophile at C3 (Scheme 5). The same intermediate has already been suggested in literature, based on stereospecific retention after hydrolysis and substitution reactions, and ring contraction to aziridinylmethyl derivatives.[40–42] Further investigation of this intermediate by ab initio calculations stated that this bicyclic intermediate is indeed the most stable one, in comparison with other possible intermediates.[43] Moreover, 1-azabicyclo[1.1.0]butanes have been converted into 3-haloazetidines and vice versa via intermediate azonia[1.1.0]bicyclobutane derivatives such as **11**.[44] The fact that thiocyanates **9o–p** are obtained, instead of the corresponding isothiocyanates, after reaction of 3-chloroazetidines **4a–g** with potassium thiocyanate is in accordance with literature data, as in most cases substitution proceeds via attack of the sulfur atom.[45] The preferential attack of the sulfur atom of the thiocyanate nucleophile can be rationalized considering the soft acid character of the intermediate bicyclic azonia[1.1.0]bicyclobutane intermediate **11**.

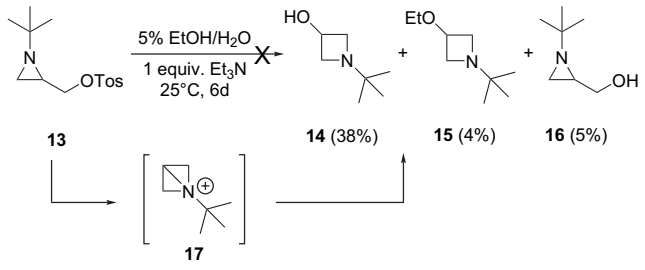


Scheme 5.

trans-2-Aryl-3-chloroazetidines **4a–g** proved to be stable in refluxing methanol for 24 h, and no isomerization to the corresponding isomeric 2-(chloromethyl)aziridines was observed. It should be remarked that upon treatment with nucleophiles the intermediate azonia[1.1.0]bicyclobutanes **11** are opened in a regioselective way at C3 towards 3-substituted azetidines instead of attack at C2 or C4 towards the corresponding aziridine derivatives. However, it has been reported previously that treatment of the analogous *trans*-1-cyclohexyl-3-mesyloxy-2-phenylazetidine with sodium hydroxide resulted in a mixture of the corresponding 3-hydroxyazetidine and both isomers of 1-cyclohexyl-2-(α-hydroxybenzyl)aziridine in a 1:1:1 ratio.[42] The same result, i.e., formation of the corresponding 3-methoxyazetidines and 2-(α-methoxybenzyl)aziridines, was obtained upon treatment of *trans*-1-cyclohexyl-3-mesyloxy-2-phenylazetidine

with sodium methoxide in methanol, which can only be explained by ring opening of the intermediate azonia[1.1.0]-bicyclobutanes at C2. Only when sodium methanethiolate and sodium benzenethiolate were used, no isomerization towards the corresponding aziridines took place.[42]

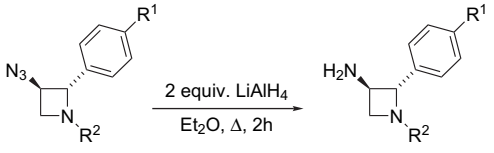
The opposite reactivity has also been reported, i.e., the conversion of a 2-(tosyloxymethyl)aziridine into an azetidine derivative via the intermediacy of an azonia[1.1.0]bicyclobutane salt. These authors described the ring expansion of 1-*tert*-butyl-2-(tosyloxymethyl)aziridine **13** into a mixture of 3-hydroxyazetidine **14** (38%), azetidine **15** (4%) and 2-(hydroxymethyl)aziridine **16** (5%) via the formation of an intermediate bicyclic aziridinium salt **17** after heating for 6 days at 25 °C in 5% EtOH/H_2O in the presence of 1 equiv of Et_3N.[15] As this result seemed to be doubtful in view of our recent study of the reactivity of 2-(bromomethyl)-aziridines, we decided to repeat the solvolysis of aziridine **13**. However, careful repetition of this reaction did not result in the formation of azetidines **14** and **15**, and the starting material was recovered completely (Scheme 6).



Scheme 6.

Although the desired substitution of 3-chloroazetidines **4a–g** was attained by reaction with sodium azide, potassium cyanide, sodium acetate and potassium thiocyanate in DMSO, treatment of *trans*-2-aryl-3-chloroazetidines **4a–g** with sodium bicarbonate, potassium cyanate, potassium fluoride and potassium iodide using the same reaction conditions (overnight heating in DMSO) resulted only in complex reaction mixtures, probably due to the less nucleophilic character of the first three nucleophiles compared to chloride. In the latter case, the higher leaving capacities of iodide in comparison to chloride can be responsible for the failure of the substitutions.

In recent years, 3-aminoazetidines have received a lot of attention because of their antibacterial activities.[46–50] Reduction of *trans*-2-aryl-3-azidoazetidines **9e–g** with 2 equiv of lithium aluminium hydride in ether yielded unprecedented 3-aminoazetidines **18a–c** after reflux for 2 h, which could be purified by performing an acid–base extraction (Scheme 7).

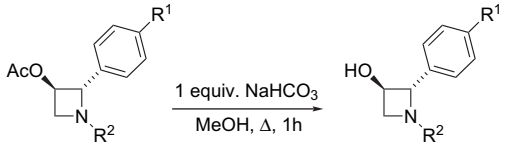


9e R^1= H, R^2= *i*Pr
9f R^1= H, R^2= *c*Hex
9g R^1= Me, R^2= *i*Pr

18a R^1= H, R^2= *i*Pr (70%)
18b R^1= H, R^2= *c*Hex (82%)
18c R^1= Me, R^2= *i*Pr (92%)

Scheme 7.

trans-3-Acetoxy-2-arylazetidines **9k–l** were stable in refluxing methanol, whereas treatment with 1 equiv of sodium bicarbonate in refluxing methanol for 1 h resulted in *trans*-2-arylazetidin-3-ols **19a–b** (Scheme 8). Azetidin-3-ols have been studied intensively in the literature since they form interesting synthons towards azetidinyl tosylates and mesylates.[15,21]



9k R^1= H, R^2= allyl
9l R^1= Cl, R^2= allyl

19a R^1= H, R^2= allyl (47%)
19b R^1= Cl, R^2= allyl (79%)

Scheme 8.

Efforts towards acidic or basic conversion of *trans*-2-aryl-3-cyanoazetidines **9h–j** to the corresponding methyl esters or carboxylic acids as analogues of the gametocide azetidine-3-carboxylic acid[7] by using 2–6 N hydrochloric acid or sulfuric acid in water or methanol only resulted in complex reaction mixtures, while treatment with 6 N aqueous sodium hydroxide yielded the starting compounds **9h–j**.

3. Conclusion

Several *trans*-2-aryl-3-chloroazetidines were synthesized in a stereoselective way by reduction of the corresponding β-lactams, which were, on their turn, formed by a Staudinger reaction between different benzaldimines and chloroketene. The formed chloroazetidines were subjected to a variety of substitution reactions with nitrogen, carbon, sulfur and oxygen nucleophiles in DMSO. Since these substitution reactions took place with retention of stereochemistry, the occurrence of a bicyclic azonio[1.1.0]bicyclobutane intermediate is proposed. These substitution reactions resulted in several *trans*-2-aryl-3-substituted azetidines as valuable new compounds with potential biological activities and as substrates for further elaboration towards e.g., 3-amino- and 3-hydroxyazetidines.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded at 270 MHz (JEOL JNM-EX 270) or at 300 MHz (JEOL ECLIPSE+) with $CDCl_3$ as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded at 68 MHz (JEOL JNM-EX 270) or at 75 MHz (JEOL ECLIPSE+) with $CDCl_3$ as solvent. Mass spectra were obtained with a mass spectrometer (VARIAN MAT 112), 70 eV using a GC–MS coupling (RSL 200, 20 m glass capillary column, i.d. 0.53 mm, He carrier gas) or AGILENT 1100, 70 eV. IR spectra were measured with a Spectrum One FT-IR spectrophotometer. Melting points were measured using a Büchi B-540 apparatus and are uncorrected. Elemental analysis was performed on a Perkin–Elmer 2400 Elemental Analyzer. Dichloromethane was distilled over calcium hydride, while diethyl ether

and THF were freshly distilled over sodium benzophenone ketyl. Other solvents were used as received from the supplier.

4.1.1. Synthesis of *trans*-4-aryl-3-chloro-β-lactams 3a–g. The synthesis of *trans*-1-allyl-3-chloro-4-phenylazetidin-2-one **3a** is given as a representative example. *N*-(Benzylidene)allylamine (7.23 g, 0.05 mol) was dissolved in benzene (150 ml) and 2,6-lutidine (16.05 g, 0.15 mol, 3 equiv) was added, after which the solution was heated to reflux. Chloroacetylchloride (8.48 g, 0.075 mol, 1.5 equiv) was added dropwise to this refluxing solution. The resulting solution was kept at reflux temperature for 22 h. After cooling, the precipitated 2,6-lutidine hydrochloride was filtered off and the filtrate was washed twice with 1 N hydrochloric acid. Drying over magnesium sulfate, filtration and evaporation of the solvent yielded *trans*-1-allyl-3-chloro-4-phenylazetidin-2-one (10.85 g, 49 mmol, 97%). Although the obtained β-lactams were pure enough for direct use in the next step, further purification was performed by flash chromatography on silica gel.

4.1.1.1. *trans*-1-Allyl-3-chloro-4-phenylazetidin-2-one 3a. Yield 85%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.41 (1H, d×d×d, J=15.5 Hz, J=7.2 Hz, J=0.8 Hz, NC(*H*)H), 4.21 (1H, d×d×t, J=15.5 Hz, J=5.0 Hz, J=1.6 Hz, NC(H)*H*), 4.54 (1H, d×d, J=1.8 Hz, J=0.8 Hz, C*H*C_6H_5), 4.58 (1H, d, J=1.8 Hz, CHCl), 5.08–5.21 (2H, m, CH=CH_2), 5.66–5.78 (1H, m, C*H*=CH_2), 7.28–7.47 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.54 (CH_2N), 63.06 (*C*HC_6H_5), 65.73 (CHCl), 119.53 (CH=*C*H_2), 126.63, 129.28 and 129.49 (C*C*$_5H_5$), 130.30 (*C*H=CH_2), 134.83 (*C*C_5H_5), 163.84 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1772. MS (70 eV): *m*/*z* (%): 224/2 (M^++1, 100), 186 (45). Anal. Calcd for $C_{12}H_{12}ClNO$ (%): C, 65.02; H, 5.46; N, 6.32. Found (%): C, 65.28; H, 5.22; N, 6.44.

4.1.1.2. *trans*-1-Allyl-3-chloro-4-(4-chlorophenyl)azetidin-2-one 3b. Yield 82%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.39 (1H, d×d, J=15.6 Hz, J=7.3 Hz, NC(*H*)H), 4.10–4.20 (1H, m, NC(H)*H*), 4.50–4.52 (2H, m, CHCl and C*H*C_6H_4Cl), 5.09–5.22 (2H, m, CH=CH_2), 5.65–5.79 (1H, m, C*H*=CH_2), 7.23–7.40 (4H, m, C_6H_4Cl). ^{13}C NMR (75 MHz, ref=$CDCl_3$): δ 43.72 (CH_2N), 63.22 and 65.11 (*C*HC_6H_4Cl and CHCl), 119.88 (CH=*C*H_2), 128.08 and 129.61 (C*C*$_4H_4CCl$), 130.29 (*C*H=CH_2), 130.99 and 135.51 (*C*C_4H_4*C*Cl), 163.50 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1774. MS (70 eV): *m*/*z* (%): 258/6 (M^++1, 100), 116 (37). Anal. Calcd for $C_{12}H_{11}Cl_2NO$ (%): C, 56.27; H, 4.33; N, 5.47. Found (%): C, 56.08; H, 4.51; N, 5.39.

4.1.1.3. *trans*-1-Benzyl-3-chloro-4-phenylazetidin-2-one 3c. Yield 75%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.32. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (1H, d, J=15.1 Hz, C(*H*)HC_6H_5), 4.38 and 4.55 (2×1H, 2×d, J=1.8 Hz, CHCl and C*H*C_6H_5), 4.86 (1H, d, J=15.1 Hz, C(H)*H*C_6H_5), 7.10–7.42 (10H, m, $CH_2C_6H_5$ and CHC_6H_5). ^{13}C NMR (75 MHz, ref=$CDCl_3$): δ 44.90 (*C*$H_2C_6H_5$), 63.19 and 65.26 (CHCl and *C*HC_6H_5), 126.65, 128.05, 128.42, 128.89, 129.24 and 129.43 (2×C*C*$_5H_5$), 134.30 and 134.68 (2×*C*C_5H_5), 163.61 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1777. MS (70 eV): *m*/*z* (%): 274/2 (M^++1, 100), 91 (14). Anal. Calcd for $C_{16}H_{14}ClNO$ (%): C, 70.72; H, 5.19; N, 5.15. Found (%): C, 70.94; H, 5.29; N, 5.01.

4.1.1.4. *trans*-1-Benzyl-3-chloro-4-(4-chlorophenyl)-azetidin-2-one 3d. Yield 90%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.44. Mp: 83 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (1H, d, J=15.0 Hz, C(*H*)HC_6H_5), 4.35 (1H, d, J=1.7 Hz, CHCl), 4.52 (1H, d×d, J=1.7 Hz, J=0.7 Hz, C*H*C_6H_4Cl), 4.84 (1H, d, J=15.0 Hz, C(H)*H*C_6H_5), 7.29–7.38 (9H, m, $CH_2C_6H_5$ and CHC_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 45.04 (*C*$H_2C_6H_5$), 63.15 and 64.61 (CHCl and *C*HC_6H_4Cl), 128.01, 128.18, 128.45, 128.97 and 129.47 (C*C*$_4H_4CCl$ and C*C*$_5H_5$), 133.28, 134.07 and 135.34 (*C*C_4H_4*C*Cl and *C*C_5H_5), 163.40 (C=O). IR (KBr, cm^{-1}): $\nu_{C=O}$=1765. MS (70 eV): *m*/*z* (%): 310/08/06 (M^++1, 100). Anal. Calcd for $C_{16}H_{13}Cl_2NO$ (%): C, 62.76; H, 4.28; N, 4.57. Found (%): C, 62.91; H, 4.18; N, 4.74.

4.1.1.5. *trans*-3-Chloro-1-isopropyl-4-phenylazetidin-2-one 3e. Yield 77%, light yellow oil. Hexane/EtOAc 3:2, R_f=0.64. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.07 and 1.31 (2×3H, 2×d, J=6.9 Hz, CH(C*H*$_3$)$_2$), 3.75 (1H, septet, J=6.9 Hz, C*H*(CH_3)$_2$), 4.46 and 4.53 (2×1H, 2×d, J=1.7 Hz, NCH and CHCl), 7.35–7.47 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 19.80 and 20.74 (CH(*C*H_3)$_2$), 45.63 (*C*H(CH$_3$)$_2$), 62.23 and 64.75 (NCH and CHCl), 126.42, 128.86 and 129.08 (C*C*$_5H_5$), 136.24 (*C*C_5H_5), 163.13 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1765. MS (70 eV): *m*/*z* (%): no M^+, 139 (57), 137 (100), 102 (46). Anal. Calcd for $C_{12}H_{14}ClNO$ (%): C, 64.43; H, 6.31; N, 6.26. Found (%): C, 64.21; H, 6.43; N, 6.34.

4.1.1.6. *trans*-3-Chloro-1-cyclohexyl-4-phenylazetidin-2-one 3f. Yield 86%, light yellow oil. Hexane/EtOAc 3:2, R_f=0.65. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.96–2.04 (10H, m, $(CH_2)_5$), 3.33–3.45 (1H, m, NCH), 4.44 and 4.54 (2×1H, 2×d, J=1.7 Hz, NCH and CHCl), 7.34–7.46 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 24.95, 25.01, 30.21, 31.15 and 32.80 ((CH_2)$_5$), 53.75 (NCH), 62.85 and 65.15 (NCH and CHCl), 126.95, 129.20 and 129.31 (C*C*$_5H_5$), 136.71 (*C*C_5H_5), 163.82 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1769. MS (70 eV): no M^+, 140/38 (100). Anal. Calcd for $C_{15}H_{18}ClNO$ (%): C, 68.30; H, 6.88; N, 5.31. Found (%): C, 68.51; H, 7.01; N, 5.19.

4.1.1.7. *trans*-3-Chloro-1-isopropyl-4-(4-methylphenyl)-azetidin-2-one 3g. Yield 81%, light yellow oil. Hexane/EtOAc 3:2, R_f=0.53. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.06 and 1.30 (2×3H, 2×d, J=6.6 Hz, CH(C*H*$_3$)$_2$), 2.38 (3H, s, C_qCH3), 3.74 (1H, septet, J=6.6 Hz, C*H*(CH$_3$)$_2$), 4.44 and 4.49 (2×1H, 2×d, J=1.7 Hz, NCH and CHCl), 7.23–7.25 (2×2H, 2×d, J=9.0 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CDCl_3$): δ 20.09, 21.08 and 21.22 (CH(*C*H_3)$_2$ and C_q*C*H3), 45.80 (*C*H(CH$_3$)$_2$), 62.53 and 64.92 (NCH and CHCl), 126.63 and 129.79 (C*C*$_cH_4$C), 133.48 and 139.37 (*C*C_4H_5*C*), 163.57 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1769. MS (70 eV): *m*/*z* (%): 238/6 (M^+, 1), 154/2 (100), 118 (44), 117 (82), 115 (75), 91 (58), 70 (42). Anal. Calcd for $C_{13}H_{16}ClNO$ (%): C, 65.68; H, 6.78; N, 5.89. Found (%): C, 65.49; H, 6.86; N, 6.02.

4.1.2. Synthesis of *trans*-2-aryl-3-chloroazetidines 4a–g. The reduction of *trans*-1-allyl-3-chloro-4-phenylazetidin-2-one **3a** to *trans*-2-aryl-3-chloroazetidine **4a** is given as a representative example. To a solution of aluminium(III) chloride (12.13 g, 0.09 mol, 3 equiv) in dry diethyl ether (150 ml) was added carefully lithium aluminium hydride (3.44 g, 0.09 mol, 3 equiv) at 0 °C. This reaction mixture was stirred at 0 °C for 10 min, and subsequently refluxed for 30 min. *trans*-1-Allyl-3-chloro-4-phenylazetidin-2-one (6.64 g, 0.03 mol) in dry diethyl ether (100 ml) was added slowly and after addition was complete, reflux was maintained for 4 h. The reaction was cooled and water (200 ml) was added carefully. The aqueous phase was extracted with dichloromethane and dried over magnesium sulfate. After filtration and evaporation of the solvent and further purification by flash chromatography on silica gel, *trans*-2-aryl-3-chloroazetidine (4.57 g, 0.022 mol, 73%) was obtained as a light yellow oil.

4.1.2.1. *trans*-1-Allyl-3-chloro-2-phenylazetidine 4a. Yield 73%, light yellow oil. Hexane/EtOAc 4:1, R_f=0.40. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97–3.01 (1H, m, C(*H*)HCHCl), 3.06 (1H, d×d, *J*=13.4 Hz, *J*=6.9 Hz, C(*H*)HCH=CH_2), 3.32 (1H, d×d×t, *J*=13.4 Hz, *J*=5.5 Hz, *J*=1.4 Hz, C(H)*H*CH=CH_2), 3.83–3.89 (1H, m, C(H)*H*CHCl), 4.05–4.12 (2H, m, CHCl and C*H*C_6H_5), 5.02–5.20 (2H, m, CH=CH_2), 5.65–5.80 (1H, m, C*H*=CH_2), 7.19–7.44 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.69 (*C*HC_6H_5), 60.80 (*C*H_2CHCl), 61.10 (*C*H_2CH=CH_2), 78.72 (CHCl), 117.66 (CH=*C*H_2), 126.59, 128.05 and 128.45 (C*C*$_5H_5$), 133.81 (*C*H=CH_2), 139.55 (*C*C_5H_5). MS (70 eV): *m*/*z* (%): 210/8 (M^++1, 100). Anal. Calcd for $C_{12}H_{14}ClN$ (%): C, 69.39; H, 6.79; N, 6.74. Found (%): C, 69.11; H, 6.95; N, 6.70.

4.1.2.2. *trans*-1-Allyl-3-chloro-2-(4-chlorophenyl)azetidine 4b. Yield 80%, light yellow oil. Hexane/EtOAc 4:1, R_f=0.60. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98–3.03 (1H, m, C(*H*)HCHCl), 3.07 (1H, d×d, *J*=13.4 Hz, *J*=6.9 Hz, C(*H*)HCH=CH_2), 3.29 (1H, d×d×t, *J*=13.4 Hz, *J*=5.6 Hz, *J*=1.0 Hz, C(H)*H*CH=CH_2), 3.84–3.88 (1H, m, C(H)*H*CHCl), 4.00–4.07 (2H, m, C*H*C_6H_4Cl and CHCl), 5.03–5.21 (2H, m, CH=CH_2), 5.64–5.77 (1H, m, C*H*=CH_2), 7.29–7.39 (4H, m, C_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.71 (CHCl), 60.80 (*C*H_2CHCl), 61.09 (*C*H_2CH=CH_2), 78.72 (*C*HC_6H_4Cl), 117.94 (CH=*C*H_2), 127.99 and 128.66 (C*C*$_4H_4$CCl), 133.70 (*C*H=CH_2), 133.82 and 138.19 (*C*C_4H_4*C*Cl). MS (70 eV): *m*/*z* (%): 246/4/2 (M^++1, 50), 116 (23). Anal. Calcd for $C_{12}H_{13}Cl_2N$ (%): C, 59.52; H, 5.41; N, 5.78. Found (%): C, 59.68; H, 5.36; N, 5.71.

4.1.2.3. *trans*-1-Benzyl-3-chloro-2-phenylazetidine 4c. Yield 87%, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.00 (1H, t, *J*=7.2 Hz, C(*H*)HCHCl), 3.47 (1H, d, *J*=12.9 Hz, C(*H*)HC_6H_5), 3.71–3.75 (1H, m, C(H)*H*CHCl), 3.89 (1H, d, *J*=12.9 Hz, C(H)*H*C_6H_5), 4.03–4.10 (1H, m, CHCl), 4.16 (1H, d, *J*=6.9 Hz, C*H*C_6H_5), 7.22–7.33 (10H, m, $CH_2C_6H_5$ and CHC_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.92 (CHCl), 60.86 (*C*H_2CHCl), 61.93 (*C*$H_2C_6H_5$), 78.52 (*C*HC_6H_5), 126.56, 127.20, 128.05, 128.28, 128.45 and 128.63 (2×C*C*$_5H_5$), 137.29 and 139.37 (2×*C*C_5H_5). MS (70 eV): *m*/*z* (%): 260/58 (M^++1, 23), 120 (100), 91 (36). Anal. Calcd for $C_{16}H_{16}ClN$ (%): C, 74.55; H, 6.26; N, 5.43. Found (%): C, 74.70; H, 6.38; N, 5.31.

4.1.2.4. *trans*-1-Benzyl-3-chloro-2-(4-chlorophenyl)-azetidine 4d. Yield 69%, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97–3.02 (1H, m, C(*H*)HCHCl), 3.48 (1H, d, *J*=12.7 Hz, C(*H*)HC_6H_5), 3.67–3.75 (1H, m, C(H)*H*CHCl), 3.82 (1H, d, *J*=12.7 Hz, C(H)*H*C_6H_5), 3.97–4.03 (1H, m, CHCl), 4.10 (1H, d, *J*=6.6 Hz, C*H*C_6H_4Cl), 7.22–7.37 (9H, m, $CH_2C_6H_5$ and CHC_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.81 (CHCl), 60.80 (*C*$H_2C_6H_5$), 61.88 (CH_2N), 77.84 (*C*HC_6H_4Cl), 127.27, 127.90, 128.13, 128.30 and 128.60 (C*C*$_4H_4$CCl and C*C*$_5H_5$), 133.73, 137.02 and 137.90 (*C*C_4H_4*C*Cl and *C*C_5H_5). MS (70 eV): *m*/*z* (%): 120 (100), 91 (38). Anal. Calcd for $C_{16}H_{15}Cl_2N$ (%): C, 65.77; H, 5.17; N, 4.79. Found (%): C, 65.89; H, 5.36; N, 4.61.

4.1.2.5. *trans*-3-Chloro-1-isopropyl-2-phenylazetidine 4e. Yield 83%, light yellow oil. ^{1}H NMR (270 MHz, C_6D_6): δ 0.60 and 0.77 (2×3H, 2×d, *J*=6.0 Hz, CH(CH_3)$_2$), 2.24 (1H, septet, *J*=6.0 Hz, C*H*(CH_3)$_2$), 2.74 (1H, d×d, *J*=6.6 Hz, *J*=6.6 Hz, NC*H*(H)), 3.51 (1H, d×d×d, *J*=6.6 Hz, *J*=6.6 Hz, *J*=1.0 Hz, NCH(*H*)), 3.79 (1H, q, *J*=6.6 Hz, CHCl), 3.93 (1H, d, *J*=6.6 Hz, NC*H*C_6H_5), 7.06–7.20 and 7.42–7.45 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, C_6D_6): δ 20.09 and 20.84 (CH(*C*H_3)$_2$), 52.99 (CHCl), 59.64 and 59.93 (*C*H(CH_3)$_2$ and NCH_2), 78.83 (N*C*HC_6H_5), 126.95–128.62 (NCHC*C*$_5H_5$), 141.96 (NCH*C*C_5H_5). MS (70 eV): *m*/*z* (%): 211/09 (M^+, 27), 196/4 ($M-Me^+$, 25), 132 (100). Anal. Calcd for $C_{12}H_{16}ClN$ (%): C, 68.73; H, 7.69; N, 6.68. Found (%): C, 68.89; H, 7.60; N, 6.82.

4.1.2.6. *trans*-3-Chloro-1-cyclohexyl-2-phenylazetidine 4f. Yield 79%, light yellow oil. ^{1}H NMR (270 MHz, C_6D_6): δ 0.75–1.57 (10H, m, (CH_2)$_5$), 2.00–2.04 (1H, m, NCH), 2.79 (1H, d×d, *J*=7.0 Hz, *J*=7.0 Hz, NC*H*(H)), 3.59 (1H, d×d, *J*=7.0 Hz, *J*=7.0 Hz, NCH(*H*)), 3.85 (1H, q, *J*=7.0 Hz, CHCl), 3.99 (1H, d, *J*=7.0 Hz, NC*H*C_6H_5), 7.07–7.20 and 7.45–7.48 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 24.29, 24.37, 25.77, 30.03 and 30.87 ((CH_2)$_5$), 52.90 (CHCl), 59.44 (NCH_2), 67.94 (NCH), 78.62 (N*C*HC_6H_5), 126.66, 127.87 and 128.34 (NCHC*C*$_5H_5$), 141.15 (NCH*C*C_5H_5). MS (70 eV): *m*/*z* (%): 252/0 (M+H^+, 100). Anal. Calcd for $C_{15}H_{20}ClN$ (%): C, 72.13; H, 8.07; N, 5.61. Found (%): C, 72.28; H, 8.22; N, 5.80.

4.1.2.7. *trans*-3-Chloro-1-isopropyl-2-(4-methylphenyl)-azetidine 4g. Yield 86%, light yellow oil. ^{1}H NMR (270 MHz, C_6D_6): δ 0.64 and 0.78 (2×3H, 2×d, *J*=6.3 Hz, CH(CH_3)$_2$), 2.11 (3H, S, C_qCH_3), 2.26 (1H, septet, *J*=6.3 Hz, C*H*(CH_3)$_2$), 2.75 (1H, d×d, *J*=7.0 Hz, *J*=7.0 Hz, NC*H*(H)), 3.53 (1H, d×d, *J*=7.0 Hz, *J*=7.0 Hz, NCH(*H*)), 3.83 (1H, q, *J*=7.0 Hz, CHCl), 3.94 (1H, d, *J*=7.0 Hz, NC*H*C_6H_4), 7.00 and 7.37 (2×2H, 2×d, *J*=7.9 Hz, C_6H_4). ^{13}C NMR (68 MHz, $CHCl_3$): δ 20.09 and 20.79 (CH(*C*H_3)$_2$), 21.11 (C_qCH_3), 52.56 (CHCl), 59.68 (*C*H(CH_3)$_2$ and NCH_2), 78.58 (N*C*HC_6H_5), 126.61 and 129.04 (NCHCC_4H_4C), 137.32 and 138.47 (NCH*C*C_4H_4*C*). MS (70 eV): *m*/*z* (%): 225/3 (M^+, 22), 210/08 ($M-Me^+$, 22), 146 (100). Anal. Calcd for $C_{13}H_{18}ClN$ (%): C, 69.79; H, 8.11; N, 6.26. Found (%): C, 69.62; H, 8.19; N, 6.11.

4.1.3. Synthesis of 3-substituted azetidines 9a–p. The transformation of *trans*-3-chloro-2-phenylazetidine **4a** into *trans*-3-azido-2-phenylazetidine **9a** is given as a representative example of substitution reactions of 3-chloroazetidines. *trans*-3-Chloro-2-phenylazetidine (1 g, 4.8 mmol) was dissolved in DMSO (40 ml). To this solution, sodium azide (1.25 g, 19.28 mmol, 4 equiv) was added carefully. This mixture was stirred overnight at 80 °C. After cooling, the reaction mixture was poured into water (40 ml) and the mixture was extracted with diethyl ether. The combined organic extracts were washed with water and brine. After drying over magnesium sulfate and evaporation of the solvent, crude *trans*-3-azido-2-phenylazetidine (1 g, 4.7 mmol, 98%) was obtained. Further purification was performed by flash chromatography on silica gel, yielding *trans*-3-azido-2-phenylazetidine (0.48 g, 2.3 mmol, 47%).

The same procedure was executed with potassium cyanide, sodium acetate and potassium thiocyanate. The reaction times and temperatures for the other derivatives are listed in Section 2.

Caution: All reactions with sodium azide were performed behind a safety shield. No (violent) decomposition of the organic azides was observed throughout.

4.1.3.1. *trans*-1-Allyl-3-azido-2-phenylazetidine 9a. Yield 47%, light yellow oil. Hexane/EtOAc 7:1, R_f=0.18. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78–2.82 (1H, m, C(*H*)HCHN$_3$), 3.00 (1H, d×d, *J*=13.2 Hz, *J*=6.9 Hz, C(*H*)HCH=CH$_2$), 3.30 (1H, d×d, *J*=13.2 Hz, *J*=5.5 Hz, C(H)*H*CH=CH$_2$), 3.67–3.77 (2H, m, C(H)*H*CHN$_3$ and CHN$_3$), 3.92 (1H, d, *J*=5.6 Hz, C*H*C$_6$H$_5$), 5.04–5.20 (2H, m, CH=C*H*$_2$), 5.67–5.80 (1H, m, C*H*=CH$_2$), 7.22–7.43 (5H, m, C$_6$H$_5$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.54 (*C*H$_2$CHN$_3$), 58.34 (CHN$_3$), 60.75 (*C*H$_2$CH=CH$_2$), 74.97 (*C*HC$_6$H$_5$), 117.63 (CH=*C*H$_2$), 126.69, 127.95 and 128.55 (C*C*$_5$H$_5$), 133.93 (*C*H=CH$_2$), 139.99 (*C*C$_5$H$_5$). IR (NaCl, cm^{-1}): ν_{N3}=2101. MS (70 eV): *m*/*z* (%): 215 (M$^+$+1, 23). Anal. Calcd for $C_{12}H_{14}N_4$ (%): C, 67.27; H, 6.59; N, 26.15. Found (%): C, 67.49; H, 6.71; N, 26.40.

4.1.3.2. *trans*-1-Allyl-3-azido-2-(4-chlorophenyl)azetidine 9b. Yield 62%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.33. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78–2.85 (1H, m, C(*H*)HCHN$_3$), 3.02 (1H, d×d, *J*=13.2 Hz, *J*=6.7 Hz, C(*H*)HCH=CH$_2$), 3.26 (1H, d×d, *J*=13.2 Hz, *J*=5.8 Hz, C(H)*H*CH=CH$_2$), 3.66–3.74 (2H, m, CHN$_3$ and C(H)*H*CCN$_3$), 3.88 (1H, d, *J*=5.5 Hz, C*H*C$_6$H$_4$Cl), 5.04–5.25 (2H, m, CH=C*H*$_2$), 5.65–5.78 (1H, m, C*H*=CH$_2$), 7.31–7.39 (4H, m, C$_6$*H*$_4$Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.52 (*C*H$_2$CHN$_3$), 58.45 (CHN$_3$), 60.71 (*C*H$_2$CH=CH$_2$), 74.36 (*C*HC$_6$H$_4$Cl), 117.82 (CH=*C*H$_2$), 128.08 and 128.76 (C*C*$_4$H$_4$CCl), 133.76 (*C*H=CH$_2$), 133.76 and 138.59 (*C*C$_4$H$_4$*C*Cl). IR (NaCl, cm^{-1}): ν_{N3}=2103. MS (70 eV): *m*/*z* (%): 251/49 (M$^+$+1, 100). Anal. Calcd for $C_{12}H_{13}ClN_4$ (%): C, 57.95; H, 5.27; N, 22.53. Found (%): C, 57.66; H, 5.42; N, 22.69.

4.1.3.3. *trans*-3-Azido-1-benzyl-2-phenylazetidine 9c. Yield 45%, light yellow oil. Hexane/EtOAc 5:1, R_f=0.53. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.80 (1H, t, *J*=6.9 Hz, C(*H*)HCHN$_3$), 3.42 (1H, d, *J*=12.8 Hz, C(*H*)HC$_6$H$_5$), 3.55–3.60 (1H, m, C(H)*H*CHN$_3$), 3.67–3.76 (1H, m, CHN$_3$), 3.88 (1H, d, *J*=12.8 Hz, C(H)*H*C$_6$H$_5$), 4.01 (1H, d, *J*=6.6 Hz, C*H*C$_6$H$_5$), 7.18–7.47 (10H, m, CH$_2$C$_6$*H*$_5$ and CHC$_6$*H*$_5$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.42 (*C*H$_2$CHN$_3$), 58.48 (CHN$_3$), 61.39 (*C*H$_2$C$_6$H$_5$), 74.63 (*C*HC$_6$H$_5$), 126.59, 127.15, 127.90, 128.25, 128.51 and 128.63 (2×C*C*$_5$H$_5$), 137.32 and 138.80 (2×*C*C$_5$H$_5$). IR (NaCl, cm^{-1}): ν_{N3}=2102. MS (70 eV): *m*/*z* (%): 265 (M$^+$+1, 22), 120 (100) and 91(20). Anal. Calcd for $C_{16}H_{16}N_4$ (%): C, 72.70; H, 6.10; N, 21.20. Found (%): C, 72.96; H, 6.01; N, 21.02.

4.1.3.4. *trans*-3-Azido-1-benzyl-2-(4-chlorophenyl)-azetidine 9d. Yield 63%, light yellow oil. Hexane/EtOAc 3:2, R_f=0.76. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81–2.85 (1H, m, C(*H*)HCHN$_3$), 3.45 (1H, d, *J*=12.8 Hz, C(*H*)HC$_6$H$_5$), 3.58–3.63 (1H, m, C(H)*H*CHN$_3$), 3.66–3.73 (1H, m, CHN$_3$), 3.83 (1H, d, *J*=12.8 Hz, C(H)*H*C$_6$H$_5$), 3.98 (1H, d, *J*=6.6 Hz, C*H*C$_6$H$_4$Cl), 7.21–7.42 (9H, m, CH$_2$C$_6$*H*$_5$ and CHC$_6$*H*$_4$Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.52 (*C*H$_2$CHN$_3$), 58.64 (CHN$_3$), 61.48 (*C*H$_2$C$_6$H$_5$), 74.14 (*C*HC$_6$H$_4$Cl), 127.34, 128.02, 128.36 and 128.73 (C*C*$_4$H$_4$CCl and C*C*$_5$H$_5$), 133.67, 137.09 and 138.36 (*C*C$_4$H$_4$*C*Cl and *C*C$_5$H$_5$). IR (NaCl, cm^{-1}): ν_{N3}=2102. MS (70 eV): *m*/*z* (%): 120 (100), 91 (18). Anal. Calcd for $C_{16}H_{15}ClN_4$ (%): C, 64.32; H, 5.06; N, 18.75. Found (%): C, 64.13; H, 5.24; N, 18.60.

4.1.3.5. *trans*-3-Azido-1-isopropyl-2-phenylazetidine 9e. Yield 62%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.3. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.72 and 0.77 (2×3H, 2×d, *J*=6.3 Hz, CH(C*H*$_3$)$_2$), 2.48 (1H, septet, *J*=6.3 Hz, C*H*(CH$_3$)$_2$), 2.76 (1H, t, *J*=7.0 Hz, NC*H*(H)), 3.61 (1H, q, *J*=7.0 Hz, CHN$_3$), 3.68 (1H, q, *J*=7.0 Hz, NCH(*H*)), 3.87 (1H, d, *J*=7.0 Hz, NC*H*C$_6$H$_5$), 7.22–7.40 and 7.43–7.47 (5H, m, C$_6$H$_5$). ^{13}C NMR (68 MHz, $CDCl_3$): δ 20.04 and 20.75 (CH(*C*H$_3$)$_2$), 55.40 (NCH$_2$), 57.68 (CHN$_3$), 59.23 (*C*H(CH$_3$)$_2$), 74.77 (N*C*HC$_6$H$_5$), 126.74, 127.71 and 128.62 (NCHC*C*$_5$H$_5$), 141.79 (NCH*C*C$_5$H$_5$). IR (NaCl, cm^{-1}): ν_{N3}=2106. MS (70 eV): *m*/*z* (%): 201 (M−Me$^+$, 25), 173 (M−N$_3$−Me$^+$, 48) 161 (100), 119 (69), 91 (100). Anal. Calcd for $C_{12}H_{16}N_4$ (%): C, 66.64; H, 7.46; N, 25.90. Found (%): C, 66.91; H, 7.59; N, 25.78.

4.1.3.6. *trans*-3-Azido-1-cyclohexyl-2-phenylazetidine 9f. Yield 70%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.3. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.68–1.77 (10H, m, (C*H*$_2$)$_5$), 2.11–2.21 (1H, m, NCH), 2.77 (1H, t, *J*=7.0 Hz, NC*H*(H)), 3.61 (1H, q, *J*=7.0 Hz, CHN$_3$), 3.69 (1H, q, *J*=7.0 Hz, NCH(*H*)), 3.90 (1H, d, *J*=7.0 Hz, NC*H*C$_6$H$_5$), 7.23–7.36 and 7.40–7.46 (5H, m, C$_6$H$_5$). ^{13}C NMR (68 MHz, $CDCl_3$): δ 24.35, 24.40, 25.84, 30.08 and 30.94 ((CH$_2$)$_5$), 55.08 (NCH$_2$), 58.17 (CHN$_3$), 67.53 (NCH), 74.64 (N*C*HC$_6$H$_5$), 126.65, 127.65 and 128.39 (NCHC*C*$_5$H$_5$), 141.88 (NCH*C*C$_5$H$_5$). IR (NaCl, cm^{-1}): ν_{N3}=2101. MS (70 eV): *m*/*z* (%): 257 (M+H$^+$, 100).

4.1.3.7. *trans*-3-Aazido-1-isopropyl-2-(4-methylphenyl)azetidine 9g. Yield 76%, light yellow oil. ^{1}H NMR (300 MHz, C$_6$D$_6$): δ 0.72 and 0.98 (2×3H, 2×d, *J*=6.3 Hz, CH(C*H*$_3$)$_2$), 2.33 (3H, S, C$_q$C*H*$_3$), 2.45 (1H, septet, *J*=6.3 Hz, C*H*(CH$_3$)$_2$), 2.74 (1H, t, *J*=7.0 Hz, NC*H*(H)),

3.69 (1H, q, J=7.0 Hz, J=7.0 Hz, NCH(*H*)), 3.83 (1H, q, J=7.0 Hz, CHN_3), 3.84 (1H, d, J=7.0 Hz, NC*H*C_6H_4), 7.15 and 7.34 (2×2H, 2×d, J=8.3 Hz, C_6H_4). ^{13}C NMR (75 MHz, $CHCl_3$): δ 20.07 and 20.79 ($CH(CH_3)_2$), 21.13 (C_qCH_3), 55.36 (NCH_2), 57.77 (CHN_3), 59.30 ($CH(CH_3)_2$), 74.68 ($NCHC_6H_4$), 126.70 and 129.14 ($NCHCC_4H_4C$), 137.36 and 138.87 ($NCHCC_4H_4C$). IR (NaCl, cm^{-1}): ν_{N3}=2105. MS (70 eV): *m*/*z* (%): No M^+, 175 (100), 133 (65), 105 (83). Anal. Calcd for $C_{13}H_{18}N_4$ (%): C, 67.80; H, 7.88; N, 24.33. Found (%): C, 67.98; H, 7.70; N, 24.47.

4.1.3.8. *trans*-1-Allyl-3-cyano-2-phenylazetidine 9h. Yield 54%, light yellow oil. Hexane/EtOAc 5:1, R_f=0.36. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.95–3.09 (3H, m, C(*H*)HCH=CH_2, CHC≡N and C(*H*)HCHC≡N), 3.27 (1H, d×d×t, J=13.4 Hz, J=5.5 Hz, J=1.4 Hz, C(H)*H*CH=CH_2), 3.65–3.70 (1H, m, C(H)*H*CHC≡N), 4.21 (1H, d, J=8.0 Hz, C*H*C_6H_5), 5.04–5.25 (2H, m, CH=CH_2), 5.62–5.77 (1H, m, C*H*=CH_2), 7.22–7.48 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.51 (*C*HC≡N), 53.50 (CH_2CHC≡N), 60.17 (CH_2CH=CH_2), 71.93 (*C*HC_6H_5), 118.05 (CH=CH_2), 118.97 (C≡N), 126.57, 128.56 and 128.68 (CC_5H_5), 133.15 (*C*H=CH_2), 139.25 (CC_5H_5). IR (NaCl, cm^{-1}): $\nu_{C\equiv N}$=2241. MS (70 eV): *m*/*z* (%): 176 (25). Anal. Calcd for $C_{13}H_{14}N_2$ (%): C, 78.75; H, 7.12; N, 14.13. Found (%): C, 78.61; H, 7.01; N, 14.29.

4.1.3.9. *trans*-1-Allyl-2-(4-chlorophenyl)-3-cyanoazetidine 9i. Yield 62%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.93–3.06 (2H, m, CHC≡N and C(*H*)HCH=CH_2), 3.10 (1H, d×d, J=9.2 Hz, J=6.5 Hz, C(*H*)HCHC≡N), 3.24 (1H, d×d×t, J=13.5 Hz, J=5.6 Hz, J=1.5 Hz, C(H)*H*CH=CH_2), 3.67–3.72 (1H, m, C(H)*H*CHC≡N), 4.19 (1H, d, J=8.3 Hz, C*H*C_6H_4Cl), 5.02–5.22 (2H, m, CH=CH_2), 5.61–5.77 (1H, m, C*H*=CH_2), 7.27–7.47 (4H, m, C_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.62 (*C*HC≡N), 53.45 (CH_2CHC≡N), 60.19 (CH_2CH=CH_2), 71.23 (*C*HC_6H_4Cl), 118.33 (CH=CH_2), 118.77 (C≡N), 128.01 and 128.89 (CC_4H_4CCl), 133.15 (*C*H=CH_2), 134.65 and 137.78 (CC_4H_4CCl). IR (NaCl, cm^{-1}): $\nu_{C\equiv N}$=2242. MS (70 eV): *m*/*z* (%): 235/3 (M^++1, 66). Anal. Calcd for $C_{13}H_{13}ClN_2$ (%): C, 67.10; H, 5.63; N, 15.23. Found (%): C, 67.30; H, 5.54; N, 15.39.

4.1.3.10. *trans*-1-Benzyl-2-(4-chlorophenyl)-3-cyanoazetidine 9j. Yield 65%, light yellow oil. Hexane/EtOAc 3:1, R_f=0.43. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.92–3.01 (1H, m, CHC≡N), 3.12 (1H, d×d, J=9.2 Hz, J=6.4 Hz, C(*H*)HCHC≡N), 3.49 (1H, d, J=12.9 Hz, C(*H*)HC_6H_5), 3.56–3.61 (1H, m, C(H)*H*CHC≡N), 3.81 (1H, d, J=12.9 Hz, C(H)*H*C_6H_5), 4.28 (1H, d, J=8.3 Hz, C*H*C_6H_4Cl), 7.22–7.42 (9H, m, $CH_2C_6H_5$ and CHC_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.83 (*C*HC≡N), 53.55 (CH_2CHC≡N), 61.10 ($CH_2C_6H_5$), 71.18 (*C*HC_6H_4Cl), 118.72 (C≡N), 127.57, 127.98, 128.47, 128.68 and 128.92 (CC_4H_4CCl and CC_5H_5), 134.44, 136.27 and 137.54 (CC_4H_4CCl and CC_5H_5). IR (NaCl, cm^{-1}): $\nu_{C\equiv N}$=2242. MS (70 eV): *m*/*z* (%): 120 (100), 91 (40). Anal. Calcd for $C_{17}H_{15}ClN_2$ (%): C, 72.21; H, 5.35; N, 9.91. Found (%): C, 72.36; H, 5.48; N, 9.73.

4.1.3.11. *trans*-3-Acetoxy-1-allyl-2-phenylazetidine 9k. Yield 52%, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (3H, s, CH_3C=O), 2.75–2.80 (1H, m, C(*H*)HCHO), 3.05 (1H, d×d, J=13.4 Hz, J=6.9 Hz, C(*H*)HCH=CH_2), 3.33 (1H, d×d×t, J=13.4 Hz, J=5.6 Hz, J=1.4 Hz, C(H)*H*CH=CH_2), 3.85 (1H, d×d×d, J=7.3 Hz, J=6.5 Hz, J=1.1 Hz, C(H)*H*CHO), 3.99 (1H, d, J=6.3 Hz, C*H*C_6H_5), 4.83–4.90 (1H, m, CHOC=O), 5.03–5.29 (2H, m, CH=CH_2), 5.68–5.80 (1H, m, C*H*=CH_2), 7.25–7.47 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.73 (CH_3), 57.91 (CH_2CHO), 61.03 (CH_2CH=CH_2), 70.68 (*C*HOC=O), 74.62 (*C*HC_6H_5), 117.52 (CH=CH_2), 127.11, 127.84 and 128.34 (CC_5H_5), 134.04 (*C*H=CH_2), 139.86 (CC_5H_5), 170.00 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1745. MS (70 eV): *m*/*z* (%): 232 (M^++1, 89), 190 (83) and 172 (84). Anal. Calcd for $C_{14}H_{17}NO_2$ (%): C, 72.70; H, 7.41; N, 6.06. Found (%): C, 72.91; H, 7.26; N, 5.94.

4.1.3.12. *trans*-3-Acetoxy-1-allyl-2-(4-chlorophenyl)-azetidine 9l. Yield 73%, light yellow oil. Hexane/EtOAc 5:2, R_f=0.48. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (3H, s, CH_3C=O), 2.80–2.84 (1H, m, C(*H*)HCHO), 3.09 (1H, d×d, J=13.2 Hz, J=6.9 Hz, C(*H*)HCH=CH_2), 3.29 (1H, d×d, J=13.2 Hz, J=6.1 Hz, C(H)*H*CH=CH_2), 3.83–3.88 (1H, m, C(H)*H*CHO), 3.97 (1H, d, J=6.3 Hz, C*H*C_6H_4Cl), 4.83–4.90 (1H, m, CHOC=O), 5.14–5.20 (2H, m, CH=CH_2), 5.65–5.78 (1H, m, C*H*=CH_2), 7.26–7.42 (4H, m, C_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.73 (CH_3), 57.61 (CH_2CHO), 60.94 (CH_2CH=CH_2), 70.52 (*C*HOC=O), 74.14 (*C*HC_6H_4Cl), 117.90 (CH=CH_2), 128.51 and 128.59 (CC_4H_4CCl), 133.61 (*C*H=CH_2), 133.72 and 138.27 (CC_4H_4CCl), 169.98 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1746. MS (70 eV): *m*/*z* (%): 268/6 (M^++1, 100). Anal. Calcd for $C_{14}H_{16}ClNO_2$ (%): C, 63.28; H, 6.07; N, 5.27. Found (%): C, 63.40; H, 6.10; N, 5.16.

4.1.3.13. *trans*-3-Acetoxy-1-benzyl-2-phenylazetidine 9m. Yield 52%, light yellow oil. Hexane/EtOAc 5:2, R_f=0.59. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (3H, s, CH_3C=O), 2.77–2.82 (1H, m, C(*H*)HCHO), 3.49 (1H, d, J=13.1 Hz, C(*H*)HC_6H_5), 3.73–3.78 (1H, m, C(H)*H*CHO), 3.91 (1H, d, J=13.1 Hz, C(H)*H*C_6H_5), 4.08 (1H, d, J=5.0 Hz, C*H*C_6H_5), 4.87–4.91 (1H, m, CHOC=O), 7.19–7.48 (10H, m, $CH_2C_6H_5$ and CHC_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.78 (CH_3), 57.90 (CH_2CHO), 61.62 ($CH_2C_6H_5$), 70.97 (*C*HOC=O), 74.34 (*C*HC_6H_5), 127.09, 127.87, 128.25, 128.34, 128.50 and 128.74 (2×CC_5H_5), 137.46 and 139.69 (2×CC_5H_5), 169.99 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1744. MS (70 eV): *m*/*z* (%): 282 (M^++1, 12), 120 (100) and 91(20). Anal. Calcd for $C_{18}H_{19}NO_2$ (%): C, 76.84; H, 6.81; N, 4.98. Found (%): C, 76.99; H, 6.70; N, 4.88.

4.1.3.14. *trans*-3-Acetoxy-1-benzyl-2-(4-chlorophenyl)-azetidine 9n. Yield 15%, light yellow oil. Hexane/EtOAc 10:3, R_f=0.43. Yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (3H, s, CH_3C=O), 2.29–2.84 (1H, m, C(*H*)HCHO), 3.50 (1H, d, J=12.9 Hz, C(*H*)HC_6H_5), 3.74 (1H, t, J=6.3 Hz, C(H)*H*CHO), 3.86 (1H, d, J=12.9 Hz, C(*H*)HC_6H_5), 4.02 (1H, d, J=6.1 Hz, C*H*C_6H_4Cl), 4.79–4.85 (1H, m, CHOC=O), 7.22–7.42 (9H, m, $CH_2C_6H_5$ and CHC_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.75

(CH_3), 57.74 (CH_2CHO), 61.67 ($CH_2C_6H_5$), 70.95 (CHOC=O), 71.49 (CHC_6H_4Cl), 127.21, 127.99, 128.28, 128.44 and 128.71 (CC_4H_4CCl and CC_5H_5), 137.29, 138.34 and 169.96 (CC_4H_4CCl and CC_5H_5), 184.16 (C=O). IR (NaCl, cm^{-1}): $\nu_{C=O}$=1745. MS (70 eV): *m/z* (%): 318/6 (M^++1, 14), 120 (100).

4.1.3.15. *trans*-1-Allyl-2-phenyl-3-thiocyanoazetidine 9o. Yield 31%, light yellow oil. Hexane/EtOAc 2:1, R_f=0.48. ^1H NMR (300 MHz, $CDCl_3$): δ 3.08 (1H, d×d, *J*=13.3 Hz, *J*=6.6 Hz, C(*H*)HCH=CH_2), 3.11–3.16 (1H, m, C(*H*)HCHSC≡N), 3.32 (1H, d×d, *J*=13.3 Hz, *J*=5.5 Hz, C(H)*H*CH=CH_2), 3.37–3.44 (1H, m, CHSC≡N), 3.85–3.90 (1H, m, C(H)*H*CHSC≡N), 4.18 (1H, d, *J*=7.7 Hz, C*H*C_6H_5), 5.06–5.38 (2H, m, CH=C*H*$_2$), 5.65–5.80 (1H, m, C*H*=CH_2), 7.25–7.48 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, ref=$CDCl_3$): δ 42.88 (*C*HSC≡N), 57.80 (CH_2CHSC≡N), 60.58 (CH_2CH=CH_2), 75.94 (CHC_6H_5), 110.57 (CH=CH_2), 118.18 (SC≡N), 126.84, 128.60 and 128.80 (CC_5H_5), 133.65 (*C*H=CH_2), 139.24 (CC_5H_5). IR (NaCl, cm^{-1}): $\nu_{C\equiv N}$=2156. MS (70 eV): *m/z* (%): 231 (M^++1, 100). Anal. Calcd for $C_{13}H_{14}N_2S$ (%): C, 67.79; H, 6.13; N, 12.16. Found (%): C, 67.96; H, 6.30; N, 12.05.

4.1.3.16. *trans*-1-Allyl-2-(4-chlorophenyl)-3-thiocyanoazetidine 9p. Yield 43%, light yellow oil. Hexane/EtOAc 10:3, R_f=0.45. ^1H NMR (300 MHz, $CDCl_3$): δ 3.12 (1H, d×d, *J*=13.2 Hz, *J*=6.6 Hz, C(*H*)HCH=CH_2), 3.11–3.17 (1H, m, C(*H*)HCHSC≡N), 3.28 (1H, d×d×t, *J*=13.2 Hz, *J*=5.8 Hz, *J*=1.4 Hz, C(H)*H*CH=CH_2), 3.32–3.40 (1H, m, CHSC≡N), 3.85–3.90 (1H, m, C(H)*H*CHSC≡N), 4.16 (1H, d, *J*=7.4 Hz, C*H*C_6H_4Cl), 5.05–5.24 (2H, m, CH=C*H*$_2$), 5.63–5.77 (1H, m, C*H*=CH_2), 7.26–7.43 (4H, m, C_6H_4Cl). ^{13}C NMR (75 MHz, $CDCl_3$): δ 42.87 (*C*HSC≡N), 57.60 (CH_2CHSC≡N), 60.41 (CH_2CH=CH_2), 75.20 (CHC_6H_4Cl), 118.22 (CH=CH_2), 128.10 and 128.89 (CC_4H_4CCl), 133.38 (*C*H=CH_2), 110.21 and 134.27 (CC_4H_4CCl), 137.74 (SC≡N). IR (NaCl, cm^{-1}): $\nu_{C\equiv N}$=2157. MS (70 eV): *m/z* (%): 267/5 (M^++1, 100). Anal. Calcd for $C_{13}H_{13}ClN_2S$ (%): C, 58.97; H, 4.95; N, 10.58. Found (%): C, 59.19; H, 4.74; N, 10.50.

4.1.4. Synthesis of 3-methoxyazetidines 9q–r. The reaction of *trans*-1-allyl-3-chloro-2-phenylazetidine **4a** to afford *trans*-1-allyl-3-methoxy-2-phenylazetidine **9q** is given as a representative example for the substitution reactions of the 3-chloroazetidines with sodium methoxide. Sodium methoxide (4 N) (5 ml, 20 mmol, 4 equiv) in methanol was added to *trans*-3-chloro-2-phenylazetidine (1 g, 4.8 mmol) and the resulting mixture was refluxed overnight (22 h). After cooling, water (10 ml) was added and the mixture was extracted with diethyl ether and dried over magnesium sulfate. After evaporation of the solvent in vacuo, *trans*-3-methoxy-2-phenylazetidine (0.69 g, 3.4 mmol, 71%) was obtained. After flash chromatography (Hexane/EtOAc 3:2) on silica gel, pure *trans*-3-methoxy-2-phenylazetidine (0.48 g, 2.35 mmol, 49%) was isolated.

4.1.4.1. *trans*-1-Allyl-3-methoxy-2-phenylazetidine 9q. Yield 49%, light yellow oil. Hexane/EtOAc 3:2, R_f=0.48. ^1H NMR (300 MHz, $CDCl_3$): δ 2.73 (1H, t, *J*=6.3 Hz, C(*H*)HCHO), 3.01 (1H, d×d, *J*=13.3 Hz, *J*=7.0 Hz, C(*H*)HCH=CH_2), 3.23 (3H, s, OCH_3), 3.24–3.32 (1H, m, C(H)*H*CH=CH_2), 3.70 (1H, t, *J*=6.3 Hz, C(H)*H*CHO), 3.79–3.83 (1H, m, CHO), 3.86 (1H, d, *J*=5.5 Hz, C*H*C_6H_5), 5.00–5.20 (2H, m, CH=C*H*$_2$), 5.67–5.80 (1H, m, C*H*=CH_2), 7.22–7.48 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.35 (OCH_3), 56.62 (CH_2CCHO), 59.95 (CH_2CH=CH_2), 75.35 (CHC_6H_5), 77.00 (*C*HO), 116.06 (CH=CH_2), 125.84, 126.30 and 127.17 (CC_5H_5), 133.28 (*C*H=CH_2), 140.12 (CC_5H_5). MS (70 eV): *m/z* (%): 204 (M^++1, 100). Anal. Calcd for $C_{13}H_{17}NO$ (%): C, 76.81; H, 8.43; N, 6.89. Found (%): C, 76.96; H, 8.56; N, 6.80.

4.1.4.2. *trans*-1-Benzyl-2-(4-chlorophenyl)-3-methoxyazetidine 9r. Yield 45%, light yellow oil. Hexane/EtOAc 5:2, R_f=0.33. ^1H NMR (300 MHz, $CDCl_3$): δ 2.73–2.77 (1H, m, C(*H*)HCHO), 3.21 (3H, s, OCH_3), 3.46 (1H, d, *J*=12.8 Hz, C(*H*)HC_6H_5), 3.59–3.60 (1H, m, C(H)*H*CHO), 3.70–3.76 (1H, m, CHO), 3.82 (1H, d, *J*=12.8 Hz, C(H)HC_6H_5), 3.92 (1H, d, *J*=5.8 Hz, C*H*C_6H_4Cl), 7.18–7.41 (9H, m, C_6H_5 and C_6H_4Cl). ^{13}C NMR (75 MHz, ref=$CDCl_3$): δ 56.73 (OCH_3), 57.97 (CH_2CHO), 61.92 ($CH_2C_6H_5$), 75.69 (CHC_6H_4Cl), 78.67 (CHO), 127.24, 128.37, 128.46, 128.63 and 128.87 (CC_4H_4CCl and CC_5H_5), 133.22, 137.88 and 139.87 (CC_4H_4CCl and CC_5H_5). MS (70 eV): *m/z* (%): 290/88 (M^++1, 20), 120 (100). Anal. Calcd for $C_{17}H_{18}ClNO$ (%): C, 70.95; H, 6.30; N, 4.87. Found (%): C, 70.79; H, 6.45; N, 4.99.

4.1.5. Synthesis of *trans*-3-amino-2-arylazetidines 18a–c. The synthesis of *trans*-3-amino-1-isopropyl-2-phenylazetidine **18a** is described as a representative example.

To *trans*-3-azido-1-isopropyl-2-phenylazetidine (1 g, 4.6 mmol) in diethyl ether (10 ml) at 0 °C was added lithium aluminium hydride (0.35 g, 9.2 mmol, 2 equiv). This reaction mixture was refluxed for 2 h and after cooling, water was added until all remaining lithium aluminium hydride had decomposed. The resulting suspension was poured into water and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried (magnesium sulfate) and, after filtration and evaporation of the solvent in vacuo, *trans*-3-amino-1-isopropyl-2-phenylazetidine (0.62 g 3.22 mmol, 70%) was obtained. Purification was performed by an acid–base extraction.

4.1.5.1. *trans*-3-Amino-1-isopropyl-2-phenylazetidine 18a. Yield 70%, yellow oil. ^1H NMR (270 MHz, $CDCl_3$): δ 0.71 and 0.98 (2×3H, 2×d, *J*=6.3 Hz, CH(CH_3)$_2$), 1.66 (2H, br s, NH_2), 2.32 (1H, septet, *J*=6.3 Hz, C*H*(CH_3)$_2$), 2.48 (1H, t, *J*=7.0 Hz, NC*H*(H)), 3.18 (1H, q, *J*=7.0 Hz CHNH_2), 3.43 (1H, d, *J*=7.0 Hz, NC*H*C_6H_5), 3.76 (1H, q, *J*=7.0 Hz, NCH(*H*)), 7.21–7.48 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 20.31 and 21.02 (CH(CH_3)$_2$), 52.96 (CHNH_2), 59.62 (*C*H(CH_3)$_2$), 60.07 (NCH_2), 80.88 (N*C*HC_6H_5), 126.57, 127.08 and 128.19 (NCHCC_5H_5), 142.98 (NCH*C*C_5H_5). IR (NaCl, cm^{-1}): ν_{NH_2}=3379, 3306. MS (70 eV): *m/z* (%): 147 (M−*i*Pr^+, 48), 106 (56). Anal. Calcd for $C_{12}H_{18}N_2$ (%): C, 75.74; H, 9.53; N, 14.72. Found (%): C, 75.99; H, 9.27; N, 14.94.

4.1.5.2. *trans*-3-Amino-1-cyclohexyl-2-phenylazetidine 18b. Yield 82%, yellow oil. ^1H NMR (270 MHz, $CDCl_3$): δ 0.95–1.97 (10H, m, (CH_2)$_5$), 2.00–2.12 (1H, m,

NCH), 2.48 (1H, t, J=7.0 Hz, NCH(H)), 3.18 (1H, q, J=7.0 Hz, CHNH$_2$), 3.45 (1H, d, J=7.0 Hz, NCHC$_6$H$_5$), 3.76 (1H, q, J=7.0 Hz, NCH(H)), 7.21–7.45 (5H, m, C$_6$H$_5$). ^{13}C NMR (68 MHz, CDCl$_3$): δ 24.49, 24.56, 25.93, 30.33 and 31.25 ((CH$_2$)$_5$), 53.58 (CHNH$_2$), 59.75 (NCH$_2$), 68.10 (NCH), 80.90 (NCHC$_6$H$_5$), 126.54, 127.10 and 128.21 (NCHCC_5H$_5$), 143.11 (NCHCC_5H$_5$). IR (NaCl, cm^{-1}): ν_{NH_2}=3379, 3307. MS (70 eV): m/z (%): 231 (M+H$^+$, 72), 112 (100). Anal. Calcd for C$_{15}$H$_{22}$N$_2$ (%): C, 78.21; H, 9.63; N, 12.16. Found (%): C, 78.47; H, 9.41; N, 12.32.

4.1.5.3. *trans*-3-Amino-1-isopropyl-2-(4-methylphenyl)-azetidine 18c. Yield 92%, yellow oil. ^{1}H NMR (270 MHz, CDCl$_3$): δ 0.71 and 0.97 (2×3H, 2×d, J=6.3 Hz, CH(CH_3)$_2$), 2.32 (3H, S, C$_q$CH_3), 2.41 (1H, septet, J=6.3 Hz, CH(CH$_3$)$_2$), 2.41 (1H, t, J=7.0 Hz, NCH(H)), 3.14 (1H, q, J=7.0 Hz, CHNH$_2$), 3.37 (1H, d, J=7.0 Hz, NCHC$_6$H$_4$), 3.73 (1H, t, J=7.0 Hz, J=7.0 Hz, NCH(H)), 7.12 and 7.32 (2×2H, 2×d, J=7.9 Hz, C$_6$H$_4$). ^{13}C NMR (68 MHz, CHCl$_3$): δ 20.34 and 21.08 (CH(CH_3)$_2$) and (C$_q$CH_3), 53.06 (CHNH$_2$), 59.68 (CH(CH$_3$)$_2$), 60.02 (NCH$_2$), 80.86 (NCHC$_6$H$_4$), 126.56 and 128.91 (NCHCC_4H$_4$C), 136.62 and 140.11 (NCHCC_4H$_4$C). IR (NaCl, cm^{-1}): ν_{NH_2}=3376. MS (70 eV): m/z (%): No M$^+$, 175 (M−NH$_2$−Me$^+$100), 133 (66), 106 (93), 84 (66). Anal. Calcd for C$_{13}$H$_{20}$N$_2$ (%): C, 76.42; H, 9.87; N, 13.71. Found (%): C, 76.66; H, 9.71; N, 13.79.

4.1.6. Synthesis of *trans*-2-arylazetidin-3-ols 19a–b. The hydrolysis of 3-acetoxy-1-allyl-2-phenylazetidine **9k** to 1-allyl-2-phenylazetidin-3-ol **19a** is described as a representative example. 3-Acetoxy-1-allyl-2-phenylazetidine **9k** (0.2 g, 0.87 mmol) was dissolved in methanol (30 ml). To this solution, sodium bicarbonate (1.89 g, 0.87 mmol, 1 equiv) was added. The resulting mixture was refluxed for 1 h. At the end of the reaction, water was added to the mixture, after which extraction was performed with diethyl ether. After drying on magnesium sulfate, filtration and evaporation of the solvent, crude 1-allyl-2-phenylazetidin-3-ol **19a** (0.28 g, 0.78 mmol, 89%, purity 95%) was obtained. Flash chromatography yielded pure 1-allyl-2-phenylazetidin-3-ol (0.15 g, 0.41 mmol, 47%).

4.1.6.1. *trans*-1-Allyl-2-phenylazetidin-3-ol 19a. Yield 47%, light yellow oil. Hexane/EtOAc 3:2, R_f=0.15. ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.63–2.68 (1H, m, C(H)HCHOH), 2.95 (1H, d×d, J=13.1 Hz, J=6.9 Hz, C(H)HCH=CH$_2$), 3.23 (1H, d×d, J=13.1 Hz, J=5.9 Hz, C(H)HCH=CH$_2$), 3.56–3.60 (1H, m, C(H)HCHOH), 3.73 (1H, d, J=6.6 Hz, CHC$_6$H$_5$), 4.01–4.07 (1H, m, CHOH), 4.98–5.13 (2H, m, CH=CH_2), 5.59–5.72 (1H, m, CH=CH$_2$), 7.21–7.32 (5H, m, C$_6H_5$). ^{13}C NMR (75 MHz, CDCl$_3$): δ 60.06 (CH_2CHOH), 61.23 (CH_2CH=CH$_2$), 69.84 (CHOH), 78.69 (CHC$_6$H$_5$), 117.67 (CH=CH_2), 126.91, 127.60 and 128.36 (CC$_5$H$_5$), 133.99 (CH=CH$_2$), 140.33 (CC$_5$H$_5$). IR (NaCl, cm^{-1}): ν_{OH}=3367, MS (70 eV): m/z (%): 190 (M$^+$+1, 100). Anal. Calcd for C$_{12}$H$_{15}$NO (%): C, 76.16; H, 7.99; N, 7.40. Found (%): C, 76.25; H, 7.50; N, 8.54.

4.1.6.2. *trans*-1-Allyl-2-(4-chlorophenyl)azetidin-3-ol 19b. Yield 79%, light yellow oil. ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.68–2.73 (1H, m, C(H)HCHOH), 3.01 (1H, d×d, J=13.2 Hz, J=6.9 Hz, C(H)HCH=CH$_2$), 3.23 (1H, d×d, J=13.2 Hz, J=5.8 Hz, C(H)HCH=CH$_2$), 3.66–3.70 (1H, m, C(H)HCHOH), 3.73 (1H, d, J=6.1 Hz, CHC$_6$H$_4$Cl), 4.03–4.09 (1H, m, CHOH), 5.00–5.21 (2H, m, CH=CH_2), 5.62–5.75 (1H, m, CH=CH$_2$), 7.26–7.33 (4H, m, C$_6H_4$Cl). ^{13}C NMR (75 MHz, CDCl$_3$): δ 60.11 (CH_2CHOH), 60.12 (CH_2CH=CH$_2$), 70.10 (CHOH), 77.96 (CHC$_6$H$_4$Cl), 117.84 (CH=CH_2), 128.24 and 128.51 (CC$_4$H$_4$CCl), 133.29 (CC_4H$_4$CCl), 133.81 (CH=CH$_2$), 138.89 (CC$_4$H$_4$CCl). IR (NaCl, cm^{-1}): ν_{OH}=3343. MS (70 eV): m/z (%): 226/4 (M$^+$+1, 100). Anal. Calcd for C$_{12}$H$_{14}$ClNO (%): C, 64.43; H, 6.31; N, 6.26. Found (%): C, 64.65; H, 6.43; N, 6.12.

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Reactions of 4-(dimethylamino)pyridinium activated pentachloropyridine with nitrogen nucleophiles and hydride

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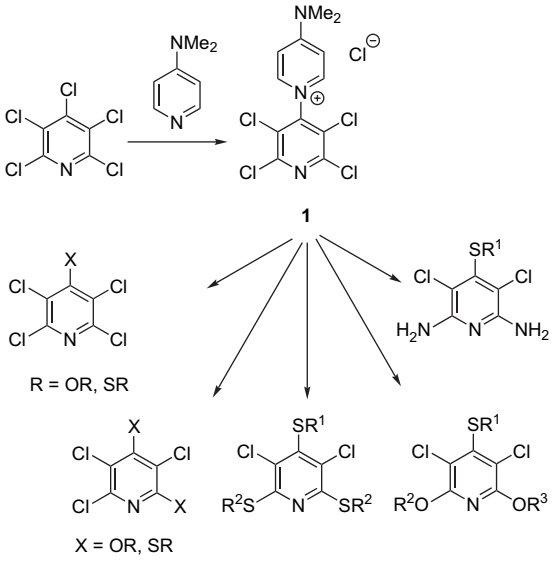
Abstract—Substitution reactions on 2′,3′,5′,6′-tetrachloro-4-dimethylamino-[1,4]bipyridinyl-1-ylium chloride with nitrogen nucleophiles such as *n*-propylamine, isopropylamine, glycine, morpholine, and piperidine were examined. Highly functionalized Cl^2,Cl^3,N^4,Cl^5,Cl^6- and N^2,Cl^3,N^4,Cl^5,Cl^6-substituted pyridines were obtained, in part possessing unsubstituted 4-amino groups due to dealkylation. Detailed NMR studies were performed in order to elucidate the regiochemistry of these dealkylations.

1. Introduction

Substituted pyridines play a crucial role in organic, bioorganic, and pharmaceutical chemistry as well as in material sciences. This is reflected in an impressive number of monographs and review articles dealing with syntheses and properties of functionalized pyridines.[1] Numerous synthetic procedures have been developed during the last decades, among these ring closure reactions from acyclic precursors,[2] Dimroth rearrangements,[3] ring contractions of 1,2-diazocines,[4] multicomponent cascade heterocyclizations,[5] Vilsmeier and the reverse Vilsmeier methods,[6] electrochemical methods,[7] pyrimidine–pyridine ring interconversions,[8] metal-mediated [2+2+2]-cycloadditions,[9] and other metal-organic syntheses.[10] Although nucleophilic substitutions on pentafluoropyridine[11] and approaches from ynamines and ynamides[12] have been studied recently and proved to be promising avenues for the synthesis of highly substituted pyridines, an astonishing large number of simply functionalized pyridines have been unavailable to date. We recently described mono- and oligocationic hetarenium salts with up to ten positive charges within the same molecule,[13] and their broad applicability in heterocyclic synthesis. Thus, 2′,3′,5′,6′-tetrachloro-4-dimethylamino-[1,4]bipyridinyl-1-ylium chloride **1**, readily available in quantitative yields from pentachloropyridine, can be used to prepare Cl^2,Cl^3,O^4,Cl^5,Cl^6- and O^2,Cl^3,O^4,Cl^5,Cl^6-pentasubstituted pyridines[14] as well as their sulfur analogs.[15] Similarly, hitherto unavailable O^2,Cl^3,O^4,Cl^5,O^6-[14] and biologically interesting S^2,Cl^3,S^4,Cl^5,S^6-pentasubstituted pyridines,[15] as well as a broad variety of symmetric and non-symmetric O^2,Cl^3,S^4,Cl^5,O^6-pentasubstituted pyridines are available starting from **1**.[16] The procedure can be extended to the synthesis of first representatives of N^2,Cl^3,S^4,Cl^5,N^6-pentasubstituted pyridines[16] (Scheme 1). The preparation of amino-substituted pyridines, however, remained challenging. 2-Amino-substituted pyridines are available by the Chichibabin reaction,[17] or by nucleophilic substitutions of suitable leaving groups at 2- or 4-position such as halogen atoms.[18] More than one- or twofold substitutions afford vigorous reaction conditions due to the decreased reactivity of chloropyridines substituted with electron-donating groups. Halogen atoms at C-3 are inert toward these substitution reactions[19] unless metal-organic procedures[10] or hetaryne mechanisms are applied.[20] As a consequence, pentasubstituted pyridines with more than one amino-substituent are very rare. According to the



Scheme 1. Synthetic potential of DMAP-activated pentachloropyridine **1**.

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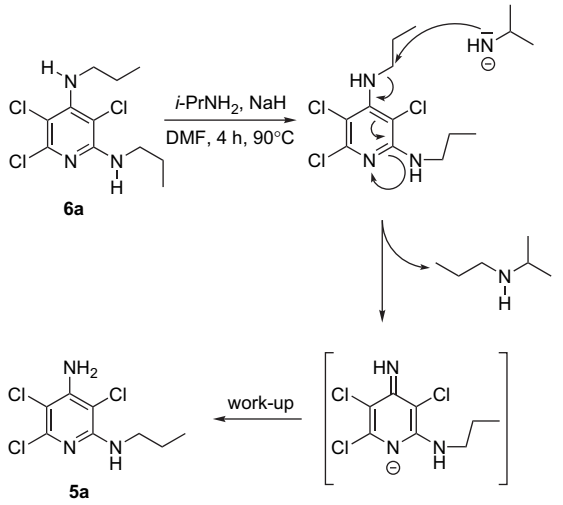
Beilstein cross-fire database, less than 10 representatives of N^2,Cl^3,N^4,Cl^5,Cl^6-pentasubstituted pyridines have been described to date in six publications[21–26] and one patent.[27] We report here our results of studies directed toward the applicability of our synthetic strategy for the preparation of functionalized pyridines starting from hetarenium salts to some nitrogen nucleophiles.

2. Results and discussion

We studied substitution reactions on 2′,3′,5′,6′-tetrachloro-4-dimethylamino-[1,4]bipyridinyl-1-ylium chloride **1** with the nitrogen nucleophiles such as *n*-propylamine, isopropylamine, glycine, morpholine, and piperidine. Nucleophilic substitutions on **1** with *n*-propylamine and isopropylamine were first performed in the presence of sodium hydride as base. Mixtures of substituted pyridines were obtained, which are presented in Table 1, among them new representatives of N^2,Cl^3,N^4,Cl^5,Cl^6-pentasubstituted species.

Surprisingly, *n*-propylamine as nucleophile resulted in the formation of 3,5,6-trichloro-N^2-propyl-pyridine-2,4-diamine **5a** as the main product (Table 1, entry 1), when the reaction was conducted at 50 °C over a period of 4 h. 3,5,6-Trichloro-N^2,N^4-dipropyl-pyridine-2,4-diamine **6a** was isolated in very low yields. The 4-isopropylamino-substituted tetrachloropyridine **4b** was found to be the main product of the reaction of hetarenium salt **1** with isopropylamine under analogous reaction conditions (Table 1, entry 3). 3,5,6-Trichloro-N^2-isopropyl-pyridine-2,4-diamine **5b** was formed in low yields as a by-product. In either case, unmodified starting material was easily separated by filtration over silica gel.

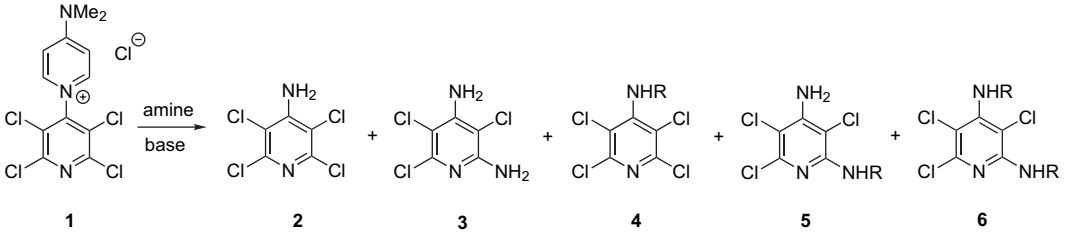
Obviously, the 4-amino groups in **5a** and **5b** were formed from propylamino- and isopropylamino groups, respectively. In a control experiment, the dipropylamino-substituted pyridine **6a** indeed reacted with isopropylamine in the presence of sodium hydride in DMF to give the dealkylated product **5a** (Scheme 2). Several mechanisms can be discussed. The lack of electron-withdrawing groups does not support an $E1_{cb}$-type mechanism and elimination of propene. Nucleophilic attack, however, of propylamide and isopropylamide on the 4-propylamino groups in the initially formed N^2,N^4-dialkyl-pyridine-2,4-diamines **6a** and **6b**, respectively, resulted in the formation of *N*,*N*-dialkylamines and 4-NH_2-substituted pyridines.



Scheme 2. Proposed mechanism for dealkylations.

The regiochemistry of this conversion was unambiguously confirmed by NMR methods. Thus, in the ^1H NMR spectra of **5a**, taken in $CDCl_3$ at rt, two H/D-exchangeable resonance frequencies in a 1:2 ratio are observable at δ=4.82 and 4.95 ppm, which were assigned to the NHR and NH_2

Table 1. Reaction of **1** with amines in the presence of bases



Entry	Amine	Temp/time	Base	Product	R	Yield %
1	*n*-$PrNH_2$	50 °C, 4 h	NaH	**5a**	*n*-Pr	32
				6a	*n*-Pr	5
2			$NaNH_2$	**2**	H	7
				3	H	18
				4a	*n*-Pr	0
				5a	*n*-Pr	40
				6a	*n*-Pr	35
3	*i*-$PrNH_2$	40 °C, 4 h	NaH	**4b**	*i*-Pr	37
				5b	*i*-Pr	5
4			$NaNH_2$	**2**	H	10
				3	H	10
				4b	*i*-Pr	15
				5b	*i*-Pr	30
				6b	*i*-Pr	0

group, respectively. In agreement to the proposed structure, the gs-HMBC (^{1}H–^{13}C) spectrum displays all the expected long range C–H couplings as presented in Figure 1.

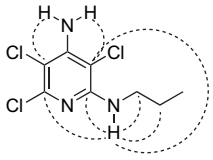


Figure 1. HMBC-detected long range C–H couplings in **5a**.

Among these, the $^{3}J_{CH}$ coupling of the hydrogen atoms of the NH_2 group with C-5 is diagnostically important. However, a 2D INADEQUATE, performed to unambiguously prove the ^{13}C peak assignments, suffered from disadvantageous relaxation times of the aromatic carbon atoms due to the absence of H atoms at the pyridine ring. We solved the problem by considering very large C–C coupling constants within this special pyridine ring (approx. 70 Hz) and by addition of 4% chromium(III) acetylacetonate as a relaxation reagent, and proved the C–C connectivities as shown in Figure 2. Analogous results were obtained with **5b**.

As a result, the ^{13}C NMR peak assignments of the N^2,Cl^3,N^4,Cl^5,Cl^6-substituted pyridines presented in this paper is as shown in Figure 3. A ^{15}N HMBC measurement allowed to assign the resonance frequency δ_N at −310.3 ppm

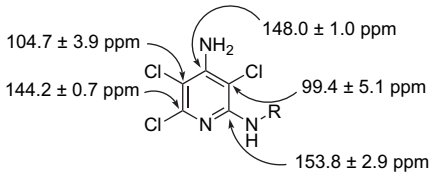


Figure 3. Peak assignments of the ^{13}C NMR resonance frequencies.

to the 4-NH_2-group of **5a**, whereas the signal at −294.5 ppm is caused by the 2-NHR group. The pyridine nitrogen atom could not be detected by ^{15}N NMR spectroscopy.

Next we used sodium amide as base for the reaction of hetarenium salt **1** with amines. This base gave best results on treatment of **1** with a broad variety of O- and S-nucleophiles.[13–16] In these reactions the amide anion never reacted as nucleophile. Indeed, we found a quantitative conversion of the starting material **1**. Again, the N^2,Cl^3,N^4,Cl^5,Cl^6-substituted pyridine **5a** was isolated as the main product of the reaction with *n*-propylamine at 50 °C (Table 1, entry 2). The diamine **6a** was found in 35% yield. Additional by-products are tetrachloropyridin-4-amine **2** and trichloropyridine-2,4-diamine **3**, isolated in 7% and 18% yield, respectively. The latter mentioned compounds formed also on treatment of pentachloropyridine with ammonia at 170–190 °C, in addition to 2-aminotetrachloropyridine.[21,22] Similar results were obtained starting from isopropylamine (Table 1, entry 4). A mixture of **2**, **3**, **4b**, and **5b** was formed, with **5b** as the

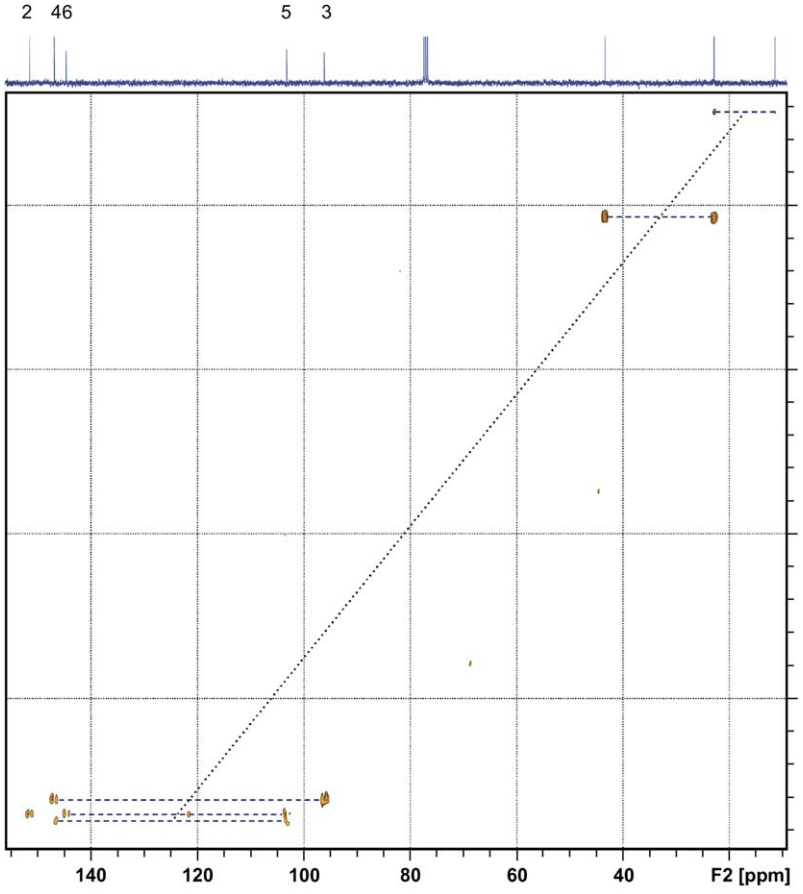
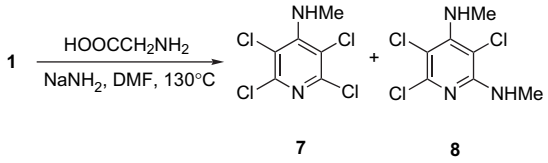


Figure 2. 2D-INADEQUATE of **5a**.

main product. All compounds were easily separated and purified by column chromatography on silica gel.

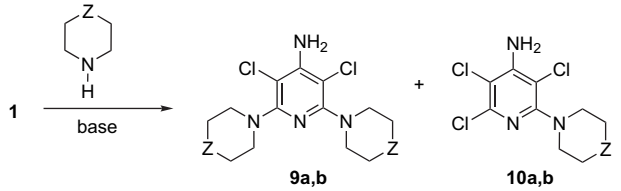
Glycine as nitrogen nucleophile yielded methyl-(2,3,5,6-tetrachloro-pyridin-4-yl)-amine **7**, which is a known compound,[24] and 3,5,6-trichloro-N^2,N^4-dimethyl-pyridine-2,4-diamine **8** in moderate and low yields, respectively (Scheme 3). Obviously, these compounds were formed on decarboxylation of the glycine moieties under the applied reaction conditions. To the best of our knowledge, **8** has never been described before. The pyridylamine **7** was obtained earlier on reaction of pentachloropyridine with methylamine in 1,4-dioxane as a mixture of 2- and 4-isomers after a reaction time of 18 h.[24]



Scheme 3.

Morpholine in the presence of sodium amide converted hetarenium salt **1** into 3,5-dichloro-(2,6-dimorpholin-4-yl)-pyridin-4-ylamine **9a** in 59% yield, which was isolated as a pure compound (Table 2, entry 2). In accordance with the structure, the ^{13}C NMR spectra showed a symmetric molecule. Sodium hydride as the base gives lower yields and considerable amounts of 2,3,5-trichloro-6-morpholin-4-yl-pyridin-4-ylamine **10a** (Table 2, entry 1), the regiochemistry of which was elucidated by HMBC measurements as described above. The morpholine-substituted trichloropyridine with N^2,Cl^3,N^4,Cl^5,Cl^6 substitution pattern is available by substitution of secondary aliphatic amines on (*Z*)-perchloro-1,3-butadiene-1-carbonitrile.[28] Pentachloropyridine reacts with morpholine at the 2-position. Twofold substitution results in the formation of N^2,Cl^3,Cl^4,Cl^5,N^6-morpholino-substituted pyridine. Some earlier published structures had to be revised.[28]

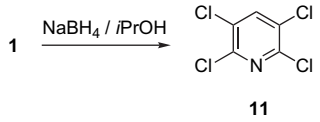
Table 2. Morpholine and piperidine as N-nucleophiles



Entry	Amine	Temp/time	Base	Product	Z	Yield %
1	Morpholine	90 °C, 3 h	NaH	**9a**	O	10
				10a		18
2			$NaNH_2$	**9a**		59
				10a		0
3	Piperidine	100 °C, 3 h	NaH	**9b**	CH_2	54
				10b		18
4			$NaNH_2$	**9b**		37
				10b		25

On reaction of hetarenium salt **1** with piperidine, **9b** and **10b** were obtained under the conditions presented in Table 2 (entries 3 and 4).

2′,3′,5′,6′-Tetrachloro-4-dimethylamino-[1,4]bipyridinyl-1-ylium chloride **1** reacts with sodium borohydride to give 2,3,5,6-tetrachlorpyridine **11** (Scheme 4). Best yields and purities were achieved when 2-propanol was used as solvent. On changing the solvent to ethanol, the yield decreased from 80 to 5% after reaction at rt over a period of 5 h. In DMF, the yield is 46% under analogous reaction conditions. In 2-propanol as solvent, the product was obtained in 95% purity. The 4-(dimethylamino)pyridine could be recovered. The chromatographically separable by-product proved to be 2,3,5-trichloropyridine, as evidenced by GC–MS analysis. Numerous procedures for the preparation of 2,3,5,6-tetrachloropyridine **11** exist, among them the reduction of pentachloropyridine with zinc, ammonium chloride, dimethylphosphonate in water at 89–90 °C,[29] electrochemical methods,[30] the chlorination of 2,3,5-trichloropyridine with hexachloroethane in the presence of *n*-Bu_4NBr in aqueous sodium hydroxide,[31] and some patented procedures.[32]



Scheme 4.

In summary, we supplement our synthetic strategy for the preparation of highly functionalized pyridines from hetarenium salts by results describing scope and limitations of the application of nitrogen nucleophiles.

3. Experimental

3.1. General

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 and DPX 200 at 400 and 200 MHz, respectively. The chemical shifts are reported in parts per million relative to internal tetramethylsilane (δ=0.00 ppm). FTIR spectra were obtained on a Bruker Vector 22 in the range of 400–4000 cm^{-1} (2.5% pellets in KBr). The GC–MS spectra were recorded on a GC Hewlett–Packard 5980, Series II in combination with a MS Hewlett–Packard 5989 B, and on a Varian GC3900 with SAT2100T mass spectrometer. Melting points are uncorrected.

3.2. General procedure for the reaction of 2′,3′,5′,6′-tetrachloro-4-dimethylamino-[1,4]bipyridinyl-1-ylium chloride 1 with amines

A suspension of **1** (10 mmol, 3.74 g) in 100 mL of the amine was treated with sodium hydride (50 mmol, 1.25 g) or sodium amide (50 mmol, 1.96 g) and heated at reflux temperature over a period of 5 h. Then, the amine was distilled off in vacuo and the residue was chromatographed (silica gel 60, EtOAc/petroleum ether=1:3).

3.2.1. 2,3,5,6-Tetrachloro-pyridin-4-ylamine (2). Pale yellow solid, mp 210 °C ($C_5H_2Cl_4N_2$ requires C, 25.90; H, 0.87; N, 12.08. Found: C, 25.87; H, 0.86; N, 12.18); δ_H (DMSO-d_6) 7.38 (s, 2H; NH_2); δ_C (DMSO-d_6) 150.8,

144.1, 111.6; ν_{max} (KBr) (cm^{-1}): 3494, 3390, 1583, 1535, 1373, 1250, 1109, 1061, 973; *m*/*z*=233 (MH^+, 100), 195 (M−Cl, 11).

3.2.2. 3,5,6-Trichloro-pyridine-2,4-diylamine (3). Pale yellow solid, mp 168 °C ($C_5H_4Cl_3N_3$ requires C, 28.27; H, 1.90; N, 19.78. Found: C, 29.06; H, 2.11; N, 19.05); δ_H (DMSO-d_6) 6.41 (s, 2H; α-NH_2), 6.31 (s, 2H; γ-NH_2); δ_C (DMSO-d_6) 153.1, 148.7, 143.5, 100.8, 94.3; ν_{max} (KBr) (cm^{-1}) 3453, 3369, 1612, 1573, 1439; *m*/*z*=213 (M, 100), 176 (M−Cl, 28).

3.2.3. Isopropyl-(2,3,5,6-tetrachloro-pyridin-4-yl)-amine (4b). Brownish solid, mp 74 °C ($C_8H_8Cl_4N_2$ requires C, 35.07; H, 2.94; N, 10.22. Found: C, 36.06; H, 2.97; N, 10.23); δ_H ($CDCl_3$) 4.85 (s, 1H; N*H*), 4.61 (h, ^3J=6.4 Hz, 1H; NC*H*), 1.27 (d, ^3J=6.4 Hz, 6H; CH_3); δ_C ($CDCl_3$) 150.0, 146.4, 115.4, 47.2, 24.4; ν_{max} (KBr) (cm^{-1}) 1556, 1539, 1413, 1353, 1259, 1236, 1140, 1021, 912, 708; *m*/*z*=275 (M, 100), 260 (M−CH_3).

3.2.4. 3,5,6-Trichloro-N^2-propyl-pyridine-2,4-diamine (5a). Yellow solid, mp 76 °C ($C_8H_{10}N_3Cl_3$ requires C, 37.75; H, 3.96; N, 16.51. Found: C, 37.56; H, 3.87; N, 16.49); δ_H ($CDCl_3$) 4.85 (br s, 2H; NH_2), 4.78 (br s, 1H; N*H*), 3.38 (dt, ^3J=7.3, 1.5 Hz, 2H; NCH_2), 1.62 (sx, ^3J=7.3 Hz, 2H; CH_2CH$_3$), 0.97 (t, ^3J=7.3 Hz, 3H; CH_3); δ_C ($CDCl_3$) 151.5, 146.9, 144.6, 103.1, 96.1, 43.3, 22.8, 11.4; ν_{max} (KBr) (cm^{-1}) 3511, 3405, 2969, 2929, 2864, 1614, 1583, 1507, 1447, 1298, 1165; *m*/*z*=255 (M, 43); 225 (M−C_3H_7, 100).

3.2.5. 3,5,6-Trichloro-N^2-isopropyl-pyridine-2,4-diamine (5b). Brownish solid, mp 26 °C ($C_8H_8Cl_3N_3$ requires C, 37.75; H, 3.96; N, 16.51. Found: C, 37.34; H, 3.75; N, 16.26); δ_H ($CDCl_3$) 4.85 (s, 2H; NH_2), 4.60 (s, 1H; N*H*), 4.19 (h, ^3J=6.4 Hz, 1H; NC*H*), 1.22 (d, ^3J=6.4 Hz, 6H; CH_3); δ_C ($CDCl_3$) 150.9, 147.0, 144.7, 103.0, 96.1, 42.9, 23.1, 11.2; ν_{max} (KBr) (cm^{-1}) 3498, 3400, 2972, 1610, 1578, 1504, 1437, 1311, 1182, 1126, 1055; *m*/*z*=254 (M, 100), 238 (M−CH_3).

3.2.6. 3,5,6-Trichloro-N^2,N^4-dipropyl-pyridine-2,4-diamine (6a). Yellow liquid ($C_{11}H_{16}Cl_3N_3$ requires C, 44.54; H, 5.44; N, 14.17. Found: C, 43.67; H, 4.95; N, 14.18); δ_H ($CDCl_3$) 4.86 (s, 1H; N*H*), 4.78 (br s, 1H; N*H*), 3.39 (t, ^3J=7.3 Hz, 2H; NCH_2), 3.36 (t, ^3J=7.3 Hz, 2H; NCH_2), 1.61 (h, ^3J=7.3 Hz, 4H; CH_2), 0.96 (t, ^3J=7.3 Hz, 6H; CH_3); δ_C ($CDCl_3$) 151.5, 146.9, 144.7, 103.2, 96.1, 43.3, 22.9, 11.4; ν_{max} (NaCl) (cm^{-1}) 3398, 2963, 2875, 1582, 1356, 1233, 1070; *m*/*z*=296 (M, 4), 260 (M−Cl, 100), 225 (M−2Cl, 27), 184 (M−2Cl−C_3H_7, 22).

3.2.7. 3,5-Dichloro-(2,6-dimorpholin-4-yl)-pyridin-4-yl-amine (9a). Colorless solid, mp 64 °C ($C_{13}H_{18}Cl_2N_4O_2$ requires C, 46.86; H, 5.44; N, 16.81. Found: C, 46.50; H, 5.70; N, 16.80); δ_H ($CDCl_3$) 4.93 (br s, 2H; NH_2), 3.80–3.85 (m, 8H), 3.26–3.31 (m, 8H); δ_C ($CDCl_3$): 154.3, 148.7, 100.1, 66.9, 49.5; ν_{max} (KBr) (cm^{-1}) 3473, 3321, 2969, 2855, 1621, 1554, 1420, 1392, 1365, 1283, 1257, 1154, 1110, 1067, 1030, 1019, 1007; *m*/*z*=333 (M, 100); 296 (M−Cl, 31).

3.2.8. 3′,5′-Dichloro-3,4,5,6,3″,4″,5″,6″-octahydro-2*H*, 2″*H*-[1,2′,6′,1″]terpyridin-4′-ylamine (9b). Yellow solid, mp 41 °C ($C_{15}H_{22}Cl_2N_4$ requires C, 54.72; H, 6.73; N, 17.02. Found: C, 54.28; H, 7.06; N, 16.81); δ_H ($CDCl_3$) 4.78 (s, 2H; NH_2), 3.10–3.30 (m, 8H; CH_2), 1.50–1.75 (m, 12H; CH_2); δ_C ($CDCl_3$) 155.3, 148.2, 99.7, 50.4, 26.0, 24.7; ν_{max} (KBr) (cm^{-1}): 3480, 3380, 2935, 2918, 2847, 2827, 1611, 1598, 1553, 1541, 1422, 1373, 1283, 1258, 1217, 1117, 1076, 1007, 866; *m*/*z*=330 (MH^+, 100); 294 (M−Cl, 45); 244 (M−$C_5H_{10}N$, 24).

3.2.9. 2,3,5-Trichloro-6-(morpholin-4-yl)-4-amino-pyridine (10a). Colorless solid, mp 144 °C ($C_9H_{10}Cl_3N_3O$ requires C, 38.26; H, 3.57; N, 14.87. Found: C, 38.23; H, 3.12; N, 14.34); δ_H ($CDCl_3$) 5.11 (s, 2H; 10H), 3.77–3.90 (m, 4H; 9H), 3.25–3.40 (m, 4H; 8H); δ_C ($CDCl_3$) 155.6, 148.9, 144.2, 108.2, 104.3, 66.8, 49.5; ν_{max} (KBr) (cm^{-1}): 3445, 3334, 1622, 1559, 1525, 1417, 1367, 1254, 1112; *m*/*z*=283 (M, 100).

3.2.10. 3′,5′,6′-Trichloro-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4′-ylamine (10b). Yellow oil. δ_H ($CDCl_3$) 5.04 (s, 2H; NH_2), 3.13–3.30 (m, 4H; CH_2), 1.50–1.77 (m, 6H; CH_2); δ_C ($CDCl_3$) 156.7, 148.7, 144.0, 107.3, 104.4, 50.4, 25.8, 24.4; *m*/*z*=281 (M, 100), 245 (M−Cl, 25); 86 ($C_5H_{10}N$, 31).

3.3. General procedure for the reaction of 2′,3′,5′,6′-tetrachloro-4-dimethylamino-[1,4]bipyridinyl-1-ylium chloride (1) with glycine

A suspension of pyridinium salt **1** (3.74 g, 10 mmol) in 100 mL of DMF was treated with sodium amide (1.96 g, 50 mmol) and glycine (1.5 g, 20 mmol) and heated for 5 h at 120 °C. After cooling, the reaction mixture was poured in water, and pH was adjusted to 5 with hydrochloric acid. The mixture was then evaporated to dryness, and the residue was chromatographed (silica gel, ethyl acetate/petroleum ether=1:4).

3.3.1. 2,3,5,6-Tetrachloro-4-(*N*-methylamino)-pyridine (7). Oil, δ_H ($CDCl_3$) 4.82 (s, 1H; 5H), 2.91 (s, 3H; 6H); δ_C ($CDCl_3$) 155.0, 148.5, 97.7, 41.5; ν_{max} (KBr) (cm^{-1}): 3498, 3397, 3945, 1601, 1553, 1484, 1396, 1325, 1045, 843, 705; *m*/*z*=247 (MH^+, 100), 214 (M−CH_4N, 75). No satisfactory elemental analysis achieved.

3.3.2. 2,3,5-Trichloro-4,6-di-(*N*-methylamino)-pyridine (8). Colorless solid, 69 °C ($C_7H_8N_3Cl_3$ requires C, 34.96; H, 3.35; N, 17.47. Found: C, 35.22; H, 2.90; N, 17.55); δ_H ($CDCl_3$) 5.07 (br s, 2H; 7H, 9H), 2.94 (s, 6H; 8H, 10H); δ_C ($CDCl_3$) 156.3, 148.8, 143.6, 106.5, 102.4, 41.4 (overlapped); ν_{max} (KBr) (cm^{-1}) 3502, 3402, 1601, 1563, 1525, 1397, 1327, 1043, 938; *m*/*z*=241 (MH^+, 100), 210 (M−CH_4N, 15).

3.3.3. 2,3,5,6-Tetrachloropyridine (11). A suspension of salt **1** (3.74 g, 10 mmol) in 100 mL of anhydrous 2-propanol was treated with sodium borohydride (0.57 g, 15 mmol) at rt and stirred for 6 h. Then, the solution was poured in diluted hydrochloric acid and extracted twice with diethyl ether. The organic layer was separated, evaporated, and chromatographed (silica gel, ethyl acetate/petroleum ether=1/1). All spectroscopic data are in agreement to an authentic sample.

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A consecutive Diels–Alder approach toward a Tet repressor directed combinatorial library

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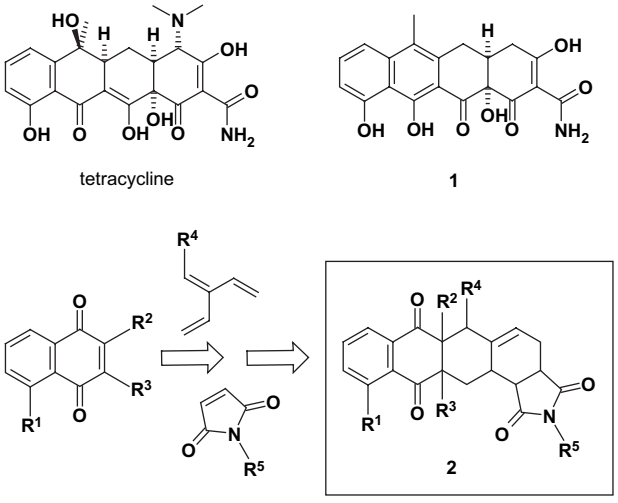
Abstract—A combinatorial library of 180 tetracycline analogs was generated by solution phase parallel synthesis applying a consecutive Diels–Alder strategy. Chemical methodology suitable for three-dimensional solution phase parallel synthesis was developed that enabled us to generate a collection of potential TetR inducers. The synthesis was built on cross-conjugated trienes as central building blocks facilitating two consecutive cycloaddition processes with different dienophiles. Upon sequential exposure to naphthoquinone and maleimide derivatives, the generation of a carbocyclic skeleton of type **2** incorporating the diversity elements R^1–R^5 was envisaged.

1. Introduction

Linking chemical synthesis of natural product derivatives with genetic evolution of biologically active proteins creates an effective bridge between a basic understanding of functional proteins and the discovery of highly potent and selective modulators for gene regulation in prokaryotes and eukaryotes.[1,2] Following this approach, we constructed a mutant of the tetracycline-inducible repressor protein TetR[3,4] displaying specificity for the tetracycline analog **1** (Scheme 1) preventing both antibiotic activity and induction of the wild-type Tet repressor.[5] As a complement to our recent efforts on solid-phase supported combinatorial synthesis,[6,7] we herein present a solution phase parallel synthesis[8] of a tetracycline related compound library of type **2**. According to preliminary studies, the tetracycline mimetic core structure of **2** could be docked into the binding pocket of TetR crystal structure.[9] To provide an efficient approach to the identical four-ring carbocyclic structure as a basic skeleton, our forward directed plan of synthesis was built on a homo-Diels–Alder (HDA) approach allowing an efficient and stereospecific construction of multiple carbon–carbon bonds. Our strategy involved cross-conjugated trienes as central building blocks facilitating two consecutive cycloaddition processes with different dienophiles.[10,11] Upon sequential exposure to naphthoquinone and maleimide derivatives, the generation of a carbocyclic skeleton of type **2** incorporating the diversity elements R^1–R^5 was envisaged.



Scheme 1. Lead compounds and plan of synthesis.

2. Results and discussion

2.1. Elaboration of the key reactions

The usage of Diels–Alder reactions represents a particularly efficient strategy for the parallel synthesis of complex molecular structures.[12] Since we aimed to generate a

Keywords: Solution phase parallel synthesis; Diels–Alder; Tetracycline; TetR.
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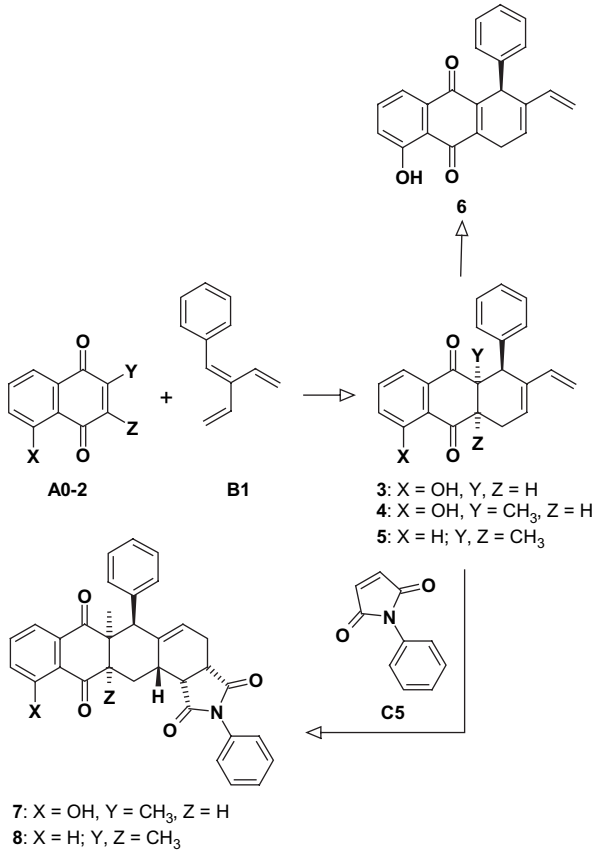
combinatorial compound collection of suitable purity and yield, appropriate reaction conditions for the consecutive Diels–Alder processes have to be elaborated by investigating a model reaction sequence. As a representative central building block, we chose the cross-conjugated triene **B1** that was expected to provide sufficient chemical stability and efficient accessibility. The preparation of **B1**[10,11] was done by γ-pentadienylation of benzaldehyde using 5-bromo-1,3-pentadiene[13] in the presence of indium dust followed by sulfonylation of the formed secondary alcohol and β-elimination. Our initial synthetic investigations were directed to the HDA reaction of the triene **B1** with 5-hydroxynaphthoquinone (**A0**) when heating both components in toluene at 40–60 °C gave a hardly separable mixture of regioisomers together with the respective dehydro-derivatives, which were obviously produced by intermolecular electron transfer. Performing the transformation under Lewis acid-catalyzed conditions at −60 °C produced the cycloaddition product **3** as a pure regioisomer. However, the formation of a substantial amount of the oxidation product **6** was observed after chromatography. To exclude this side reaction, we subjected the 2-methyl substituted hydroxynaphthoquinone **A1** (plumbagin) as well as the 2,3-dimethyl derivative **A2**[14,15] being devoid of an activating 5-hydroxy group to the above mentioned reaction conditions resulting in the formation of the tricyclic products **4** and **5**, respectively. Besides the thermally induced HDA processes, we investigated Lewis acid-mediated conditions at low temperature (−60 °C) using scandium trifluoromethanesulfonate[16] and, alternatively, boron trifluoride diethyl etherate[17] when pure cycloaddition products could be isolated in 70–85% yield. Due to economic reasons, we chose the BF_3 promoted variant as the method of choice. To accommodate conditions in a parallel reactor, the reaction temperature was raised to −30 °C which proved to be possible without significant loss of yield and purity. For the transformation of the hydroxynaphthoquinone derivative **A1**, complete regioselectivity was observed, which is obviously due to secondary orbital interactions. The regiochemical outcome of the reaction could be unambiguously determined by NMR spectroscopy when HMBC experiments clearly indicated that the carbonyl carbon located in β-position to the hydroxy substituent showed cross-peaks to both methylene hydrogens whereas the C═O with the remote hydroxyl group displaying coupling via three bonds with an aromatic hydrogen interacted with the proton adjacent to the phenyl substituent. Since the sterically demanding phenyl group of **B1** was expected to predominantly adopt a trans-orientation with respect to the reactive diene substructure and substantial *endo*-selectivity was assumed for the Diels–Alder process, we anticipated a trans-disposition between the phenyl and the vicinally positioned methyl group, which was confirmed at the final product stage.

An efficient approach of the pentacyclic model compounds **7** and **8** by cycloaddition of the synthetic intermediates **4** and **5**, respectively, with *N*-phenylmaleimide (**C5**) was investigated employing both microwave technology (80 °C for 20 min without use of a solvent) and conventional heating (toluene at 68 °C for 3 days). In fact, application of both techniques resulted in the formation of the final products **7** and **8** in >80% yield (Scheme 2). According to diagnostic NOEs, complete diastereospecifity was observed indicating a cis-selective *endo*-approach of the dienophile opposite to the phenyl substituent to avoid steric interactions. The molecular structure of the racemic pentacyclic target compound **8** could be elucidated by X-ray crystallography (Fig. 1). Interestingly, the pentacyclic scaffold shows a concave shape when the phenyl moiety is located in a sandwich-like manner over the aromatic system of the former naphthoquinone.



Scheme 2. Representative model reactions.

Based on our model studies, reaction conditions could be found that seemed to be robust and reproducible and, thus, suitable for an efficient production of a liquid organic phase supported 3D-library. Since the parallelization of the conventional heating requires substantially less technical sophistication than parallel microwave assisted synthesis, we decided to perform the library production without microwave technology.

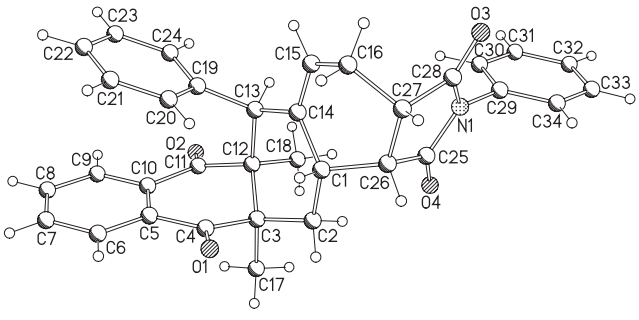
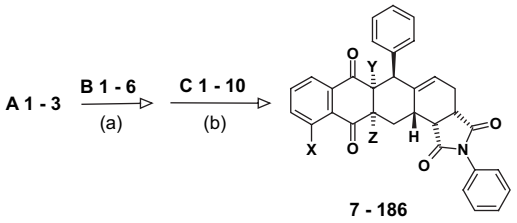


Figure 1. Crystal structure of (3a*S*,6*R*,6a*S*,12a*R*,13a*S*,13b*R*)-**8**.

2.2. Library production

Following the procedure described above, 180 tetracycline analogs should be prepared starting from the naphthoquinones **A1–3**, the cross-conjugated trienes **B1–6** and the maleimides **C1–10** (Scheme 3, Fig. 2).



Scheme 3. Solution phase parallel synthesis. (a) **B1–6**, $BF_3 \cdot Et_2O$, CH_2Cl_2, −30 °C, 16 h. (b) **C1–10**, toluene, 68 °C, 3 days.

Thus, each of the quinones **A1–3** was reacted with the set of cross-conjugated trienes **B1–6** in the presence of boron trifluoride diethyl etherate at −30 °C. After extraction with water, the organic layer had to be separated. This could be done in a very practical manner by adding *n*-hexane to convey the organic layer to the top followed by freezing the aqueous layer at −60 °C and sucking off the liquid organic part. After removal of nonpolar impurities on a short pad of silica gel, each of the 18 tricyclic compounds {**A1–3B1–6**} was distributed to 10 reaction vessels and stirred with the maleimides {**C1–10**} in toluene to afford the 180 tetracycline analogs **7–186** {**A1–3B1–6C1–10**}. After preparative HPLC, the members of the compound collection were obtained in 26.7–86.4% yield. Ninety-five percent of the final products showed purities higher than 90% (Table 1).

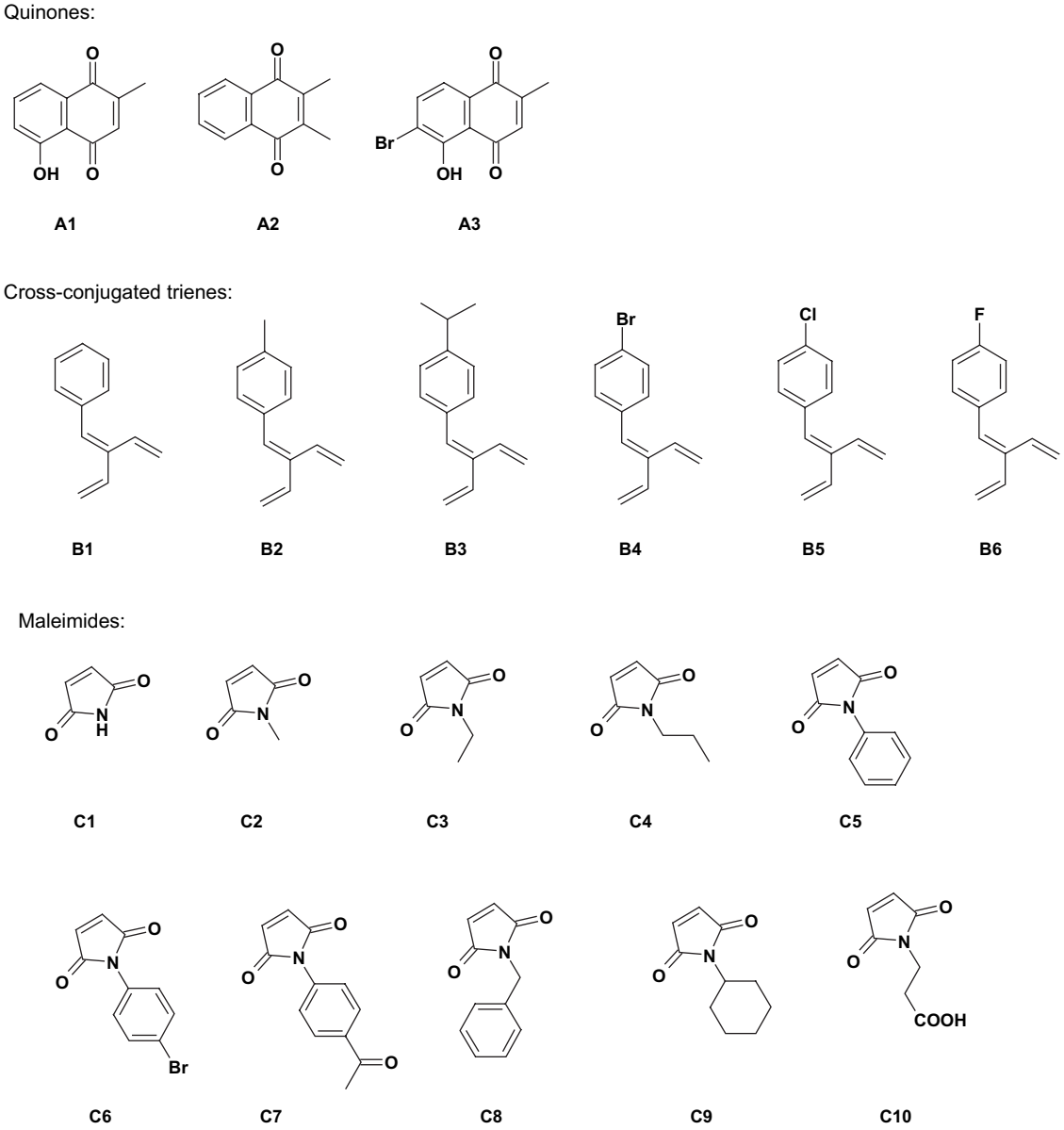


Figure 2. Building blocks.

Table 1. Purities and yields of library products

No.	Building blocks	Purity (LC–MS) (%)	Yield (over two steps) (%)	No.	Building blocks	Purity (LC–MS) (%)	Yield (over two steps) (%)
9	A1B1C1	>99.0	62.4	**97**	A2B4C1	89.9	55.9
10	A1B1C2	>99.0	84.0	**98**	A2B4C2	>99.0	29.8
11	A1B1C3	>99.0	86.4	**99**	A2B4C3	>99.0	54.1
12	A1B1C4	>99.0	83.5	**100**	A2B4C4	>99.0	55.1
7	A1B1C5	>99.0	73.5	**101**	A2B4C5	>99.0	56.9
13	A1B1C6	89.1	82.6	**102**	A2B4C6	98.6	59.2
14	A1B1C7	>99.0	76.6	**103**	A2B4C7	81.7	69.9
15	A1B1C8	>99.0	73.8	**104**	A2B4C8	>99.0	59.2
16	A1B1C9	>99.0	70.4	**105**	A2B4C9	>99.0	42.8
17	A1B1C10	94.0	77.2	**106**	A2B4C10	93.8	65.2
18	A1B2C1	98.0	61.7	**107**	A2B5C1	88.0	54.6
19	A1B2C2	>99.0	70.1	**108**	A2B5C2	>99.0	65.9
20	A1B2C3	>99.0	72.2	**109**	A2B5C3	>99.0	59.9
21	A1B2C4	>99.0	71.8	**110**	A2B5C4	98.9	49.4
22	A1B2C5	>99.0	67.9	**111**	A2B5C5	>99.0	38.0
23	A1B2C6	>99.0	43.4	**112**	A2B5C6	>99.0	67.1
24	A1B2C7	>99.0	61.1	**113**	A2B5C7	88.7	55.7
25	A1B2C8	>99.0	57.4	**114**	A2B5C8	>99.0	57.0
26	A1B2C9	>99.0	71.0	**115**	A2B5C9	>99.0	48.8
27	A1B2C10	>99.0	57.8	**116**	A2B5C10	91.0	58.9
28	A1B3C1	97.7	69.5	**117**	A2B6C1	98.9	64.2
29	A1B3C2	>99.0	85.2	**118**	A2B6C2	94.7	52.0
30	A1B3C3	>99.0	76.1	**119**	A2B6C3	>99.0	61.7
31	A1B3C4	>99.0	61.2	**120**	A2B6C4	>99.0	57.6
32	A1B3C5	97.1	81.7	**121**	A2B6C5	>99.0	47.7
33	A1B3C6	91.1	76.8	**122**	A2B6C6	98.4	54.7
34	A1B3C7	>99.0	75.9	**123**	A2B6C7	95.4	53.5
35	A1B3C8	96.0	58.8	**124**	A2B6C8	>99.0	56.9
36	A1B3C9	94.6	72.3	**125**	A2B6C9	>99.0	68.4
37	A1B3C10	>99.0	56.6	**126**	A2B6C10	95.6	62.9
38	A1B4C1	>99.0	68.6	**127**	A3B1C1	95.5	33.0
39	A1B4C2	>99.0	60.6	**128**	A3B1C2	96.1	47.9
40	A1B4C3	97.4	74.1	**129**	A1B1C3	97.3	58.6
41	A1B4C4	>99.0	60.5	**130**	A3B1C4	96.7	45.7
42	A1B4C5	>99.0	69.6	**131**	A3B1C5	97.4	53.9
43	A1B4C6	96.6	40.3	**132**	A3B1C6	95.7	41.1
44	A1B4C7	>99.0	58.3	**133**	A3B1C7	93.6	31.7
45	A1B4C8	>99.0	84.3	**134**	A3B1C8	92.3	26.7
46	A1B4C9	>99.0	73.7	**135**	A3B1C9	95.1	43.5
47	A1B4C10	87.3	71.4	**136**	A3B1C10	98.2	48.2
48	A1B5C1	>99.0	80.2	**137**	A3B2C1	93.3	45.8
49	A1B5C2	98.4	75.7	**138**	A3B2C2	96.5	33.5
50	A1B5C3	>99.0	53.8	**139**	A3B2C3	>99.0	31.1
51	A1B5C4	>99.0	66.6	**140**	A3B2C4	94.6	27.3
52	A1B5C5	>99.0	55.8	**141**	A3B2C5	93.4	35.8
53	A1B5C6	96.7	43.1	**142**	A3B2C6	96.5	28.2
54	A1B5C7	>99.0	41.0	**143**	A3B2C7	96.0	25.4
55	A1B5C8	>99.0	83.2	**144**	A3B2C8	97.7	30.8
56	A1B5C9	>99.0	61.8	**145**	A3B2C9	95.9	38.3
57	A1B5C10	82.0	60.9	**146**	A3B2C10	98.7	43.3
58	A1B6C1	>99.0	58.7	**147**	A3B3C1	92.1	31.4
59	A1B6C2	97.1	49.5	**148**	A3B3C2	97.6	32.3
60	A1B6C3	>99.0	63.4	**149**	A3B3C3	95.4	28.4
61	A1B6C4	>99.0	76.5	**150**	A3B3C4	98.0	38.5
62	A1B6C5	>99.0	72.3	**151**	A3B3C5	96.3	27.7
63	A1B6C6	95.7	59.6	**152**	A3B3C6	97.4	30.1
64	A1B6C7	>99.0	76.3	**153**	A3B3C7	>99.0	40.2
65	A1B6C8	>99.0	68.1	**154**	A3B3C8	96.6	37.1
66	A1B6C9	>99.0	79.4	**155**	A3B3C9	90.6	31.8
67	A1B6C10	88.6	73.5	**156**	A3B3C10	92.7	20.5
68	A2B1C1	>99.0	61.3	**157**	A3B4C1	>99.0	42.2
69	A2B1C2	>99.0	65.4	**158**	A3B4C2	>99.0	35.8
70	A2B1C3	>99.0	68.0	**159**	A3B4C3	>99.0	38.2
71	A2B1C4	>99.0	63.4	**160**	A3B4C4	>99.0	32.2
8	A2B1C5	98.9	52.4	**161**	A3B4C5	>99.0	38.1
72	A2B1C6	94.9	63.7	**162**	A3B4C6	>99.0	37.6
73	A2B1C7	>99.0	62.2	**163**	A3B4C7	>99.0	48.1
74	A2B1C8	>99.0	59.3	**164**	A3B4C8	>99.0	39.2
75	A2B1C9	>99.0	73.3	**165**	A3B4C9	95.6	32.2
76	A2B1C10	>99.0	71.1	**166**	A3B4C10	93.9	46.5
77	A2B2C1	86.0	58.9	**167**	A3B5C1	95.2	38.7

(*continued*)

Table 1. (*continued*)

No.	Building blocks	Purity (LC–MS) (%)	Yield (over two steps) (%)	No.	Building blocks	Purity (LC–MS) (%)	Yield (over two steps) (%)
78	A2B2C2	>99.0	35.2	**168**	A3B5C2	>99.0	36.0
79	A2B2C3	98.5	80.0	**169**	A3B5C3	>99.0	44.5
80	A2B2C4	94.4	56.8	**170**	A3B5C4	>99.0	34.4
81	A2B2C5	98.2	57.2	**171**	A3B5C5	96.9	43.2
82	A2B2C6	>99.0	54.7	**172**	A3B5C6	>99.0	37.1
83	A2B2C7	>99.0	58.4	**173**	A3B5C7	>99.0	43.8
84	A2B2C8	>99.0	61.4	**174**	A3B5C8	>99.0	36.3
85	A2B2C9	98.7	47.3	**175**	A3B5C9	>99.0	38.7
86	A2B2C10	94.1	55.1	**176**	A3B5C10	94.2	33.7
87	A2B3C1	>99.0	64.7	**177**	A3B6C1	>99.0	40.3
88	A2B3C2	>99.0	64.5	**178**	A3B6C2	>99.0	36.2
89	A2B3C3	>99.0	44.4	**179**	A3B6C3	>99.0	33.4
90	A2B3C4	>99.0	57.0	**180**	A3B6C4	>99.0	37.9
91	A2B3C5	>99.0	59.7	**181**	A3B6C5	>99.0	37.6
92	A2B3C6	95.1	54.3	**182**	A3B6C6	>99.0	29.6
93	A2B3C7	96.1	41.0	**183**	A3B6C7	>99.0	32.1
94	A2B3C8	>99.0	58.2	**184**	A3B6C8	>99.0	38.3
95	A2B3C9	97.1	55.2	**185**	A3B6C9	>99.0	42.2
96	A2B3C10	>99.0	53.9	**186**	A3B6C10	98.5	39.6

3. Summary

In summary, a consecutive Diels–Alder approach was exploited for a highly regio- and stereoselective two-step synthesis of a TetR directed library of carbocyclic scaffolds in racemic form. Chemical methodology suitable for three-dimensional solution phase parallel synthesis was developed that enabled us to generate a collection of 180 potential TetR inducers.

4. Experimental

4.1. General

Absolute solvents (over molecular sieves) and starting materials obtained from commercial source were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were determined on a BRUKER AVANCE 360 or a BRUKER AVANCE 600 spectrometer in solution. COSY, HSQC, HMBC and NOE spectra (600 MHz) spectra were determined in solution using instrument BRUKER AVANCE 600. LC–MS analyses were conducted using an Agilent Binary Gradient System in combination with ChemStation Software (MeOH/0.1 N aq HCOOH 10/90–90/10) applying a Zorbax SB-C8 (4.6 mm×150 mm, 5 μm) column, UV detection at 254 nm and a flow rate of 0.5 mL/min. Mass detection was pointed out with a Bruker Esquire 2000 ion-trap-mass spectrometer using an APC ionization source. EIMS spectra were recorded on FINNIGAN MAT TSQ 700 spectrometer. HRMS were determined on a JOEL GCmateII at a resolution of $M/\Delta M>5000$. CHN elementary analyses were done at the laboratory of Ilse Beetz, Kronach. Silica gel (40–63 μm) was used for a purification step. Preparative HPLC was conducted using an Agilent 1100 Series system applying a Eurospher-C18 (4.6 mm×250 mm, 7 μm) with H_2O and acetonitrile both containing 0.1% of trifluoroacetic acid (gradient 0–80% acetonitrile) with a flow rate of 20 mL/min.

4.2. Preparation of the quinones A1–3

A2 was readily prepared by a chromium-(VI)-oxide catalyzed oxidation of 2,3-dimethylnaphthalene with periodic acid.[14] **A3** was obtained by the bromination of commercially available **A1**.[18]

4.3. Preparation of the cross-conjugated trienes B1–6[10,11,19]

Indium powder (100 mesh, 282 mg, 2.46 mmol) was added in portions to a solution of 5-bromo-1,3-pentadiene (657 mg, 4.47 mmol) and aromatic aldehyde (2.23 mmol) in DMF (2.23 mL) at 0 °C. After stirring for 5 h at 0 °C, temperature was allowed to rise to 10 °C. The mixture was diluted with CH_2Cl_2 (15 mL) and poured into diethyl ether (190 mL). The resulting turbid mixture was filtered through a pad of silica gel and washed with an additional amount of ether. Evaporation of the solvent and purification of the crude intermediate by flash chromatography on silica gel using *n*-hexane/ethyl acetate (10:1) resulted in the desired secondary alcohol. Mesyl chloride (1.35 mL, 17.5 mmol) was added dropwise to a solution of this intermediate (13.1 mmol) and triethylamine (2.74 mL, 19.7 mmol) in CH_2Cl_2 (100 mL) at −50 °C. The resulting mixture was allowed to warm to −30 °C over 45 min and poured into a half saturated aqueous solution of $NaHCO_3$ (100 mL). Subsequent extraction with diethyl ether (3×100 mL), treatment with Na_2SO_4, filtration and evaporation of the solvent resulted in the crude methane sulfonate, which was dissolved in dry benzene (100 mL), treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (2.4 mL, 15.7 mmol) and gently heated at 44 °C for 3 h. Flash column chromatography of the concentrated crude mixture employing *n*-hexane furnished the trienes **B1–6** in 54–66% overall yield.

4.3.1. 1-Isopropyl-4-(2-vinylbuta-1,3-dienyl)benzol B3. MS *m/z* 198 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.29 (d, J=7.0 Hz, 6H), 2.94 (sept, J=7.0 Hz, 1H), 5.22 (d,

J=10.7 Hz, 1H), 5.37 (d, J=11.1 Hz, 1H), 5.47 (d, J=17.7 Hz, 1H), 5.55 (d, J=17.3 Hz, 1H), 6.58 (dd, J=17.3 Hz, 10.7 Hz, 1H), 6.65 (br s, 1H), 6.76 (dd, J=17.7 Hz, 11.1 Hz, 1H), 7.20–7.25 (m, 2H), 7.32–7.36 (m, 2H).

4.3.2. 1-Brom-4-(2-vinylbuta-1,3-dienyl)benzol B4. MS m/z 234 (M^+ ^{79}Br). ^1H NMR (360 MHz, $CDCl_3$): δ 5.26 (dd, J=10.8 Hz, 1.0 Hz, 1H), 5.41 (dt, J=11.0 Hz, 1.4 Hz, 1H), 5.49 (d, J=17.7 Hz, 1.5 Hz, 1H), 5.57 (dd, J=17.3 Hz, 1.4 Hz, 1H), 6.56 (ddd, J=17.3 Hz, 10.8 Hz, 1.0 Hz, 1H), 6.57 (br s, 1H), 6.66 (dd, J=17.7 Hz, 11.1 Hz, 1H), 7.24–7.29 (m, 2H), 7.41–7.46 (m, 2H).

4.3.3. 1-Chlor-4-(2-vinylbuta-1,3-dienyl)benzol B5. MS m/z 190 (M^+). ^1H NMR (360 MHz, $CDCl_3$): δ 5.26 (dd, J=10.7 Hz, 1.0 Hz, 1H), 5.41 (dt, J=11.0 Hz, 0.9 Hz, 1H), 5.49 (dd, J=17.6 Hz, 1.6 Hz, 1H), 5.57 (dd, J=17.4 Hz, 1.5 Hz, 1H), 6.57 (ddd, J=17.4 Hz, 10.7 Hz, 1.0 Hz, 1H), 6.60 (br s, 1H), 6.66 (ddd, J=17.6 Hz, 11.1 Hz, 0.9 Hz, 1H), 7.31–7.35 (m, 4H).

4.3.4. 1-Fluor-4-(2-vinylbuta-1,3-dienyl)benzol B6. MS m/z 174 (M^+). ^1H NMR ($CDCl_3$): δ 5.23 (br d, J=10.6 Hz, 1H), 5.38 (br d, J=11.1 Hz, 1H), 5.47 (dd, J=17.8 Hz, 1.3 Hz, 1H), 5.54 (dd, J=17.2 Hz, 1.3 Hz, 1H), 6.55 (br dd, J=17.2 Hz, 10.6 Hz, 1H), 6.60 (br s, 1H), 6.65 (br dd, J=17.8 Hz, 11.1 Hz, 1H), 7.01–7.06 (m, 2H), 7.33–7.37 (m, 2H).

4.4. General procedure for synthesis of tricyclic compounds {A1–3/B1–6}

A solution of quinone **A1–3** (1.5 mmol) and cross-conjugated triene **B1–6** (1.5 mmol) in dichloromethane (6 mL) was cooled to −30 °C under inert atmosphere. Boron trifluoride diethyl etherate (0.5 mL) was added dropwise within 5 min. After stirring for 1 h, the reaction mixture was treated with an additional amount of **B1–6** (0.75 mmol). The solution was allowed to stir overnight. After addition of H_2O (6 mL) the reaction mixture was allowed to warm to 0 °C under vigorous stirring. Treatment with *n*-hexane (6 mL) followed by cooling to −60 °C resulted in freezing of the aqueous layer. The supernatant organic phase was removed by pipetting and evaporated under reduced pressure. Crude purification of the resulting residue was performed by adsorption to silica gel, whereas the un-reacted triene and non-polar side-products were removed by washing with *n*-hexane (500 mL). Elution using dichloromethane (500 mL) and subsequent evaporation of the solvent afforded the tricyclic compounds {**A1–3/B1–6**}, which were used for the next step without further purification.

4.4.1. (1*RS*,4a*RS*,9a*SR*)-5-Hydroxy-9a-methyl-1-phenyl-2-vinyl-1,4,4a,9a-tetrahydroanthracene-9,10-dione 4. MS m/z 344 (M^+). ^1H NMR (360 MHz, acetone-d_6): δ 1.65 (s, 3H), 2.53 (m, 1H), 3.42 (br d, J=8.2 Hz, 1H), 3.62 (br dd, J=20.3 Hz, 4.5 Hz, 1H), 3.79 (s, 1H), 4.77 (d, J=10.7 Hz, 1H), 4.78 (d, J=17.7 Hz, 1H), 6.21 (m, 1H), 6.34 (dd, J=17.7 Hz, 10.7 Hz, 1H), 6.76–6.87 (m, 5H), 6.91 (dd, J=8.1 Hz, 1.2 Hz, 1H), 7.34 (dd, J=7.7 Hz, 1.2 Hz, 1H), 7.42 (dd, J=8.1 Hz, 7.7 Hz, 1H), 11.72 (s, 1H). ^{13}C NMR (90 MHz, acetone-d_6): δ 20.1, 23.4, 47.6, 49.8, 52.0, 112.1, 117.6, 117.9, 122.0, 127.0, 127.6 (2C), 128.1, 129.2 (2C), 135.0, 135.2, 135.6, 138.2, 138.5, 159.7, 199.2, 203.2.

4.4.2. (1*RS*,4a*RS*,9a*SR*)-4a,9a-Dimethyl-1-phenyl-2-vinyl-1,4,4a,9a-tetrahydroanthracene-9,10-dione 5. MS m/z 342 (M^+). ^1H NMR (360 MHz, $CDCl_3$): δ 1.16 (s, 3H), 1.65 (s, 3H), 2.10 (m, 1H), 3.62 (br dd, J=19.8 Hz, 4.8 Hz, 1H), 3.84 (s, 1H), 4.65 (d, J=17.6 Hz, 1H), 4.78 (d, J=11.0 Hz, 1H), 6.15 (m, 1H), 6.26 (dd, J=17.6 Hz, 11.0 Hz, 1H), 6.68–6.83 (m, 5H), 7.39 (m, 2H), 7.55–7.61 (m, 1H), 7.77–7.81 (m, 1H).

4.5. General procedure for synthesis of pentacyclic compounds {A1–3/B1–6/C1–10}

Each of the compounds {**A1–3/B1–6**} was divided into 10 parts. Every part was placed in a sealed glass tube with the maleimides **C1–10** (5 equiv) under nitrogen. Toluene (1 mL) was added to each reaction vessel. The solution was stirred for 3 days at 68 °C. Removal of the solvent under reduced pressure and further purification by HPLC afforded the pentacyclic compounds {**A1–3/B1–6/C1–10**}.

4.5.1. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-11-Hydroxy-6a-methyl-6-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 9 {A1,B1,C1}. MS m/z 441 (M^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.45 (s, 3H), 2.16 (m, 1H), 2.57–2.62 (2H), 2.85 (m, 1H), 2.99 (ddd, J=14.4 Hz, 10.0 Hz, 6.0 Hz, 1H), 3.18 (ddd, J=10.1 Hz, 8.3 Hz, 1.4 Hz, 1H), 3.24–3.27 (2H), 3.53 (br s, 1H), 5.46 (m, 1H), 6.99–7.03 (m, 2H), 7.04–7.07 (m, 1H), 7.07–7.11 (m, 2H), 7.10 (br d, J=8.2 Hz, 1H), 7.33 (br d, J=7.5 Hz, 1H), 7.49 (dd, J=8.2 Hz, 7.5 Hz, 1H), 7.97 (br s, 1H), 11.92 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 22.9, 24.9, 25.2, 32.6, 41.2, 45.2, 52.8, 53.2, 56.1, 117.2, 118.6, 122.9, 124.1, 127.1, 127.9 (2C), 130.6 (2C), 135.8, 136.6, 139.3, 140.3, 160.7, 178.2, 179.1, 199.4, 204.4. Exact mass (EI^+) m/z calcd for $C_{27}H_{23}NO_5$: 441.1576. Found: 441.1577.

4.5.2. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Ethyl-11-hydroxy-6a-methyl-6-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 11 {A1,B1,C3}. MS m/z 469 (M^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.11 (t, J=7.2 Hz, 3H), 1.45 (s, 3H), 2.13 (m, 1H), 2.63 (br dd, J=15.8 Hz, 7.4 Hz, 1H), 2.66 (ddd, J=14.0 Hz, 8.0 Hz, 5.7 Hz, 1H), 2.84 (m, 1H), 3.01–3.14 (2H), 3.17 (dd, J=8.5 Hz, 5.4 Hz, 1H), 3.29 (dd, J=5.7 Hz, 5.7 Hz, 1H), 3.45 (br s, 1H), 3.52 (q, J=7.2 Hz, 2H), 5.39 (m, 1H), 6.98–7.13 (m, 5H), 7.13 (dd, J=8.4 Hz, 8.0 Hz, 1H), 7.33 (dd, J=7.6 Hz, 1.0 Hz, 1H), 7.53 (dd, J=8.4 Hz, 7.6 Hz, 1H), 11.97 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.1, 23.2, 25.2 (2C), 33.0, 33.7, 40.0, 44.1, 52.9, 53.4, 56.2, 117.2, 118.7, 122.9, 123.9, 127.2, 127.9 (2C), 130.8 (2C), 136.0, 136.8, 139.0, 140.4, 160.8, 178.3, 179.3, 199.6, 204.6. Exact mass (EI^+) m/z calcd for $C_{29}H_{27}NO_5$: 469.1889. Found: 469.1890.

4.5.3. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-11-Hydroxy-6a-methyl-2,6-diphenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 7 {A1,B1,C5}. MS m/z 517 (M^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.44 (s, 3H), 2.24 (m, 1H), 2.66–2.76 (2H), 2.93

(m, 1H), 3.04 (ddd, 15.0 Hz, 9.5 Hz, 6.0 Hz, 1H), 3.29–3.33 (2H), 3.39 (dd, *J*=8.6 Hz, 5.5 Hz, 1H), 3.50 (br s, 1H), 5.50 (m, 1H), 7.03–7.07 (m, 2H), 7.08–7.19 (6H), 7.35 (br d, *J*=7.5 Hz, 1H), 7.40–7.44 (m, 1H), 7.45–7.51 (m, 2H), 7.54 (dd, *J*=8.1 Hz, 7.5 Hz, 1H), 11.98 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 23.5, 25.2, 25.5, 33.1, 40.3, 44.4, 52.9, 53.5, 56.5, 117.1, 118.6, 122.9, 123.9, 126.5 (2C), 127.2, 128.0 (2C), 128.8 (2C), 129.3 (2C), 130.7, 131.8, 136.0, 136.8, 138.8, 140.6, 160.8, 177.6, 178.5, 199.4, 204.5. Exact mass (EI^+) *m*/*z* calcd for $C_{33}H_{27}NO_5$: 517.1889. Found: 517.1884. Anal. Calcd for $C_{33}H_{27}NO_5$: C, 76.58; H, 5.26; N, 2.71. Found: C, 76.58; H, 5.43; N, 2.40.

4.5.4. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Ethyl-11-hydroxy-6a-methyl-6-(4-methylphenyl)-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 20 {A1,B2,C3}. MS *m*/*z* 483 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.09 (t, *J*=7.2 Hz, 3H), 1.43 (s, 3H), 2.11 (m, 1H), 2.22 (s, 3H), 2.61 (br dd, *J*=15.5 Hz, 7.2 Hz, 1H), 2.65 (ddd, *J*=14.4 Hz, 7.9 Hz, 5.7 Hz, 1H), 2.80 (m, 1H), 3.04 (ddd, *J*=14.4 Hz, 9.8 Hz, 5.8 Hz, 1H), 3.09 (br dd, *J*=8.6 Hz, 7.7 Hz, 1H), 3.15 (dd, *J*=8.6 Hz, 5.7 Hz, 1H), 3.27 (dd, *J*=5.8 Hz, 5.7 Hz, 1H), 3.40 (br s, 1H), 3.50 (q, *J*=7.2 Hz, 2H), 5.38 (m, 1H), 6.85–6.89 (m, 2H), 6.89–6.94 (m, 2H), 7.14 (br d, *J*=8.2 Hz, 1H), 7.32 (br d, *J*=7.6 Hz, 1H), 7.53 (dd, *J*=8.2 Hz, 7.6 Hz, 1H), 12.00 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.1, 20.9, 23.3, 25.2, 25.3, 33.1, 33.7, 40.0, 44.0, 52.9, 53.4, 55.8, 117.2, 118.6, 122.7, 123.7, 126.5, 128.6 (2C), 130.5 (2C), 135.6, 136.1, 136.7, 136.8, 140.5, 160.8, 178.3, 179.2, 199.6, 204.6. Exact mass (EI^+) *m*/*z* calcd for $C_{30}H_{29}NO_5$: 483.2046. Found: 483.2045.

4.5.5. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-11-Hydroxy-6a-methyl-6-(4-methylphenyl)-2-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 22 {A1,B2,C5}. MS *m*/*z* 531 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (s, 3H), 2.21 (m, 1H), 2.25 (s, 3H), 2.65–2.75 (2H), 2.90 (m, 1H), 3.02 (m, 1H), 3.25–3.32 (2H), 3.37 (dd, *J*=8.5 Hz, 5.7 Hz, 1H), 3.46 (br s, 1H), 5.49 (m, 1H), 6.69–6.94 (m, 2H), 6.94–6.99 (m, 2H), 7.12–7.20 (3H), 7.34 (br d, *J*=7.6 Hz, 1H), 7.39–7.45 (m, 1H), 7.45–7.51 (m, 2H), 7.54 (dd, *J*=8.3 Hz, 7.6 Hz, 1H), 12.01 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.9, 23.7, 25.3, 25.5, 33.2, 40.3, 44.4, 53.0, 53.4, 56.0, 117.2, 118.6, 122.8, 123.6, 126.5 (2C), 128.7 (2C), 128.8, 129.3 (2C), 130.6 (2C), 131.8, 135.4, 136.1, 136.8, 136.9, 140.8, 160.8, 177.6, 178.5, 199.6, 204.6. Exact mass (EI^+) *m*/*z* calcd for $C_{34}H_{29}NO_5$: 531.2046. Found: 531.2039.

4.5.6. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-(4-Bromophenyl)-11-hydroxy-6-(4-isopropylphenyl)-6a-methyl-3a,4,6,6a,12a,13,13a,13b-octa-hydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 34 {A1,B3,C7}. MS *m*/*z* 637 (M^+ ^{79}Br). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.12 (d, *J*=6.8 Hz, 3H), 1.13 (d, *J*=6.8 Hz, 3H), 1.46 (s, 3H), 2.27 (m, 1H), 2.67–2.77 (m, 3H), 2.94–3.05 (2H), 3.24 (dd, *J*=5.5 Hz, 4.8 Hz, 1H), 3.33 (ddd, *J*=10.4 Hz, 8.6 Hz, 2.0 Hz, 1H), 3.40 (br dd, *J*=8.6 Hz, 5.5 Hz, 1H), 3.52 (br s, 1H), 5.54 (m, 1H), 6.85–6.87 (m, 2H), 6.89–6.92 (m, 2H), 7.07 (br d, *J*=8.3 Hz, 1H), 7.08–7.11 (m, 2H), 7.32 (br d, *J*=7.6 Hz, 1H), 7.47 (dd, *J*=8.3 Hz, 7.6 Hz, 1H), 7.60–7.63 (m, 2H), 11.89 (s, 1H). Exact mass (EI^+) *m*/*z* calcd for $C_{36}H_{32}^{79}BrNO_5$ (M^+): 637.1464. Found: 637.1464.

4.5.7. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Bromophenyl)-2-cyclohexyl-11-hydroxy-6a-methyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 46 {A1,B4,C9}. MS *m*/*z* 601 (M^+ ^{79}Br). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.19–1.34 (m, 3H), 1.36 (s, 3H), 1.42–1.48 (m, 2H), 1.65–1.69 (m, 1H), 1.80–1.85 (m, 2H), 2.01–2.16 (m, 3H), 2.51–2.61 (m, 2H), 2.71 (m, 1H), 2.96 (ddd, *J*=14.5 Hz, 8.5 Hz, 6.1 Hz, 1H), 3.04–3.11 (m, 2H), 3.28–3.32 (m, 2H), 3.86–3.93 (m, 1H), 5.30 (m, 1H), 6.95–7.01 (m, 2H), 7.19 (dd, *J*=8.3 Hz, 0.9 Hz, 1H), 7.23–7.27 (m, 2H), 7.33 (dd, *J*=7.7 Hz, 0.9 Hz, 1H), 7.58 (dd, *J*=8.3 Hz, 7.7 Hz, 1H), 11.97 (s, 1H). Exact mass (EI^+) *m*/*z* calcd for $C_{33}H_{32}^{79}BrNO_5$ (M^+): 601.1464. Found: 601.1464.

4.5.8. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Chlorophenyl)-2-ethyl-11-hydroxy-6a-methyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 50 {A1,B5,C3}. MS *m*/*z* 503 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.08 (t, *J*=7.1 Hz, 3H), 1.37 (s, 3H), 2.11 (m, 1H), 2.54–2.65 (m, 2H), 2.74 (m, 1H), 3.03 (ddd, *J*=14.6 Hz, 9.0 Hz, 6.2 Hz, 1H), 2.99–3.06 (m, 1H), 3.11–3.15 (m, 1H), 3.30 (dd, *J*=6.4 Hz, 6.2 Hz, 1H), 3.36 (br s, 1H), 3.49 (q, *J*=7.1 Hz, 2H), 5.32 (m, 1H), 6.99–7.03 (m, 2H), 7.09–7.13 (m, 2H), 7.18 (br d, *J*=8.4 Hz, 1H), 7.32 (br d, *J*=7.6 Hz, 1H), 7.56 (dd, *J*=8.4 Hz, 7.6 Hz, 1H), 11.96 (s, 1H). Exact mass (EI^+) *m*/*z* calcd for $C_{29}H_{26}ClNO_5$: 503.1499. Found: 503.1499.

4.5.9. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Benzyl-6-(4-fluorophenyl)-11-hydroxy-6a-methyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 65 {A1,B6,C8}. MS *m*/*z* 549 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.25 (s, 3H), 2.12 (m, 1H), 2.52 (ddd, *J*=14.6 Hz, 6.6 Hz, 6.4 Hz, 1H), 2.64 (br dd, *J*=15.3 Hz, 7.6 Hz, 1H), 2.74 (m, 1H), 2.94 (ddd, *J*=14.6 Hz, 9.0 Hz, 6.2 Hz, 1H), 3.11–3.18 (3H), 3.24 (dd, *J*=6.4 Hz, 6.2 Hz, 1H), 4.58 (d, *J*=14.2 Hz, 1H), 4.63 (d, *J*=14.2 Hz, 1H), 5.28 (m, 1H), 6.77–6.82 (m, 2H), 6.87–6.91 (m, 2H), 7.16 (br d, *J*=8.3 Hz, 1H), 7.24–7.32 (m, 5H), 7.29 (br d, *J*=7.6 Hz, 1H), 7.54 (dd, *J*=8.3 Hz, 7.6 Hz, 1H), 11.94 (s, 1H). Exact mass (EI^+) *m*/*z* calcd for $C_{34}H_{28}FNO_5$: 549.1952. Found: 549.1951.

4.5.10. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6a,12a-Dimethyl-6-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 68 {A2,B1,C1}. MS *m*/*z* 439 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.22 (s, 3H), 1.59 (s, 3H), 2.17 (m, 1H), 2.54–2.60 (2H), 2.78 (dd, *J*=13.9 Hz, 12.3 Hz, 1H), 3.19 (m, 1H), 3.21 (ddd, *J*=8.6 Hz, 8.3 Hz, 2.1 Hz, 1H), 3.31 (dd, *J*=8.6 Hz, 6.1 Hz, 1H), 3.74 (br s, 1H), 5.46 (m, 1H), 6.60–6.65 (m, 2H), 6.82–6.87 (m, 3H), 7.47 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.1 Hz, 1H), 7.52 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.1 Hz, 1H), 7.60 (br d, *J*=7.5 Hz, 1H), 7.82 (br d, *J*=7.5 Hz, 1H), 8.25 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.8, 25.1, 26.9, 29.4, 33.8, 41.3, 44.5, 51.7, 56.9, 57.3, 125.3, 125.9, 126.8, 126.8, 127.7 (2C), 130.1 (2C), 133.3, 133.6, 133.8, 135.7, 139.7, 141.1, 178.6, 179.5, 200.5, 201.2. Exact mass (EI^+) *m*/*z* calcd for $C_{28}H_{25}NO_4$: 439.1784. Found: 439.1784.

4.5.11. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Ethyl-6a,12a-dimethyl-6-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 70 {A2,B1,C3}. MS *m/z* 467 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.10 (t, *J*=7.2 Hz, 3H), 1.23 (s, 3H), 1.57 (s, 3H), 2.13 (m, 1H), 2.55–2.61 (2H), 2.86 (dd, *J*=14.0 Hz, 12.2 Hz, 1H), 3.13 (ddd, *J*=8.5 Hz, 8.4 Hz, 1.6 Hz, 1H), 3.14 (m, 1H), 3.20 (dd, *J*=8.5 Hz, 5.9 Hz, 1H), 3.51 (q, *J*=7.2 Hz, 1H), 3.63 (br s, 1H), 5.37 (m, 1H), 6.57–6.61 (m, 2H), 6.85–6.91 (m, 3H), 7.50 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.54 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.61 (br d, *J*=7.5 Hz, 1H), 7.85 (br d, *J*=7.5 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.1, 20.9, 25.3, 26.7, 29.4, 33.6, 34.3, 40.1, 43.2, 51.8, 56.9, 57.2, 125.3, 126.0, 126.9, 126.9, 127.6 (2C), 130.1 (2C), 133.4, 133.6, 133.8, 135.9, 139.8, 140.6, 178.4, 179.3, 200.4, 201.1. Exact mass (EI^+) *m/z* calcd for $C_{30}H_{29}NO_4$: 467.2097. Found: 467.2098.

4.5.12. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6a,12a-Dimethyl-2,6-diphenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 8 {A2,B1,C5}. MS *m/z* 515 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.23 (s, 3H), 1.58 (s, 3H), 2.26 (m, 1H), 2.64 (dd, *J*=13.9 Hz, 7.4 Hz, 1H), 2.67 (ddd, *J*=16.0 Hz, 6.8 Hz, 1.9 Hz, 1H), 2.86 (dd, *J*=13.9 Hz, 12.3 Hz, 1H), 3.25 (m, 1H), 3.33 (ddd, *J*=8.5 Hz, 8.3 Hz, 1.9 Hz, 1H), 3.43 (dd, *J*=8.5 Hz, 5.9 Hz, 1H), 3.70 (br s, 1H), 5.49 (m, 1H), 6.63–6.67 (m, 2H), 6.88–6.93 (m, 3H), 7.16–7.19 (m, 2H), 7.39–7.44 (m, 1H), 7.45–7.50 (m, 2H), 7.52 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.1 Hz, 1H), 7.56 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.1 Hz, 1H), 7.65 (br d, *J*=7.5 Hz, 1H), 7.87 (br d, *J*=7.5 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.9, 25.5, 26.8, 29.7, 34.3, 40.3, 43.5, 51.8, 57.0, 57.6, 125.2, 126.0, 126.5 (2C), 126.9, 127.0, 127.7 (2C), 128.7, 129.2 (2C), 130.1 (2C), 133.4, 133.7, 133.8, 135.8, 139.9, 140.5, 177.5, 178.6, 200.4, 201.1. Exact mass (EI^+) *m/z* calcd for $C_{34}H_{29}NO_4$: 515.2097. Found: 515.2097. Anal. Calcd for $C_{34}H_{29}NO_4$: C, 79.20; H, 5.67; N, 2.72. Found: C, 79.20; H, 5.64; N, 2.78.

4.5.13. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6a,12a-Dimethyl-6-(4-methylphenyl)-2-propyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra-[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 80 {A2,B2,C4}. MS *m/z* 495 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.82 (t, *J*=7.4 Hz, 3H), 1.22 (s, 3H), 1.46–1.56 (2H), 1.54 (s, 3H), 2.14 (m, 1H), 2.53–2.59 (2H), 2.86 (dd, *J*=14.1 Hz, 12.1 Hz, 1H), 3.08 (m, 1H), 3.12 (ddd, *J*=8.4 Hz, 8.2 Hz, 1.8 Hz, 1H), 3.18 (dd, *J*=8.6 Hz, 5.8 Hz, 1H), 3.42 (m, 2H), 3.58 (br s, 1H), 5.36 (m, 1H), 6.43–6.47 (m, 2H), 6.65–6.69 (m, 2H), 7.51 (ddd, *J*=8.6 Hz, 7.6 Hz, 1.1 Hz, 1H), 7.55 (ddd, *J*=8.6 Hz, 7.6 Hz, 1.1 Hz, 1H), 7.60 (br dd, *J*=7.6 Hz, 1.1 Hz, 1H), 7.85 (br dd, *J*=7.6 Hz, 1.1 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{32}H_{33}NO_4$: 495.2410. Found: 495.2410.

4.5.14. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6a,12a-Dimethyl-6-(4-methylphenyl)-2-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra-[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 81 {A2,B2,C5}. MS *m/z* 529 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.22 (s, 3H), 1.54 (s, 3H), 2.11 (s, 3H), 2.23 (m, 1H), 2.61 (dd, *J*=14.1 Hz, 7.2 Hz, 1H), 2.67 (ddd, *J*=15.9 Hz, 6.8 Hz, 2.1 Hz, 1H), 2.84 (dd, *J*=14.1 Hz, 12.1 Hz, 1H), 3.19 (m, 1H), 3.31 (ddd, *J*=8.4 Hz, 8.2 Hz, 2.1 Hz, 1H), 3.41 (dd, *J*=8.4 Hz, 5.9 Hz, 1H), 3.63 (br s, 1H), 5.47 (m, 1H), 6.49–6.53 (m, 2H), 6.70–6.74 (m, 2H), 7.13–7.17 (m, 2H), 7.38–7.43 (m, 1H), 7.44–7.49 (m, 2H), 7.53 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.57 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.65 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H), 7.87 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{35}H_{31}NO_4$: 529.2253. Found: 529.2253.

4.5.15. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-(4-Acetylphenyl)-6-(4-isopropylphenyl)-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 92 {A2,B3,C6}. MS *m/z* 599 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.02 (d, *J*=6.8 Hz, 6H), 1.19 (s, 3H), 1.57 (s, 3H), 2.31 (m, 1H), 2.57–2.67 (2H), 2.65 (s, 3H), 2.68 (ddd, *J*=16.0 Hz, 6.8 Hz, 2.2 Hz, 1H), 2.76 (dd, *J*=14.1 Hz, 12.1 Hz, 1H), 3.33 (m, 1H), 3.36 (ddd, *J*=8.5 Hz, 8.3 Hz, 2.2 Hz, 1H), 3.47 (dd, *J*=8.5 Hz, 6.0 Hz, 1H), 3.73 (br s, 1H), 5.55 (m, 1H), 6.52–6.57 (m, 2H), 6.65–6.70 (m, 2H), 7.36–7.40 (m, 2H), 7.44 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.48 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.63 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H), 7.78 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H), 8.06–8.09 (m, 2H). Exact mass (EI^+) *m/z* calcd for $C_{39}H_{37}NO_5$: 599.2672. Found: 599.2672.

4.5.16. 3-[(3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Isopropylphenyl)-6a,12a-dimethyl-1,3,7,12-tetraoxo-1,3,3a,4,6,6a,7,12,12a,13,13a,13b-dodecahydro-2*H*-anthra[2,3-*e*]isoindol-2-yl]propanoic acid 96 {A2,B3,C10}. MS *m/z* 553 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.01 (d, *J*=6.8 Hz, 6H), 1.19 (s, 3H), 1.56 (s, 3H), 2.18 (m, 1H), 2.54–2.63 (3H), 2.64 (t, *J*=7.2 Hz, 2H), 2.79 (dd, *J*=14.1 Hz, 12.0 Hz, 1H), 3.17 (ddd, *J*=8.4 Hz, 8.2 Hz, 2.1 Hz, 1H), 3.21 (m, 1H), 3.25 (dd, *J*=8.4 Hz, 5.9 Hz, 1H), 3.71 (br s, 1H), 3.80 (m, 2H), 5.43 (m, 1H), 6.49–6.53 (m, 2H), 6.62–6.66 (m, 2H), 7.42 (ddd, *J*=7.6 Hz, 7.6 Hz, 1.2 Hz, 1H), 7,45 (ddd, *J*=7.6 Hz, 7.6 Hz, 1.2 Hz, 1H), 7.59 (br dd, *J*=7.6 Hz, 1.2 Hz, 1H), 7.75 (br dd, *J*=7.6 Hz, 1.2 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{34}H_{35}NO_6$: 553.2464. Found: 553.2463.

4.5.17. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Bromophenyl)-2,6a,12a-trimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 98 {A2,B4,C2}. MS *m/z* 531 (M^+ ^{79}Br). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.23 (s, 3H), 1.56 (s, 3H), 2.15 (m, 1H), 2.54–2.61 (2H), 2.85 (dd, *J*=14.1 Hz, 12.0 Hz, 1H), 2.95 (s, 3H), 3.13 (m, 1H), 3.17 (ddd, *J*=8.4 Hz, 8.2 Hz, 2.1 Hz, 1H), 3.23 (dd, *J*=8.4 Hz, 5.9 Hz, 1H), 3.65 (br s, 1H), 5.36 (m, 1H), 6.44–6.50 (m, 2H), 6.93–6.99 (m, 2H), 7.53 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.57 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.60 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H), 7.82 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{29}H_{26}^{79}BrNO_4$ (M^+): 531.1045. Found: 531.1041.

4.5.18. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Bromophenyl)-2-cyclohexyl-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 105 {A2,B4,C9}. MS *m/z* 599 (M^+ ^{79}Br). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.18–1.36 (m, 3H), 1.23 (s, 3H), 1.42–1.50 (m, 2H), 1.55 (s, 3H), 1.62–1.71 (m, 1H), 1.78–1.88 (m, 2H), 2.00–2.17 (3H), 2.52–2.58 (2H), 2.80 (dd, *J*=14.1 Hz, 12.1 Hz, 1H), 3.06 (m, 1H), 3.08

(ddd, *J*=15.9 Hz, 6.8 Hz, 2.1 Hz, 1H), 3.14 (dd, 8.4 Hz, 5.9 Hz, 1H), 3.56 (br s, 1H), 3.90 (m, 1H), 5.33 (m, 1H), 6.42–6.49 (m, 2H), 6.97–7.03 (m, 2H), 7.56 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.60 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.63 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H), 7.87 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{34}H_{34}^{79}BrNO_4$ (M^+): 599.1671. Found: 599.1675.

4.5.19. 3-[(3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Bromophenyl)-6a,12a-dimethyl-1,3,7,12-tetra-oxo-1,3,3a,4,6,6a,7,12,12a,13,13a,13b-dodeca-hydro-2*H*-anthra-[2,3-*e*]isoindol-2-yl]propanoic acid 106 {A2,B4,C10}. MS *m/z* 589 (M^+ ^{79}Br). ^1H NMR (600 MHz, $CDCl_3$): δ 1.23 (s, 3H), 1.56 (s, 3H), 2.14 (m, 1H), 2.54–2.60 (2H), 2.61 (dd, *J*=7.2 Hz, *J*=7.2 Hz, 2H), 2.84 (dd, *J*=14.1 Hz, 12.1 Hz, 1H), 3.12 (m, 1H), 3.16 (ddd, *J*=8.5 Hz, 8.3 Hz, 1.6 Hz, 1H), 3.22 (dd, *J*=8.5 Hz, 5.7 Hz, 1H), 3.64 (br s, 1H), 3.78 (m, 2H), 5.35 (m, 1H), 6.45–6.50 (m, 2H), 6.95–6.99 (m, 2H), 7.54 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.56–7.62 (2H), 7.84 (dd, *J*=7.5 Hz, 1.2 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{31}H_{28}^{79}BrNO_6$ (M^+): 589.1100. Found: 589.1098.

4.5.20. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-6-(4-Chlorophenyl)-6a,12a-dimethyl-2-phenyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra-[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 111 {A2,B5,C5}. MS *m/z* 549 (M^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.23 (s, 3H), 1.56 (s, 3H), 2.25 (m, 1H), 2.62 (dd, *J*=14.1 Hz, 7.2 Hz, 1H), 2.69 (ddd, *J*=15.9 Hz, 6.8 Hz, 2.1 Hz, 1H), 2.85 (dd, *J*=14.1 Hz, 12.1 Hz, 1H), 3.22 (m, 1H), 3.33 (ddd, *J*=8.4 Hz, 8.2 Hz, 2.1 Hz, 1H), 3.42 (dd, *J*=8.4 Hz, 5.9 Hz, 1H), 3.68 (br s, 1H), 5.46 (m, 1H), 6.54–6.59 (m, 2H), 6.84–6.88 (m, 2H), 7.14–7.18 (m, 2H), 7.39–7.43 (m, 1H), 7.44–7.50 (m, 2H), 7.56 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.60 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.2 Hz, 1H), 7.65 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H), 7.87 (br dd, *J*=7.5 Hz, 1.2 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{34}H_{28}ClNO_4$: 549.1707. Found: 549.1705.

4.5.21. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Ethyl-6-(4-fluorophenyl)-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 119 {A2,B6,C3}. MS *m/z* 485 (M^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.09 (t, *J*=7.2 Hz, 3H), 1.22 (s, 3H), 2.55 (s, 3H), 2.12 (m, 1H), 2.57 (dd, *J*=14.2 Hz, 7.6 Hz, 1H), 2.59 (ddd, *J*=15.8 Hz, 6.8 Hz, 2.1 Hz, 1H), 2.85 (dd, *J*=14.2 Hz, 12.0 Hz, 1H), 3.10 (m, 1H), 3.13 (ddd, *J*=8.4 Hz, 8.2 Hz, 2.1 Hz, 1H), 3.19 (dd, *J*=8.4 Hz, 5.7 Hz, 1H), 3.51 (q, *J*=7.2 Hz, 2H), 3.63 (br s, 1H), 5.35 (m, 1H), 6.51–6.59 (m, 4H), 7.53 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.1 Hz, 1H), 7.57 (ddd, *J*=7.5 Hz, 7.5 Hz, 1.1 Hz, 1H), 7.62 (br dd, *J*=7.5 Hz, 1.1 Hz, 1H), 7.85 (br dd, *J*=7.5 Hz, 1.1 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{30}H_{28}FNO_4$: 485.2002. Found: 485.1999.

4.5.22. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Benzyl-6-(4-fluorophenyl)-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 124 {A2,B6,C8}. MS *m/z* 547 (M^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.16 (s, 3H), 1.37 (s, 3H), 2.13 (m, 1H), 2.51 (dd, *J*=14.1 Hz, 7.6 Hz, 1H), 2.62 (ddd, *J*=15.8 Hz, 7.1 Hz, 2.1 Hz, 1H), 2.69 (dd, *J*=14.1 Hz, 12.0 Hz, 1H), 3.12 (m, 1H), 3.18 (ddd, *J*=8.4 Hz, 8.2 Hz, 2.1 Hz, 1H), 3.23 (dd, *J*=8.4 Hz, 5.9 Hz, 1H), 3.41 (br s, 1H), 4.58 (d, *J*=14.0 Hz, 1H), 4.65 (d, *J*=14.0 Hz, 1H), 5.32 (m, 1H), 6.41–6.47 (m, 2H), 6.47–6.53 (m, 2H), 7.25–7.32 (m, 5H), 7.50 (br dd, *J*=7.6 Hz, 7.6 Hz, 1H), 7.55 (br dd, *J*=7.6 Hz, 7.6 Hz, 1H), 7.55 (br d, *J*=7.6 Hz, 1H), 7.83 (br d, *J*=7.6 Hz, 1H). Exact mass (EI^+) *m/z* calcd for $C_{35}H_{30}FNO_4$: 547.2159. Found: 547.2159.

4.5.23. 3-[(3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-10-Bromo-11-hydroxy-6a,12a-dimethyl-1,3,7,12-tetraoxo-6-phenyl-1,3,3a,4,6,6a,7,12,12a,13,13a,13b-dodeca-hydro-2*H*-anthra[2,3-*e*]isoindol-2-yl]propanoic acid 136 {A3,B1,C10}. MS *m/z* 591 (M^+ ^{79}Br). ^1H NMR (600 MHz, $CDCl_3$): δ 1.46 (s, 3H), 2.16 (m, 1H), 2.61 (t, *J*=7.2 Hz, 2H), 2.61 (dd, 1H), 2.65 (ddd, *J*=14.5 Hz, 8.1 Hz, 5.4 Hz, 1H), 2.86 (m, 1H), 3.05 (ddd, *J*=14.5 Hz, 10.0 Hz, 6.0 Hz, 1H), 3.15 (ddd, *J*=8.5 Hz, 8.3 Hz, 1.3 Hz, 1H), 3.20 (dd, *J*=8.5 Hz, 5.4 Hz, 1H), 3.29 (dd, *J*=6.0 Hz, 5.4 Hz, 1H), 3.50 (br s, 1H), 3.78 (t, *J*=7.2 Hz, 2H), 5.42 (m, 1H), 6.94–7.00 (m, 2H), 7.04–7.12 (m, 3H), 7.21 (d, *J*=8.1 Hz, 1H), 7.76 (d, *J*=8.1 Hz, 1H), 12.61 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{30}H_{26}^{79}BrNO_7$ (M^+): 591.0893. Found: 591.0896.

4.5.24. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-10-Bromo-2-ethyl-11-hydroxy-6a,12a-dimethyl-6-(4-methylphenyl)-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra-[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 139 {A3,B2,C3}. MS *m/z* 561 (M^+ ^{79}Br). ^1H NMR (600 MHz, $CDCl_3$): δ 1.10 (t, *J*=7.2 Hz, 3H), 1.45 (s, 3H), 2.13 (m, 1H), 2.19 (s, 3H), 2.62 (ddd, *J*=15.7 Hz, 7.2 Hz, 1.4 Hz, 1H), 2.67 (ddd, *J*=14.4 Hz, 8.1 Hz, 5.2 Hz, 1H), 2.86 (m, 1H), 3.05 (ddd, *J*=14.4 Hz, 10.1 Hz, 6.0 Hz, 1H), 3.12 (ddd, *J*=9.4 Hz, 8.1 Hz, 1.4 Hz, 1H), 3.17 (dd, *J*=8.5 Hz, 5.8 Hz, 1H), 3.27 (dd, *J*=6.0 Hz, 5.2 Hz, 1H), 3.44 (br s, 1H), 3.51 (q, *J*=7.2 Hz, 2H), 5.42 (m, 1H), 6.75–6.79 (m, 2H), 6.85–6.89 (m, 2H), 7.21 (d, *J*=8.1 Hz, 1H), 7.76 (d, *J*=8.1 Hz, 1H), 12.64 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{30}H_{28}^{79}BrNO_5$ (M^+): 561.1151. Found: 561.1151.

4.5.25. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-10-Bromo-2-(4-bromophenyl)-11-hydroxy-6-(4-isopropylphenyl)-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 153 {A3,B3,C7}. MS *m/z* 717 (M^+ $^{79}Br/^{81}Br$). ^1H NMR (600 MHz, $CDCl_3$): δ 1.10 (d, *J*=6.8 Hz, 3H), 1.10 (d, *J*=6.8 Hz, 3H), 1.51 (s, 3H), 2.29 (m, 1H), 2.66–2.79 (3H), 3.01 (ddd, *J*=14.7 Hz, 10.5 Hz, 6.0 Hz, 1H), 3.11 (m, 1H), 3.25 (dd, *J*=5.5 Hz, 4.4 Hz, 1H), 3.35 (ddd, *J*=8.5 Hz, 8.5 Hz, 2.1 Hz, 1H), 3.43 (dd, *J*=8.7 Hz, 6.0 Hz, 1H), 3.60 (br s, 1H), 5.59 (m, 1H), 6.75–6.79 (m, 2H), 6.83–6.87 (m, 2H), 7.10–7.14 (m, 2H), 7.21 (d, *J*=8.2 Hz, 1H), 7.60–7.64 (m, 5H), 7.68 (d, *J*=8.2 Hz, 1H), 12.50 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{36}H_{31}^{79}Br_2NO_5$ (M^+): 715.0569. Found: 715.0570.

4.5.26. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Benzyl-10-bromo-11-hydroxy-6-(4-isopropylphenyl)-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra-[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 154 {A3,B3,C8}. MS *m/z* 651 (M^+ ^{79}Br). ^1H NMR (600 MHz, $CDCl_3$): δ 1.08 (d, *J*=6.8 Hz, 3H), 1.09 (d, *J*=6.8 Hz, 3H), 1.32

(s, 3H), 2.17 (m, 1H), 2.63–2.71 (3H), 2.94 (m, 1H), 2.99 (m, 1H), 3.17 (dd, *J*=5.9 Hz, 4.4 Hz, 1H), 3.20 (ddd, *J*=9.4 Hz, 8.1 Hz, 1.4 Hz, 1H), 3.24 (dd, *J*=8.7 Hz, 5.8 Hz, 1H), 3.42 (br s, 1H), 4.62 (d, *J*=14.0 Hz, 1H), 4.67 (d, *J*=14.0 Hz, 1H), 5.48 (m, 1H), 6.64–6.68 (m, 2H), 6.78–6.82 (m, 2H), 7.16 (d, *J*=8.2 Hz, 1H), 7.24–7.32 (m, 5H), 7.65 (d, *J*=8.2 Hz, 1H), 12.49 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{37}H_{34}^{79}BrNO_5$ (M^+): 651.1620. Found: 651.1624.

4.5.27. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-10-Bromo-6-(4-bromophenyl)-11-hydroxy-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 157 {A3,B4,C1}. MS *m/z* 599 (M^+ $^{79}Br/^{81}Br$). ^1H NMR (600 MHz, $CDCl_3$): δ 1.41 (s, 3H), 2.16 (m, 1H), 2.56 (ddd, *J*=14,8 Hz, 8.3 Hz, 6.1 Hz, 1H), 2.62 (ddd, *J*=16.1 Hz, 7.2 Hz, 1.4 Hz, 1H), 2.78 (m, 1H), 3.00 (ddd, *J*=14.8 Hz, 9.3 Hz, 6.4 Hz, 1H), 3.19 (ddd, *J*=8.4 Hz, 8.3 Hz, 1.4 Hz, 1H), 3.24 (dd, *J*=8.7 Hz, 5.5 Hz, 1H), 3.31 (dd, *J*=6.4 Hz, 6.1 Hz, 1H), 3.45 (br s, 1H), 5.44 (m, 1H), 6.93–6.97 (m, 2H), 7.23 (d, *J*=8.1 Hz, 1H), 7.24–7.27 (m, 2H), 7.67 (br s, 1H), 7.83 (d, *J*=8.1 Hz, 1H), 12.60 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{27}H_{21}^{79}Br_2NO_5$ (M^+): 596.9786. Found: 596.9783.

4.5.28. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-(4-Acetylphenyl)-10-bromo-6-(4-chlorophenyl)-11-hydroxy-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 172 {A3,B5,C6}. MS *m/z* 673 (M^+ ^{81}Br). ^1H NMR (600 MHz, $CDCl_3$): δ 1.38 (s, 3H), 2.27 (m, 1H), 2.62 (m, 1H), 2.64 (s, 3H), 2.74 (br dd, *J*=15.9 Hz, 7.1 Hz, 1H), 2.87 (m, 1H), 3.02 (ddd, *J*=14.9 Hz, 8.8 Hz, 6.2 Hz, 1H), 3.29 (ddd, *J*=9.0 Hz, 8.6 Hz, 1.9 Hz, 1H), 3.34 (dd, *J*=6.4 Hz, 6.2 Hz, 1H), 3.39 (dd, *J*=8.6 Hz, 5.3 Hz, 1H), 3.42 (br s, 1H), 5.48 (m, 1H), 6.99–7.04 (m, 2H), 7.23–7.26 (m, 2H), 7.25 (d, *J*=8.1 Hz, 1H), 7.30–7.34 (m, 2H), 7.84 (d, *J*=8.1 Hz, 1H), 8.05–8.08 (m, 2H), 12.62 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{35}H_{27}^{79}BrClNO_6$ (M^+): 671.0710. Found: 671.0710.

4.5.29. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-10-Bromo-2-(4-bromophenyl)-6-(4-fluorophenyl)-11-hydroxy-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 183 {A3,B6,C7}. MS *m/z* 693 (M^+ $^{79}Br/^{81}Br$). ^1H NMR (600 MHz, $CDCl_3$): δ 1.38 (s, 3H), 2.25 (m, 1H), 2.62 (ddd, *J*=14.8 Hz, 8.3 Hz, 6.4 Hz, 1H), 2.73 (ddd, *J*=15.8 Hz, 7.1 Hz, 1.4 Hz, 1H), 2.87 (m, 1H), 3.02 (ddd, *J*=14.8 Hz, 9.0 Hz, 6.1 Hz, 1H), 3.30 (ddd, *J*=9.1 Hz, 8.4 Hz, 1.4 Hz, 1H), 3.34 (dd, *J*=6.4 Hz, 6.1 Hz, 1H), 3.36 (dd, *J*=8.4 Hz, 5.3 Hz, 1H), 3.43 (br s, 1H), 5.46 (m, 1H), 6.83–6.89 (m, 2H), 7.00–7.05 (m, 2H), 7.04–7.08 (m, 2H), 7.25 (d, *J*=8.1 Hz, 1H), 7.58–7.63 (m, 2H), 7.83 (d, *J*=8.1 Hz, 1H), 12.63 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{33}H_{24}^{79}Br_2FNO_5$ (M^+): 691.0005. Found: 691.0019.

4.5.30. (3a*RS*,6*SR*,6a*RS*,12a*SR*,13a*RS*,13b*SR*)-2-Benzyl-10-bromo-6-(4-fluorophenyl)-11-hydroxy-6a,12a-dimethyl-3a,4,6,6a,12a,13,13a,13b-octahydro-1*H*-anthra[2,3-*e*]isoindole-1,3,7,12(2*H*)-tetrone 184 {A3,B6,C8}. MS *m/z* 627 (M^+ ^{79}Br). ^1H NMR (600 MHz, $CDCl_3$): δ 1.25 (s, 3H), 2.13 (m, 1H), 2.25 (ddd, *J*=14.7 Hz, 8.6 Hz, 6.4 Hz, 1H), 2.65 (br dd, *J*=15.8 Hz, 7.3 Hz, 1H), 2.75 (m, 1H), 2.95 (ddd, *J*=14.7 Hz, 8.8 Hz, 6.1 Hz, 1H), 3.13–3.19 (3H), 3.27 (dd, *J*=6.4 Hz, 6.1 Hz, 1H), 4.59 (d, *J*=14.1 Hz, 1H), 4.63 (d, *J*=14.1 Hz, 1H), 5.30 (m, 1H), 6.77–6.82 (m, 2H), 6.84–6.88 (m, 2H), 7.19 (d, *J*=8.1 Hz, 1H), 7.25–7.31 (m, 5H), 7.81 (d, *J*=8.1 Hz, 1H), 12.62 (s, 1H). Exact mass (EI^+) *m/z* calcd for $C_{34}H_{27}^{79}BrFNO_5$ (M^+): 627.1057. Found: 627.1059.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.092.

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Construction of C-nucleosides diversified by [3+2] cycloaddition from a sugar-based mesoionic ring

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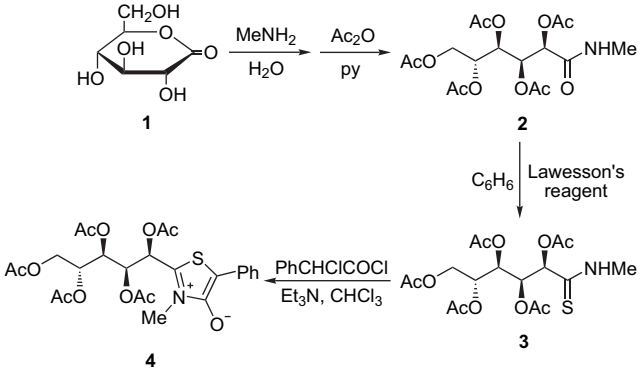
Abstract—A mesoionic acyclic C-nucleoside (**4**), serves as the starting chiron to construct highly functionalized 2-aza-7-thiabicyclo[2.2.1]-heptanes and heptenes by means of a [3+2] cycloaddition with acetylenic and olefinic dipolarophiles. Further elimination of either sulfur or hydrogen sulfide leads to acyclic C-nucleosides bearing a heterocyclic moiety of 2-pyridone.

1. Introduction

The synthesis of modified nucleosides and glycoconjugates has come of age.[1,2] Construction of a heterocyclic aglycon or spacer by means of cycloaddition reactions represents a convenient atom-economy strategy. Of particular relevance are stereocontrolled [3+2] cycloadditions, which can be employed to access numerous natural products and pharmaceuticals.[3] Renewed interest in [3+2] cycloadditions emerges from a few reactions of orthogonal functionalization, well suited for biological applications, such as in click chemistry repertories.[4] Our research group has been largely involved in asymmetric [3+2] cycloadditions of 1,3-thiazolium-4-olates (thioisomünchnones) using carbohydrates as stereodifferentiating elements.[5] In a recent study we were able to synthesize a mesoionic nucleus with an acyclic carbohydrate chain as substituent and we explored the reactivity of the heterocyclic moiety.[6] The present work extends such results to other unsaturated dipolarophiles to produce new C-nucleosides containing highly functionalized and polycyclic fragments derived from pyrid-2-ones. It has been recently demonstrated that C-nucleosides bearing the pyrid-2-one core serve as a non-disruptive pyrimidine analog in DNA duplex.[7] Moreover, the pyridone chromophore absorbs in the near UV range (λ>300 nm) where common nucleic acid bases are transparent, and exhibit room temperature fluorescence. These properties enabling selective excitation in an oligonucleotide chain make these compounds interesting as potential fluorescent and/or photochemical probes in nucleic acids.[8]

2. Results and discussion

As reported in a previous communication,[6] the 1,3-thiazolium-4-olate system **4** (Scheme 1), can easily be prepared from *N*-methyl-δ-thiogluconamide[9] (**3**) in a few step sequence using δ-gluconolactone (**1**) as raw material.



Scheme 1.

Despite **4** turned out to be air sensitive and slowly decomposed at room temperature, it underwent 1,3-dipolar cycloadditions in dry solvents with several types of acetylenic and olefinic dipolarophiles.

Thioisomünchnones are known to react with acetylenic dipolarophiles to form either pyrid-2-ones or thiophenes,[10]

Keywords: 1,3-Dipolar cycloaddition; Mesoionic heterocycle; C-nucleoside; Pyridone; Thioisomünchnone.
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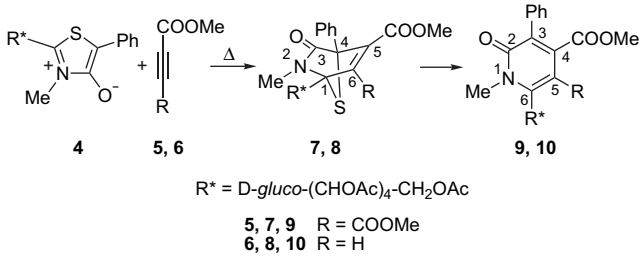
by extrusion of sulfur or isocyanate, respectively, from the initially generated cycloadducts, which to the best of our knowledge have never been isolated. However, the thioisomünchnone **4** reacted with acetylenic dipolarophiles such as dimethyl acetylenedicarboxylate (**5**) and methyl propiolate (**6**) in refluxing toluene and CH_2Cl_2, respectively, to afford single products, which were identified as cycloadducts **7** and **8** (Scheme 2).



Scheme 2.

The main structural features of these initial bicyclic systems were established by NMR spectroscopy and combustion analysis. Particularly, in the ^{13}C NMR spectra of **7**, the resonances at δ 83.3 and 69.4 ppm were attributed to the bridgehead carbons C-4 and C-1, respectively. The (1*R*,4*R*) configuration proposed for **7** was established on the basis of its ^1H NMR spectrum, having a close similarity to those of **23b** and **25b** (see below). On the other hand, cycloadduct **8** was not stable enough when it was subjected to chromatographic purification on SiO_2 and spontaneously evolved into **10** after sulfur extrusion.

Desulfuration of **7** and **8** by $Hg(OAc)_2$ in acetic acid–acetone at room temperature also afforded the C-nucleosides **9** and **10**, respectively. ^{13}C NMR data supported the pyrid-2-one structure and NOE experiments on compound **10** confirmed the regiochemistry suggested for the methyl propiolate reaction. Both H-1′ and H-2′, located at the sugar moiety, showed NOEs with H-5 (Fig. 1).

The bimolecular cycloaddition of 2-phenylthioisomünchnones with electron-deficient olefins provides a series of bicyclic adducts, which were further reacted with $NaOCH_3$ to yield pyrid-2-one derivatives.[11] However, the 2-(*N*,*N*′-dialkylamino)thioisomünchnones showed a different behavior leading to 4,5-dihydrothiophenes by opening of the initial 1:1 cycloadduct.[12]

Thioisomünchnone **4** was found to undergo dipolar cycloaddition with asymmetric and symmetrically substituted olefinic dipolarophiles. Stable cycloadducts (**14**–**16**) were obtained after reaction with methyl acrylate (**11**), methyl vinyl ketone (**12**), and acrylonitrile (**13**) (Scheme 3).

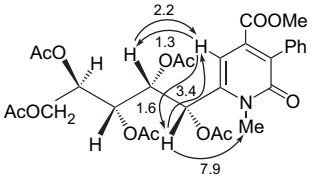
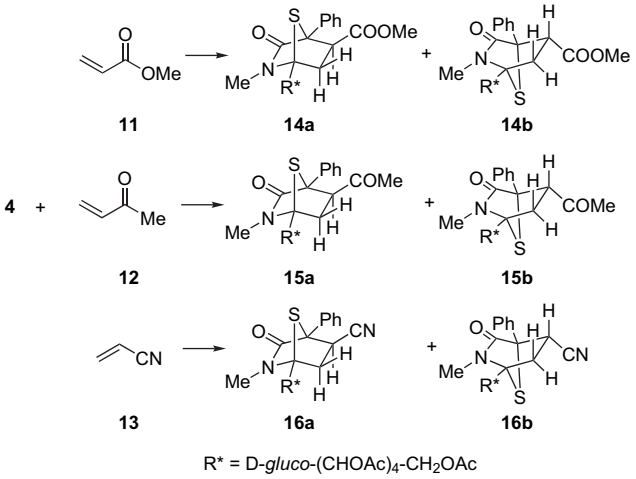


Figure 1. NOEs measured for compound **10**.



Scheme 3.

The structure of **16a** could unequivocally be established by single-crystal X-ray analysis,[6] which shows the *exo* disposition of the cyano group. In the ^1H NMR spectrum of **16a**, the resonances of H-5$_{endo}$, H-6$_{endo}$, and H-6$_{exo}$ appeared as double doublets. The more deshielding signal centered at δ 3.56 ppm contains a trans coupling (J=3.6 Hz) and a cis coupling (J=8.0 Hz) and it is therefore assigned to H-5$_{endo}$. The signal at δ 2.85 ppm showed a trans coupling as above and a geminal one (J=12.7 Hz), consistent with the H-6$_{exo}$ proton. Finally, the resonance at 2.74 ppm with cis and geminal couplings was attributed to H-6$_{endo}$. Although H-5$_{endo}$, H-6$_{endo}$, and H-6$_{exo}$ of the cycloadduct **16b** isolated from the same reaction, resonated at slightly different chemical shifts: δ 3.51 ppm (H-5$_{endo}$) and 2.80 ppm (H-6$_{exo}$ and H-6$_{endo}$), they showed a similar coupling pattern, thus allowing us to suggest that **16b** was obtained from the approach of the dipolarophile to the opposite face of the dipole. Moreover, the chemical shifts for C-5 and C-6 of **16a** and **16b** proved that the CN group is located at C-5 in both cases. The regio- and stereochemistry of the cycloadducts **14** and **15**, assigned by analogy with the NMR data of **16a** and **16b** (Tables 1 and 2) was confirmed by the existence of NOEs between the H-6$_{endo}$ proton and the *N*-CH_3 groups in these compounds. This evidence together with the cis coupling observed for the signal of the H-6$_{endo}$ proton suggest that the H-5 proton lies in an *endo* disposition in all cases.

Likewise, the optical rotatory power can be helpful for the assignment of the configuration of the bridgehead carbon atoms in these cycloadducts. Compound **16a** showed an optical rotation (+6.0°) as low as they were found for **14a** (−0.5°) and **15a** (+4.0°). However, higher values were measured for the cycloadducts **14b**–**16b** ($+56.0<[\alpha]_D<+67.5$).[13]

None of these reactions showed appreciable facial diastereoselectivity and diastereomeric mixtures (ca. 1:1 ratio) of *exo* cycloadducts arose from the approach of the dipolarophile to both faces of the dipole (Scheme 4). However, these cycloadditions proceeded with complete regioselectivity, which was in turn opposite to that observed in the case of 2-(*N*,*N*′-dialkylamino)thioisomünchnones, largely explored by our research group.[5,12,14] Although this distinctive behavior could be attributed to the presence of an amino substituent in the latter mesoionics, exerting a profound

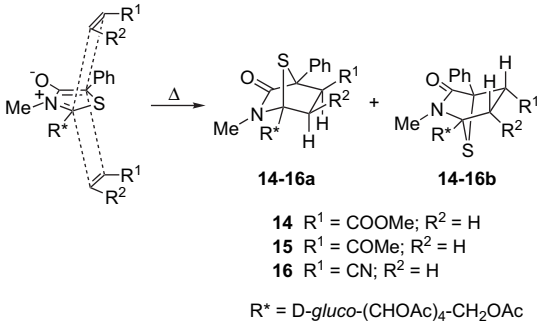
Table 1. ^{1}H NMR chemical shift (ppm) data of compounds **14–16** ($CDCl_3$)

Compound	H-5$_{endo}$	H-6$_{exo}$	H-6$_{endo}$	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″
14a	3.30	2.70	2.60	5.76	5.62	5.44	5.12	4.37	4.15
14b	3.28	2.78	2.56	5.84	5.51	5.42	5.15	4.38	4.19
15a	3.44	2.70	2.50	5.75	5.63	5.45	5.15	4.38	4.17
15b	3.39	2.76	2.44	5.82	5.51	5.42	5.15	4.37	4.19
16a	3.56	2.85	2.74	5.77	5.60	5.44	5.13	4.39	4.17
16b	3.51	2.80	2.80	5.82	5.46	5.41	5.14	4.38	4.19

Table 2. ^{13}C NMR chemical shift (ppm) data of compounds **14–16** ($CDCl_3$)

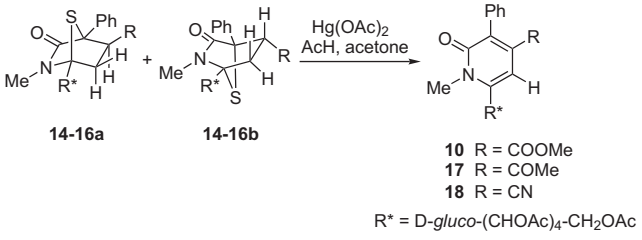
Compound	C-1	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′
14a	70.63	174.73	79.40	50.39	40.69	67.50	68.26	69.56	69.11	61.66
14b	70.91	174.48	78.21	49.31	40.72	67.57	67.66	69.42	69.42	61.47
15a	70.16	174.79	79.53	56.95	39.68	67.24	68.12	69.57	69.03	61.71
15b	70.50	174.55	78.22	55.59	39.76	67.53	67.53	69.35	69.35	61.45
16a	69.90	173.12	80.09	69.41	42.45	66.84	67.93	69.65	69.15	61.73
16b	70.24	172.72	78.79	69.22	42.45	67.05	67.42	69.22	69.22	61.43

stereodirecting effect, a satisfactory rationale has not yet been provided and additional studies will be required.



Scheme 4.

Cycloadducts **14–16** were transformed into the corresponding pyrid-2-one C-nucleosides **10**, **17**, and **18**, by elimination of hydrogen sulfide using mercury(II) acetate in acetic acid–acetone (Scheme 5). The formation of **10**, previously obtained from **4** and methyl propiolate, evidences the regiochemical control of these cycloadditions. The structures for pyrid-2-ones **17** and **18** were assigned on the basis of their analytical data and by comparison of their NMR spectra with those of **10** (Table 3).

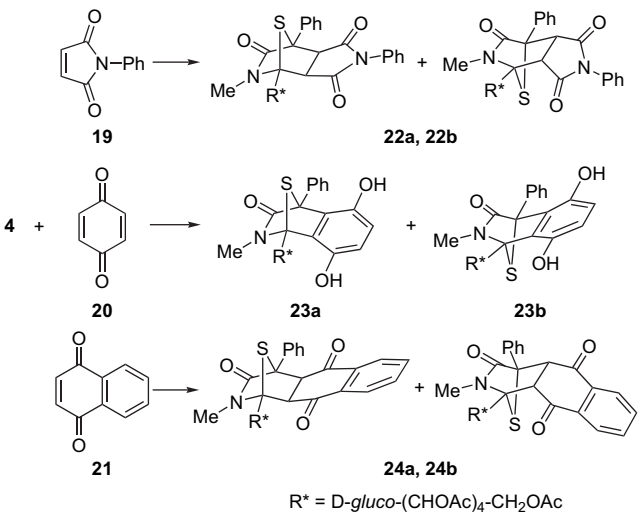


Scheme 5.

Table 3. Selected ^{13}C NMR chemical shift (ppm) data of compounds **10**, **17**, and **18** ($CDCl_3$)

Compound	C-2	C-3	C-4	C-5	C-6
10	162.41	134.88	142.67	105.11	138.57
17	162.48	134.41	146.40	104.57	142.73
18	160.93	132.62	144.31	105.73	137.31

Diastereomeric mixtures of cycloadducts **22–24** were obtained when **4** was reacted with symmetrically substituted cyclic olefins such as *N*-phenylmaleimide (**19**), 1,4-benzoquinone (**20**), and 1,4-naphthoquinone (**21**) (Scheme 6).



Scheme 6.

Suitable crystals for X-ray diffraction analysis could also be obtained in the case of **23a** (Fig. 2).[15] It is interesting to note that the tricyclic aglycon of **23a**, unlike **22** and **24**, contains only two stereogenic centers due to aromatization of the quinone moiety. This structural simplification proves that diastereomers **23a** and **23b** do not emerge from *endo*/*exo* approaches of the dipolarophile to the same face of the dipole, but rather by *exo* attack of the dipolarophile to both faces of the thioisomünchnone.

The nature of the rings fused to the 2-aza-7-thiabicyclo[2.2.1]-heptane system of **22** and **24** has a remarkable influence on their spectroscopic data, and prevents the unequivocal assignment of their configurations by means of ^{1}H- and ^{13}C-chemical shift correlations with those of **14–16**. However, aromatization of the naphthoquinone system of **24a** and **24b**, easily achieved by treatment of such a diastereomeric mixture with SiO_2 (Scheme 7), led to a mixture of

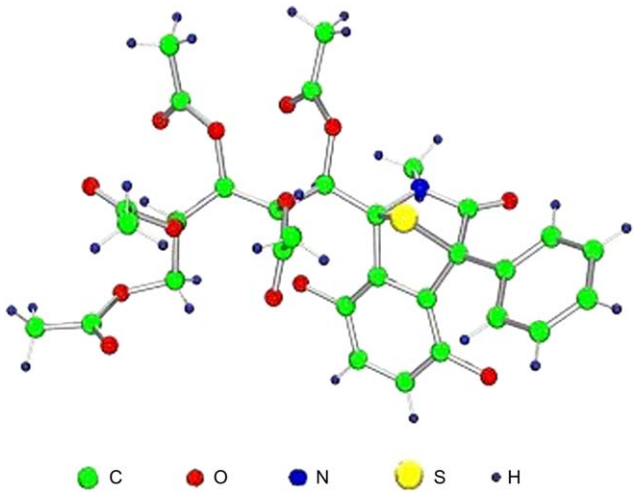
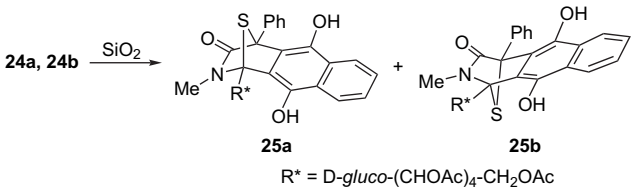


Figure 2. Perspective view of the structure of **23a**.

25a and **25b**, whose spectroscopic data were very similar to those of **23a** and **23b**, respectively. Moreover, the ^{1}H NMR spectrum of **7**, which also contains an endocyclic double bond between C-5 and C-6 atoms, exhibits the same proton pattern of ^{1}H NMR of **23b** and **25b**. This fact points to a (1*R*,4*R*) configuration for **7** (Table 4).

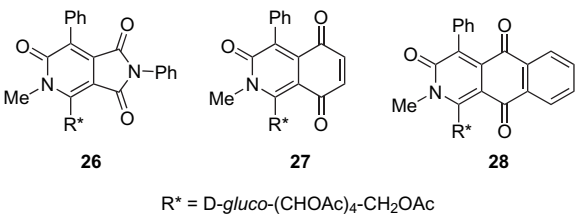


Scheme 7.

Table 4. ^{1}H NMR chemical shift (ppm) data of compounds **7**, **23**, and **25** ($CDCl_3$)

Compound	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″
7	6.14	5.54	5.37	5.20	4.41	4.14
23a	6.51	5.89	5.33	5.64	4.99	4.01
23b	6.27	5.61	5.41	5.27	4.43	4.14
25a	6.62	5.82	5.59	5.33	4.76	4.30
25b	6.47	5.58	5.49	5.30	4.40	4.18

As above, cycloadducts **22** and **23** gave rise to pyrid-2-one C-nucleosides **26** and **27** upon treatment with $Hg(OAc)_2$ in acetic acid at room temperature. However, desulfuration of **24** to give **28** could only be carried out using Raney nickel in refluxing 2-butanol.



3. Conclusions

In conclusion, this paper reports a rapid access to enantiomerically pure acyclic C-nucleosides containing a pyrid-2-one moiety based on the 1,3-dipolar cycloadditions of a carbohydrate-derived 1,3-thiazolium-4-olate system with acetylenic and olefinic dipolarophiles. To the best of our knowledge, this is the first time stable cycloadducts have been isolated in the reaction of activated triple bonds (e.g., dimethyl acetylenedicarboxylate) with thioisomünchnones. Despite these [3+2] cycloadditions processes were found to be regiospecific, no appreciable facial selectivity was induced by appended chiral carbohydrate substituent. Efforts to understand this stereochemical outcome by theoretical studies as well as further pursuits to improve the diastereoselection are currently under way in our laboratories.

4. Experimental

4.1. General methods

Melting points were determined on a capillary apparatus and are uncorrected. Optical rotations were measured at the sodium line at 18±2 °C. Analytical and preparative TLC were performed on silica gel with monitoring by means of UV light at 254 and 360 nm and iodine vapors. Flash chromatography was performed with silica gel (400–230 mesh). IR spectra were recorded on KBr pellets. ^{1}H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, in $CDCl_3$ (Me_4Si as internal standard) unless otherwise specified. Compounds **2** and **3** were prepared according to literature procedures.[9]

4.2. Synthesis and characterization

4.2.1. 2-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-3-methyl-5-phenyl-1,3-thiazolium-4-olate (4). To a solution of *N*-methyl-D-thiogluconamide (**3**) (1.0 g, 2.3 mmol) in dry chloroform (10 mL) was added dropwise a solution of α-chlorophenylacetyl chloride (0.7 mL, 4.6 mmol) in dry chloroform (5 mL). After stirring at room temperature for 15 min and refluxing for 30 min, the reaction mixture was washed with water. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was washed with petroleum ether and treated with a mixture of chloroform–diethyl ether–petroleum ether to give **4** (0.8 g, 63%) as a yellowish solid; mp 65–70 °C (dec); $[\alpha]_D$ +128 (*c* 0.5, chloroform); IR (KBr, cm^{-1}): ν_{max} 1751, 1624, 1371, 1217; ^{1}H NMR ($CDCl_3$) δ 7.90–7.16 (m, 5H), 6.30 (d, 1H, *J*=6.3 Hz), 5.69 (dd, 1H, *J*=4.3 and 6.2 Hz), 5.34 (dd, 1H, *J*=4.3 and 6.6 Hz), 5.02 (m, 1H), 4.38 (dd, 1H, *J*=2.5 and 12.7 Hz), 4.09 (dd, 1H, *J*=5.6 and 12.7 Hz), 3.81 (s, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.72, 169.72, 169.51, 169.26, 169.01, 159.78, 143.60, 132.60, 128.62, 125.66, 124.07, 99.87, 68.95, 68.50, 68.24, 67.06, 61.38, 33.08, 20.62, 20.49, 20.35, 20.25.

4.2.2. (1*R*,4*R*)-1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-2-methyl-5,6-dimethoxycarbonyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]hept-5-ene (7). To a solution of **4** (1.0 g, 1.8 mmol) in dry toluene (15 mL) dimethyl

acetylenedicarboxylate (0.3 mL, 2.2 mmol) was added, and the mixture was refluxed for 5 h. After removal of the solvent, the crude product was purified by flash chromatography (diethyl ether) to give **7** (0.5 g, 40%); mp 60–65 °C (dec); $[\alpha]_D$ +66 (*c* 0.5, chloroform); IR (KBr, cm^{-1}): ν_{max} 2959, 1750, 1624, 1440, 1373, 1213, 1128, 1044, 976; ^1H NMR ($CDCl_3$) δ 7.66–7.37 (m, 5H), 6.14 (d, 1H, *J*=2.9 Hz), 5.54 (dd, 1H, *J*=5.9 and 5.4 Hz), 5.37 (t, 1H, *J*=5.7 Hz), 5.20 (m, 1H), 4.41 (dd, 1H, *J*=3.5 and 12.4 Hz), 4.14 (dd, 1H, *J*=5.3 and 12.4 Hz), 3.79 (s, 3H), 3.51 (s, 3H), 3.04 (s, 3H), 2.17 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.52, 170.49, 169.75, 169.69, 169.23, 169.12, 163.01, 162.50, 150.31, 149.80, 130.93, 128.96, 128.29, 128.11, 83.28, 69.36, 68.08, 67.81, 65.29, 52.94, 52.45, 33.08, 20.76, 20.59, 20.33. Anal. Calcd for $C_{31}H_{35}NO_{15}S$: C, 53.68; H, 5.08; N, 2.02; S, 4.62. Found: C, 53.96; H, 5.06; N, 1.82; S, 4.47.

4.2.3. 6-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-4,5-dimethoxycarbonyl-1-methyl-3-phenylpyrid-2-one (9). To a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) compound **7** (0.1 g, 0.2 mmol) was added and the reaction mixture was stirred at room temperature. Acetone (3 mL) was mixed with the resultant gel and the insoluble material was filtered off and washed with acetone. The filtrate was diluted with water, filtered, adjusted to pH 5 with sodium hydrogen carbonate, and extracted with chloroform. The organic layer was washed with sodium hydrogen carbonate solution (1 M) followed by water, dried ($MgSO_4$), and concentrated in vacuo. Purification of the residue by thin layer preparative chromatography (diethyl ether) afforded **9**, which crystallized as a white solid from diethyl ether–petroleum ether (0.02 g, 30%); mp 75 °C (dec); $[\alpha]_D$ +74.5 (*c* 0.5, chloroform); IR ν_{max} (KBr, cm^{-1}) 3418, 2955, 1751, 1655, 1541, 1439, 1371, 1304, 1219, 1045; ^1H NMR ($CDCl_3$) δ 7.41–7.22 (m, 5H), 6.49 (m, 1H), 5.91 (dd, 1H, *J*=3.2 and 5.7 Hz), 5.31 (dd, 1H, *J*=3.4 and 6.2 Hz), 5.09 (m, 1H), 4.29 (dd, 1H, *J*=3.7 and 12.3 Hz), 4.10 (dd, 1H, *J*=6.0 and 12.2 Hz), 3.78 (s, 3H), 2.72 (s, 3H), 3.46 (s, 3H), 2.09 (s, 9H), 2.02 (s, 3H), 1.98 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.30, 168.79, 168.58, 165.33, 164.99, 160.66, 143.13, 139.52, 133.83, 128.76, 127.71, 127.24, 110.79, 70.33, 69.70, 68.82, 68.32, 60.97, 52.23, 51.60, 33.73, 19.84, 19.68. Anal. Calcd for $C_{31}H_{35}NO_{15}$: C, 56.28; H, 5.33; N, 2.12. Found: C, 55.50; H, 5.20; N, 2.07.

4.2.4. 6-(1′,2′,3′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-4-methoxycarbonyl-2-methyl-3-phenylpyrid-2-one (10). *Method A*: A solution of **4** (1.0 g, 1.81 mmol) and methyl propiolate (0.2 mL, 2.17 mmol) in dry dichloromethane (15 mL) was heated at reflux for 10 h. The solvent was then evaporated under reduced pressure and the crude product was purified by flash chromatography (diethyl ether) and then poured into a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) at room temperature and processed as described for **9** to give **10** (0.2 g, 19%) as a white solid; mp 80 °C; $[\alpha]_D$ 56.5 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 3472, 2960, 1749, 1653, 1555, 1454, 1373, 1217, 1049; ^1H NMR ($CDCl_3$) δ 7.40–7.27 (m, 5H), 6.41 (s, 1H), 6.07 (d, 1H, *J*=4.6 Hz), 5.60 (t, 1H, *J*=4.7 Hz), 5.42 (t, 1H, *J*=5.3 Hz), 5.07 (m, 1H), 4.36 (dd, 1H, *J*=2.9 and 12.6 Hz), 4.10 (dd, 1H, *J*=6.2 and 12.6 Hz), 3.75 (s, 3H), 3.54 (s, 3H), 2.20 (s, 3H), 2.13 (s, 6H), 2.08 (s, 3H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.63, 169.80, 169.49, 167.36, 162.41, 142.67, 138.57, 134.88, 131.62, 129.12, 128.10, 127.87, 105.11, 70.11, 69.19, 68.95, 68.41, 61.48, 52.31, 31.90, 20.65, 20.68, 20.62, 20.22. Anal. Calcd for $C_{29}H_{33}NO_{13}$: C, 57.71; H, 5.51; N, 2.32. Found: C, 57.54; H, 5.53; N, 2.23. *Method B*: A mixture of cycloadducts **14a** and **14b** (0.13 g, 0.2 mmol) was poured into a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) at room temperature and processed as described for **9** to give **10** (0.3 g, 35%).

4.2.5. (1*S*,4*R*,5*R*)-1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-5-methoxycarbonyl-2-methyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]heptane (14a) and (1*R*,4*S*,5*S*)-1-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-5-methoxycarbonyl-2-methyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]heptane (14b). To a solution of **4** (1.0 g, 1.8 mmol) in dry chloroform (15 mL) methyl acrylate (0.2 mL, 2.2 mmol) was added, and the mixture was refluxed for 12 h. After removal of the solvent, the crude product was purified by flash chromatography (diethyl ether) and the two diastereomeric cycloadducts **14a** (0.2 g, 12%) and **14b** (0.2 g, 12%) were separated by thin layer preparative chromatography (diethyl ether). **14a**: mp 83 °C (dec); $[\alpha]_D$ −0.5 (*c* 0.5, chloroform); IR (KBr, cm^{-1}): ν_{max} 2965, 1750, 1447, 1373, 1213, 1045; ^1H NMR ($CDCl_3$) δ 7.42–7.26 (m, 5H), 5.76 (d, 1H, *J*=5.1 Hz), 5.62 (t, 1H, *J*=4.9 Hz), 5.44 (t, 1H, *J*=4.9 Hz), 5.12 (m, 1H), 4.37 (dd, 1H, *J*=3.3 and 12.4 Hz), 4.15 (dd, 1H, *J*=5.3 and 12.4 Hz), 3.30 (m, 1H, *J*=4.3 and 8.0 Hz), 3.25 (s, 3H), 2.84 (s, 3H), 2.70 (dd, 1H, *J*=4.4 and 12.3 Hz), 2.60 (dd, 1H, *J*=8.0 and 12.3 Hz), 2.19 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.73, 170.57, 170.39, 169.80, 169.42, 169.27, 131.75, 128.39, 127.85, 79.40, 70.63, 69.56, 69.11, 68.26, 67.50, 61.66, 51.76, 50.39, 40.69, 28.75, 20.96, 20.65, 20.55. Anal. Calcd for $C_{29}H_{35}NO_{13}S$: C, 54.62; H, 5.53; N, 2.20; S, 5.03. Found: C, 54.57; H, 5.58; N, 2.14; S, 5.07. **14b**: mp 80 °C (dec); $[\alpha]_D$ +56.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2955, 1751, 1447, 1373, 1213, 1043; ^1H NMR ($CDCl_3$) δ 7.43–7.27 (m, 5H), 5.84 (d, 1H, *J*=3.3 Hz), 5.51 (dd, 1H, *J*=3.7 and 4.9 Hz), 5.42 (t, 1H, *J*=4.9 Hz), 5.15 (m, 1H), 4.38 (dd, 1H, *J*=2.9 and 12.5 Hz), 4.19 (dd, 1H, *J*=5.8 and 12.4 Hz), 3.28 (m, 1H), 3.26 (s, 3H), 2.88 (s, 3H), 2.78 (dd, 1H, *J*=4.4 and 12.8 Hz), 2.56 (dd, 1H, *J*=8.2 and 12.7 Hz), 2.25 (s, 3H), 2.17 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.48, 170.78, 170.63, 169.96, 169.72, 169.39, 131.85, 128.52, 128.31, 127.93, 78.21, 70.91, 69.42, 67.66, 67.57, 61.47, 51.80, 49.31, 40.72, 28.22, 20.89, 20.71, 20.57. Anal. Calcd for $C_{29}H_{35}NO_{13}S$: C, 54.62; H, 5.53; N, 2.20; S, 5.03. Found: C, 54.63; H, 5.47; N, 2.28; S, 4.84.

4.2.6. (1*S*,4*R*,5*R*)-5-Acetyl-1-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-2-methyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]heptane (15a) and (1*R*,4*S*,5*S*)-5-acetyl-1-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-2-methyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]-heptane (15b). To a solution of **4** (1.0 g, 1.8 mmol) in dry chloroform (15 mL) methyl vinyl ketone (0.3 mL, 2.2 mmol) was added, and the mixture was refluxed for 12 h. After removal of the solvent, the crude product was purified by flash chromatography (eluant: diethyl ether) and the two

diastereomeric cycloadducts **15a** (0.2 g, 18%) and **15b** (0.2 g, 18%) were separated by preparative thin layer chromatography (diethyl ether). **15a**: mp 100 °C (dec); $[\alpha]_D$ +4.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}): ν_{max} 2980, 1751, 1707, 1371, 1215, 1043; ^1H NMR ($CDCl_3$) δ 7.51–7.27 (m, 5H), 5.75 (d, 1H, *J*=5.3 Hz), 5.63 (t, 1H, *J*=4.9 Hz), 5.45 (dd, 1H, *J*=4.6 and 6.5 Hz,), 5.15 (m, 1H), 4.38 (dd, 1H, *J*=3.1 and 12.5 Hz), 4.17 (dd, 1H, *J*=5.3 and 12.5 Hz), 3.44 (dd, 1H, *J*=4.3 Hz), 2.85 (s, 3H), 2.70 (dd, 1H, *J*=4.3 and 12.3 Hz), 2.50 (dd, 1H, *J*=8.0 and 12.3 Hz), 2.20 (s, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 1.68 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 204.02, 174.79, 170.60, 170.48, 169.82, 169.42, 169.34, 131.92, 128.76, 128.41, 79.53, 70.16, 69.57, 69.03, 68.12, 67.24, 61.71, 56.95, 39.68, 30.19, 28.62, 20.69, 20.55. Anal. Calcd for $C_{29}H_{35}NO_{12}S$: C, 56.03; H, 5.67; N, 2.25; S, 5.16. Found: C, 55.85; H, 5.70; N, 2.49; S, 5.18. **15b**: mp 80 °C (dec); $[\alpha]_D$ +62.5 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2961, 1744, 1707, 1373, 1215, 1043; ^1H NMR ($CDCl_3$) δ 7.49–7.28 (m, 5H), 5.82 (d, 1H, *J*=3.5 Hz), 5.51 (t, 1H, *J*=4.8 Hz), 5.42 (dd, 1H, *J*=5.2 and 6.3 Hz), 5.15 (m, 1H), 4.37 (dd, 1H, *J*=2.9 and 12.5 Hz), 4.19 (dd, 1H, *J*=5.8 and 12.4 Hz), 3.39 (dd, 1H, *J*=4.3 and 8.0), 2.87 (s, 3H), 2.76 (dd, 1H, *J*=4.4 and 12.8 Hz), 2.44 (dd, 1H, *J*=8.1 and 12.8 Hz), 2.25 (s, 3H), 2.17 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 1.69 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 204.36, 174.55, 170.60, 169.89, 169.85, 169.75, 169.40, 131.97, 128.77, 128.44, 128.26, 78.22, 70.50, 69.35, 67.53, 61.45, 55.59, 39.76, 30.50, 28.15, 20.85, 20.65, 20.59. Anal. Calcd for $C_{29}H_{35}NO_{12}S$: C, 56.03; H, 5.67; N, 2.25; S, 5.16. Found: C, 55.94; H, 5.83; N, 2.32; S, 5.18.

4.2.7. (1*S*,4*R*,5*R*)-1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-5-cyano-2-methyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]heptane (16a) and (1*R*,4*S*,5*S*)-1-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-aza-5-cyano-2-methyl-3-oxo-4-phenyl-7-thiabicyclo[2.2.1]-heptane (16b). To a solution of **4** (1.0 g, 1.8 mmol) in dry chloroform (15 mL) acrylonitrile (0.1 mL, 2.2 mmol) was added, and the mixture was refluxed for 12 h. After removal of the solvent, the crude product was purified by flash chromatography (diethyl ether) and the two diastereomeric cycloadducts **16a** (0.1 g, 9%) and **16b** (0.1 g, 9%) were separated by preparative thin layer chromatography (diethyl ether). **16a**: mp 97 °C (dec); $[\alpha]_D$ +6.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2949, 1753, 1715, 1448, 1373, 1215, 1047; ^1H NMR ($CDCl_3$) δ 7.47–7.43 (m, 5H), 5.77 (d, 1H, *J*=5.4 Hz), 5.60 (t, 1H, *J*=4.8 Hz), 5.44 (dd, 1H, *J*=4.6 and 6.5 Hz), 5.13 (m, 1H), 4.39 (dd, 1H, *J*=3.0 and 12.6 Hz), 4.17 (dd, 1H, *J*=5.1 and 12.6 Hz), 3.56 (dd, 1H, *J*=3.6 and 8.0 Hz), 2.85 (dd, 1H, *J*=8.0 and 12.7 Hz), 2.83 (s, 3H), 2.74 (dd, 1H, *J*=3.7 and 12.7 Hz), 2.22 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.12, 171.15, 170.69, 170.55, 169.78, 169.32, 130.71, 129.44, 128.77, 128.26, 118.28, 80.09, 69.90, 69.65, 69.41, 69.15, 67.93, 66.84, 61.73, 42.45, 39.09, 28.54, 20.72, 20.60, 20.52, 20.46. Anal. Calcd for $C_{28}H_{32}N_2O_{11}S$: C, 55.62; H, 5.33; N, 4.63; S, 5.30. Found: C, 55.31; H, 5.28; N, 4.64; S, 5.48. **16b**: mp 93.2 °C (dec); $[\alpha]_D$ +67.5° (*c* 0.5, chloroform); IR (KBr, cm^{-1}): ν_{max} 2976, 1755, 1719, 1448, 1373, 1213, 1047; ^1H NMR ($CDCl_3$) δ 7.48–7.43 (m, 5H), 5.82 (d, 1H, *J*=3.8 Hz), 5.46 (t, 1H, *J*=4.5 Hz), 5.41 (dd, 1H, *J*=4.9 and 6.3 Hz), 5.14 (m, 1H), 4.38 (dd, 1H, *J*=2.92 and 12.5 Hz, H-5′), 4.19 (dd, 1H, *J*=5.6 and 12.5 Hz), 3.51 (dd, 1H, *J*=3.9 and 8.0 Hz), 2.85 (s, 3H), 2.80 (m, 2H), 2.25 (s, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.09 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 172.72, 170.62, 169.85, 169.66, 169.36, 130.78, 129.45, 128.77, 128.16, 118.19, 78.79, 70.24, 69.22, 67.42, 67.05, 61.43, 42.45, 37.87, 28.02, 20.99, 20.76, 20.67, 20.52. Anal. Calcd for $C_{28}H_{32}N_2O_{11}S$: C, 55.62; H, 5.33; N, 4.63; S, 5.30. Found: C, 55.33; H, 5.43; N, 4.77; S, 4.81.

4.2.8. 4-Acetyl-6-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-1-methyl-3-phenylpyrid-2-one (17). A mixture of cycloadducts **15a** and **15b** (0.12 g, 0.2 mmol) was poured into a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) at room temperature and processed as described for **9** to give **17** (0.05 g, 53%); mp 72 °C (dec); $[\alpha]_D$ +118.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 3472, 2964, 1751, 1719, 1659, 1501, 1447, 1373, 1215, 1045; ^1H NMR ($CDCl_3$) δ 7.42–7.30 (m, 5H), 6.22 (s, 1H), 6.05 (d, 1H, *J*=4.7 Hz), 5.61 (t, 1H, *J*=4.5 Hz), 5.39 (t, 1H, *J*=5.1 Hz), 5.08 (m, 1H), 4.35 (dd, 1H, *J*=2.8 and 12.4 Hz), 4.09 (dd, 1H, *J*=6.1 and 12.4 Hz), 3.77 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 203.05, 170.58, 170.43, 169.76, 169.57, 169.52, 162.48, 146.40, 142.73, 134.41, 130.02, 129.15, 128.89, 128.41, 104.57, 70.36, 69.14, 68.87, 68.39, 61.47, 31.88, 29.72, 20.67, 20.25. Anal. Calcd for $C_{29}H_{33}NO_{12}$: C, 59.28; H, 5.66; N, 2.38. Found: C, 59.14; H, 5.61; N, 2.19.

4.2.9. 6-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-4-cyano-1-methyl-3-phenylpyrid-2-one (18). A mixture of cycloadducts **16a** and **16b** (0.12 g, 0.2 mmol) was poured into a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) at room temperature and processed as described for **9** to give **18** (0.03 g, 33%); mp 86.5 °C; $[\alpha]_D$ +114.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2353, 1732, 1695, 1454, 1373, 1217, 1049; ^1H NMR ($CDCl_3$) δ 7.55–7.44 (m, 5H), 6.34 (s, 1H), 6.06 (d, 1H, *J*=4.0 Hz), 5.57 (t, 1H, *J*=4.6 Hz), 5.45 (t, 1H, *J*=5.5 Hz), 5.06 (m, 1H), 4.38 (dd, 1H, *J*=2.8 and 12.5 Hz), 4.11 (dd, 1H, *J*=5.9 and 12.5 Hz), 3.76 (s, 3H), 2.23 (s, 3H), 2.14 (s, 6H), 2.08 (s, 6H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.71, 169.81, 169.48, 160.93, 144.31, 137.31, 132.62, 129.62, 128.29, 119.18, 116.12, 105.73, 69.62, 69.24, 69.02, 68.20, 61.46, 32.14, 20.64, 20.22. Anal. Calcd for $C_{28}H_{30}N_2O_{11}$: C, 58.94; H, 5.30; N, 4.91. Found: C, 58.93; H, 5.27; N, 5.03.

4.2.10. (3a*S*,4*S*,7*R*,7a*R* and 3a*R*,4*R*,7*S*,7a*S*)-4-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-4,7-epithio-2,3a,4,5,7,7a-hexahydro-5-methyl-2,7-diphenyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3,6-trione (22a and 22b). To a suspension of **4** (1.0 g, 1.8 mmol) in dry toluene (15 mL) *N*-phenylmaleimide (0.4 g, 2.2 mmol) was added, and the mixture was refluxed for 12 h. After removal of the solvent, the crude product was purified by flash chromatography (diethyl ether) and the two diastereomeric cycloadducts were separated by preparative thin layer chromatography (diethyl ether). Fast-moving diastereomer: 0.4 g (30%), mp 175–6 °C (dec); $[\alpha]_D$ −33.5 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2980, 1723, 1499, 1373, 1217, 1044, 955; ^1H NMR ($CDCl_3$) δ 7.43–7.19 (m, 10H), 6.35 (m, 1H), 6.10

(d, 1H, *J*=2.0 Hz), 5.56 (t, 1H, *J*=2.0 Hz), 5.10 (m, 1H), 4.50 (dd, 1H, *J*=2.6 and 12.5 Hz), 4.19 (dd, 1H, *J*=6.8 and 12.5 Hz), 4.03 (d, 1H, *J*=6.8 Hz), 4.03 (d, 1H, *J*=6.8 Hz), 3.79 (d, 1H, *J*=6.8 Hz), 3.10 (s, 3H), 2.20 (s, 3H), 2.18 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.58, 171.75, 170.53, 170.43, 169.99, 169.75, 169.55, 164.46, 131.19, 129.99, 128.99, 128.67, 128.17, 126.33, 80.67, 70.62, 69.55, 69.25, 68.51, 61.45, 55.91, 51.05, 30.69, 21.05, 20.73, 20.60. Anal. Calcd for $C_{35}H_{36}N_2O_{13}S$: C, 58.00; H, 5.01; N, 3.87; S, 4.42. Found: C, 57.81; H, 4.81; N, 3.63; S, 4.01. Slow-moving diastereomer: 0.4 g (30%), mp 120–1 °C (dec); $[\alpha]_D$ +3.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2994, 1719, 1499, 1373, 1215, 1051; ^{1}H NMR ($CDCl_3$) δ 7.50–7.21 (m, 10H), 5.71–5.63 (m, 3H), 5.10 (m, 1H), 4.28 (dd, 1H, *J*=2.4 and 12.5 Hz), 4.17 (dd, 1H, *J*=4.2 and 12.5 Hz), 3.92 (d, 1H, *J*=6.7 Hz), 3.77 (d, 1H, *J*=6.7 Hz), 3.09 (s, 3H), 2.26 (s, 3H), 2.17 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 175.05, 171.12, 170.85, 170.48, 169.87, 169.68, 167.27, 131.20, 129.84, 129.11, 128.96, 128.77, 128.26, 126.44, 79.06, 70.33, 68.96, 68.13, 66.80, 66.72, 61.66, 53.86, 51.04, 30.57, 20.83, 20.64, 20.45, 20.37. Anal. Calcd for $C_{35}H_{36}N_2O_{13}S$: C, 58.00; H, 5.01; N, 3.87; S, 4.42. Found: C, 57.70; H, 4.85; N, 3.73; S, 4.21.

4.2.11. (1*S*,4*S*)-1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-1,4-epithio-1,2,4-trihydro-5,8-dihydroxy-2-methyl-4-phenylisoquinolin-3-one (23a) and (1*R*,4*R*)-1-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-gluco-pentitol-1-yl)-1,4-epithio-1,2,4-trihydro-5,8-dihydroxy-2-methyl-4-phenyl-isoquinolin-3-one (23b). From 1,4-benzoquinone (0.2 g, 2.2 mmol) compounds **23** were obtained as white solids as described for **22**. **23a**: 0.2 g (20%); mp 210 °C (dec); $[\alpha]_D$ +1.0 (c 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 2920, 1744, 1696, 1493, 1435, 1375, 1287, 1213, 1065, 955; ^{1}H NMR ($CDCl_3$) δ 8.09–7.43 (m, 5H), 7.23 (s, 1H), 6.58 (d, 1H, *J*=8.9 Hz), 6.51 (s, 1H), 6.42 (d, 1H, *J*=8.9 Hz), 5.88 (d, 1H, *J*=8.5 Hz), 5.64 (dd, 1H, *J*=2.2 and 7.7 Hz), 5.33 (dd, 1H, *J*=2.2 and 8.5 Hz), 4.99 (dd, 1H, *J*=2.5 and 12.4 Hz), 4.38 (s, 1H), 4.01 (dd, 1H, *J*=10.0 and 12.4 Hz), 2.62 (s, 3H), 2.27 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 192.45, 191.92, 177.91, 172.77, 171.03, 170.04, 169.63, 169.21, 145.34, 141.67, 132.77, 130.38, 129.51, 128.57, 128.27, 119.40, 118.82, 81.80, 70.54, 68.96, 68.17, 66.26, 63.71, 62.16, 30.84, 21.31, 21.07, 20.83, 20.46. Anal. Calcd for $C_{31}H_{33}NO_{13}S$: C, 56.44; H, 5.04; N, 2.12; S, 4.86. Found: C, 56.29; H, 5.16; N, 2.03; S, 4.65. **23b**: 0.09 g (7%); mp 115 °C; $[\alpha]_D$ +4.5 (*c* 0.5, chloroform); IR (KBr, cm^{-1}): ν_{max} 3430, 1753, 1493, 1371, 1217, 1047, 955; ^{1}H NMR ($CDCl_3$) δ 8.13–7.46 (m, 5H), 6.56 (d, 1H, *J*=8.9 Hz), 6.43 (d, 1H, *J*=8.9 Hz), 6.27 (d, 1H, *J*=2.4 Hz), 5.61 (dd, 1H, *J*=2.4 and 5.8 Hz), 5.41 (dd, 1H, *J*=5.8 Hz), 5.27 (m, 1H), 4.43 (dd, 1H, *J*=3.8 and 12.3 Hz), 4.14 (dd, 1H, *J*=5.5 and 12.3 Hz), 2.78 (s, 3H), 2.14 (s, 3H), 2.12 (s, 6H), 2.10 (s, 3H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.69, 170.08, 169.87, 169.08, 145.25, 143.02, 132.00, 129.74, 129.56, 128.62, 127.44, 119.31, 119.13, 81.51, 69.58, 68.73, 63.44, 61.42, 30.47, 21.00, 20.85, 20.66. Anal. Calcd for $C_{31}H_{33}NO_{13}S$: C, 56.44; H, 5.04; N, 2.12; S, 4.86. Found: C, 56.22; H, 5.25; N, 2.05; S, 4.58.

4.2.12. (1*S*,4*R*,4a*R*,10a*S* and 1*R*,4*S*,4a*S*,10a*R*)-1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-1,4-epithio-1,2,4,4a,10a-pentahydro-2-methyl-4-phenylbenzo[*h*]isoquinolin-3,5,10-trione (24a and 24b). From 1,4-naphtoquinone (0.3 g, 2.2 mmol) compounds **24** were obtained as white solids as described for **22**. Fast-moving diastereomer: 0.2 g (10%); mp 105–110 °C; $[\alpha]_D$ +47 (*c* 0.5, chloroform), IR (KBr, cm^{-1}): ν_{max} 2955, 1750, 1686, 1449, 1371, 1215, 1049; ^{1}H NMR ($CDCl_3$) δ 7.89–7.39 (m, 9H), 5.56 (dd, 1H, *J*=1.2 and 8.2 Hz), 5.47 (dd, 1H, *J*=1.3 and 9.1 Hz), 5.24 (d, 1H, *J*=8.2 Hz), 55.02 (m, 1H), 4.22 (dd, 1H, *J*=2.6 and 12.5 Hz), 4.00 (m, 2H), 3.63 (d, 1H, *J*=7.3 Hz), 3.11 (s, 3H), 2.30 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 192.45, 191.92, 175.12, 170.60, 170.42, 169.87, 169.57, 168.15, 137.42, 137.12, 134.46, 129.87, 129.23, 128.84, 128.08, 126.89, 126.20, 79.43, 71.69, 69.11, 68.08, 67.14, 67.01, 61.68, 58.34, 56.79, 30.75, 20.82, 20.61, 20.47. Anal. Calcd for $C_{35}H_{35}NO_{13}S$: C, 59.23; H, 4.97; N, 1.97; S, 4.52. Found: C, 58.76; H, 4.86; N, 1.64; S, 4.55. Slow-moving diastereomer: 0.06 g (3%); mp 135 °C; $[\alpha]_D$ −32 (*c* 0.25, chloroform); IR (KBr, cm^{-1}) ν_{max} 2930, 1760, 1684, 1446, 1371, 1213, 1045; ^{1}H NMR ($CDCl_3$) δ 7.94–7.36 (m, 9H), 5.93 (d, 1H, *J*=2.9 Hz), 5.80 (t, 1H, *J*=3.1 Hz), 5.46 (dd, 1H, *J*=4.9 and 6.2 Hz), 5.15 (m, 1H), 4.44 (dd, 1H, *J*=3.1 and 12.4 Hz), 4.14 (dd, 1H, *J*=5.6 and 12.4 Hz), 3.91 (d, 1H, *J*=7.4 Hz), 3.66 (d, 1H, *J*=7.3 Hz), 3.11 (s, 1H), 2.13 (s, 6H), 2.08 (s, 6H), 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 192.30, 191.30, 175.42, 170.60, 170.09, 169.75, 169.15, 168.57, 137.41, 136.21, 134.60, 134.23, 130.26, 129.04, 128.90, 127.98, 126.73, 81.73, 69.91, 69.66, 69.18, 67.90, 67.72, 61.48, 58.93, 56.62, 30.53, 20.83, 20.70, 20.59.

4.2.13. 1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-1,4-epithio-1,2,4-trihydro-5,10-dihydroxy-2-methyl-4-phenylbenzo[*h*]isoquinoline-3-one (25a) and 1-(1′,2′,3′,4′,5′-penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-1,4-epithio-1,2,4-trihydro-5,10-dihydroxy-2-methyl-4-phenylbenzo[*h*]isoquinoline-3-one (25b). A mixture of diastereomers **24a** and **24b** (0.1 g, 0.14 mmol) was treated with silica gel (3.0 g) for 10 days. The reaction mixture was filtered and after washing three times the silica gel with ethyl acetate, combined solutions were concentrated in vacuo to give a mixture of **25a** and **25b**, which were separated by preparative thin layer chromatography (diethyl ether). **25a**: 0.03 g (25%), mp 85 °C (dec); $[\alpha]_D$ −216 (*c* 0.25, chloroform); IR (KBr, cm^{-1}) ν_{max} 1753, 1667, 1593, 1371, 1049; ^{1}H NMR ($CDCl_3$) δ 8.12–7.42 (m, 10H), 6.62 (d, 1H, *J*=0.8 Hz), 5.82 (dd, 1H, *J*=0.8 and 7.1 Hz), 5.59 (dd, 1H, *J*=4.3 and 7.1 Hz), 5.33 (m, 1H), 4.76 (dd, 1H, *J*=2.7 and 12.4 Hz), 4.30 (dd, 1H, *J*=7.9 and 12.5 Hz), 2.84 (s, 3H), 2.23 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 183.04, 179.04, 177.70, 170.75, 170.32, 170.03, 169.70, 169.45, 156.75, 153.07, 134.69, 134.11, 132.25, 131.50, 131.02, 128.93, 128.68, 128.32, 127.98, 126.89, 126.73, 82.57, 70.45, 69.93, 69.08, 65.80, 66.04, 61.44, 32.57, 21.39, 20.78, 20.55. Anal. Calcd for $C_{35}H_{35}NO_{13}S$: C, 59.23; H, 4.97; N, 1.97; S, 4.52. Found: C, 59.45; H, 4.82; N, 1.82; S, 4.48. **25b**: 0.04 g (40%), $[\alpha]_D$ +45 (*c* 0.25, chloroform); IR (KBr, cm^{-1}) ν_{max} 1755, 1666, 1593, 1371, 1049; ^{1}H NMR ($CDCl_3$) δ 8.03–7.26 (m, 10H), 6.47 (d, 1H, *J*=3.8 Hz),

5.58 (t, 1H, J=4.2 Hz), 5.49 (dd, 1H, J=4.8 and 6.1 Hz), 5.30 (m, 1H), 4.40 (dd, 1H, J=3.4 and 12.4 Hz), 4.18 (dd, 1H, J=5.2 and 12.4 Hz), 2.97 (s, 3H), 2.22 (s, 3H), 2.09 (s, 9H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 181.79, 179.03, 178.74, 170.54, 170.27, 169.75, 169.36, 168.90, 156.81, 153.50, 134.45, 134.08, 132.25, 131.70, 131.08, 128.98, 128.75, 128.02, 126.77, 126.35, 82.49, 69.50, 68.99, 68.69, 65.60, 61.51, 30.89, 21.01, 20.75, 20.68, 20.49.

4.2.14. 4-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2,5-dihydro-5-methyl-2,7-diphenyl-1*H*-pyrrolo [3,4-*c*]pyridine-1,3,6-trione (26). A mixture of cycloadducts **22a** and **22b** (0.1 g, 0.2 mmol) was poured into a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) at room temperature and processed as described for **9** to give **26** (0.03 g, 30%) as a white solid; mp 95 °C (dec); $[\alpha]_D$ +47.0 (*c* 0.5, chloroform); IR (KBr, cm^{-1}) ν_{max} 3472, 2964, 1751, 1719, 1659, 1501, 1447, 1373, 1215, 1045; ^{1}H NMR ($CDCl_3$) δ 7.52–7.35 (m, 5H), 7.59 (d, 1H, J=8.6 Hz), 6.00 (dd, 1H, J=2.3 and 8.7 Hz), 5.19 (dd, 1H, J=2.5 and 7.6 Hz), 5.08 (m, 1H), 4.25 (dd, 1H, J=3.0 and 12.6 Hz), 4.03 (dd, 1H, J=5.6 and 12.6 Hz), 3.88 (s, 3H), 2.16 (s, 6H), 2.08 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.48, 170.24, 169.99, 169.63, 168.66, 164.56, 163.81, 163.69, 142.97, 132.78, 131.50, 131.08, 130.15, 129.95, 129.53, 128.86, 128.59, 127.71, 126.89, 126.50, 108.09, 68.70, 68.60, 67.44, 66.96, 61.35, 35.38, 21.01, 20.73, 20.62, 20.46. Anal. Calcd for $C_{35}H_{34}N_2O_{13}$: C, 60.13; H, 4.96; N, 4.06. Found: C, 60.11; H, 5.02; N, 4.09.

4.2.15. 1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-methyl-4-phenyl-2*H*-isoquinoline-3,5,6-trione (27). A mixture of cycloadducts **23a** and **23b** (0.1 g, 0.2 mmol) was poured into a suspension of $Hg(OAc)_2$ (0.1 g, 0.3 mmol) in acetic acid (3 mL) at room temperature and processed as described for **9** to give **27** (0.02 g, 20%) as a yellowish solid; mp 85 °C (dec); $[\alpha]_D$ −17.0 (*c* 0.25, chloroform); IR (KBr, cm^{-1}) ν_{max} 3480, 2980, 1751, 1643, 1510, 1447, 1373, 1217, 1074; ^{1}H NMR ($CDCl_3$) δ 7.46–7.75 (m, 5H), 7.53 (d, 1H, J=4.1 Hz), 6.01 (t, 1H, J=4.5 Hz), 5.62 (t, 1H, J=5.6 Hz), 5.19 (m, 1H), 4.53 (dd, 1H, J=2.4 and 12.5 Hz), 4.32 (dd, 1H, J=6.7 and 12.5 Hz), 3.81 (s, 3H), 2.71 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 2.08 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 184.22, 183.71, 170.64, 170.02, 169.69, 169.30, 169.21, 163.45, 150.44, 141.72, 138.18, 134.87, 134.75, 133.44, 111.88, 70.07, 69.73, 69.32, 69.24, 61.56, 37.20, 20.77, 20.55. Anal. Calcd for $C_{31}H_{31}NO_{13}$: C, 59.52; H, 4.99; N, 2.24. Found: C, 59.14; H, 4.91; N, 2.19.

4.2.16. 1-(1′,2′,3′,4′,5′-Penta-*O*-acetyl-D-*gluco*-pentitol-1-yl)-2-ethoxy-5-(1,2-diethoxycarbonyl hydrazine)-5-phenyl-3-methylthiazolidin-4-one (28). A suspension of Raney nickel (0.03 g) in acetone (5 mL) was refluxed for 2 h. After cooling at room temperature and decanting the solvent, a solution of cycloadducts **24a** and **24b** (0.1 g, 0.1 mmol) in 2-butanol was added and the reaction mixture was heated at reflux for 2 h. After three washings of the catalyst with 2-butanol, the combined solutions were filtered and the solvent was concentrated in vacuo. The crude mixture was purified by preparative thin layer chromatography (acetonitrile–chloroform 1:9 as eluent) to give **28** (0.03 g, 32%); mp 95 °C; $[\alpha]_D$ −9.5 (*c* 0.25, chloroform); IR (KBr, cm^{-1}) ν_{max} 2970, 1751, 1641, 1447, 1371, 1217, 1049; ^{1}H NMR ($CDCl_3$) δ 8.08–6.88 (m, 9H), 8.17 (d, 1H, J=4.0 Hz), 629.55 (t, 1H, J=4.5 Hz), 6.07 (t, 1H, J=5.7 Hz), 5.59 (m, 1H), 4.83 (d, 1H, J=12.3 Hz), 4.48 (dd, 1H, J=6.5 and 12.3 Hz), 3.84 (s, 3H), 1.74 (s, 6H), 1.68 (s, 3H), 1.60 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 183.31, 182.61, 170.61, 170.02, 169.66, 169.27, 150.79, 136.81, 135.75, 135.23, 134.51, 133.93, 133.78, 128.32, 127.83, 127.36, 126.67, 113.40, 70.61, 69.97, 69.50, 69.33, 61.67, 37.65, 21.01, 20.78. Anal. Calcd for $C_{35}H_{33}NO_{13}$: C, 62.22; H, 4.92; N, 2.07. Found: C, 61.85; H, 4.98; N, 2.19.

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15. Crystal data for compound **23a**: monoclinic, space group $P2_1$, a=13.137(2), b=7.9233(8), c=15.190(2) Å, V=1580.9(4) Å^3, Z=2, d_{calcd}=1.382 Mg/m^3, θ range for data collection=3.00–25.02°, index ranges=$-15\leq h\leq 15$, $-9\leq k\leq 9$, $-14\leq l\leq 17$, (Mo Kα)=0.171 mm^{-1}. For a total of 5779 collected reflections, 4481 were independent reflections [R_{int}=0.0755]. The final R indices were R_1=0.0697, wR_2=0.1330 [$F^2>2\sigma(F^2)$], R_1=0.1312, wR_2=0.1624 (all data). The final difference electron density map contains maximum and minimum peak heights of 0.303 and −0.387 e/Å^3. These data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 146528. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB12 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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Synthesis of coumarins by Pt-catalyzed hydroarylation of propiolic acids with phenols

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Abstract—Synthesis of coumarins from phenols and propiolic acids was examined by using a Pt catalyst such as $PtCl_2$/AgOTf, K_2PtCl_4/AgOTf, and K_2PtCl_4/AgOAc. Propiolic acid reacted even with less reactive phenols in trifluoroacetic acid to give coumarins and dihydrocoumarins. In the case of substituted propiolic acids, phenylpropiolic acid and 2-octynoic acid, the reactions proceeded selectively to afford coumarins in good to high yields.

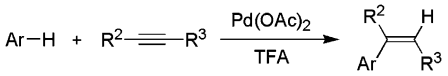
1. Introduction

Coumarin derivatives exist widely in nature, especially in plants, and many of them show a wide range of biological activities.[1,2] To date, many synthetic methods for coumarins have been developed due to their useful properties.[1,3] The representative methods are the Perkin, Pechmann and Knoevenagel reactions. However, there are still limitations such as severe reaction conditions, requirement of a stoichiometric amount of condensing agents, and difficulty of getting the starting materials.

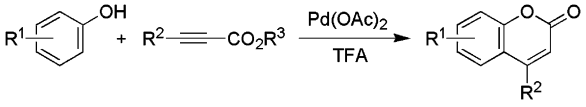
Much effort has been paid to the development of coumarin synthesis through the reaction utilizing a transition metal catalyst.[4–11] However, most of the syntheses require halogenated substrates such as iodophenols and iodoarenes as starting materials for the construction of the coumarin skeleton. These synthetic reactions involve bond cleavage of the C–X bonds by transition metals and produce waste halides. When the atom-economy of the reaction is considered, the use of halogenated substrates is not favorable. If a direct construction of a C–C bond from the C–H bond in simple arenes is possible then this strategy will become a straightforward efficient process.

Trost et al. have developed an atom-economic synthesis of coumarins from the reaction of propiolic acids and phenols in the presence of $Pd_2(dba)_3CHCl_3$ or $Pd(OAc)_2$ catalysts in formic acid.[9] Their reaction did not need any halogenated phenols. Shi and He also reported a direct synthesis of coumarins by the reaction of aryl propiolates with $AuCl_3$/AgOTf catalyst.[10]

We have reported that the hydroarylation of alkynes proceeded by using $Pd(OAc)_2$ or $PtCl_2$/AgOAc catalyst in trifluoroacetic acid (TFA) to give aryl-substituted alkenes (Eq. 1).[12] This direct functionalization of the C–H bonds in arenes was expanded to the synthesis of coumarins by the intra- or intermolecular hydroarylation of propiolates with phenols (Eqs. 2 and 3).[11] Recently, $PtCl_2$/AgOTf and K_2PtCl_4/AgOTf were found to be effective catalysts for hydroarylation of propiolic acids, affording the corresponding cinnamic acids selectively.[13] Especially, the K_2PtCl_4/AgOTf catalyst was the most effective in application to the hydroarylation of propiolic acid with less reactive benzene. The effectiveness of the Pt catalysts encouraged us to investigate the synthesis of coumarin.



(1)



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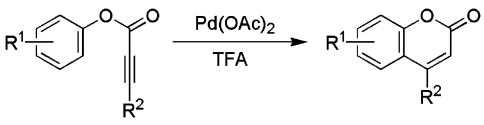

(3)

Keywords: Coumarin; Hydroarylation; Propiolic acid; Platinum catalyst; Silver triflate; C–H bond functionalization; Trifluoroacetic acid.
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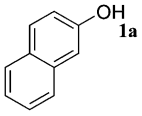
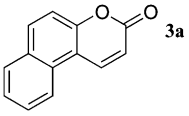
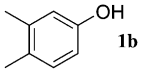
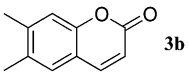
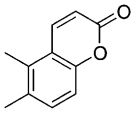
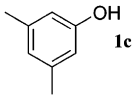
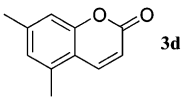
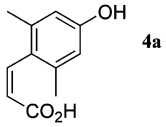
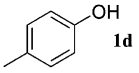
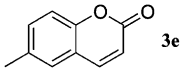
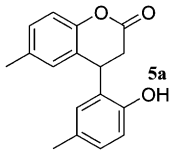
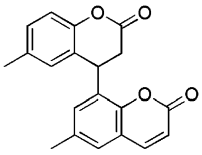
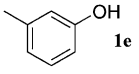
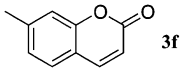
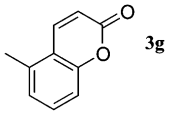
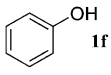
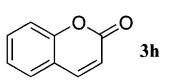
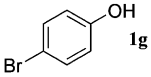
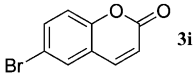
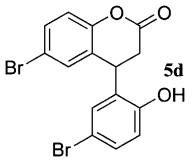
2. Results and discussion

First, we examined the reaction of propiolic acid (**2a**) (R^2=H, R^3=H) or ethyl propiolate (**2b**) (R^2=H, R^3=Et) under similar conditions to the previous reports (Eq. 4, Table 1).[13] The reaction with 2-naphthol (**1a**) proceeded at room temperature to give coumarin **3a** selectively in high yield (entries 1 and 2). 3,4-Dimethylphenol (**1b**) gave 6,7-dimethylcoumarin (**3b**) and 5,6-dimethylcoumarin (**3c**) in 46 and 31% yields, respectively (entry 3). In the case of 3,5-dimethylphenol (**1c**), (2*Z*)-cinnamic acid derivative **4a** was obtained as the major product along with coumarin **3d** (entry 4). *p*-Cresol (**1d**) also reacted to give 6-methylcoumarin (**3e**) and dihydrocoumarin **5a** although the reaction was slow at room temperature and required higher temperature (entries 5–7). Furthermore, a small amount of dihydrocoumarin **5b** was formed, which might be derived from the further reaction of **5a** and **2a**. The reaction using an excess

Table 1. The reaction of propiolic acid (**2a**) or its ethyl ester (**2b**) with phenols **1**[a]

Entry	Phenol	R^3	Cat.[b]	Temp (°C)	Time (h)	Products and yields/%[c]			
1	 **1a**	H	A	rt	25	 **3a**	86[d,e]		
2		H	B	rt	25		86[e]		
3	 **1b**	Et	A	rt	45	 **3b**	46[f]	 **3c**	31[f]
4	 **1c**	Et	A	rt	26	 **3d**	37	 **4a**	50
5	 **1d**	Et	A	50	48	 **3e**	35	 **5a**	44
6		H	C	40	48		51		24
7		H	C	rt	90		51		21
8		H	C	40	12		27[g]	 **5b**	15[g]
9	 **1e**	H	C	rt	25	 **3f**	18[f,h]	 **3g**	17[f,h]
10	 **1f**	H	C	40	48	 **3h**	33		
11	 **1g**	H	C	40	90	 **3i**	7	 **5d**	34

[a] Reaction conditions: phenol **1** (4 mmol), **2a** or **2b** (2 mmol), catalyst, and TFA (1 mL).
[b] Catalyst A: $PtCl_2$ (0.05 mmol) and AgOTf (0.10 mmol). B: K_2PtCl_4 (0.05 mmol) and AgOTf (0.10 mmol). C: K_2PtCl_4 (0.02 mmol) and AgOTf (0.08 mmol).
[c] Isolated yields based on **2**.
[d] Compound **1a** (3 mmol) was used.
[e] CH_2Cl_2 (0.75 mL) was added.
[f] The products were obtained as a mixture of the isomeric coumarins. The product ratios were determined by ^1H NMR.
[g] Compound **1d** (2 mmol) and **2a** (3 mmol) were used.
[h] Compound **5c** was also isolated in 27% yield.

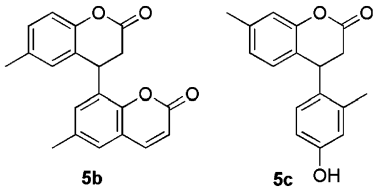
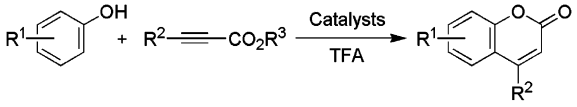


Figure 1.

amount of **2a** was carried out to improve the selectivity of **3e**, resulting in the decrease in the yield of **3e** and the formation of **5b** (entry 8). In contrast to **1d**, the reaction of 3-methylphenol (**1e**) proceeded smoothly at room temperature to give a mixture of 7-methylcoumarin (**3f**) and 5-methylcoumarin (**3g**) along with dihydrocoumarin **5c** (entry 9). The yields were low although most of **2a** was consumed after the reaction. The low yields are attributed to the low selectivity toward the formation of coumarin **3**, similar to the reaction of **1d**. Unsubstituted, simple phenol **1f** reacted to give coumarin (**3h**) in 33% yield (entry 10). Interestingly, 4-bromophenol (**1g**) also participated in this reaction to afford dihydrocoumarin **5d** and 6-bromocoumarin (**3i**) in 34 and 7% yields, respectively (entry 11, Fig. 1).

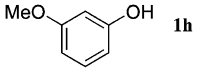
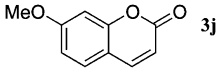
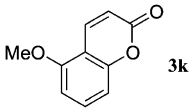
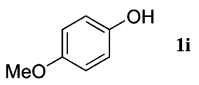
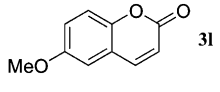
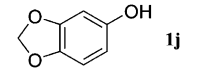
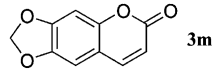
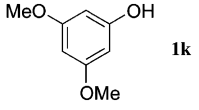
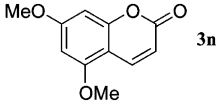


(4)

Next, alkoxyphenols having a strong electron-donating group were examined (Table 2). The reaction of 3-methoxyphenol (**1h**) proceeded at room temperature to give 7-methoxycoumarin (**3j**) and 5-methoxycoumarin (**3k**) in 38 and 10% yields, respectively (entry 1). The yields were not sufficient although most of **2a** used was consumed. ^{1}H NMR analysis of the reaction mixture indicated the formation of dihydrocoumarins. Therefore, further investigation was carried out to improve the selectivity. However, using an excess amount of **2a** or use of AgOAc did not improve the yields (entries 2–5). The reaction of 4-methoxyphenol (**1i**) was slower than that of **1h** (entry 6). The low reactivity of **1i** is in accord with the general concept that a methoxy group activates *ortho* and *para* positions but deactivates the *meta* position for electrophilic aromatic substitution. Sesamol (**1j**) also gave coumarin **3m** (entry 7). Interestingly, the reaction of 3,5-dimethoxyphenol (**1k**) proceeded selectively to afford coumarin **3n** in high yield while **1h**, **1i**, and **1j** gave the corresponding coumarins in low yields (entries 8 and 9).

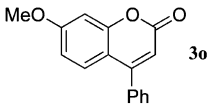
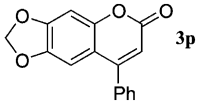
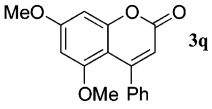
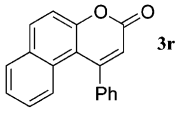
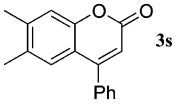
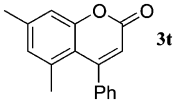
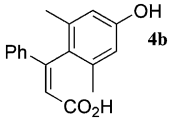
Next, we examined the reaction of the substituted propiolic acids. In contrast to **2a** and **2b**, the reaction of substituted propiolic acids proceeded selectively to give the corresponding coumarins in good to high yields. Table 3 shows the results of the reaction of phenylpropiolic acid (**2c**) (R^2=Ph, R^3=H). In contrast to **2a**, the reaction of **1h** proceeded selectively to give coumarin **3o** in high yield (entries 1–3). Furthermore, higher yield was obtained when the reaction was carried out at room temperature instead of 40 °C. Compound **1j** also gave coumarin **3p** in high yield (entries 4 and 5). 3,5-Dimethoxyphenol (**1k**) gave coumarin **3q** in good yield although the yield was a little bit lower than those of **1h** and **1j** (entry 6). The lower yield of **3q** is possibly attributed to the formation of **7** from the further reaction of coumarin **3q** with **2c**. The formation of **7** analog has been observed in Pd-catalyzed coumarin synthesis.[11b] The reaction of **1a** gave coumarin **3r** in high yield (entries 7 and 8). The reaction of **1b** at 40 °C gave coumarin **3s** in moderate yield because of the lower reactivity of **1b** (entry 9). The reaction of **1c** gave coumarin **3t** and cinnamate **4b**, being similar to the reaction of **2a** (entry 10, Fig. 2).

Table 2. The reaction of propiolic acid (**2a**) with alkoxyphenols[a]

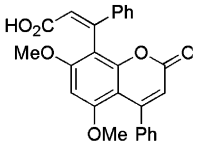
Entry	Phenol	Time (h)	Products and yields %[b]			
1	 **1h**	15	 **3j**	38	 **3k**	10
2		18		28[c]		8[c]
3		11		28[d]		9[d]
4		11		34[d,e]		8[d,e]
5		15		34[f]		11[f]
6	 **1i**	72	 **3l**	22[g]		
7	 **1j**	45	 **3m**	38[g]		
8	 **1k**	15	 **3n**	77[g]		
9		15		81[f,g]		

[a] Reaction conditions: phenol **1** (4 mmol), **2a** (2 mmol), K_2PtCl_4 (0.02 mmol), AgOTf (0.08 mmol), and TFA (1 mL) at rt.
[b] Isolated yields based on **2a**.
[c] Compound **1h** (2 mmol) and **2a** (3 mmol) were used.
[d] Compound **1h** (2 mmol) and **2a** (2.4 mmol) were used.
[e] CH_2Cl_2 (1 mL) was added.
[f] AgOAc (0.08 mmol) was used instead of AgOTf.
[g] CH_2Cl_2 (0.5 mL) was added.

Table 3. The reaction of phenylpropiolic acid (**2c**) with phenol **1**[a]

Entry	Phenol	Cat.[b]	Temp (°C)	Time (h)	Products and yields %[c]		
1	**1h**	B	40	40	 **3o**	71[d]	
2	**1h**	B	rt	40		81	
3	**1h**	C	rt	45		82	
4	**1j**	B	rt	40	 **3p**	76	
5	**1j**	C	rt	45		77	
6	**1k**	C	rt	45	 **3q**	69	
7	**1a**	B	rt	40	 **3r**	84[e]	
8	**1a**	C	rt	45		84	
9	**1b**	C	40	45	 **3s**	58[d]	
10	**1c**	C	40	45	 **3t**	50[d]	 **4b** 29[d]

[a] Reaction conditions: phenol **1** (4 mmol), **2c** (2 mmol), catalyst, TFA (1 mL), and CH_2Cl_2 (0.5 mL).
[b] Catalysts B: K_2PtCl_4 (0.05 mmol) and AgOTf (0.10 mmol). C: K_2PtCl_4 (0.02 mmol) and AgOTf (0.08 mmol).
[c] Isolated yields based on **2c**.
[d] $Cl(CH_2)_2Cl$ (0.5 mL) was added instead of CH_2Cl_2.
[e] $Cl(CH_2)_2Cl$ (1 mL) was added instead of CH_2Cl_2.



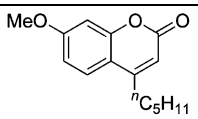
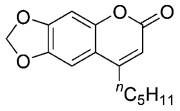
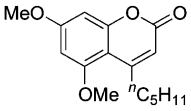
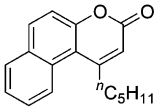
7

Figure 2.

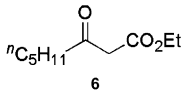
2-Octynoic acid (**2d**) ($R^2={}^nC_5H_{11}$, R^3=H) also reacted with phenols to give the coumarins although the reactivity of **2d** seemed to be lower than that of **2c** (Table 4). The reaction of **1h** gave coumarin **3u** in high yield even at room temperature (entries 1–3). In the case of **1j**, coumarin **3v** was obtained in very low yield when the reaction was carried out at room temperature (entry 4). The elevation of temperature to 40 °C increased the yield but it was not sufficient (entry 5). The ^{1}H NMR analysis of the reaction mixture revealed that the methylenedioxy moiety of **1j** was not present probably because the acetal moiety was hydrolyzed by a strong acid derived from AgOTf. Actually, coumarin **3v** was obtained in high yield when AgOAc was used as co-catalyst instead of AgOTf (entry 6). The reaction of **1k** with ethyl 2-octynoate (**2e**) ($R^2={}^nC_5H_{11}$, R^3=Et) also gave coumarin **3w** in high yield (entry 7). The reaction of **1a** resulted in low yield of coumarin **3x** in contrast to the reaction of **1a** with **2a** or **2c**, indicating that reactivity of **2d** and **2e** was lower than that of **2a**, **2b**, and **2c** (entries 8–13). The reaction also gave ethyl 3-oxooctanoate (**6**) derived from the hydration of **2e** (entry 8). Elongation of the reaction time did not improve the yield (entry 9). The yield of **3x** was somewhat improved when **2d** was used instead of **2e** (entry 10). In this case, the formation of 2-heptanone derived from hydration and decarboxylation of **2d** was observed. The elevation of temperature and the use of AgOAc did not increase the yield (entries 11–13). This result suggests that the coumarin formation competes with the decomposition of **2d** and **2e** (Fig. 3).

The reaction is thought to proceed via the hydroarylation of propiolic acids with phenols, which affords *ortho*-hydroxy substituted cinnamic acids (**A**) followed by the intramolecular esterification of intermediate **A** as depicted in Scheme 1. A similar mechanism has been proposed for Pd(II)-catalyzed reaction of propiolates with phenols giving coumarins.[11b] A very recent report by Tunge and Foresee suggested that the hydroarylation proceeds via aromatic electrophilic substitution, not C–H bond activation.[14] As regards the formation of hydroxyphenyl-substituted dihydrocoumarins **5** in the reaction of **2a**, it was expected that the dihydrocoumarins were formed by the further reaction of coumarins with phenols. However, the reaction of **3e** with **1d** under reaction conditions did not afford dihydrocoumarin **5a**, resulting in the recovery of **3e** and **1d**. This result shows that **3e** formed in the reaction is stable under the reaction conditions. It has been reported that cinnamate derivatives react with phenols in TFA via the formation of carbocation intermediates, affording dihydrocoumarins.[15] A possible route is that dihydrocoumarin **5** is generated by

Table 4. The reaction of 2-octynoic acid (**2d**) or its ethyl ester (**2e**) with phenols **1**[a]

Entry	Phenol	R^3	Cat.[b]	Temp (°C)	Time (h)	Products and yields %[c]	
1	**1h**	H	A	40	45	 **3u**	93
2	**1h**	H	C	40	45		83
3	**1h**	H	C	rt	45		83
4	**1j**	H	C	rt	45	 **3v**	8[d]
5	**1j**	H	C	40	45		41[d]
6	**1j**	H	D	40	45		72[d]
7	**1k**	Et	C	rt	45	 **3w**	87[d]
8	**1a**	Et	C	40	45		13[e]
9	**1a**	Et	C	40	77		16[f]
10	**1a**	H	C	40	45	 **3x**	28[g,h]
11	**1a**	H	D	40	45		28[g]
12	**1a**	H	C	50	45		26[g]
13	**1a**	H	C	60	45		22[g]

[a] Reaction conditions: phenol **1** (4 mmol), **2d** or **2e** (2 mmol), catalyst, and TFA (1 mL) for 45 h.
[b] Catalysts A: $PtCl_2$ (0.05 mmol) and AgOTf (0.10 mmol). C: K_2PtCl_4 (0.02 mmol) and AgOTf (0.08 mmol). D: K_2PtCl_4 (0.02 mmol) and AgOAc (0.08 mmol).
[c] Isolated yields based on **2**.
[d] CH_2Cl_2 (0.5 mL) was added.
[e] CH_2Cl_2 (0.85 mL) was added. Ethyl 3-oxooctanate (**6**) was isolated in 47% yield.
[f] CH_2Cl_2 (1 mL) was added. Compound **6** was isolated in 55% yield.
[g] Compound **1a** (3 mmol) was used. $Cl(CH_2)_2Cl$ (0.5 mL) was added.
[h] 2-Heptanone was formed in 39% GC yield.



6

Figure 3.

the reaction of intermediate **A** with phenols prior to the cyclization of **A**.

In conclusion, we have demonstrated the synthesis of coumarins from phenols and propiolic acids by using Pt catalysts such as $PtCl_2$/AgOTf, K_2PtCl_4/AgOTf, and K_2PtCl_4/AgOAc. The reaction of propiolic acid and its ethyl ester (**2a** and **2b**) proceeded to give coumarin and dihydrocoumarin even in the reaction with less reactive, non-activated phenols. In the cases of substituted propiolic acids **2c**, **2d**, and **2e**, the reactions proceeded selectively to afford coumarins **3** in good to high yields.

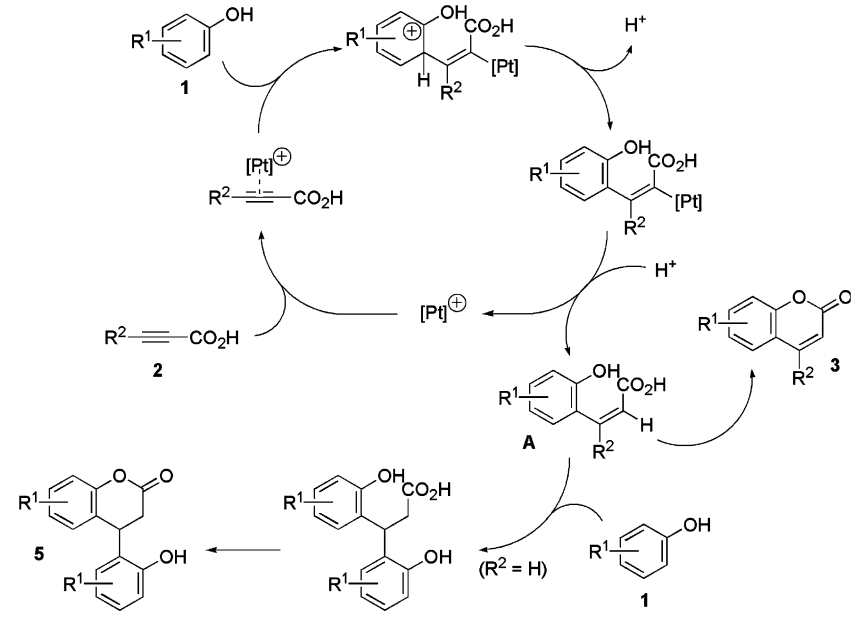
3. Experimental

3.1. General

All solvents and reagents were commercially available and used as received without further purification. All the reactions were conducted in a dry Pyrex tube with a rubber septum. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-AL 300 FT-NMR using tetramethylsilane (TMS) as internal standard. Melting points were measured with YANACO micro melting apparatus and are uncorrected. Mass spectra were performed on a Shimadzu GC/MS 5020A. Elemental analyses were performed by the Service Centre of the Elementary Analysis of Organic Compounds, Faculty of Science, Kyushu University.

3.2. The procedure for the synthesis of coumarins by the Pt-catalyzed hydroarylation of propiolic acids with phenols. Typical example: the reaction of 3-methoxyphenol (1h) and phenylpropiolic acid (2c) by using K_2PtCl_2/AgOTf catalyst (Table 3, entry 3)

After a mixture of K_2PtCl_2 (0.02 mmol), AgOTf (0.08 mmol), and trifluoroacetic acid (TFA) (1 mL) was stirred at room temperature for 1 h, **1h** (4 mmol) and **2c** (2 mmol) was added



Scheme 1. The plausible mechanism of the reaction.

to the mixture. The mixture was continuously stirred at a room temperature (27–32 °C) for 45 h. After the reaction, the mixture was poured into water (20 mL), neutralized by $NaHCO_3$, and extracted with diethyl ether (20 mL×4). The ethereal layer was washed with 2 N NaOH aqueous solution (10 mL×3), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a mixture of ethyl acetate and hexane as eluent to give 4-phenyl-7-methoxycoumarin (**3o**) in 82%.

In some reactions, cinnamic acid **4** and dihydrocoumarin **5** were obtained from the water layer. The procedure is as follows: the combined water layer was acidified by concd HCl aqueous solution (ca. 36%) on ice/water bath and extracted with CH_2Cl_2 (20 mL×3). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel.

3.2.1. 3*H*-Naphtho[2,1-*b*]pyran-3-one (3a).[9b,11d,16] Pink crystals. Mp 117–118 °C (AcOEt/hexane).

3.2.2. 6,7-Dimethylcoumarin (3b).[17,18] This compound was partially isolated from the mixture of **3b** and **3c**. Colorless crystals. Mp 146–149 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 6.33 (d, J=9.6 Hz, 1H, vinyl), 7.11 (s, 1H, aryl), 7.21 (s, 1H, aryl), 7.62 (d, J=9.6 Hz, 1H, vinyl). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.04, 20.21, 115.32, 116.54, 117.28, 127.83, 133.07, 141.81, 143.23, 152.39, 161.22.

3.2.3. 5,6-Dimethylcoumarin (3c).[17] This compound was obtained as a mixture of **3b** and **3c**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.40 (d, J=9.9 Hz, 1H, vinyl), 7.09 (d, J=8.4 Hz, 1H, aryl), 7.31 (d, J=8.4 Hz, 1H, aryl), 7.99 (d, J=9.9 Hz, 1H, vinyl).

3.2.4. 5,7-Dimethylcoumarin (3d).[11d,18,19] Colorless crystals. Mp 133–135 °C (CH_2Cl_2/hexane).

3.2.5. 6-Methylcoumarin (3e).[16] Colorless crystals. Mp 73.5–74 °C (CH_2Cl_2/hexane).

3.2.6. 7-Methycoumarin (3f).[16,17] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3), 6.35 (d, J=9.6 Hz, 1H, vinyl), 7.10 (d, J=7.8 Hz, 1H, aryl), 7.14 (s, 1H, aryl), 7.36 (d, J=7.8 Hz, 1H, aryl), 7.67 (d, J=9.6 Hz, 1H, vinyl). 7-Methylcoumarin (**3f**) and 5-methylcoumarin (**3g**) were obtained as an inseparable mixture.

3.2.7. 5-Methycoumarin (3g).[17] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.53 (s, 3H, CH_3), 6.43 (d, J=9.6 Hz, 1H, vinyl), 7.10 (d, J=8.1 Hz, 1H, aryl), 7.18 (d, J=8.1 Hz, 1H, aryl), 7.40 (app t, J=8.1 Hz, 1H, aryl), 7.92 (d, J=9.6 Hz, 1H, vinyl). 7-Methylcoumarin (**3f**) and 5-methylcoumarin (**3g**) were obtained as an inseparable mixture.

3.2.8. Coumarin (3h).[16,20] Colorless crystals. Mp 67–68 °C (hexane).

3.2.9. 6-Bromocoumarin (3i).[21] Colorless crystals. Mp 160–163 °C (CH_2Cl_2/hexane).

3.2.10. 7-Methoxycoumarin (3j).[9b,11c,d] Colorless crystals. Mp 117–118 °C (CH_2Cl_2/hexane).

3.2.11. 5-Methoxycoumarin (3k).[9b,11d] Colorless crystals. Mp 82–83 °C (CH_2Cl_2/hexane).

3.2.12. 6-Methoxycoumarin (3l).[11c,d] Yellow crystals. Mp 101–103 °C (CH_2Cl_2/hexane).

3.2.13. 6,7-Methylenedioxycoumarin (3m).[9b,11d] Dark crystals. Mp 225–227 °C (CH_2Cl_2/hexane).

3.2.14. 5,7-Dimethoxycoumarin (3n).[11c,d] Colorless crystals. Mp 147.5–149 °C (CH_2Cl_2/hexane).

3.2.15. 7-Methoxy-4-phenylcoumarin (3o).[11c,d] White powder. Mp 111–111.5 °C (CH_2Cl_2/hexane).

3.2.16. 6,7-Methylenedioxy-4-phenylcoumarin (3p).[11c,d] Slightly green crystals. Mp 142–144 °C (CH_2Cl_2/hexane).

3.2.17. 5,7-Dimethoxy-4-phenylcoumarin (3q).[9b,11c,d] Colorless crystals. Mp 169–171 °C (CH_2Cl_2/hexane).

3.2.18. 1-Phenyl-3*H*-naphtho[2,1-*b*]pyran-3-one (3r).[11c,d] Light yellow crystals. Mp 160–161 °C (CH_2Cl_2/hexane).

3.2.19. 6,7-Dimethy-4-phenylcoumarin (3s).[11c,d] Colorless crystals. Mp 144–146 °C (CH_2Cl_2/hexane).

3.2.20. 5,7-Dimethy-4-phenylcoumarin (3t).[11a,c] White powder. Mp 94–95 °C (hexane).

3.2.21. 7-Methoxy-4-*n*-pentylcoumarin (3u).[11d,22] Colorless crystals. Mp 68–69.5 °C (CH_2Cl_2/hexane).

3.2.22. 6,7-Methylenedioxy-4-*n*-pentylcoumarin (3v).[11d] Slightly yellow crystals. Mp 106–107.5 °C (CH_2Cl_2/hexane).

3.2.23. 5,7-Dimethoxy-4-*n*-pentylcoumarin (3w).[11c,d] Colorless crystals. Mp 102–103 °C (CH_2Cl_2/hexane).

3.2.24. 1-*n*-Pentyl-3*H*-naphtho[2,1-*b*]pyran-3-one (3x). Colorless crystals. Mp 98.5–99.5 °C (CH_2Cl_2/hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J=7.2 Hz, 3H, CH_3), 1.37–1.56 (m, 4H, CH_2), 1.77–1.87 (m, 2H, CH_2), 3.23 (t, J=7.7 Hz, 2H, CH_2), 6.41 (s, 1H, vinyl), 7.47 (d, J=9.0 Hz, 1H, aryl), 7.55 (dd, J=6.9, 8.1 Hz, 1H, aryl), 7.65 (ddd, J=1.5, 6.9, 8.7 Hz, 1H, aryl), 7.92 (dd, J=1.5, 8.1 Hz, 1H, aryl), 7.97 (d, J=9.0 Hz, 2H, aryl), 8.47 (d, J=8.7 Hz, 1H, aryl). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 13.90, 22.33, 28.33, 31.51, 37.47, 113.91, 115.19, 117.90, 124.89, 125.26, 127.76, 129.64, 129.66, 131.30, 133.49, 154.78, 158.17, 160.51. MS (EI, *m*/*z*): 266 (M^+). Anal. Calcd for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 80.97; H, 6.85.

3.2.25. (2*Z*)-3-(4-Hydroxy-2,6-dimethylphenyl)propenoic acid (4a). Slightly orange crystals. Mp 170–172 °C (AcOEt/hexane). ^{1}H NMR (300 MHz, CD_3OD): δ 2.12 (s, 6H, CH_3), 6.08 (d, J=12.0 Hz, 1H, vinyl), 6.45 (s, 2H, aryl), 7.01 (d, J=12.0 Hz, 1H, vinyl). ^{13}C NMR (75.5 MHz, CD_3OD): δ 20.53, 114.90, 123.88, 128.39, 137.40, 145.21,

157.27, 169.38. MS (EI, *m*/*z*): 192 (M^+). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.58; H, 6.31.

3.2.26. (2*Z*)-3-(4-Hydroxy-2,6-dimethylphenyl)cinnamic acid (4b). Light yellow crystals. Mp 219–221 °C (AcOEt/hexane). ^{1}H NMR (300 MHz, CD_3OD): δ 1.97 (s, 6H, CH_3), 6.53 (s, 2H, aryl), 6.64 (s, 1H, vinyl), 7.32 (app s, 5H, phenyl). ^{13}C NMR (75.5 MHz, CD_3OD): δ 20.20, 115.08, 119.26, 128.00, 129.73, 130.56, 130.86, 137.36, 140.26, 156.35, 157.37, 169.35. MS (EI, *m*/*z*): 268 (M^+). Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 75.96; H, 6.00. The stereochemistry of **4b** was determined by NOE experiments.

3.2.27. 4-(2-Hydroxy-5-methylphenyl)-6-methyl-3,4-dihydrocoumarin (5a). Colorless crystals. Mp 166–168 °C (CH_2Cl_2/hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H, CH_3), 2.26 (s, 6H, CH_3), 2.95 (dd, *J*=6.6, 16.1 Hz, 1H, $COCH_2$), 3.19 (dd, *J*=5.7, 16.1 Hz, 1H, $COCH_2$), 4.59 (dd, *J*=5.7, 6.6 Hz, 1H, CH), 6.05 (br s, 1H, OH), 6.54 (d, *J*=2.1 Hz, 1H, aryl), 6.61 (d, *J*=8.1 Hz, 1H, aryl), 6.85 (dd, *J*=2.1, 8.1 Hz, 1H, aryl), 6.86 (d, *J*=2.1 Hz, 1H, aryl), 7.01 (d, *J*=8.1 Hz, 1H, aryl), 7.09 (dd, *J*=2.1, 8.1 Hz, 1H, aryl). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.52, 20.71, 35.17, 35.26, 115.51, 116.66, 124.55, 126.48, 128.76, 129.02, 129.13, 129.97, 134.44, 149.84, 151.03, 169.83, 169.86. MS (EI, *m*/*z*): 268 (M^+). Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 75.87; H, 5.97. The structure of **5a** was determined by NOE experiments.

3.2.28. 6,6′-Dimethyl-3,4-dihydro-[4,8′]bichromenyl-2,2′-dione (5b). Colorless crystals. Mp 244–246 °C (CH_2Cl_2/hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.08 (dd, *J*=6.0, 15.9 Hz, 1H, $COCH_2$), 3.14 (dd, *J*=4.5, 15.9 Hz, 1H, $COCH_2$), 4.98 (dd, *J*=4.5, 6.0 Hz, 1H, CH), 6.45 (d, *J*=9.6 Hz, 1H, vinyl), 6.77 (d, *J*=1.8 Hz, 1H, aryl), 6.92 (d, *J*=1.8 Hz, 1H, aryl), 7.08 (d, *J*=8.1 Hz, 1H, aryl), 7.16 (dd, *J*=1.8, 8.1 Hz, 1H, aryl), 7.19 (d, *J*=1.8 Hz, 1H, aryl), 7.68 (d, *J*=9.6 Hz, 1H, vinyl). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.74, 20.84, 33.69, 35.80, 116.66, 117.05, 118.93, 123.44, 127.34, 128.49, 128.84, 129.81, 131.26, 134.51, 134.72, 143.75, 149.23, 150.19, 160.36, 167.53. MS (EI, *m*/*z*): 320 (M^+). Anal. Calcd for $C_{20}H_{16}O_4$: C, 74.99; H, 5.03. Found: C, 74.74; H, 5.05.

3.2.29. 4-(4-Hydroxy-2-methylphenyl)-7-methyl-3,4-dihydrocoumarin (5c). Colorless crystals. Mp 183–185 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.88 (dd, *J*=7.2, 15.9 Hz, 1H, $COCH_2$), 2.99 (dd, *J*=5.7, 15.9 Hz, 1H, $COCH_2$), 4.51 (dd, *J*=5.7, 7.2 Hz, 1H, CH), 6.48 (dd, *J*=2.1, 8.4 Hz, 1H, aryl), 6.54 (d, *J*=8.4 Hz, 1H, aryl), 6.63 (d, *J*=2.1 Hz, 1H, aryl), 6.78 (d, *J*=7.8 Hz, 1H, aryl), 6.91 (d, *J*=7.8 Hz, 1H, aryl), 7.00 (s, 1H, aryl), 9.26 (s, 1H, OH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 19.15, 20.55, 34.78, 35.82, 113.16, 116.74, 117.39, 123.58, 125.16, 127.46, 127.86, 129.29, 137.02, 138.12, 151.58, 156.08, 167.94. MS (EI, *m*/*z*): 268 (M^+). Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 75.98; H, 6.01. The structure of **5c** was determined by NOE experiments.

3.2.30. 4-(5-Bromo-2-hydroxyphenyl)-6-bromo-3,4-dihydrocoumarin (5d). Colorless crystals. Mp 237.5–238.5 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.04 (dd, *J*=5.7, 16.2 Hz, 1H, $COCH_2$), 3.14 (dd, *J*=6.9, 16.2 Hz, 1H, $COCH_2$), 4.62 (dd, *J*=5.7, 6.9 Hz, 1H, CH), 6.81 (d, *J*=8.4 Hz, 1H, aryl), 6.96 (d, *J*=2.4 Hz, 1H, aryl), 7.12 (d, *J*=8.7 Hz, 1H, aryl), 7.25 (d, *J*=2.1 Hz, 1H, aryl), 7.28 (dd, *J*=2.4, 8.4 Hz, 1H, aryl), 7.50 (dd, *J*=2.1, 8.7 Hz, 1H, aryl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 33.45, 34.79, 110.23, 116.07, 117.65, 118.88, 127.08, 129.45, 130.51, 130.83, 131.26, 131.36, 150.82, 154.41, 167.03. MS (EI, *m*/*z*): 396, 398, 400 (M^+). Anal. Calcd for $C_{15}H_{10}Br_2O_3$: C, 45.26; H, 2.53. Found: C, 45.36; H, 2.46.

3.2.31. Ethyl 3-oxooctanoate (6).[23] Yellow liquid.

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Total synthesis of actinobolin from D-glucose by way of the stereoselective three-component coupling reaction

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Abstract—The total synthesis of (−)-actinobolin **3**, an antipode of the natural product, starting from D-glucose is described. A three-component coupling reaction of functionalized cyclohexenone (+)-**6** derived from D-glucose by way of Ferrier's carbocyclization reaction, with vinyl cuprate and 2-alkoxypropanal **7** effectively constructed the carbon framework of **3** in a highly stereoselective manner. In an aldol process of the three-component coupling reaction, stereochemical control (chelation and Felkin–Anh conditions) was achieved by the choice of the protecting groups of a hydroxy function in 2-hydroxypropanal and the reaction solvents. The formal synthesis of the natural enantiomer, (+)-actinobolin **1**, starting from D-glucose was also accomplished.

1. Introduction

(+)-Actinobolin **1**, isolated from the culture broth of *Streptomyces griceoviridus* by Haskell and Bartz in 1959, has a broad antibacterial spectrum as well as moderate antitumor activity.[1] The substance is a hydrophilic, amphoteric, water soluble base, and it chelates with iron, aluminum, and other metal ions. It has also been reported that actinobolin, which suppresses antibody production, has a therapeutic effect on autoimmune encephalomyelitis[2] and serves to increase the hardness of human enamel.[3] The structure elucidation study revealed that actinobolin has a highly oxygenated bicyclic γ-lactone (tetrahydroisochromane) framework with five contiguous chiral centers including an L-alanine residue (Fig. 1).[4] Later, in 1979, a structurally related natural product, (−)-bactobolin **2** was discovered in *Pseudomonas* BMG13-A7 and found to show more potent activities than actinobolin.[5] Such interesting and challenging structures with potent biological properties have naturally received considerable attention from the synthetic community, and several reports on total syntheses,[6] synthetic approach,[7] and chemical modification[8] of actinobolin and bactobolin have been described. In 1984, Ohno and co-workers reported the first and elegant total synthesis of (+)-actinobolin **1** starting from L-threonine using an intramolecular Diels–Alder reaction as the key reaction.[6a] Second synthesis was accomplished in 1985 by Weinreb and co-workers.[6b] The features of Weinreb's work were shorter synthetic route than Ohno's synthesis and the preparation of common bicyclic olefinic γ-lactone, which could be utilized for the synthesis of both actinobolin and bactobolin. In 1986, Kozikowski and co-workers also completed the synthesis of actinobolin from L-threonine.[6c,d] Most recently, Ward and co-workers carried out the total synthesis in 1993[6e,f] in which they used the novel diastereoselective 6-*endo-trig* radical cyclization of a thiocarbamate derived from D-glucose. On the other hand, *N*-acetylactinobolin was prepared by two groups.[7] Rahman and Fraser-Raid synthesized it in an optically active form via a [4+2] cycloaddition.[7a] Danishefsky and co-workers achieved a synthesis of racemic *N*-acetylactinobolin utilizing a key siloxy Cope rearrangement.[7b]

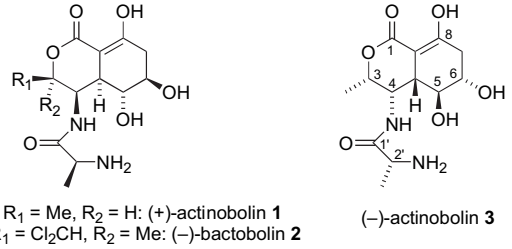

Figure 1. Structures of actinobolin and bactobolin.

We report here the new synthesis of (−)-actinobolin **3**, the antipode of the natural product expected to show some biological activity, starting from D-glucose using stereoselective three-component coupling reactions as the key

Keywords: Actinobolin; Ferrier's carbocyclization; Three-component coupling reaction.
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transformation.[9] The formal synthesis of natural enantiomer **1** from D-glucose is also presented.

2. Results and discussion

2.1. Retrosynthesis

Our retrosynthetic analysis for (−)-actinobolin **3** suggested that bicyclic γ-lactone possessing an azide function **4** would be a promising intermediate for the total synthesis (Fig. 2). Lactone **4** was expected to arise from cyclohexanone derivative **5**, which we planned to prepare by way of a one-pot three-component coupling reaction[10] of cyclohexenone (+)-**6**, a vinyl metal species, and 2-hydroxypropanal derivative **7**. The aldol process in the three-component coupling reaction of an enolate generated from (+)-**6** with (*R*)- or (*S*)-**7** was expected to show stereoselectivities, since both partners in the reaction are chiral, therefore, the reaction would proceed under 'double diastereoselection conditions'.[11] The cyclohexenone (+)-**6**, in turn, was envisioned to be synthesized in optically pure form starting from D-glucose utilizing Ferrier's carbocyclization[12] as the key transformation.

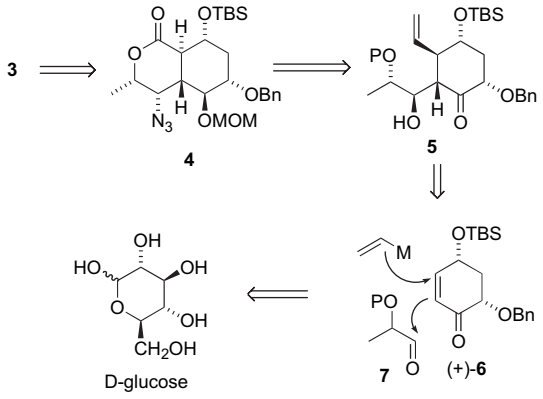
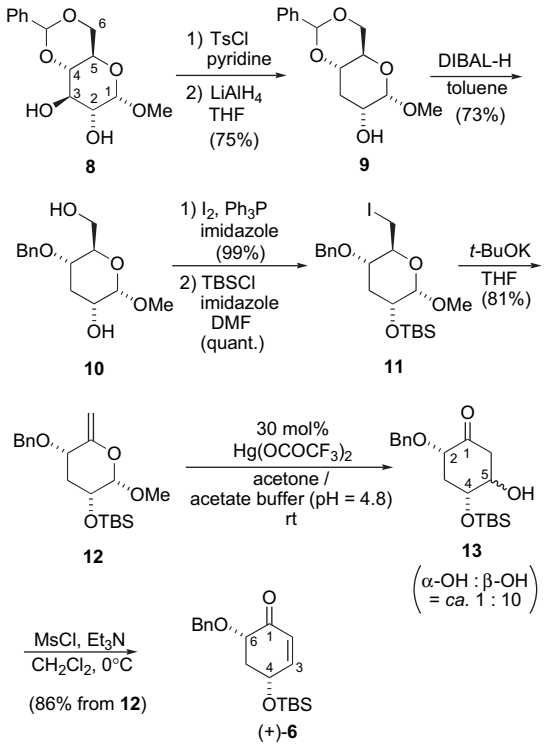


Figure 2. Retrosynthetic analysis of (−)-actinobolin.

2.2. Preparation of subunits

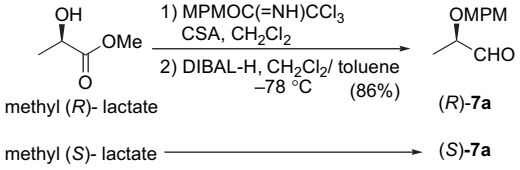
Synthesis of cyclohexenone (+)-**6** commenced from the known 3-deoxy-D-glucose derivative[13] **9** prepared from the commercially available methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **8** in two steps (Scheme 1). Cleavage of a benzylidene acetal in **9** with diisobutylaluminum hydride (DIBAL-H) gave **10**, whose primary hydroxy group was selectively iodinated and the remaining hydroxy function was protected as a TBS ether to afford **11**. Treatment of **11** with *t*-BuOK provided 5-enopyranoside **12**. Catalytic Ferrier's carbocyclization reaction[14] of **12** in acetone–acetate buffer (pH 4.8) gave **13** as a mixture of diastereomer (α-OH/β-OH=ca. 1:10). Use of acetate buffer was effective to suppress the partial hydrolysis of the *O*-TBS group due to the acidity of the catalyst, and greatly improved the cyclization yields. β-Elimination of the hydroxy function cleanly generated cyclohexenone (+)-**6**.

The other requisite subunit, aldehyde **7a**, was synthesized in optically active forms and as a racemate from methyl lactate (Scheme 2). Acid catalyzed *p*-methoxybenzylation of the hydroxy group in methyl (*R*)-lactate,[15] followed by



Scheme 1. Bn=–CH_2Ph, TBS=–$SiMe_2(t\text{-}Bu)$.

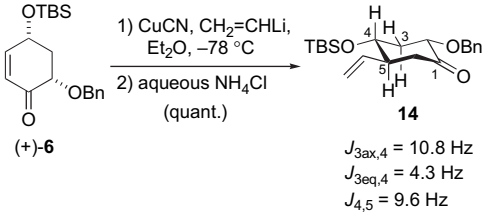
reduction with DIBAL-H afforded (*R*)-**7a** in 86% overall yield. Similar treatment of methyl (*S*)-lactate gave enantiomeric aldehyde (*S*)-**7a**.



Scheme 2. MPM=–$CH_2C_6H_4(p\text{-}OMe)$.

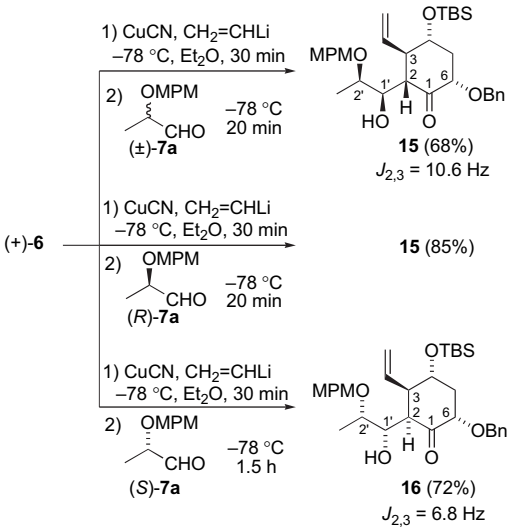
2.3. Three-component coupling reaction

With chiral cyclohexenone (+)-**6** and aldehydes (*R*)-, (*S*)-, and (±)-**7a** in hand, the crucial three-component coupling reaction was investigated using vinyl metal species as the nucleophile (Scheme 3). At first, 1,4-addition of a vinyl group to (+)-**6** was attempted. Treatment of (+)-**6** with higher order vinyl cuprate[16] in Et_2O at −78 °C followed by quenching the reaction with aqueous NH_4Cl, afforded **14** as a single isomer in a quantitative yield. The observed large coupling constants ($J_{3ax,4}$=10.8 Hz, $J_{4,5}$=9.6 Hz) in ^1H NMR spectra of **14** clearly revealed that the vinyl group was introduced from the less crowded β-face.



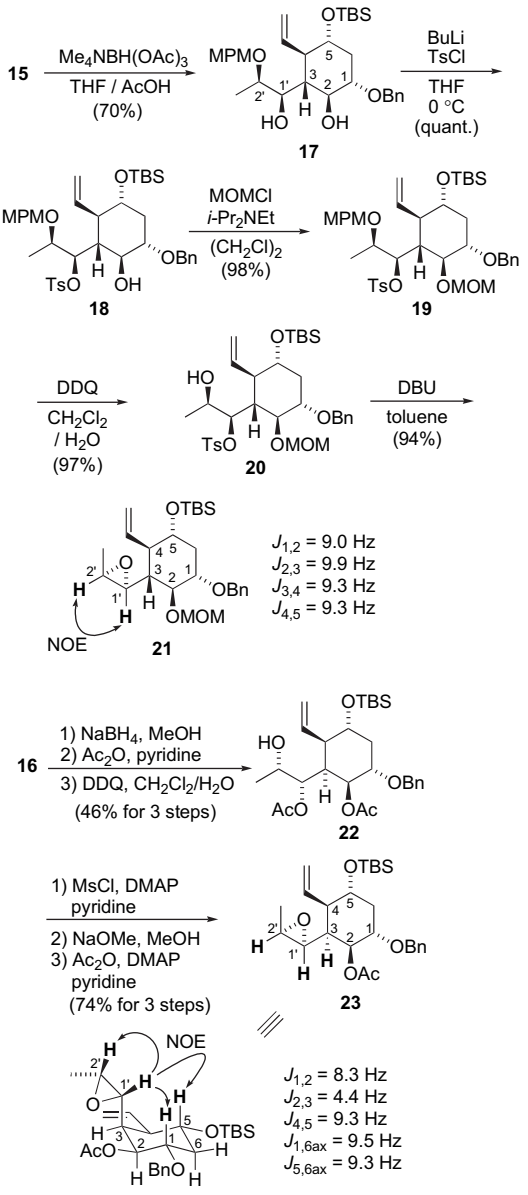
Scheme 3.

Then, the intermediate enolate was trapped with excess amount (6 equiv to (+)-**6**) of racemic aldehyde (±)-**7a** in Et_2O to give **15** as the major isomer in 68% isolated yield after chromatographic separation (Scheme 4). The coupling constant ($J_{2,3}$=10.6 Hz) in ^{1}H NMR spectrum of **15** showed the stereochemical relationship between C-2 and C-3 substituents to be *trans*. The similar reaction employing vinylmagnesium bromide as a nucleophile in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) and CuI in THF gave less satisfactory results to afford **15** in 48% yield. When chiral aldehyde (*R*)-**7a** was employed as an electrophile, the same diastereomer **15** was obtained in 85% yield. Interestingly with another chiral aldehyde (*S*)-**7a**, the aldol process was found to proceed much slower than the reaction with (*R*)-**7a**, and a different diastereomer **16**, in which the stereochemistry of substituents at C-2 and C-3 were *cis* ($J_{2,3}$=6.8 Hz), was formed in 72% yield.



Scheme 4.

The stereochemistry at C-1′ of **15** and **16** was determined by NOE experiments of epoxides **21** and **23** derived from **15** and **16**, respectively (Scheme 5). Treatment of **15** with $Me_4NBH(OAc)_3$[17] at room temperature stereoselectively reduced the carbonyl group to give *syn* diol **17** in 70% yield. The hydroxy group at C-2 in **17** was anticipated to show less reactivity than that at C-1′ due to the steric congestion. Indeed, reaction of **17** with BuLi (3 equiv) at 0 °C, followed by treatment with TsCl (2.8 equiv) generated 1′-OTs derivative **18**, quantitatively. The remaining hydroxy function was then masked as a MOM ether to afford **19** in 92% yield. The MPM group in **19** was deprotected by DDQ to afford **20**, which was then converted into epoxide **21** by treatment with DBU. The observed NOE between H-1′ and H-2′ in **21** revealed the relationship of these protons to be cis, showing that the stereochemistry at C-1′ in **15** should be *R*. On the other hand, reduction of **16** with $NaBH_4$ gave β-alcohol, which was converted into diacetate **22** possessing (1′*S*,2*R*) configuration by O-acetylation and deprotection of the *O*-MPM group. Methanesulfonylation of the hydroxy group in **22** followed by treatment with NaOMe provided an epoxide, whose O-acetylation gave **23**. The observed coupling constants and NOE experiments unambiguously supported the whole structure of **23**, indicating the stereochemistry at C-1′ in **16** to be *S*.



Scheme 5. Ts=–$SO_2C_6H_4$(*p*-Me).

The predominant formation of 1′,2′-*syn* isomers (**15** and **16**) in the three-component coupling reactions suggested that the chelation control (chelation between the alkoxy and aldehyde oxygens in (*R*)-**7a** and (*S*)-**7a**) should be an important factor in the aldol process.[11] Reaction of the intermediate enolate with chelated (*R*)-**7a** would proceed in a 'matched pair' manner (route *a* in Fig. 3) to give **15** smoothly, whereas combination of (*S*)-**7a** and the enolate

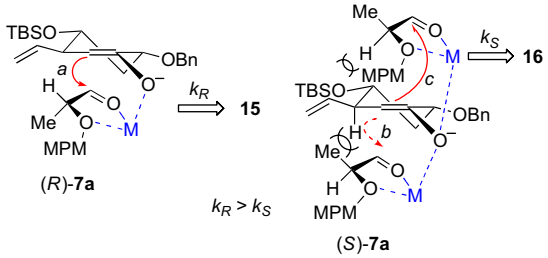


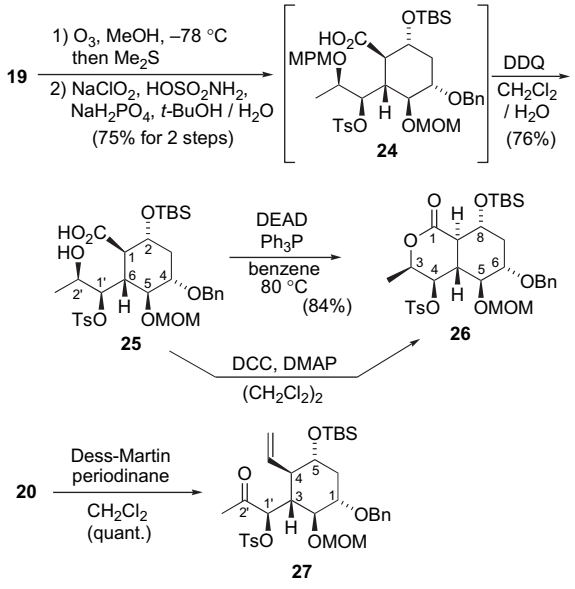
Figure 3.

would be 'mismatched'. The steric repulsion between chelated (*S*)-**7a** and the enolate (routes *b* and *c*) rendered the aldol reaction much sluggish, but gave 2,3-*cis*-adduct **16** stereoselectively via less crowded pathway (route *c*) when (*S*)-**7a** was employed as the electrophile. Apparently, a kinetic resolution had taken place in the aldol process with racemic aldehyde (±)-**7a**, providing **15** as the major product, since the aldol reaction of the intermediate enolate with (*R*)-**7a** was much faster than that with (*S*)-**7a**.

The stereoselective formation of three-component adduct **15** led us to use this compound as the precursor for the synthesis of (−)-actinobolin. Successful conversion of **15** into **19** (Scheme 5) revealed that the required transformations into the desired lactone would be (1) introduction of a nitrogen function at C-1′ via S_N2 fashion and (2) formation of γ-lactone with inversion of the configuration at the C-2′ hydroxy group.

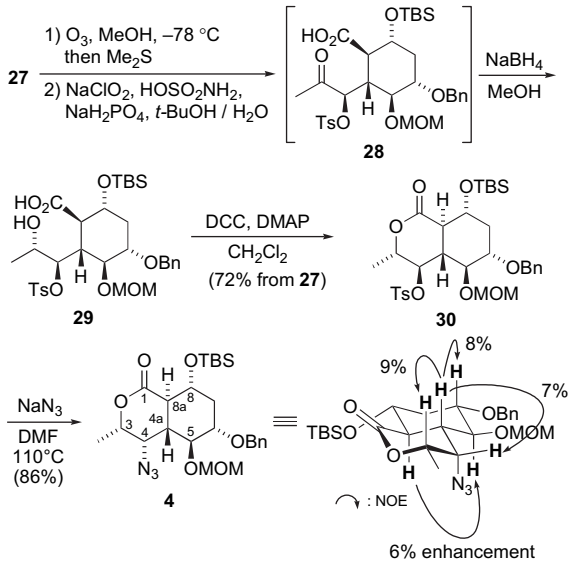
2.4. Total synthesis of (−)-actinobolin

Ozonolysis of a vinyl group in **19** and further oxidation with $NaClO_2$ afforded **24** in 75% yield (Scheme 6). The MPM protecting group in **24** was removed to give hydroxycarboxylic acid **25**. To obtain a γ-lactone with inversion of the hydroxy group, compound **25** was subjected to the intramolecular Mitsunobu reaction[18] (DEAD, PPh_3). However, it was found that the product obtained in 80% yield was γ-lactone **26** with retention of the C-2′ stereochemistry. The structure of **26** was confirmed by the fact that DCC-mediated lactonization of **25** afforded the same lactone **26**. These results revealed that Mitsunobu reagent did not activate the alcohol moiety in **25** probably due to the steric congestion, but activated the carboxylic acid moiety. A similar phenomenon has been documentated,[18b] and mechanistic study on Mitsunobu lactonization of hydroxycarboxylic acid with hindered alcohol system has been reported in detail by DeShong.[19]



Scheme 6. MOM=–CH_2OMe.

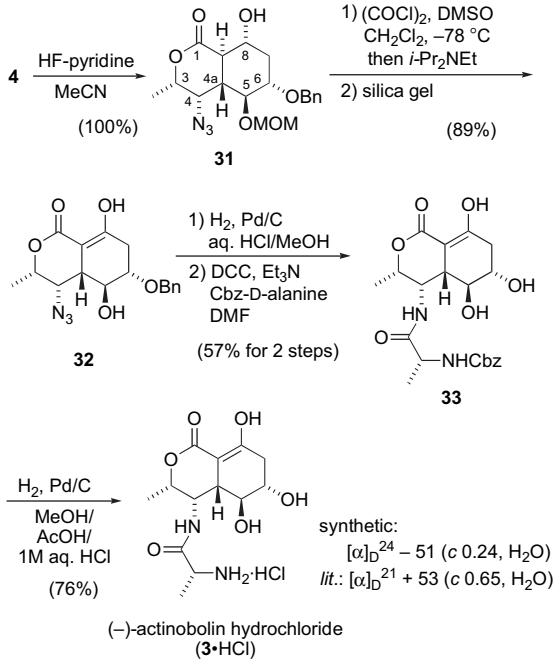
Attempted inversion of a hydroxy function in **20** by intermolecular Mitsunobu reaction proved also fruitless, so we next adopted an oxidation–reduction procedure. Dess–Martin oxidation of **20** provided methyl ketone **27** (Scheme 6). Reduction of **27** with various reducing reagents was carried out, however, the desired inverted alcohol could not be obtained as the major isomer. After several attempts, it was found that the stereoselective reduction successfully proceeded when carboxylic acid **28** was employed as the substrate (Scheme 7). Thus, ozonolysis of **27**, followed by further oxidation gave **28**. Treatment of **28** with $NaBH_4$ provided desired product **29** as the major isomer, and bicyclic compound **30** was obtained in 72% yield from **27** after lactonization followed by separation with silica gel chromatography. Azidolysis of **30** with NaN_3 cleanly provided advanced intermediate **4** in 86% yield, whose structure was confirmed by NOE experiments.



Scheme 7.

With the desired lactone **4** possessing proper functionalities and stereochemistry in hand, the final transformation to **3** was then explored. Deprotection of the *O*-TBS moiety in **4** gave **31**, whose Swern oxidation afforded β-ketoester **32** in 89% yield from **4** (Scheme 8). Interestingly, the methoxymethyl group was unexpectedly removed during the purification process with silica gel chromatography. Hydrogenation of **32** in the presence of Pd catalyst in aqueous HCl reduced the azide function as well as induced removal of the *O*-benzyl group to provide an amine hydrochloride, which, without isolation, was condensed with *N*-benzyloxycarbonyl-D-alanine (Z-D-alanine) by the action of DCC to give protected actinobolin **33** in 57% yield. Finally, removal of the benzyloxycarbonyl group by hydrogenolysis in MeOH–AcOH–aqueous HCl, followed by purification with Sephadex LH-20 (MeOH) furnished (−)-actinobolin hydrochloride (**3**·HCl) in 76% yield. The spectral (^1H and ^{13}C NMR) data of synthetic **3**·HCl were fully identical with those of natural (+)-actinobolin hydrochloride, kindly provided by Dr. Y. Nishimura, and the $[\alpha]_D$ value of the synthetic compound {$[\alpha]_D^{24}$ −51 (*c* 0.24, H_2O): lit.[6c,d] $[\alpha]_D^{21}$ +53 (*c* 0.65, H_2O)} confirmed its unnatural absolute configuration.

Thus, total synthesis of (−)-actinobolin (**3**) starting from D-glucose was accomplished. The high stereoselectivities observed in the three-component coupling reaction under

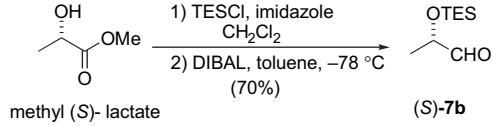


Scheme 8. Cbz=–C(O)OCH$_2$Ph.

the chelation control led us to explore the possibility of the coupling reaction under non-chelation conditions. If a three-component coupling reaction with (*S*)-hydroxypropanal derivative proceeded via Felkin–Anh conditions, the product possessing the proper stereochemistries for the synthesis of actinobolin was anticipated to be obtained as the major isomer, which would bring about the shorter step synthesis of actinobolin.

2.5. Three-component coupling with a TES-aldehyde: a shorter route to (−)-actinobolin

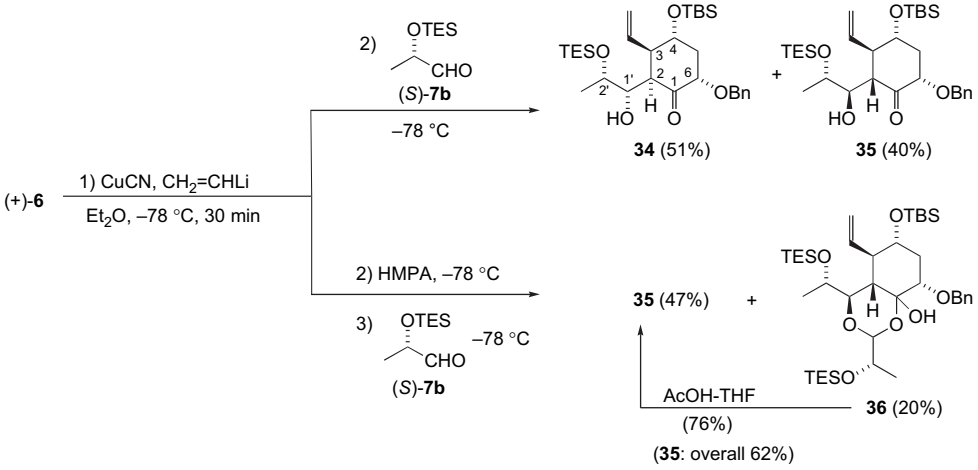
For the aldol reaction under Felkin–Anh conditions, aldehyde **7b** possessing *O*-TES protecting group was employed for three-component coupling reaction. It has been reported that *O*-silyl protecting group does not form chelation between alkoxy and carbonyl oxygens.[20] Aldehyde (*S*)-**7b** was prepared from methyl lactate by protection of the hydroxy group with TESCl, followed by reduction (Scheme 9).[21]



Scheme 9. TES=–SiEt$_3$.

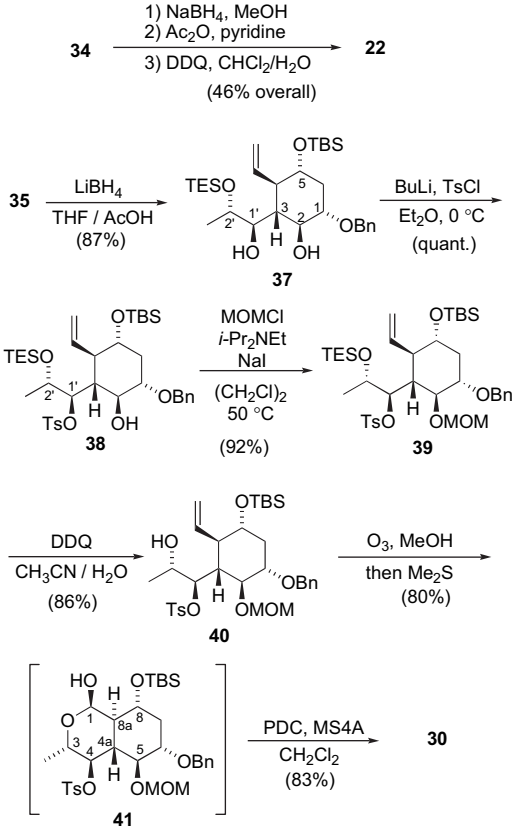
Three-component coupling reaction of (+)-**6**, vinyl cuprate, and aldehyde (*S*)-**7b** at −78 °C resulted in the formation of **34** (chelation product, 51%) and **35** (Felkin–Anh product, 40%) (Scheme 10). Contrary to our expectation, the aldol process provided a 5:4 mixture of chelation and Felkin–Anh products, in spite of using an *O*-TES protecting group. To reduce the degree of the chelation in (*S*)-**7b**, hexamethylphosphoramide (HMPA) was added[22] to the reaction mixture. To our delight, in the presence of HMPA, the three-component coupling reaction with (*S*)-**7b** proceeded stereoselectively to provide Felkin–Anh products **35** and **36** in 47% and 20% isolated yields, respectively, and chelation product **34** could not be isolated in this case. The ketal product **36**, presumably formed by the further reaction of **35** with excess (*S*)-**7b**, was converted into **35** in 76% yield by treatment with acetic acid. Thus, desired compound **35** was obtained in 62% overall yield from (+)-**6** after acetolysis of **36**.

The structure of compound **34** was assigned by comparison of its NMR spectra with those of the structurally related compound **16**, and finally confirmed by its conversion into the known compound **22**, by the similar procedures as described for the preparation of **22** from **16**. On the other hand, the structure of **35** was verified by its transformation into the known synthetic intermediate **30** of actinobolin as shown in Scheme 11. Thus, treatment of **35** with LiBH$_4$, in THF/AcOH at room temperature reduced the carbonyl group stereoselectively to give *syn* diol **37** in 87% yield. Reduction of **35** with NaBH(OAc)$_3$[23] and Me$_4$NBH(OAc)$_3$[17] also gave **37** as a major isomer, but the yields (76% and 25%, respectively) were less satisfactory. Reaction of **37** with BuLi in Et$_2$O at 0 °C, followed by treatment with TsCl generated the 1′-OTs derivative **38**, quantitatively. The remaining



Scheme 10.

hydroxy function was then masked as a MOM ether to afford **39** in 92% yield. The TES protecting group was removed by the action of DDQ[24] to give **40** in 86% yield. Ozonolysis of **40** followed by reductive workup afforded lactol **41** as a single anomer. Oxidation of **41** by PDC provided lactone **30**, which was fully identical with the compound prepared in previous route as shown in Scheme 7. This new synthetic sequence to actinobolin from **35**, which has no necessity to invert the configuration at C-2′ hydroxy group, is three steps shorter than the previous route using *O*-MPM aldehyde (*R*)-**7a**.

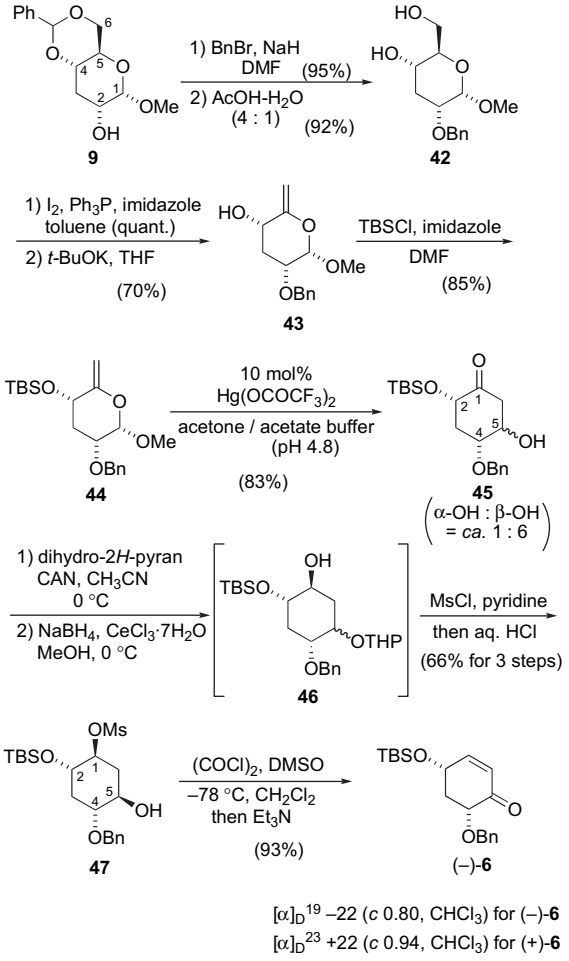


Scheme 11.

2.6. Formal synthesis of (+)-actinobolin

Having established the new synthetic pathways to (−)-actinobolin from D-glucose, we turned our attention to the synthesis of the natural enantiomer **1**, also starting from D-glucose. It is interesting and important issue for synthetic chemistry to prepare both enantiomers from the same starting material. For this purpose, 3-deoxy-D-glucose derivative **9** was again chosen as a building block, and its transformation into the enantiomer of (+)-**6** was investigated.

Benzylation of a hydroxy group in **9**, followed by acetal hydrolysis afforded **42** in 87% overall yield (Scheme 12). Selective iodination of primary alcohol of **42** and subsequent treatment with base gave 5-enopyranoside **43** in 70% yield. Protection of the remaining hydroxy function as a TBS ether afforded **44** in 85% yield. Catalytic Ferrier's carbocyclization of **44** in acetone–acetate buffer generated **45** as a diastereomeric mixture (α:β=ca. 1:6) in 83% yield. Protection of a hydroxy group in **45** as a THP ether and subsequent reduction of the ketone carbonyl gave **46**. Methanesulfonylation of the hydroxy function in **46** followed by acidic workup afforded **47** as the major product in 66% isolated yield. The observed large coupling constants in **47** ($J_{1,2}$=8.8, $J_{4,5}$=9.0 Hz) clearly showed that both OMs and OH groups were in the equatorial positions. Swern oxidation of **47** was accompanied by the β-elimination of the OMs group to furnish (−)-**6** in 93% yield. The spectral data and the absolute value of $[\alpha]_D$ of (−)-**6** {$[\alpha]_D^{19}$ −22 (*c* 0.80, $CHCl_3$)} were fully identical with those of (+)-**6** {$[\alpha]_D^{23}$ +22 (*c* 0.94, $CHCl_3$)}, representing a formal synthesis of (+)-actinobolin.



Scheme 12.

3. Conclusion

In this work, a new synthesis route to both (−)- and (+)-actinobolin starting from D-glucose has been established. This synthesis demonstrated that the methodology involving the three-component coupling reaction on chiral cyclohexenones, derived from D-glucose by way of catalytic Ferrier's carbocyclization, is effective for the chiral and stereoselective synthesis of natural products possessing highly functionalized cyclohexane units. The stereochemical control in three-component coupling reaction by way of the choice of the protecting groups on aldehydes and the reaction

solvents would be an important finding and could be applicable to the stereoselective synthesis of other natural products.

4. Experimental

4.1. General

Melting points were determined on a Mitamura–Riken micro hot stage and were not corrected. Optical rotations were recorded using a sodium lamp (589 nm) with a JASCO DIP-370 instrument with 1 dm tube. Infrared (IR) spectra were measured with a JASCO FT/IR-200 spectrometer. ^{1}H NMR spectra were recorded at 300 MHz on a JEOL Lambda 300 or on a Varian MVX-300 spectrometer. Chemical shifts are reported as δ values in parts per million relative to tetramethylsilane (δ=0) or chloroform (δ=7.26). Coupling constants (J) are reported in Hertz. Abbreviations used are br (broad peak), s (singlet), d (doublet), t (triplet), q (quartet), and m (complex multiplet). ^{13}C NMR spectra were recorded at 75 MHz on the JEOL Lambda 300 spectrometer. Chemical shifts are reported as d values in parts per million relative to chloroform-d (δ=77.00) or methanol-d_4 (δ=49.00) as internal references. Mass spectra are measured by a JEOL GC Mate spectrometer with EI (70 eV) or FAB mode. Organic extracts were dried over solid anhydrous Na_2SO_4 and concentrated below 40 °C under reduced pressure. Column chromatography was carried out with silica gel (Merck Kieselgel 60 F_{254}; 230–400 mesh) or alumina powder (WAKO alumina, activated; 300 mesh) for purification. Preparative TLC (PLC) was performed with Merck PLC plate (Kieselgel 60 F_{254}, 0.5 mm thickness).

4.2. Total synthesis of (−)-actinobolin

4.2.1. Methyl 4-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranoside (10). To a solution of methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside[13] **9** (1.57 g, 5.90 mmol) in toluene (10 mL) under Ar at 0 °C was slowly added 1.01 mol/L solution of DIBAL-H in toluene (23.4 mL, 23.6 mmol). After being stirred at room temperature for 6 h, the reaction mixture was quenched with water, and the products were extracted with EtOAc. The organic layer was washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was recrystallized from EtOAc/hexane (2:5, 28 mL), to afford **10** (1.15 g, 73%) as colorless crystals: R_f 0.12 (EtOAc/toluene=1:1); mp 130.0–130.5 °C; $[\alpha]_D^{20}$ +184 (c 0.95, $CHCl_3$); IR (KBr disk) 3240, 2900, 1120, 1095, 1075, 1055, 1030, 1000, 715 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.40–7.24 (5H, m), 4.65 (1H, d, J=11.6 Hz), 4.64 (1H, d, J=3.5 Hz), 4.47 (1H, d, J=11.6 Hz), 3.83 (1H, dd, J=3.3, 11.5 Hz), 3.73 (1H, dd, J=4.4, 11.5 Hz), 3.63 (1H, ddd, J=3.5, 4.3, 11.7 Hz), 3.59 (1H, ddd, J=3.3, 4.4, 9.8 Hz), 3.45 (1H, ddd, J=4.6, 9.8, 11.3 Hz), 3.44 (3H, s), 2.38 (1H, ddd, J=4.3, 4.6, 11.5 Hz), 1.68 (1H, ddd, J=11.3, 11.5, 11.7 Hz); ^{13}C NMR (75 MHz) δ 137.8, 128.5, 127.8, 127.7, 98.4, 71.9, 70.9, 70.7, 67.3, 62.2, 55.0, 33.3; LRMS (EI) m/z 268 (M$^+$, 14.5%), 236 (53), 146 (100); HRMS (EI) m/z calcd for $C_{14}H_{20}O_5$ (M$^+$) 268.1311, found 268.1310. Anal. Calcd for $C_{14}H_{20}O_5$: C, 62.67; H, 7.51. Found: C, 62.47; H, 7.21.

4.2.2. Methyl 4-*O*-benzyl-2-*O*-(*tert*-butyldimethylsilyl)-3,6-dideoxy-6-iodo-α-D-*ribo*-hexopyranoside (11). To a solution of **10** (1.04 g, 3.88 mmol) in toluene (25 mL) were added PPh_3 (1.63 g, 6.22 mmol), imidazole (0.840 g, 12.3 mmol), and iodine (1.97 g, 7.76 mmol), and the reaction mixture was stirred at room temperature for 28 h. The reaction mixture was diluted with EtOAc, and washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 60 g, EtOAc/toluene=1:5) to give methyl 4-*O*-benzyl-3,6-dideoxy-6-iodo-α-D-*ribo*-hexopyranoside (1.16 g, 99%) as a colorless syrup: R_f 0.55 (EtOAc/toluene=1:1); $[\alpha]_D^{22}$ +124 (c 0.85, $CHCl_3$); IR (neat) 3440, 2940, 1600, 1455, 1030 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.40–7.27 (5H, m), 4.67 (1H, s), 4.65 and 4.47 (each 1H, 2d, J=11.2 Hz), 3.68 (1H, ddd, J=4.9, 10.5, 11.5 Hz), 3.56 (1H, dd, J=2.0, 10.5 Hz), 3.49 (3H, s), 3.41 (1H, ddd, J=2.0, 6.8, 9.4 Hz), 3.30 (1H, dd, J=6.8, 10.5 Hz), 3.29 (1H, ddd, J=4.4, 9.4, 11.2 Hz), 2.38 (1H, ddd, J=4.4, 4.9, 11.5 Hz), 1.95 (1H, br d, J=10.5 Hz), 1.68 (1H, ddd, J=11.2, 11.5, 11.5 Hz); ^{13}C NMR (75 MHz) δ 137.7, 128.5, 127.94, 127.88, 98.6, 75.5, 70.8, 70.1, 67.3, 55.3, 33.1, 7.6; LRMS (EI) m/z 378 (M$^+$, 0.8%), 346 (1.4), 219 (37.6), 201 (41.9) 175 (84.6), 117 (100); HRMS (EI) m/z calcd for $C_{14}H_{19}O_4I$, (M$^+$) 378.0328, found 378.0328. Anal. Calcd for $C_{14}H_{19}IO_4$: C, 44.46; H, 5.06. Found: C, 44.65; H, 5.05.

To a solution of the 6-iodide derivative (10.3 g, 27.2 mmol) in DMF (200 mL) were added imidazole (11.1 g, 163 mmol) and TBSCl (6.20 g, 41.1 mmol), and the reaction mixture was stirred at room temperature for 8.5 h. The reaction mixture was diluted with EtOAc, washed with H_2O, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 300 g, EtOAc/toluene=1:20) to give 2-*O*-silyl ether **11** (13.4 g, 100%) as a colorless syrup: R_f 0.81 (EtOAc/toluene=1:8); $[\alpha]_D^{25}$ +89.4 (c 0.93, $CHCl_3$); IR (neat) 2960, 2930, 2860, 1100, 1030, 840 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.27 (5H, m), 4.66 (1H, d, J=11.3 Hz), 4.55 (1H, d, J=3.4 Hz), 4.47 (1H, d, J=11.3 Hz), 3.73 (1H, ddd, J=3.4, 4.1, 11.6 Hz), 3.55 (1H, dd, J=2.1, 10.2 Hz), 3.47 (3H, s), 3.43 (1H, ddd, J=2.1, 6.6, 9.1 Hz), 3.31 (1H, dd, J=6.6, 10.2 Hz), 3.29 (1H, ddd, J=4.6, 9.1, 11.6 Hz), 2.15 (1H, ddd, J=4.1, 4.6, 11.5 Hz), 1.88 (1H, ddd, J=11.5, 11.6, 11.6 Hz), 0.89 (9H, s), 0.08 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.8, 128.5, 128.2, 127.9, 127.8, 127.7, 99.4, 75.9, 70.7, 69.6, 68.5, 55.3, 32.6, 25.8, 18.2, 8.1, −4.6; LRMS (EI) m/z 461 (M$^+$−OMe, 2.7%), 435 (34.3), 329 (22.8), 313 (33.6), 201 (96.9), 91 (100); HRMS (EI) m/z calcd for $C_{19}H_{30}IO_3Si$, (M$^+$−OMe) 461.1009, found 461.1007.

4.2.3. Methyl 4-*O*-benzyl-2-*O*-(*tert*-butyldimethylsilyl)-3,6-dideoxy-α-D-*erythro*-hex-5-enopyranoside (12). To a solution of **11** (13.4 g, 27.2 mmol) in THF (260 mL) was added *t*-BuOK (12.3 g, 81.6 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with EtOAc, washed successively with H_2O and brine, and then dried over Na_2CO_3. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 200 g, EtOAc/hexane=1:30 containing 1 vol % Et_3N) to give **12** (8.0 g, 81%) as a colorless

syrup: R_f 0.68 (EtOAc/hexane=1:5); $[\alpha]_D^{24}$ +42.0 (*c* 0.61, $CHCl_3$); IR (neat) 2960, 2930, 2855, 1660, 1260, 1100, 1030, 840 cm^{-1}; ^1H NMR (300 MHz) δ 7.42–7.24 (5H, m), 4.83 (1H, br s), 4.69 and 4.64 (each 1H, 2d, *J*=12.3 Hz), 4.60 and 4.59 (each 1H, 2br s), 3.94–3.82 (2H, m), 3.47 (3H, s), 2.15–2.05 (1H, m), 2.01 (1H, m), 0.89 (9H, s), 0.08 (6H, s); ^{13}C NMR (75 MHz) δ 154.9, 138.2, 128.4, 127.7, 127.4, 100.8, 95.1, 72.4, 71.2, 68.4, 55.4, 34.2, 25.8, 18.2, −4.6; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{20}H_{32}O_4Si$, $(M+H)^+$ 365.2152, found 365.2148.

4.2.4. A mixture of (2*S*,4*R*,5*R*)-2-benzyloxy-4-(*tert*-butyldimethylsilyloxy)-5-hydroxy-cyclohexen-1-one and its (5*S*)-isomer (13). To a solution of **12** (4.46 g, 12.2 mmol) in acetone (300 mL) and acetate buffer (0.1 mol/L solution, prepared from 0.2 mol/L aqueous sodium acetate and acetic acid, pH 4.8, 150 mL) was added $Hg(OCOCF_3)_2$ (1.56 g, 3.66 mmol), and the mixture was stirred at room temperature for 14 h. The reaction mixture was partially concentrated and then extracted with EtOAc. The organic layer was washed successively with 10% aqueous KI solution and 20% aqueous $Na_2S_2O_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 90 g, EtOAc/hexane=1:5) to give **13** as a diastereomeric mixture (5*S*:5*R*=ca. 1:10, 3.97 g, 93%) as a colorless syrup: R_f 0.51 (EtOAc/hexane=1:5); ^1H NMR (300 MHz, for the major isomer) δ 7.40–7.28 (5H, m), 4.87 and 4.49 (each 1H, 2d, *J*=11.6 Hz), 3.98 (1H, dd, *J*=6.1, 12.7 Hz), 3.75 (1H, ddd, *J*=4.1, 8.3, 10.6 Hz), 3.60 (1H, ddd, *J*=5.1, 8.3, 12.1 Hz), 2.76 (1H, dd, *J*=5.1, 13.8 Hz), 2.51 (1H, br s), 2.39 (1H, dd, *J*=12.1, 13.8 Hz), 2.35 (1H, ddd, *J*=4.1, 6.1, 12.1 Hz), 1.69 (1H, ddd, *J*=10.6, 12.1, 12.7 Hz), 0.90 (9H, s), 0.13 and 0.11 (each 3H, 2s); ^{13}C NMR (75 MHz, for the major isomer) δ 204.9, 137.5, 128.5, 128.0, 127.9, 78.6, 73.8, 73.4, 72.2, 43.8, 36.5, 25.7, 17.9, −4.3, −4.6; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{19}H_{30}O_4Si$, $(M+H)^+$ 351.1992, found 351.2002.

4.2.5. (4*R*,6*S*)-6-Benzyloxy-4-(*tert*-butyldimethylsilyloxy)-2-cyclohexenone [(+)-6]. To a solution of **13** (3.97 g, 11.3 mmol) in CH_2Cl_2 (80 mL) were added MsCl (3.50 mL, 25.2 mmol) and Et_3N (12.6 mL, 90.4 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2.5 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 70 g, EtOAc/hexane=1:6) to give (+)-**6** (3.50 g, 86% from **12**) as a white solid: R_f 0.57 (EtOAc/hexane=1:4); mp 46.5–47.5 °C; $[\alpha]_D^{23}$ +22.0 (*c* 0.94, $CHCl_3$); IR (KBr disk) 2955, 2930, 2860, 1700, 1255, 1100, 1070, 965, 940 cm^{-1}; ^1H NMR (300 MHz) δ 7.45–7.28 (5H, m), 6.77 (1H, ddd, *J*=1.9, 2.1, 10.2 Hz), 5.96 (1H, dd, *J*=2.4, 10.2 Hz), 4.99 and 4.66 (each 1H, 2d, *J*=11.7 Hz), 4.58 (1H, dddd, *J*=2.1, 2.4, 5.1, 10.0 Hz), 3.91 (1H, dd, *J*=4.9, 13.7 Hz), 2.51 (1H, dddd, *J*=1.9, 4.9, 5.1, 11.9 Hz), 2.09 (1H, ddd, *J*=10.0, 11.9, 13.7 Hz), 0.90 (9H, s), 0.12 and 0.11 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 198.2, 153.8, 137.8, 128.4, 127.9, 127.8, 127.3, 76.6, 72.4, 67.7, 40.8, 25.7, 18.0, −4.6, −4.8; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{19}H_{28}O_3Si$, $(M+H)^+$ 333.1886, found 333.1883. Anal. Calcd for $C_{19}H_{28}O_3Si$: C, 68.63; H, 8.49. Found: C, 68.46; H, 8.52.

4.2.6. (2*R*)-2-(4-Methoxybenzyloxy)propanal [(*R*)-7a] and its enantiomer [(*S*)-7a]. To a solution of methyl (*R*)-lactate (1.14 g, 11.0 mmol) in CH_2Cl_2 (20 mL) were added (4-methoxybenzyl)trichloroacetimidate (6.17 g, 21.8 mmol) and CSA (0.50 g, 2.15 mmol) at room temperature, and the reaction mixture was stirred for 15 h. The reaction mixture was quenched with MeOH and the products were extracted with EtOAc. The organic layer was washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (EtOAc/hexane=1:20) to give methyl (*R*)-lactate *p*-methoxybenzyl ether (2.40 g) as a colorless syrup: ^1H NMR (300 MHz) δ 7.29 and 6.88 (each 2H, 2d, *J*=8.6 Hz), 4.62 and 4.39 (each 1H, 2d, *J*=11.4 Hz), 4.05 (1H, q, *J*=6.9 Hz), 3.80 and 3.75 (each 3H, 2s), 1.42 (3H, d, *J*=6.9 Hz); ^{13}C NMR (75 MHz) δ 173.7, 159.3, 129.58, 129.48, 113.7, 73.5, 71.6, 55.2, 51.8, 18.7.

To a solution of methyl (*R*)-lactate *p*-methoxybenzyl ether (2.40 g, 10.7 mmol) in CH_2Cl_2 (50 mL) was added 1.01 mol/L solution of DIBAL-H in toluene (13.7 mL, 13.9 mmol) at −78 °C, and the reaction mixture was stirred for 20 min at −78 °C. The reaction mixture was quenched with water, and the products were extracted with EtOAc. The organic layer was washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (EtOAc/hexane=1:15) to give (*R*)-**7a** (1.82 g, 86% for two steps) as a colorless liquid: $[\alpha]_D^{20}$ +26.0 (*c* 0.93, $CHCl_3$); IR (neat) 2940, 2840, 2480, 1730, 1615, 1515, 1250, 1035 cm^{-1}; ^1H NMR (300 MHz) δ 9.63 (1H, d, *J*=1.7 Hz), 7.29 and 6.89 (each 2H, 2d, *J*=8.6 Hz), 4.58 and 4.53 (each 1H, 2d, *J*=11.5 Hz), 3.87 (1H, qd, *J*=6.8, 1.7 Hz), 3.81 (3H, s), 1.31 (3H, d, *J*=6.8 Hz); ^{13}C NMR (75 MHz,) δ 203.5, 159.5, 129.6, 129.3, 113.9, 79.1, 71.7, 55.2, 15.3; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{11}H_{14}O_3$, $(M+H)^+$ 195.1040, found 195.1021.

The similar treatment of methyl (*S*)-lactate (1.14 g, 11.0 mmol) as described for the preparation of (*R*)-**7a** afforded (2*S*)-2-(4-methoxybenzyloxy)propanal [(*S*)-**7a**] (1.26 g, 60% for two steps) as a colorless liquid: $[\alpha]_D^{20}$ −25.0 (*c* 0.95, $CHCl_3$).

4.2.7. (3*R*,4*R*,6*S*)-6-Benzyloxy-4-(*tert*-butyldimethylsilyloxy)-3-vinylcyclohexan-1-one (14). To a suspension of copper(I) cyanide (CuCN, 18.0 mg, 0.20 mmol) in ether (0.6 mL) at −78 °C was added dropwise vinyllithium (freshly prepared from tetravinyltin and PhLi, according to the procedure reported by Seyferth and Weiner,[16] 1.0 mol/L solution in ether, 0.45 mL, 0.45 mmol) under Ar. After being stirred at −78 °C for 3 min, the mixture was allowed to warm to 0 °C and further stirred at 0 °C for 1 min. The resulting clear solution was cooled to −78 °C, and to this solution was added slowly a solution of enone (+)-**6** (50.0 mg, 0.15 mmol) in ether (0.6 mL) via a cannula. After being stirred at −78 °C for 15 min, the reaction mixture was quenched with saturated aqueous NH_4Cl solution, and the

products were extracted with EtOAc. The organic layer was washed successively with saturated aqueous $NaHCO_3$, brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:10) to give **14** (54.1 mg, 100%) as a colorless syrup: $[\alpha]_D^{23}$ −93.1 (*c* 1.00, $CHCl_3$); IR (KBr disk) 2960, 2930, 2860, 1730, 1260, 1100, 860, 840, 780, 700 cm^{-1}; ^1H NMR (300 MHz) δ 7.42–7.28 (5H, m), 5.77 (1H, ddd, *J*=6.9, 10.5, 17.4 Hz), 5.08 (1H, dd, *J*=0.9, 10.5 Hz), 5.20 (1H, dd, *J*=0.9, 17.4 Hz), 4.87 and 4.49 (each 1H, 2d, *J*=11.9 Hz), 3.98 (1H, dd, *J*=6.3, 12.9 Hz), 3.74 (1H, ddd, *J*=4.3, 9.6, 10.8 Hz), 2.50–2.36 (3H, m), 2.25 (1H, m), 1.85 (1H, ddd, *J*=10.8, 12.3, 12.9 Hz), 0.87 (9H, s), 0.06 and 0.05 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 207.5, 138.8, 137.8, 128.6, 116.1, 78.3, 72.1, 71.7, 50.0, 41.9, 41.6, 25.9, 18.2, −4.2, −4.4; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{21}H_{33}O_3Si$, $(M+H)^+$ 361.2199, found 361.2197.

4.2.8. Three-component coupling reaction with (±)-7a. To a suspension of copper(I) cyanide (CuCN, 112 mg, 1.25 mmol) in ether (3.0 mL) at −78 °C was added dropwise vinyllithium (freshly prepared from tetravinyltin and PhLi, 1.0 mol/L solution in ether, 2.51 mL, 2.51 mmol) under Ar. After being stirred at −78 °C for 10 min, the mixture was allowed to warm to 0 °C and further stirred at 0 °C for 2 min. The resulting clear solution was cooled to −78 °C, and to this solution was added slowly a solution of enone (+)-**6** (209 mg, 0.627 mmol) in ether (1.0 mL) via a cannula. After being stirred at −78 °C for 30 min, a solution of aldehyde (±)-**7a** (740 mg, 3.76 mmol) in ether (1.0 mL) was added to the mixture dropwise via a cannula, and the mixture was stirred for 20 min at −78 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl solution, and the products were extracted with EtOAc. The organic layer was washed successively with saturated aqueous $NaHCO_3$, brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 35 g, EtOAc/hexane=1:8) to give (2*S*,3*R*,4*R*,6*S*)-6-benzyloxy-4-(*tert*-butyldimethylsilyloxy)-2-[(1′*R*,2′*R*)-1′-hydroxy-2′-(4-methoxybenzyloxy)propyl]-3-vinylcyclohexan-1-one (**15**) (228.9 mg, 68%) as a colorless syrup: R_f 0.59 (EtOAc/hexane=1:3); $[\alpha]_D^{23}$ −69.0 (*c* 0.56, $CHCl_3$); IR (neat) 3420, 2960, 2930, 2860, 2360, 1715, 1615 cm^{-1}; ^1H NMR (300 MHz) δ 7.36–7.26 (7H, m), 6.84 (2H, d, *J*=8.8 Hz), 5.40 (1H, ddd, *J*=11.7, 15.0, 17.2 Hz), 5.21 (1H, dd, *J*=1.6, 11.7 Hz), 5.20 (1H, dd, *J*=1.6, 17.2 Hz), 4.67 (1H, d, *J*=11.5 Hz), 4.57 (1H, d, *J*=11.2 Hz), 4.43 (1H, d, *J*=11.5 Hz), 4.31 (1H, d, *J*=11.2 Hz), 3.95 (1H, dd, *J*=6.3, 12.7 Hz), 3.90–3.75 (1H, m), 3.85–3.70 (2H, m), 3.81 (3H, s), 2.68 (1H, ddd, *J*=10.6, 12.4, 15.0 Hz), 2.44 (1H, ddd, *J*=4.6, 6.1, 10.8 Hz), 2.28 (1H, d, *J*=10.6 Hz), 1.85 (1H, ddd, *J*=10.8, 12.6, 12.7 Hz), 1.18 (3H, d, *J*=6.1 Hz), 0.84 (9H, s), 0.03 (6H, s); ^{13}C NMR (75 MHz) δ 210.9, 159.1, 137.5, 130.6, 129.4, 128.5, 127.8, 119.9, 113.7, 113.6, 78.2, 77.3, 73.9, 71.7, 71.1, 70.3, 55.1, 54.6, 48.3, 42.0, 25.7, 17.9, 16.1, −4.4; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{32}H_{47}O_6Si$, $(M+H)^+$ 555.3142, found 555.3151. Anal. Calcd for $C_{32}H_{46}O_6Si$: C, 69.28; H, 8.36. Found: C, 69.36; H, 8.42.

4.2.9. Three-component coupling reaction with (*R*)-7a. The similar treatment of (+)-**6** (600 mg, 1.81 mmol) with copper(I) cyanide (CuCN, 259 mg, 2.89 mmol), vinyllithium (5.80 mmol), and aldehyde (*R*)-**7a** (1.09 g, 5.61 mmol) afforded **15** (855.1 mg, 85%).

4.2.10. Three-component coupling reaction with (*S*)-7a. The similar treatment of (+)-**6** (78.7 mg, 0.237 mmol) with copper(I) cyanide (CuCN, 42.4 mg, 0.474 mmol) and vinyllithium (1.0 mol/L solution in ether, 0.95 mL, 0.95 mmol) as described for the preparation of **15** afforded 1,4-addition intermediate. To an ethereal solution of the enolate (5 mL) at −78 °C was added a solution of aldehyde (*S*)-**7a** (184 mg, 0.948 mmol) in ether (1.0 mL) dropwise via a cannula. After being stirred at −78 °C for 1.5 h, the reaction mixture was quenched and processed similarly as described for the preparation of **15**. Purification by column chromatography (silica gel: 18 g, EtOAc/hexane=1:10) gave (2*R*,3*R*,4*R*,6*S*)-6-benzyloxy-4-(*tert*-butyldimethylsilyloxy)-2-[(1′*S*,2′*S*)-1′-hydroxy-2′-(4-methoxybenzyloxy)propyl]-3-vinylcyclohexan-1-one (**16**) (91.9 mg, 72%) as a white solid: R_f 0.69 (EtOAc/hexane=1:2); mp 118–119 °C; $[\alpha]_D^{19}$ −15.0 (*c* 0.66, $CHCl_3$); IR (neat) 3420, 2930, 2860, 1700, 1255, 1140, 1090, 860, 840 cm^{-1}; ^1H NMR (300 MHz) δ 7.40–7.24 (5H, m), 7.21 and 6.84 (each 2H, 2d, *J*=8.8 Hz), 5.80 (1H, ddd, *J*=9.3, 10.0, 16.7 Hz), 5.13 (1H, dd, *J*=1.7, 10.0 Hz), 5.09 (1H, dd, *J*=1.7, 16.7 Hz), 4.62 and 4.56 (each 1H, 2d, *J*=11.3 Hz), 4.41 (1H, ddd, *J*=4.6, 9.3, 10.1 Hz), 4.29 (1H, dd, *J*=6.1, 12.3 Hz), 4.24 and 4.23 (each 1H, 2d, *J*=11.4 Hz), 3.72–3.68 (1H, m), 3.71 (3H, s), 3.19 (1H, qd, *J*=6.1, 7.6 Hz), 3.00 (1H, br s), 2.69 (1H, dd, *J*=3.2, 6.8 Hz), 2.57 (1H, ddd, *J*=6.8, 9.3, 9.3 Hz), 2.33 (1H, ddd, *J*=4.6, 6.1, 12.4 Hz), 1.74 (1H, ddd, *J*=10.1, 12.3, 12.4 Hz), 1.20 (3H, d, *J*=6.1 Hz), 0.83 (9H, s), 0.04 and 0.02 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 210.0, 159.4, 138.1, 136.9, 129.8, 129.6, 128.3, 127.9, 127.7, 118.6, 113.9, 80.3, 75.8, 72.1, 72.1, 70.3, 68.8, 55.2, 54.6, 53.9, 42.9, 25.8, 18.1, 15.1, −4.2, −4.4; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{32}H_{47}O_6Si$, $(M+H)^+$ 555.3142, found 555.3139.

4.2.11. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*R*)-1′-hydroxy-2′-(4-methoxybenzyloxy)propyl]-4-vinylcyclohexan-2-ol (17). To a solution of **15** (164 mg, 0.296 mmol) in AcOH/THF (2:1, 3.3 mL) was added $Me_4NBH(OAc)_3$ (479 mg, 1.82 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was quenched with saturated aqueous Rochelle salt solution, and the products were extracted with EtOAc. The organic layer was washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 6 g, EtOAc/hexane=1:8) to give **17** (114 mg, 70%) as a white solid: R_f 0.59 (EtOAc/hexane=1:2); $[\alpha]_D^{21}$ −15.0 (*c* 0.91, $CHCl_3$); mp 81–84 °C; IR (neat) 3500, 2960, 2930, 2860, 2360, 1615 cm^{-1}; ^1H NMR (300 MHz) δ 7.36–7.32 (5H, m), 7.26 and 6.86 (each 2H, 2d, *J*=8.7 Hz), 5.28 (1H, ddd, *J*=9.9, 10.3, 16.6 Hz), 5.11 (1H, dd, *J*=2.2, 9.9 Hz), 5.08 (1H, dd, *J*=2.2, 16.6 Hz), 4.68 and 4.53 (each 1H, 2d, *J* =11.7 Hz), 4.58 and 4.35 (each 1H, 2d, *J*=11.0 Hz), 4.00 (1H, qd, *J*=6.1, 8.2 Hz), 3.82–3.76 (1H, m), 3.79 (3H, s), 3.63 (1H, dd, *J*=2.1, 8.2 Hz), 3.32 (1H, ddd, *J*=3.9, 9.8, 10.7 Hz), 3.24 (1H, ddd, *J*=3.9, 9.0, 12.9 Hz), 2.83 (1H, d, *J*=2.0 Hz), 2.73 (1H, d, *J*=2.1 Hz), 2.25 (1H,

ddd, J=9.8, 10.3, 10.7 Hz), 2.19 (1H, ddd, J=3.9, 3.9, 11.8 Hz), 1.39 (1H, ddd, J=10.7, 11.8, 12.9 Hz), 1.32 (1H, m), 1.13 (3H, d, J=6.1 Hz), 0.83 (9H, s), −0.04 (6H, s); ^{13}C NMR (75 MHz) δ 159.2, 139.0, 138.4, 130.6, 129.6, 128.4, 127.7, 119.2, 113.8, 80.0, 78.4, 74.6, 71.6, 71.1, 71.0, 70.7, 55.2, 51.9, 43.0, 37.5, 25.8, 18.0, 15.3, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{32}H_{49}O_6Si$, $(M+H)^+$ 557.3298, found 557.3304. Anal. Calcd for $C_{32}H_{48}O_6Si$: C, 69.03; H, 8.69. Found: C, 69.20; H, 8.63.

4.2.12. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*R*)-2′-(4-methoxybenzyloxy)-1′-(*p*-toluenesulfonyloxy)propyl]-4-vinylcyclohexan-2-ol (18). To a solution of **17** (413 mg, 0.742 mmol) in THF (8.3 mL) were added 1.59 mol/L solution of BuLi in hexane (1.40 mL, 2.23 mmol) and TsCl (293 mg, 2.08 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 20 min. The mixture was diluted with EtOAc, and washed successively with saturated aqueous NH_4Cl solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 11 g, EtOAc/hexane=1:7) to give **18** (534 mg, 100%) as a colorless oil: R_f 0.59 (EtOAc/hexane=1:2); $[\alpha]_D^{22}$ +3.0 (c 1.05, $CHCl_3$); IR (neat) 3560, 2960, 2930, 2860, 1615 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.78 (2H, d, J=8.4 Hz), 7.39–7.27 (5H, m), 7.19 and 7.12 (each 2H, 2d, J=8.6 Hz), 6.79 (2H, d, J=8.4 Hz), 5.30–5.20 (3H, m), 4.86 (1H, d, J=7.5 Hz), 4.67 and 4.52 (each 1H, 2d, J=11.6 Hz), 4.30 and 4.16 (each 1H, 2d, J=11.8 Hz), 3.90 (1H, qd, J=6.1, 7.5 Hz), 3.78 (3H, s), 3.67 (1H, ddd, J=1.5, 8.5, 10.2 Hz), 3.29 (1H, ddd, J=4.4, 12.0, 12.2, Hz), 3.19 (1H, ddd, J=4.4, 8.5, 12.2 Hz), 2.95 (1H, d, J=1.5 Hz), 2.37 (3H, s), 2.12 (1H, m), 2.08–2.00 (1H, m), 1.46 (1H, dd, J=10.2, 11.9 Hz), 1.26 (1H, m), 1.06 (3H, d, J=6.1 Hz), 0.83 (9H, s), −0.03 and −0.05 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 159.0, 143.9, 138.3, 135.1, 130.5, 129.4, 128.5, 127.8, 120.6, 113.5, 86.1, 79.5, 75.6, 72.1, 71.3, 71.1, 70.9, 55.2, 51.1, 43.5, 37.4, 25.7, 21.6, 18.0, 16.7, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{39}H_{54}NaO_8SSi$, $(M+Na)^+$ 733.3207, found 733.3207.

4.2.13. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*R*)-2′-(4-methoxybenzyloxy)-1′-(*p*-toluenesulfonyloxy)propyl]-2-methoxymethyloxy-4-vinylcyclohexane (19). To a solution of **18** (536 mg, 0.754 mmol) in $(CH_2Cl)_2$ (11 mL) were added i-Pr_2NEt (1.30 mL, 7.54 mmol) and MOMCl (0.570 mL, 7.54 mmol) at 0 °C, and the reaction mixture was stirred at 50 °C for 3.5 h. The reaction mixture was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 20 g, EtOAc/hexane=1:10) to give **19** (557 mg, 98%) as a colorless oil: R_f 0.67 (EtOAc/hexane=1:2); $[\alpha]_D^{23}$ +16.0 (c 1.0, $CHCl_3$); IR (neat) 2980, 2930, 2860, 1615 1515 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.76 (2H, d, J=8.0 Hz), 7.36–7.27 (5H, m), 7.16 (2H, d, J=8.0 Hz), 7.08 and 6.79 (each 2H, 2d, J=8.7 Hz), 5.30–5.26 (3H, m), 4.90 (1H, d, J=8.7 Hz), 4.87 and 4.75 (each 1H, 2d, J=5.3 Hz), 4.59 and 4.53 (each 1H, 2d, J=11.6 Hz), 4.25 and 4.13 (each 1H, 2d, J=11.6 Hz), 4.00 (1H, qd, J=6.3, 8.7 Hz), 3.77 (3H, s), 3.58 (1H, dd, J=10.4, 10.5 Hz), 3.37–3.28 (1H, m), 3.32 (3H, s), 3.22 (1H, ddd, J=3.9, 10.4, 11.6 Hz), 2.36 (3H, s), 2.20–2.10 (1H, m), 2.11 (1H, ddd, J=3.9, 3.9, 12.4 Hz), 1.62 (1H, dd, J=10.5, 10.5 Hz), 1.29 (1H, ddd, J=11.6, 11.6, 12.4 Hz), 1.05 (3H, d, J=6.3 Hz), 0.81 (9H, s), −0.04 and −0.05 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 158.2, 143.8, 138.3, 138.2, 135.1, 130.7, 129.2, 129.1, 128.3, 127.7, 127.6, 113.4, 98.9, 86.2, 80.0, 78.5, 75.2, 71.5, 70.8, 70.7, 57.0, 55.1, 51.3, 43.0, 38.3, 25.7, 21.5, 17.9, 16.8, −4.3, −4.5; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{41}H_{58}O_9SSiK$, $(M+K)^+$ 793.3208, found 793.3197.

4.2.14. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*R*)-2′-hydroxy-1′-(*p*-toluenesulfonyloxy)propyl]-2-methoxymethyloxy-4-vinylcyclohexane (20). To a solution of **19** (674 mg, 0.89 mmol) in CH_2Cl_2/H_2O (10:1, 11 mL) was added DDQ (405 mg, 1.78 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 18 g, EtOAc/hexane=1:4) to give **20** (549 mg, 97%) as a colorless oil: R_f 0.34 (EtOAc/hexane=1:2); $[\alpha]_D^{22}$ −9.0 (c 1.1, $CHCl_3$); IR (neat) 3500, 2960, 2930, 2860, 2360 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.83 (2H, d, J=8.0 Hz), 7.36–7.27 (7H, m), 5.32–5.25 (3H, m), 4.89 and 4.78 (each 1H, 2d, J=5.1 Hz), 4.83 (1H, d, J=4.6 Hz), 4.59 and 4.54 (each 1H, 2d, J=11.6 Hz), 3.93 (1H, qd, J=4.6, 5.8 Hz), 3.64 (1H, br s), 3.51 (1H, dd, J=10.3, 10.9 Hz), 3.37 (1H, ddd, J=4.0, 11.9, 11.9 Hz), 3.32 (3H, s), 3.25 (1H, ddd, J=4.0, 10.9, 12.8 Hz), 2.44 (3H, s), 2.22 (1H, ddd, J=11.5, 11.9 Hz), 2.14 (1H, ddd, J=4.0, 4.1, 12.2 Hz), 1.72 (1H, dd, J=10.3, 11.5 Hz), 1.33 (1H, ddd, J=11.9, 12.2, 12.8 Hz), 1.04 (3H, d, J=4.6 Hz), 0.81 (9H, s), −0.04 and −0.05 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 144.9, 138.3, 138.1, 133.7, 129.7, 128.4, 127.8, 127.7, 120.6, 99.0, 85.9, 79.9, 78.0, 71.6, 70.5, 69.1, 57.3, 52.2, 44.2, 38.3, 25.7, 21.6, 19.7, 17.9, −4.4, −4.5; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{33}H_{51}O_8SSi$, $(M+H)^+$ 635.3074, found 635.3084.

4.2.15. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-2-methoxymethyloxy-3-[(1′*S*,2′*R*)-2′-methyloxiranyl]-4-vinylcyclohexane (21). To a solution of **20** (78.5 mg, 0.124 mmol) in toluene (1.6 mL) was added DBU (0.15 mL, 0.99 mmol) at 0 °C, and the reaction mixture was stirred at 50 °C for 20 h. The reaction mixture was diluted with EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:6) to give **21** (49.7 mg, 94%) as a colorless syrup; $[\alpha]_D^{24}$ −14.0 (c 0.53, $CHCl_3$); IR (neat) 2960, 2920, 2860, 1260, 1080, 1020 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.36–7.27 (5H, m), 5.80 (1H, ddd, J=9.3, 10.5, 15.9 Hz), 5.26 (1H, dd, J=1.8, 10.5 Hz), 5.10 (1H, dd, J=1.8, 15.9 Hz), 4.97 and 4.69 (each 1H, 2d, J=6.3 Hz), 4.61 and 4.59 (each 1H, 2d, J=11.4 Hz), 3.48 (1H, dd, J=9.0, 9.9 Hz), 3.29–3.45 (2H, m), 3.36 (3H, s), 3.01 (1H, qd, J=4.2, 5.7 Hz), 2.78 (1H, dd, J=4.2, 9.3 Hz), 2.26 (1H, ddd, J=4.2, 4.2, 11.1 Hz), 2.14 (1H, ddd, J=9.3, 9.3, 9.3 Hz), 1.47 (1H, ddd, J=11.1,

11.1, 11.1 Hz), 1.28–1.40 (1H, m), 1.30 (3H, d, J=5.7 Hz), 0.84 (9H, s), −0.01 and −0.02 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 138.72, 138.40, 128.38, 127.65, 117.01, 98.24, 79.87, 79.58, 71.78, 70.24, 59.39, 56.48, 55.10, 52.62, 42.21, 39.35, 25.83, 18.09, 14.75, −4.37; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{26}H_{43}O_5Si$, $(M+H)^+$ 463.2880, found 463.2875.

4.2.16. (1*S*,2*S*,3*R*,4*R*,5*R*)-2-Acetoxy-1-benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*S*,2′*S*)-1′-acetoxy-2′-hydroxypropyl]-4-vinylcyclohexane (22). To a solution of **16** (32.8 mg, 0.0591 mmol) in MeOH (1.2 mL) was added $NaBH_4$ (22.4 mg, 0.592 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 35 min. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:5) to give an alcohol (21.7 mg, 66%) as a colorless syrup.

To a solution of the alcohol (21.7 mg, 0.0390 mmol) in pyridine (1 mL) were added Ac_2O (0.5 mL) and DMAP (1.0 mg) at 0 °C, and the reaction mixture was stirred at 50 °C for 4 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was dissolved in CH_2Cl_2/H_2O (10:1, 1.0 mL). To this solution was added DDQ (42.0 mg, 0.185 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc and washed successively with 20% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:5) to give **22** (14.3 mg, 46% from **16**) as a colorless syrup: $[\alpha]_D^{26}$ +3.7 (c 0.57, $CHCl_3$); IR (neat) 3500, 2970, 2860, 1740, 1250 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.24–7.38 (5H, m), 6.10 (1H, ddd, J=10.2, 10.5, 16.5 Hz), 5.13–5.07 (2H, m), 5.08 (1H, dd, J=1.5, 9.0 Hz), 4.93 (1H, dd, J=1.5, 11.1 Hz), 4.66 and 4.59 (each 1H, 2d, J=12.6 Hz), 3.99 (1H, qd, J=1.5, 6.6 Hz), 3.81 (1H, ddd, J=3.0, 3.0, 4.2 Hz), 3.55 (1H, ddd, J=3.0, 3.0, 9.0 Hz), 2.98 (1H, ddd, J=3.3, 3.9, 11.1 Hz), 2.56 (1H, ddd, J=3.0, 3.9, 10.2 Hz), 2.06 and 2.01 (each 3H, 2s), 1.90 (1H, ddd, J=2.7, 2.7, 15.3 Hz), 1.72 (1H, ddd, J=3.0, 4.2, 15.3 Hz), 1.10 (3H, d, J=6.6 Hz), 0.87 (9H, s), 0.07 and 0.06 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 170.74, 170.40, 138.89, 137.87, 128.10, 127.38, 127.18, 118.24, 74.35, 73.74, 71.29, 70.75, 69.14, 65.15, 48.43, 32.17, 28.52, 25.72, 21.28, 20.80, 20.32, 17.99, −4.73, −4.99; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{28}H_{44}O_7SiNa$, $(M+Na)^+$ 543.2754, found 543.2757.

4.2.17. (1*S*,2*S*,3*R*,4*R*,5*R*)-2-Acetoxy-1-benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*S*,2′*R*)-2′-methyloxiranyl]-4-vinylcyclohexane (23). To a solution of **22** (16.8 mg, 0.0323 mmol) in pyridine (1 mL) at 0 °C were added MsCl (0.025 mL, 0.32 mmol) and DMAP (1.0 mg), and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was roughly purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:5) to give mesylate (17.9 mg, 93%) as a colorless syrup.

To a solution of the mesylate (17.9 mg, 0.0300 mmol) in MeOH (1 mL) was added NaOMe (5.1 mg, 0.15 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was neutralized with acidic resin (Amberlite 120B, H^+ form) and filtered. Removal of the solvent gave a residue, which was dissolved in pyridine (1 mL). To this solution were added Ac_2O (0.5 mL) and DMAP (1.0 mg) at room temperature, and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:15) to give **23** (11.0 mg, 74% from **22**) as a colorless syrup: $[\alpha]_D^{24}$ −2.5 (c 0.22, $CHCl_3$); IR (neat) 2960, 2940, 2860, 1820 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.39–7.24 (5H, m), 5.69 (1H, ddd, J=9.8, 9.8, 16.8 Hz), 5.10 (1H, dd, J=1.5, 16.8 Hz), 5.07 (1H, dd, J=1.5, 9.8 Hz), 4.95 (1H, dd, J=4.4, 8.3 Hz), 4.72 and 4.64 (each 1H, 2d, J =11.9 Hz), 3.85 (1H, ddd, J=4.6, 8.3, 9.5 Hz), 3.74 (1H, ddd, J=4.1, 9.3, 9.3 Hz), 2.96 (1H, qd, J=3.9, 5.6 Hz), 2.86 (1H, dd, J=3.9, 5.6 Hz), 2.41–2.26 (2H, m), 2.20 (1H, ddd, J=4.1, 4.6, 13.1 Hz), 2.08 (3H, s), 1.62 (1H, ddd, J=9.3, 9.5, 13.1 Hz), 1.18 (3H, d, J=5.6 Hz), 0.83 (9H, s), 0.02 and 0.00 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 170.76, 138.67, 137.50, 128.30, 127.50, 127.45, 118.09, 76.31, 73.74, 72.23, 68.39, 53.86, 51.20, 50.93, 37.68, 36.70, 25.71, 21.28, 18.00, 13.76, −4.29, −4.61; HRMS (FAB$^+$, NBA matrix) m/z calcd for $C_{26}H_{40}O_5SiNa$, $(M+Na)^+$ 483.2543, found 483.2543.

4.2.18. (4*S*,5*S*,6*S*,1*R*,2*R*)-4-Benzyloxy-2-(*tert*-butyldimethylsilyloxy)-6-[(1′*R*,2′*R*)-2′-hydroxy-1′-(*p*-toluenesulfonyloxy)propyl]-5-methoxymethyloxy-cyclohexancarboxylic acid (25). Ozone was introduced to a solution of **19** (7.9 mg, 0.010 mmol) in MeOH (1 mL) at −78 °C for 5 min. To the reaction mixture was added Me_2S (0.070 mL, 0.95 mmol) at −78 °C. After being stirred at room temperature for 12 h, the reaction mixture was diluted with Et_2O. The organic layer was washed with brine, and then dried. Removal of the solvent gave a crude aldehyde, which was used for the next reaction without purification.

To a solution of the crude aldehyde in t-BuOH/H_2O (1:1, 0.2 mL) were added $NaH_2PO_4\cdot H_2O$ (16 mg, 0.10 mmol), $HOSO_2NH_2$ (9.7 mg, 0.10 mmol), and $NaClO_2$ (9.1 mg, 0.10 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:1) to give carboxylic acid **24** (6.1 mg, 75% for two steps) as a colorless syrup.

To a solution of carboxylic acid **24** (6.1 mg, 0.0081 mmol) in CH_2Cl_2/H_2O (10:1, 0.5 mL) was added DDQ (3.7 mg,

0.016 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 20 min. The reaction mixture was diluted with EtOAc and washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:2) to give **25** (3.9 mg, 76% from **19**) as a colorless syrup: R_f 0.34 (EtOAc/hexane=1:1); IR (neat) 3480, 2955, 2930, 1730, 1715, 1255, 1180, 1100, 850 cm^{-1}; ^1H NMR (300 MHz) δ 7.86 (2H, d, J=8.3 Hz), 7.39–7.25 (7H, m), 4.91 and 4.76 (each 1H, 2d, J=6.1 Hz), 4.72 (1H, br), 4.56 (2H, s), 4.17 (1H, qd, J=6.4, 3.4 Hz), 3.71 (1H, ddd, J=4.0, 10.0, 11.7 Hz), 3.47–3.27 (2H, m), 3.36 (3H, s), 2.85 (1H, dd, J=10.0, 10.5 Hz), 2.42 (3H, s), 2.23 (1H, ddd, J=2.2, 10.5, 10.5, Hz), 2.11 (1H, ddd, J=4.0, 4.0, 12.7 Hz), 1.34 (1H, ddd, J=11.7, 11.7, 12.7 Hz), 1.26 (1H, s), 1.06 (3H, d, J=6.4 Hz), 0.82 (9H, s), −0.02 (6H, s); HRMS (FAB^+, NBA matrix) m/z calcd for $C_{32}H_{48}O_{10}SSiNa$, $(M+Na)^+$ 675.2636, found 675.2627.

4.2.19. (3*R*,4*R*,4a*S*,5*S*,6*S*,8*R*,8a*R*)-6-Benzyloxy-8-(*tert*-butyldimethylsilyloxy)-5-methoxymethyloxy-3-methyl-4-(*p*-toluenesulfonyloxy)-perhydroisochroman-1-one (26). *By Mitsunobu reaction*: To a solution of **25** (3.2 mg, 0.0049 mmol) in benzene (1 mL) were added Ph_3P (3.2 mg, 0.012 mmol) and DEAD (0.0022 mL, 0.012 mmol) at 0 °C, and the reaction mixture was stirred at 80 °C for 30 h. The reaction mixture was diluted with Et_2O and washed successively with brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:4) to give **26** (2.6 mg, 84%) as a colorless oil: R_f 0.16 (EtOAc/hexane=1:5); $[\alpha]_D^{23}$ +22.6 (c 0.69, $CHCl_3$); IR (neat) 2960, 2930, 2860, 1760, 1360, 1180, 1015, 940, 850 cm^{-1}; ^1H NMR (300 MHz) δ 7.81 (2H, d, J=8.4 Hz), 7.40–7.22 (7H, m), 5.29 (1H, s), 5.12 and 4.71 (each 1H, 2d, J=6.3 Hz), 4.59 and 4.54 (each 1H, 2d, J=11.4 Hz), 4.53 (1H, q, J=6.3 Hz), 3.84 (1H, ddd, J=4.7, 9.3, 9.3 Hz), 3.54 (3H, s), 3.48 (1H, dd, J=8.4, 11.4 Hz), 3.31 (1H, ddd, J=4.2, 8.4, 11.4 Hz), 2.44 (3H, s), 2.32–2.18 (2H, m), 1.78 (1H, dd, J=11.4, 12.9 Hz), 1.44 (3H, d, J=6.3 Hz), 1.50–1.33 (1H, m), 0.82 (9H, s), 0.09 and 0.08 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 169.7, 144.6, 138.1, 134.8, 129.5, 128.5, 127.8, 127.7, 127.6, 98.3, 80.0, 79.3, 78.9, 73.9, 72.0, 66.1, 57.1, 47.3, 44.6, 39.2, 29.7, 25.8, 21.7, 18.0, 16.4, −4.7, −4.8; HRMS (FAB^+, NBA matrix) m/z calcd for $C_{32}H_{46}O_9SSiNa$, $(M+Na)^+$ 657.2529, found 657.2521.

By DCC-mediated lactonization: To a solution of **25** (1.9 mg, 0.0029 mmol) in $(CH_2Cl)_2$ (0.3 mL) were added DCC (2.0 mg, 0.0096 mmol) and DMAP (1 mg) at 0 °C, and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with EtOAc, and successively washed with 1 mol/L aqueous HCl solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:4) to give **26** (1.1 mg, 60%).

4.2.20. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-2-methoxymethyloxy-3-[(*R*)-1′-(*p*-toluenesulfonyloxy)-2′-oxopropyl]-4-vinylcyclohexane (27). To a solution of **20** (131 mg, 0.206 mmol) in CH_2Cl_2 (2.6 mL) was added Dess–Martin periodinane (874 mg, 2.06 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 23 h. The reaction mixture was diluted with EtOAc and washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 2 g, EtOAc/hexane=1:9) to give **27** (130 mg, 100%) as a colorless oil: R_f 0.65 (EtOAc/hexane=1:2); $[\alpha]_D^{19}$ −6.0 (c 0.56, $CHCl_3$); IR (neat) 2960, 2930, 2860, 1720, 1600 cm^{-1}; ^1H NMR (300 MHz) δ 7.85 (2H, d, J=8.0 Hz), 7.36–7.30 (7H, m), 5.41 (1H, ddd, J=8.8, 10.3, 17.1 Hz), 5.26 (1H, dd, J=2.0, 10.3 Hz), 5.15 (1H, dd, J=2.0, 17.1 Hz), 4.69 and 4.57 (each 1H, 2d, J=5.1 Hz), 4.56 and 4.20 (each 1H, 2d, J=11.5 Hz), 3.47 (1H, dd, J=9.0, 10.8 Hz), 3.36 (1H, ddd, J=4.1, 9.0, 11.6 Hz), 3.30 (1H, ddd, J=4.4, 8.8, 11.6 Hz), 3.23 and 2.45 (each 3H, 2s), 2.23 (1H, dd, J=10.8, 11.2 Hz), 2.18 (3H, s), 2.15 (1H, ddd, J=4.1, 4.4, 12.4 Hz), 2.01 (1H, ddd, J=8.8, 8.8, 11.2 Hz), 1.32 (1H, ddd, J=8.8, 11.6, 12.4 Hz), 0.82 (9H, s), −0.04 and −0.05 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 204.4, 145.0, 138.1, 137.7, 133.9, 129.7, 128.4, 127.9, 127.8, 127.7, 120.8, 98.1, 82.8, 79.4, 76.9, 71.5, 71.0, 56.8, 50.3, 45.8, 38.5, 26.5, 25.7, 21.6, 17.9, −4.3, −4.5; HRMS (FAB^+, NBA matrix) m/z calcd for $C_{33}H_{48}O_8SSiNa$, $(M+Na)^+$ 655.2737, found 655.2740.

4.2.21. (3*S*,4*R*,4a*S*,5*S*,6*S*,8*R*,8a*R*)-6-Benzyloxy-8-(*tert*-butyldimethylsilyloxy)-5-methoxymethyloxy-3-methyl-4-(*p*-toluenesulfonyloxy)-perhydroisochroman-1-one (30). Ozone was introduced to a solution of **27** (300 mg, 0.473 mmol) in MeOH (30 mL) at −78 °C for 10 min. To the reaction mixture was added Me_2S (3.00 mL, 40.8 mmol) at −78 °C, and the reaction mixture was stirred at room temperature for 3 h. The products were extracted with Et_2O, and the organic layer was washed with brine, and then dried. Removal of the solvent gave a crude aldehyde (311 mg), which was used for the next reaction without purification.

To a solution of the crude aldehyde (311 mg) in t-BuOH/H_2O (1:1, 6 mL) were added $NaH_2PO_4 \cdot H_2O$ (382 mg, 2.45 mmol), $HOSO_2NH_2$ (238 mg, 2.45 mmol), and $NaClO_2$ (222 mg, 2.45 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution and brine, and then dried. Removal of the solvent gave a residue, which was roughly purified by column chromatography (silica gel: 7 g, EtOAc/hexane=1:4, containing 1 vol % AcOH) to give carboxylic acid **28** (308 mg) as a colorless syrup. This was used for the next reaction without further purification.

To a solution of **28** (308 mg, 0.473 mmol) in MeOH (6.5 mL) was added $NaBH_4$ (35.8 mg, 0.946 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was diluted with EtOAc, and successively washed with 1 mol/L aqueous HCl solution and brine, and then dried. Removal of the solvent gave a residue, which was roughly purified by column chromatography (silica gel: 7 g, EtOAc/hexane=1:4, containing 1 vol % AcOH) to give a colorless syrup, which was dissolved in CH_2Cl_2 (6 mL).

To this solution were added DCC (231 mg, 1.12 mmol) and DMAP (9.1 mg, 0.074 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 6 g, EtOAc/hexane=1:5) to give lactone **30** (216 mg, 72% from **27**) as a colorless syrup: R_f 0.54 (EtOAc/toluene=1:6); $[\alpha]_D^{19}$ +3.0 (*c* 0.91, $CHCl_3$); IR (neat) 2960, 2930, 2860, 1760, 1600 cm^{-1}; ^1H NMR (300 MHz) δ 7.78 (2H, d, *J*=8.3 Hz), 7.36–7.28 (7H, m), 5.15 (1H, d, *J*=3.2 Hz), 4.99 (1H, d, *J*=6.4 Hz), 4.82 (1H, q, *J*=6.9 Hz), 4.62–4.52 (3H, m), 3.85 (1H, ddd, *J*=4.9, 9.5, 10.4 Hz), 3.53 (1H, dd, *J*=8.4, 10.5 Hz), 3.46 (3H, s), 3.35 (1H, ddd, *J*=4.7, 8.4, 10.7 Hz), 2.44 (3H, s), 2.39 (1H, dd, *J*=9.5, 13.7 Hz), 2.26 (1H, ddd, *J*=4.7, 4.9, 13.4 Hz), 1.90 (1H, ddd, *J*=3.2, 10.5, 13.7 Hz), 1.53 (3H, d, *J*=6.9 Hz), 1.44 (1H, ddd, *J*=10.4, 10.7, 13.4 Hz), 0.84 (9H, s), 0.11 and 0.09 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 169.1, 144.8, 138.0, 134.4, 129.7, 128.4, 127.7, 127.6, 127.5, 97.8, 80.0, 79.5, 79.2, 77.7, 71.9, 66.5, 56.6, 45.6, 43.2, 38.9, 25.9, 21.6, 18.7, 18.0, −4.7, −5.0; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{32}H_{46}NaO_9SSi$, $(M+Na)^+$ 657.2529, found 657.2522. Anal. Calcd for $C_{32}H_{46}O_9SSi$: C, 60.54; H, 7.30. Found: C, 60.61; H, 7.39.

4.2.22. (3*S*,4*S*,5*S*,6*S*,8*R*,9*R*,10*S*)-4-Azido-6-benzyloxy-8-(*tert*-butyldimethylsilyloxy)-5-methoxymethyloxy-3-methyl-perhydroisochroman-1-one (4). To a solution of lactone **30** (25.0 mg, 0.0394 mmol) in DMF (1 mL) was added NaN_3 (25.0 mg, 0.385 mmol) at room temperature, and the reaction mixture was stirred at 110 °C for 4 h. The reaction mixture was diluted with EtOAc and washed with brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/toluene=1:7) to give **4** (17.2 mg, 86%) as a colorless oil: R_f 0.33 (EtOAc/toluene=1:6); $[\alpha]_D^{21}$ −96.0 (*c* 0.70, $CHCl_3$); IR (neat) 2960, 2930, 2860, 2110, 1745 cm^{-1}; ^1H NMR (300 MHz) δ 7.40–7.19 (5H, m), 4.97 and 4.76 (each 1H, 2d, *J*=5.9 Hz), 4.63 and 4.56 (each 1H, 2d, *J*=11.7 Hz), 4.47 (1H, qd, *J*=2.4, 6.6 Hz), 4.11 (1H, dd, *J*=1.9, 2.4 Hz), 3.80 (1H, ddd, *J*=4.6, 9.6, 11.2 Hz), 3.59 (1H, dd, *J*=8.9, 10.2 Hz), 3.41 (3H, s), 3.30 (1H, ddd, *J*=3.9, 8.9, 12.9 Hz), 2.50 (1H, dd, *J*=9.6, 13.5 Hz), 2.25 (1H, ddd, *J*=3.9, 4.6, 12.5 Hz), 1.79 (1H, ddd, *J*=1.9, 10.2, 13.5 Hz), 1.50 (3H, d, *J*=6.6 Hz), 1.40 (1H, ddd, *J*=11.2, 12.5, 12.5 Hz), 0.85 (9H, s), 0.12 and 0.06 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 168.8, 138.2, 128.4, 127.8, 127.5, 98.7, 79.3, 78.8, 78.6, 71.8, 67.0, 59.4, 56.0, 43.8, 42.7, 39.0, 25.9, 18.2, 18.1, −4.6, −4.8; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{25}H_{39}N_3O_6SiCs$, $(M+Cs)^+$ 638.1659, found 638.1633.

4.2.23. (3*S*,4*S*,5*S*,6*S*,10*S*)-4-Azido-6-benzyloxy-5,8-dihydroxy-3-methyl-5,6,7,4a-tetrahydroisochroman-1-one (32). To a solution of azide **4** (41.3 mg, 0.0817 mmol) in CH_3CN (3 mL) in a polyethylene vial was added HF/pyridine (ca. 0.1 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. The reaction mixture was diluted with EtOAc and neutralized with saturated aqueous $NaHCO_3$ solution. The organic layer was washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave crude alcohol **31** (33.4 mg) as a white solid. A solution of $(COCl)_2$ (2.0 mol/L solution in CH_2Cl_2, 1.28 mL, 2.56 mmol) and DMSO (0.400 mL, 5.17 mmol) in CH_2Cl_2 (1 mL) was stirred at −78 °C for 10 min under Ar. To this mixture was added a solution of the crude alcohol **31** (33.4 mg) in CH_2Cl_2 (1 mL) at −78 °C. After stirring at −78 °C for 2 h, to the reaction mixture was added *i*-Pr_2NEt (1.32 mL, 7.66 mmol) at −78 °C. The resulting mixture was further stirred at room temperature for 2 h and then diluted with Et_2O. The mixture was washed with brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:4) to give **32** (25.2 mg, 89% from **4**) as a colorless syrup: R_f 0.47 (EtOAc/hexane=1:1); $[\alpha]_D^{23}$ −16.0 (*c* 0.92, $CHCl_3$); IR (neat) 3460, 3030, 2900, 2120, 1650 cm^{-1}; ^1H NMR (300 MHz) δ 13.04 (1H, s), 7.44–7.30 (5H, m), 4.73 and 4.54 (each 1H, 2d, *J*=11.4 Hz), 4.49 (1H, qd, *J*=1.5, 6.6 Hz), 4.04 (1H, dd, *J*=1.5, 2.9 Hz), 3.74 (1H, dd, *J*=9.3, 9.5 Hz), 3.62 (1H, ddd, *J*=6.4, 9.5, 9.5 Hz), 2.93 (1H, ddd, *J*=1.1, 6.4, 18.3 Hz), 2.88 (1H, s), 2.72 (1H, dddd, *J*=1.1, 2.5, 2.9, 9.3 Hz), 2.45 (1H, ddd, *J*=2.5, 9.5, 18.3 Hz), 1.52 (3H, d, *J*=6.6 Hz); ^{13}C NMR (75 MHz) δ 172.6, 169.7, 137.2, 128.7, 128.3, 128.0, 90.0, 77.3, 76.3, 71.7, 70.1, 58.3, 42.2, 34.6, 18.4; LRMS (EI) *m/z* 345 (M^+, 1.3%), 239 (9.0), 191 (5.6), 91 (100); HRMS (EI) *m/z* calcd for $C_{17}H_{19}N_3O_5$, (M^+) 345.1325, found 345.1326.

4.2.24. *N*-Benzyloxycarbonyl-(−)-actinobolin (33). To a solution of **32** (6.5 mg, 0.019 mmol) in MeOH (1 mL) were added 10% Pd/C (2.0 mg) and 4 mol/L HCl in dioxane (0.0095 mL, 0.038 mmol). The reaction mixture was stirred under an atmospheric pressure of H_2 at room temperature for 18 h. The catalyst was removed by filtration through a pad of Celite and the filtrate was concentrated to give a residue, which was dissolved in DMF (0.5 mL). To this solution were added DCC (19.4 mg, 0.0940 mmol), *N*-benzyloxycarbonyl-D-alanine (12.6 mg, 0.0564 mmol), and Et_3N (0.100 mL, 0.717 mmol). The mixture was stirred at room temperature for 14 h, and then concentrated to give a residue, which was purified by preparative TLC (acetone/toluene=1:2) to give **33** (4.7 mg, 57% from **32**) as a colorless oil: R_f 0.34 (acetone/toluene=1:1); $[\alpha]_D^{24}$ +34.0 (*c* 0.17, $CHCl_3$); IR (neat) 3330, 2980, 2930, 1650, 1540, 1230, 1050 cm^{-1}; ^1H NMR (300 MHz) δ 13.05 (1H, s), 7.40–7.30 (5H, m), 6.78 (1H, br d, *J*=8.9 Hz), 5.19 (1H, br d, *J*=6.9 Hz), 5.11 and 5.04 (each 1H, 2d, *J*=11.7 Hz), 4.70–4.60 (1H, br s), 4.61 (1H, qd, *J*=1.5, 6.6 Hz), 4.33 (1H, ddd, *J*=1.5, 1.8, 8.9 Hz), 4.27 (1H, qd, *J*=6.9, 7.2 Hz), 3.89 (1H, ddd, *J*=6.9, 9.6, 9.9 Hz), 3.14 (1H, dd, *J*=9.6, 9.6 Hz), 3.05 (1H, br s), 2.93 (1H, dd, *J*=6.9, 18.9 Hz), 2.65 (1H, dd, *J*=1.8, 9.6 Hz), 2.47 (1H, dd, *J*=9.9, 18.9 Hz), 1.41 (3H, d, *J*=7.2 Hz), 1.34 (3H, d, *J*=6.6 Hz); ^{13}C NMR (75 MHz) δ 175.9, 175.6, 170.4, 156.0, 135.6, 128.6, 128.4, 128.3, 89.8, 76.8, 71.6, 68.0, 67.5, 50.9, 46.3, 43.4, 36.4, 17.9, 17.6; LRMS (EI) *m/z* 434 (M^+, 9.1%), 416 (6.8), 325 (3.8), 223 (100); HRMS (EI) *m/z* calcd for $C_{21}H_{26}N_2O_8$, (M^+) 434.1689, found 434.1696.

4.2.25. (−)-Actinobolin hydrochloride (3·HCl). To a solution of **33** (3.4 mg, 0.0078 mmol) in MeOH (0.6 mL) and AcOH (0.5 mL) were added 10% Pd/C (3 mg) and 1 mol/L aqueous HCl solution (0.023 mL, 0.023 mmol). The reaction mixture was stirred under an atmospheric pressure of

H_2 at room temperature for 30 min. The catalyst was removed by filtration through a pad of Celite, and the filtrate was concentrated. The residue was purified by column chromatography (Sephadex LH-20, MeOH as an eluent) to give **3**·HCl (2.0 mg, 76%) as an amorphous solid: $[\alpha]_D^{24}$ −51.2 (*c* 0.24, H_2O), {lit.[6c,d] for (+)-actinobolin hydrochloride: $[\alpha]_D^{21}$ +53 (*c* 0.65, H_2O)}; ^{1}H NMR (300 MHz, MeOH-d_4) δ 4.70 (1H, qd, *J*=1.8, 6.6 Hz), 4.56 (1H, dd, *J*=1.8, 3.5 Hz), 4.01 (1H, q, *J*=6.9 Hz), 3.78 (1H, ddd, *J*=6.6, 9.6, 9.8 Hz), 3.13 (1H, dd, *J*=9.6, 9.6 Hz), 2.82–2.74 (1H, m), 2.81 (1H, dd, *J*=6.6, 18.9 Hz), 2.35 (1H, ddd, *J*=2.7, 9.6, 18.9 Hz), 1.50 (3H, d, *J*=6.9 Hz), 1.33 (3H, d, *J*=6.6 Hz); ^{13}C NMR (75 MHz, MeOH-d_4) δ 175.1, 172.7, 172.1, 91.8, 79.3, 72.8, 70.1, 50.2, 47.0, 43.3, 38.0, 18.5, 18.1; HRMS (FAB$^+$, NBA matrix) *m*/*z* calcd for $C_{13}H_{21}N_2O_6$, (M+H)$^+$ 301.1400, found 301.1400. The ^{1}H and ^{13}C NMR spectra of the synthetic compound were fully identical with those of natural (+)-actinobolin hydrochloride.

4.3. An improved route to (−)-actinobolin

4.3.1. (2*S*)-2-(Triethylsilyloxy)propanal [(*S*)-7b]. To a solution of methyl (*S*)-lactate (335 mg, 3.22 mmol) in CH_2Cl_2 (7 mL) were added TESCl (0.620 mL, 3.69 mmol) and imidazole (285 mg, 4.19 mmol) at 0 °C, and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (EtOAc/hexane=1:20) to give methyl (*S*)-lactate triethylsilyl ether (702 mg, 100%) as a colorless syrup: R_f 0.77 (EtOAc/hexane=1:3); $[\alpha]_D^{22}$ −26.9 (*c* 1.06, $CHCl_3$); IR (neat) 2955, 2880, 1760, 1740, 1145, 1005, 745 cm^{-1}; ^{1}H NMR (300 MHz) δ 4.34 (1H, q, *J*=6.6 Hz), 3.73 (3H, s), 1.41 (3H, d, *J*=6.6 Hz), 0.96 (9H, t, *J*=7.8 Hz), 0.62 (6H, q, *J*=7.8 Hz); ^{13}C NMR (75 MHz) δ 174.5, 68.0, 51.8, 21.5, 6.6, 4.5.

To a solution of methyl (*S*)-lactate triethylsilyl ether (702 mg, 3.21 mmol) in toluene (10 mL) was added 1.01 mol/L solution of DIBAL-H in toluene (3.76 mL, 3.80 mmol) at −78 °C, and the mixture was stirred at −78 °C for 2 h. The reaction mixture was quenched with water, and the products were extracted with EtOAc. The organic layer was washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (EtOAc/hexane=1:50) to give (*S*)-**7b** (426 mg, 70%) as a colorless syrup: R_f 0.77 (EtOAc/hexane=1:3); $[\alpha]_D^{23}$ −6.5 (*c* 1.1, $CHCl_3$); IR (neat) 2960, 2880, 1740, 1140, 1010 cm^{-1}; ^{1}H NMR (300 MHz) δ 9.62 (1H, s), 4.08 (1H, q, *J*=6.8 Hz), 1.29 (3H, d, *J*=6.8 Hz), 0.97 (9H, t, *J*=7.8 Hz), 0.64 (6H, q, *J*=7.8 Hz); ^{13}C NMR (75 MHz,) δ 204.2, 73.5, 18.6, 6.6, 4.7; HRMS (FAB$^+$, NBA matrix) *m*/*z* calcd for $C_{11}H_{14}O_3$, (M+H)$^+$ 189.1325, found 189.1311.

4.3.2. Three-component coupling reaction with (*S*)-7b. To a suspension of copper(I) cyanide (CuCN, 43.3 mg, 0.483 mmol) in ether (1.0 mL) at −78 °C was added dropwise vinyllithium (1.0 mol/L solution in ether, 0.75 mL, 0.75 mmol) under Ar. After being stirred at −78 °C for 10 min, the mixture was allowed to warm to 0 °C and further stirred at 0 °C for 1 min. The resulting clear solution was cooled to −78 °C, and to this solution was added slowly a solution of enone (+)-**6** (100 mg, 0.302 mmol) in ether (1.0 mL) via a cannula. After being stirred at −78 °C for 30 min, a solution of aldehyde (*S*)-**7b** (265 mg, 1.40 mmol) in ether (1.5 mL) was added to the mixture dropwise via a cannula, and the mixture was stirred for 2 h at −78 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl solution, and the products were extracted with EtOAc. The organic layer was washed successively with saturated aqueous $NaHCO_3$, brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 7 g, EtOAc/hexane=1:40) to give (2*R*,3*R*,4*R*,6*S*)-6-benzyloxy-4-(*tert*-butyldimethylsilyloxy)-2-[(1′*S*,2′*S*)-1′-hydroxy-2′-(triethylsilyloxy)propyl]-3-vinylcyclohexan-1-one (**34**) (84.8 mg, 51%) and its (1′*R*)-isomer (**35**) (66.7 mg, 40%) as a colorless syrup. Data for **34**: R_f 0.62 (EtOAc/hexane=1:6); mp 82.5–83.0 °C; $[\alpha]_D^{23}$ −35.5 (*c* 1.02, $CHCl_3$); IR (KBr disk) 3500, 2960, 2880, 1710, 1260, 1090, 1020 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.44–7.25 (5H, m), 5.83 (1H, ddd, *J*=9.0, 9.0, 16.8 Hz), 5.15 (1H, dd, *J*=1.4, 9.0 Hz), 5.11 (1H, dd, *J*=1.4, 16.8 Hz), 4.82 (1H, d, *J*=11.4 Hz), 4.49–4.36 (2H, m), 4.40 (1H, d, *J*=11.4 Hz), 3.60 (1H, dd, *J*=1.5, 6.4 Hz), 3.52 (1H, qd, *J*=5.9, 6.4 Hz), 3.00 (1H, s), 2.68 (1H, dd, *J*=1.5, 9.0 Hz), 2.58 (1H, ddd, *J*=9.0, 9.0, 9.2 Hz), 2.39 (1H, ddd, *J*=4.6, 6.4, 12.4 Hz), 1.76 (1H, ddd, *J*=10.5, 10.5, 12.4 Hz), 1.16 (3H, d, *J*=5.9 Hz), 0.95 (9H, t, *J*=7.8 Hz), 0.85 (9H, s), 0.60 (6H, q, *J*=7.8 Hz), 0.06 and 0.05 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 210.0, 138.1, 137.0, 128.3, 127.8, 127.7, 118.4, 80.5, 73.0, 72.2, 70.5, 68.7, 54.2, 53.8, 41.0, 25.8, 19.7, 18.0, 6.8, 5.0, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) *m*/*z* calcd for $C_{30}H_{53}O_5Si_2$, (M+H)$^+$ 549.3432, found 549.3435. Anal. Calcd for $C_{30}H_{52}O_5Si_2$: C, 65.64; H, 9.55. Found: C, 65.36; H, 9.36. Data for **35**: R_f 0.52 (EtOAc/hexane=1:6); $[\alpha]_D^{23}$ −64.3 (*c* 0.71, $CHCl_3$); IR (neat) 3540, 2960, 2930, 1715, 1090, 835, 775 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.42–7.25 (5H, m), 5.44 (1H, ddd, *J*=8.0, 10.7, 16.0 Hz), 5.22 (1H, dd, *J*=1.7, 8.0 Hz), 5.21 (1H, dd, *J*=1.7, 16.0 Hz), 4.81 and 4.39 (each 1H, 2d, *J*=11.4 Hz), 4.05–3.96 (2H, m), 3.82 (1H, ddd, *J*=4.4, 8.4, 11.2 Hz), 3.28 (1H, dd, *J*=8.8, 12.2 Hz), 3.10 (1H, d, *J*=12.2 Hz), 2.76–2.60 (2H, m), 2.47 (1H, ddd, *J*=4.4, 6.1, 12.7 Hz), 1.85 (1H, ddd, *J*=11.2, 12.7, 12.7 Hz), 1.27 (3H, d, *J*=6.1 Hz), 0.93 (9H, t, *J*=7.9 Hz), 0.85 (9H, s), 0.63–0.47 (6H, m), 0.06 and 0.03 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 212.5, 138.6, 137.6, 128.5, 127.9, 127.8, 119.7, 78.6, 75.7, 72.0, 70.6, 69.9, 54.2, 47.0, 42.3, 25.7, 21.6, 18.0, 7.0, 5.1, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) *m*/*z* calcd for $C_{30}H_{53}O_5Si_2$, (M+H)$^+$ 549.3432, found 549.3423. Anal. Calcd for $C_{30}H_{52}O_5Si_2$: C, 65.64; H, 9.55. Found: C, 65.42; H, 9.56.

4.3.3. Three-component coupling reaction with (*S*)-7b in the presence of HMPA. The similar treatment of (+)-**6** (96.8 mg, 0.291 mmol) with copper(I) cyanide (CuCN, 36.5 mg, 0.408 mmol) and vinyllithium (1.0 mol/L solution in ether, 0.65 mL, 0.65 mmol) as described for the preparation of **34** afforded 1,4-addition intermediate. To an ethereal solution of the enolate (4 mL) at −78 °C was added HMPA (0.345 mL, 1.98 mmol) and, after 5 min, a solution of

aldehyde (*S*)-**7b** (232 mg, 1.23 mmol) in ether (1.2 mL) was added to the mixture dropwise over 60 min via a cannula. After being stirred at −78 °C for 2 h, the reaction mixture was quenched and processed similarly as described for the preparation of **34**. Purification by column chromatography (silica gel: 3 g, EtOAc/hexane=1:40) gave **35** (75.2 mg, 47%) and bicyclic ketal (**36**) (42.2 mg, 20%) as a colorless syrup. Data for **36**: R_f 0.65 (EtOAc/hexane=1:6); $[\alpha]_D^{24}$ −49.1 (*c* 1.21, $CHCl_3$); IR (neat) 3440, 2955, 2880, 1100, 1075, 1000 cm^{-1}; ^1H NMR (300 MHz) δ 7.38–7.25 (5H, m), 5.34 (1H, ddd, *J*=9.6, 9.6, 17.4 Hz), 5.13–5.00 (3H, m), 4.95 and 4.65 (each 1H, 2d, *J*=11.6 Hz), 4.52 (1H, q, *J*=6.0 Hz), 4.03 (1H, d, *J*=10.2 Hz), 3.86 (1H, qd, *J*=3.9, 6.0 Hz), 3.39 (1H, dd, *J*=4.8, 11.7 Hz), 3.24 (1H, s), 3.22 (1H, ddd, *J*=4.8, 9.9, 11.4 Hz), 2.19 (1H, ddd, *J*=9.6, 9.9, 11.4 Hz), 2.02 (1H, ddd, *J*=4.8, 4.8, 12.6 Hz), 1.72 (1H, ddd, *J*=11.4, 11.7, 12.6 Hz), 1.39 (1H, dd, *J*=10.2, 11.4 Hz), 1.16 and 1.06 (each 3H, 2d, *J*=6.0 Hz), 0.94 and 0.89 (each 9H, 2t, *J*=7.8 Hz), 0.81 (9H, s), 0.60 and 0.53 (each 6H, 2q, *J*=7.8 Hz), −0.02 and −0.07 (each 3H, 2s); HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{39}H_{72}O_7Si_3Na$, $(M+Na)^+$ 759.4484, found 759.4493.

4.3.4. Conversion of 36 to 35. A solution of **36** (20.8 mg, 0.0282 mmol) in THF (1.5 mL) and AcOH (1.5 mL) was stirred at room temperature for 3 days. The reaction mixture was diluted with EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:6) to give **35** (11.8 mg, 76%).

4.3.5. Conversion of 34 to 22. To a solution of **34** (52.1 mg, 0.0949 mmol) in MeOH (1 mL) was added $NaBH_4$ (16.0 mg, 0.423 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. The mixture was diluted with EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried over Na_2SO_4. Removal of the solvent gave a residue, which was roughly purified by column chromatography (EtOAc/hexane=1:15) to give diol (34.5 mg, 66%). To a solution of the diol (34.5 mg, 0.0626 mmol) in pyridine (1 mL) were added Ac_2O (0.5 mL) and DMAP (1.0 mg) at 0 °C, and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc, and washed successively with 1 mol/L aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was dissolved in CH_2Cl_2/H_2O (10:1, 1.0 mL). To this solution was added DDQ (15.3 mg, 0.0673 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:7) to give **22** (22.7 mg, 46% from **34**).

4.3.6. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*S*)-1′-hydroxy-2′-(triethylsilyloxy)propyl]-4-vinylcyclohexan-2-ol (37). To a solution of **35** (22.0 mg, 0.0401 mmol) in AcOH/THF [1:5 (v/v), 1 mL] was added $LiBH_4$ (12.0 mg, 0.551 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. The mixture was diluted with EtOAc and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried over Na_2CO_3. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:7) to give **37** (19.2 mg, 87%) as a colorless syrup: R_f 0.40 (EtOAc/toluene=1:6); $[\alpha]_D^{21}$ +6.5 (*c* 0.895, $CHCl_3$); IR (neat) 3420, 2955, 2930, 2880, 1255, 1090, 1070, 840 cm^{-1}; ^1H NMR (300 MHz) δ 7.44–7.20 (5H, m), 5.30 (1H, ddd, *J*=9.3, 10.2, 16.8 Hz), 5.14 (1H, dd, *J*=2.4, 10.2 Hz), 5.10 (1H, dd, *J*=2.4, 16.8 Hz), 4.78 and 4.68 (each 1H, 2d, *J* =12.3 Hz), 4.28 (1H, d, *J*=1.8 Hz), 4.03 (1H, qd, *J*=5.1, 6.3 Hz), 3.74–3.61 (2H, m), 3.40–3.26 (2H, m), 2.32 (1H, d, *J*=9.0 Hz), 2.18 (1H, ddd, *J*=4.2, 4.2, 12.0 Hz), 2.10 (1H, ddd, *J*=9.3, 9.6, 11.4 Hz), 1.56 (1H, dd, *J*=9.6, 9.9 Hz), 1.46 (1H, ddd, *J*=11.4, 11.4, 12.0 Hz), 1.15 (3H, d, *J*=6.3 Hz), 0.97 (9H, t, *J*=7.8 Hz), 0.83 (9H, s), 0.63 (6H, q, *J*=7.8 Hz), −0.01 and −0.04 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 139.4, 139.0, 128.3, 127.7, 127.5, 119.1, 79.3, 75.2, 72.5, 72.0, 71.5, 71.1, 53.2, 42.9, 38.8, 25.8, 19.3, 18.0, 6.7, 4.8, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{30}H_{55}O_5Si_2$, $(M+H)^+$ 551.3588, found 551.3590. Anal. Calcd for $C_{30}H_{54}O_5Si_2$: C, 65.40; H, 9.88. Found: C, 65.48; H, 10.02.

4.3.7. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*S*)-1′-(*p*-toluenesulfonyloxy)-2′-(triethylsilyloxy)propyl]-4-vinylcyclohexan-2-ol (38). To a solution of **37** (39.0 mg, 0.0708 mmol) in THF (2 mL) were added 1.59 mol/L solution of BuLi in hexane (0.140 mL, 0.223 mmol) and TsCl (81 mg, 0.42 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. The mixture was diluted with EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 10 g, EtOAc/hexane=1:10) to give **38** (50.0 mg, 100%) as a white solid: R_f 0.71 (EtOAc/toluene=1:6); $[\alpha]_D^{26}$ +7.7 (*c* 0.79, $CHCl_3$); IR (KBr disk) 3410, 2960, 1360, 1175, 1090, 1070, 925 cm^{-1}; ^1H NMR (300 MHz) δ 7.81 (2H, d, *J*=8.4 Hz), 7.39–7.27 (7H, m), 5.32–5.15 (2H, m), 5.06 (1H, dd, *J*=3.6, 15.6 Hz), 4.74 (1H, d, *J*=12.0 Hz), 4.68 (1H, d, *J*=5.7 Hz), 4.66 (1H, d, *J*=12.0 Hz), 4.30 (1H, qd, *J*=5.7, 6.3 Hz), 4.15 (1H, s), 3.62 (1H, dd, *J*=8.7, 8.7 Hz), 3.29–3.16 (2H, m), 2.43 (3H, s), 2.06 (1H, ddd, *J*=3.9, 3.9, 12.0 Hz), 1.81–1.74 (2H, m), 1.22 (1H, ddd, *J*=11.4, 11.4, 12.0 Hz), 1.05 (3H, d, *J*=6.3 Hz), 0.94 (9H, t, *J*=7.8 Hz), 0.81 (9H, s), 0.61 (6H, q, *J*=7.8 Hz), −0.05 and −0.08 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 144.7, 138.9, 134.2, 129.7, 128.3, 127.9, 127.8, 127.5, 120.1, 84.4, 79.4, 71.9, 71.6, 71.0, 69.9, 51.6, 42.4, 38.3, 25.7, 21.6, 20.5, 17.9, 6.7, 4.7, −4.3, −4.5; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{37}H_{61}O_7SSi_2$, $(M+H)^+$ 705.3677, found 705.3684.

4.3.8. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*S*)-1′-(*p*-toluenesulfonyloxy)-2′-(triethylsilyloxy)propyl]-2-methoxymethyloxy-4-vinylcyclohexane (39). To a solution of **38** (33.6 mg, 0.0477 mmol) in $(CH_2Cl)_2$ (1 mL) were added *i*-Pr_2NEt (0.088 mL, 0.51 mmol) and MOMCl (0.039 mL, 0.52 mmol) at 0 °C, and the reaction mixture was stirred at 50 °C for 3.5 h. The mixture was diluted with EtOAc and washed successively

with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:10) to give **39** (35.8 mg, 92%) as a pale yellow syrup: R_f 0.71 (EtOAc/toluene=1:6); $[\alpha]_D^{22}$ −10.5 (*c* 0.92, $CHCl_3$); IR (neat) 2955, 2930, 1360, 1180, 1100, 1075, 1000, 925, 840 cm^{-1}; ^1H NMR (300 MHz) δ 7.80 (2H, d, *J*=7.8 Hz), 7.40–7.22 (7H, m), 5.38–5.10 (3H, m), 4.85 and 4.74 (each 1H, 2d, *J*=5.0 Hz), 4.66–4.52 (1H, m), 4.58 (2H, s), 4.26 (1H, qd, *J*=6.0, 9.0 Hz), 3.52 (1H, dd, *J*=9.3, 9.3 Hz), 3.30 (3H, s), 3.36–3.20 (1H, m), 3.15 (1H, ddd, *J*=3.9, 11.7, 12.0 Hz), 2.43 (3H, s), 2.11 (1H, ddd, *J*=3.9, 4.3, 12.3 Hz), 2.16–1.92 (2H, m), 1.27 (1H, ddd, *J*=12.0, 12.0, 12.3 Hz), 1.02 (3H, d, *J*=6.0 Hz), 0.95 (9H, t, *J*=7.8 Hz), 0.83 (9H, s), 0.60 (6H, q, *J*=7.8 Hz), −0.05 and −0.06 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 144.5, 138.7, 138.5, 134.5, 129.6, 128.3, 127.8, 127.7, 127.5, 119.7, 98.7, 85.0, 80.3, 78.9, 71.6, 70.8, 69.0, 57.0, 52.0, 38.8, 25.8, 21.6, 21.2, 18.0, 6.8, 5.1, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{39}H_{64}O_8SSi_2Na$, $(M+Na)^+$ 771.3758, found 771.3767.

4.3.9. (1*S*,2*S*,3*S*,4*R*,5*R*)-1-Benzyloxy-5-(*tert*-butyldimethylsilyloxy)-3-[(1′*R*,2′*S*)-2′-hydroxy-1′-(*p*-toluenesulfonyloxy)propyl]-2-methoxymethyloxy-4-vinylcyclohexane (40). To a solution of **39** (31.7 mg, 0.0423 mmol) in CH_3CN/H_2O (10:1, 3 mL) was added DDQ (10.6 mg, 0.0467 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and washed successively with 20% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:4) to give **40** (23.1 mg, 86%) as a white solid: R_f 0.40 (EtOAc/hexane=1:2); mp 107.8–108.5 °C; $[\alpha]_D^{26}$ −31.7 (*c* 0.89, $CHCl_3$); IR (neat) 3420, 2930, 2860, 1360, 1180, 1100, 1075, 840 cm^{-1}; ^1H NMR (300 MHz) δ 7.79 (2H, d, *J*=8.0 Hz), 7.37–7.28 (7H, m), 5.30 (1H, ddd, *J*=9.6, 10.2, 16.6 Hz), 5.17 (1H, dd, *J*=2.3, 10.2 Hz), 5.13 (1H, dd, *J*=2.3, 16.6 Hz), 4.88 (2H, s), 4.85 (1H, d, *J*=5.4 Hz), 4.60 and 4.55 (each 1H, 2d, *J*=11.8 Hz), 3.94 (1H, qd, *J*=6.3, 5.4 Hz), 3.63 (1H, dd, *J*=9.8, 10.4 Hz), 3.41 (3H, s), 3.31 (1H, ddd, *J*=4.0, 9.8, 11.7 Hz), 3.21 (1H, ddd, *J*=4.0, 11.2, 11.7 Hz), 2.44 (3H, s), 2.16 (1H, ddd, *J*=9.6, 11.2, 11.4 Hz), 2.16 (1H, ddd, *J*=4.0, 4.0, 11.9 Hz), 1.89 (1H, dd, *J*=10.4, 11.7 Hz), 1.36 (1H, ddd, *J*=11.7, 11.7, 11.9 Hz), 1.03 (3H, d, *J*=6.3 Hz), 0.82 (9H, s), −0.04 and −0.06 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 144.7, 138.6, 138.2, 134.3, 129.7, 128.4, 127.8, 127.7, 119.6, 99.5, 84.3, 79.7, 77.2, 71.7, 70.4, 68.2, 57.1, 51.6, 38.7, 25.7, 21.6, 21.4, 18.0, −4.3, −4.4; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{33}H_{50}O_8SSiNa$, $(M+Na)^+$ 657.2894, found 657.2893.

4.3.10. Lactone (30) from 40. Ozone was introduced to a solution of **40** (45.2 mg, 0.0712 mmol) in MeOH (4.5 mL) at −78 °C for 6 min. To the reaction mixture was added Me_2S (0.5 mL) at −78 °C, and the reaction mixture was stirred at room temperature for 3 h and then diluted with Et_2O. The organic layer was washed with brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:6) to give lactol **41** (36.2 mg, 80%) as a colorless syrup: R_f 0.56 (EtOAc/hexane=1:2); IR (neat) 3420, 2960, 2930, 2860, 1720, 1600 cm^{-1}; ^1H NMR (300 MHz) δ 7.81 (2H, d, *J*=8.3 Hz), 7.50–7.20 (7H, m), 5.23 (1H, d, *J*=2.5 Hz), 4.75 (1H, d, *J*=6.7 Hz), 4.67–4.53 (3H, m), 4.33 (1H, dd, *J*=9.5, 10.0 Hz), 4.08 (1H, qd, *J*=6.7, 9.5 Hz), 3.83 (1H, dd, *J*=7.4, 7.6 Hz), 3.72 (1H, ddd, *J*=5.4, 9.3, 9.8 Hz), 3.59 (1H, ddd, *J*=4.6, 7.6, 8.4 Hz), 3.28 (3H, s), 2.43 (3H, s), 2.42–2.33 (1H, m), 2.20 (1H, ddd, *J*=4.6, 5.4, 13.4 Hz), 1.85 (1H, ddd, *J*=2.5, 9.8, 10.2 Hz), 1.47 (1H, ddd, *J*=8.4, 9.3, 13.4 Hz), 0.87 (3H, d, *J*=6.7 Hz), 0.82 (9H, s), 0.02 (6H, s); ^{13}C NMR (75 MHz) δ 144.4, 138.8, 135.3, 129.5, 128.3, 127.8, 127.7, 127.4, 96.2, 90.1, 86.0, 79.1, 77.9, 71.2, 67.1, 66.7, 55.9, 48.1, 38.7, 37.4, 25.7, 21.6, 18.3, 17.6, −4.2, −5.0.

To a solution of lactol **41** (15.1 mg, 0.0237 mmol) in CH_2Cl_2 (1 mL) were added PDC (178 mg, 0.473 mmol) and MS4A (300 mg) at 0 °C, and the reaction mixture was stirred at room temperature for 12 h. The insoluble material was removed by filtration through a pad of Celite, and the filtrate was diluted with EtOAc. The organic layer was washed with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/toluene=1:19) to give **30** (12.5 mg, 83%).

4.4. Formal synthesis of (+)-actinobolin

4.4.1. Methyl 2-*O*-benzyl-3-deoxy-α-D-glucopyranoside (42). To a suspension of NaH (18.0 mg, 0.450 mmol) in DMF (0.5 mL) at 0 °C was added a solution of **9** (20.0 mg, 0.0751 mmol) in DMF (0.5 mL). After being stirred for 10 min, to the mixture was added BnBr (0.0180 mL, 0.151 mmol) at 0 °C, and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 0.5 g, EtOAc/hexane=1:6) to give methyl 4,6-*O*-benzylidene-2-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranoside (25.4 mg, 95%) as a white solid: R_f 0.55 (EtOAc/hexane =1:2); mp 102.0–102.5 °C; $[\alpha]_D^{20}$ +21.4 (*c* 0.99, $CHCl_3$); IR (KBr disk) 2940, 2900, 2860, 1500, 1100, 1050, 990 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50–7.27 (5H, m), 5.49 (1H, s), 4.68 (1H, d, *J*=3.3 Hz), 4.67 and 4.58 (each 1H, 2d, *J*=12.6 Hz), 4.25 (1H, dd, *J*=4.5, 10.2 Hz), 3.77 (1H, ddd, *J*=4.5, 9.6, 10.2 Hz), 3.66 (1H, dd, *J*=10.2, 10.6 Hz), 3.60 (1H, ddd, *J*=3.3, 4.2, 11.7 Hz), 3.49 (1H, ddd, *J*=4.5, 9.6, 11.7 Hz), 3.45 (3H, s), 2.27 (1H, ddd, *J*=4.2, 4.5, 11.4 Hz), 2.05 (ddd, *J*=11.4, 11.7, 11.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 137.4, 129.1, 128.5, 128.3, 127.91, 127.86, 126.2, 101.8, 98.0, 76.7, 73.8, 71.0, 69.4, 63.9, 55.1, 30.1; LRMS (EI) *m/z* 356 (M^+, 1.7%), 265 (4.5), 233 (3.4), 218 (81.6), 162 (61.9), 105 (100), 91 (100); HRMS (EI) *m/z* calcd for $C_{19}H_{30}IO_3Si$ 356.1624, found 356.1629. Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79. Found: C, 70.56; H, 6.93.

A solution of methyl 4,6-*O*-benzylidene-2-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranoside (25.4 mg, 0.0713 mmol) in 80% AcOH (1 mL) was stirred at 80 °C for 1 h. The reaction

mixture was concentrated to give a residue, which was purified by column chromatography (silica gel: 0.6 g, EtOAc/toluene=1:1) to afford **42** (17.5 mg, 92%) as a white solid: R_f 0.1 (EtOAc/toluene=1:1); mp 101.0–102.0 °C; $[\alpha]_D^{20}$ +65.1 (*c* 0.71, $CHCl_3$); IR (KBr disk) 3320, 2900, 2880, 1105, 1050, 1030, 1000 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.28 (5H, m), 4.64 (1H, d, *J*=4.2 Hz), 4.64 and 4.56 (each 1H, 2d, *J*=12.3 Hz), 3.85 (1H, dd, *J*=3.7, 11.5 Hz), 3.75 (1H, dd, *J*=3.6, 11.5 Hz), 3.62 (1H, ddd, *J*=4.8, 10.0, 11.2 Hz), 3.56–3.45 (2H, m), 3.42 (3H, s), 2.11 (1H, ddd, *J*=4.7, 4.8, 11.4 Hz), 1.87 (1H, ddd, *J*=11.2, 11.4, 11.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 128.5, 127.90, 127.85, 97.1, 73.5, 71.9, 71.9, 66.1, 62.6, 55.0, 33.1; HRMS (FAB$^+$, NBA matrix) *m/z* (M+H)$^+$ calcd for $C_{14}H_{21}O_5$ 269.1389, found 269.1384. Anal. Calcd for $C_{14}H_{20}O_5$: C, 62.67; H, 7.51. Found: C, 62.79; H, 7.50.

4.4.2. Methyl 2-*O*-benzyl-3,6-dideoxy-α-D-*xylo*-hex-5-enopyranoside (43). To a solution of **42** (997 mg, 3.72 mmol) in toluene (20 mL) were added PPh_3 (1.56 g, 5.95 mmol), imidazole (1.01 g, 14.8 mmol), and iodine (1.51 g, 5.95 mmol), and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with EtOAc, and washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 30 g, EtOAc/hexane=1:2) to give methyl 2-*O*-benzyl-3,6-dideoxy-6-iodo-α-D-glucopyranoside (1.40 g, 100%) as a colorless syrup: R_f 0.55 (EtOAc/hexane=1:1); $[\alpha]_D^{22}$ +64.2 (*c* 1.3, $CHCl_3$); IR (neat) 3430, 2940, 2900, 1090, 1060, 980 cm^{-1}; ^1H NMR (300 MHz) δ 7.38–7.27 (5H, m), 4.69 (1H, d, *J*=3.4 Hz), 4.63 and 4.56 (each 1H, 2d, *J*=12.4 Hz), 3.62–3.23 (6H, m), 3.48 (3H, s), 2.19 (1H, ddd, *J*=4.6, 4.6, 11.5 Hz), 1.89 (1H, ddd, *J*=11.5, 11.5, 11.5 Hz); ^{13}C NMR (75 MHz) δ 137.9, 128.5, 127.9, 127.8, 97.3, 73.7, 71.5, 71.2, 69.5, 55.3, 33.4, 7.3; LRMS (EI) *m/z* 378 (M$^+$, 0.9%), 347 (1.1), 272 (13.9), 240 (100), 135 (89.5), 91 (100); HRMS (EI) *m/z* calcd for $C_{14}H_{19}IO_4$, (M$^+$) 378.0328, found 378.0335.

To a solution of the iodide (2.40 g, 9.59 mmol) in THF (45 mL) was added *t*-BuOK (2.14 g, 28.8 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc, and successively washed with H_2O and brine, and then dried over Na_2CO_3. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 40 g, EtOAc/hexane=1:10 contain 1 vol % Et_3N) to give enopyranoside **43** (1.10 g, 70%) as a colorless syrup: R_f 0.54 (EtOAc/hexane=1:1); $[\alpha]_D^{20}$ +62.3 (*c* 0.23, $CHCl_3$); IR (neat) 3440, 2940, 1680, 1085, 1055, 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.22 (5H, m), 4.72 (1H, d, *J*=3.3 Hz), 4.70 (1H, br s), 4.67 (1H, d, *J*=12.6 Hz), 4.65 (1H, br s), 4.61 (1H, d, *J*=12.6 Hz), 4.09 (1H, m), 3.67 (1H, ddd, *J*=3.3, 4.7, 11.1 Hz), 3.49 (3H, s), 2.25 (1H, ddd, *J*=4.7, 5.7, 11.3 Hz), 2.04 (1H, d, *J*=8.7 Hz), 1.94 (1H, ddd, *J*=11.3, 11.1, 10.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 137.8, 128.5, 128.0, 127.9, 99.3, 94.6, 73.1, 71.5, 66.0, 55.5, 34.0; HRMS (FAB$^+$, glycerol matrix) *m/z* (M+H)$^+$ calcd for $C_{14}H_{19}O_4$: 251.1283, found 251.1289.

4.4.3. Methyl 2-*O*-benzyl-4-*O*-(*tert*-butyldimethylsilyl)-3,6-dideoxy-α-D-*xylo*-hex-5-enopyranoside (44). To a solution of enopyranoside **43** (26.7 mg, 0.107 mmol) in DMF (1 mL) were added imidazole (21.8 mg, 0.320 mmol) and TBSCl (24.1 mg, 0.160 mmol), and the mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with EtOAc, washed with H_2O, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel; 4 g, EtOAc/hexane=1:50 containing 1 vol % Et_3N) to give **44** (33.1 mg, 85%) as a white solid: R_f 0.70 (EtOAc/hexane =1:20); mp 78–79 °C; $[\alpha]_D^{23}$ +41.0 (*c* 1.1, $CHCl_3$); IR (KBr disk) 2955, 2860, 1665, 1115, 1060, 1000, 855, 840 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.24 (5H, m), 4.77 (1H, d, *J*=2.4 Hz), 4.74 and 4.72 (each 1H, 2br s), 4.65 and 4.58 (each 1H, 2d, *J*=12.6 Hz), 3.68 (1H, ddd, *J*=2.4, 6.9, 9.6 Hz), 3.56–3.30 (1H, m), 3.42 (3H, s), 2.23–1.90 (2H, m), 0.92 (9H, s), 0.10 and 0.08 (each 3H, 2s); HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{20}H_{33}O_4Si$, (M+H)$^+$ 365.2148, found 365.2149.

4.4.4. A mixture of (2*S*,4*R*,5*R*)-4-benzyloxy-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-cyclohexan-1-one and its (5*S*)-isomer (45). To a solution of **44** (2.30 g, 6.31 mmol) in acetone (120 mL) and acetate buffer (0.1 mol/L solution, pH 4.8, 120 mL) was added $Hg(OCOCF_3)_2$ (0.81 g, 1.9 mmol), and the mixture was stirred at room temperature for 48 h. The reaction mixture was partially concentrated and the products were extracted with EtOAc. The organic layer was washed successively with 10% aqueous KI solution, 20% $Na_2S_2O_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 45 g, EtOAc/hexane=1:5) to give **45** (1.84 g, 83%) as a diastereomeric mixture (5*S*:5*R*=ca. 1:6): R_f 0.41 (EtOAc/hexane=1:2); IR (neat) 3450, 2930, 2860, 1740, 1250, 1150, 1075, 840 cm^{-1}; ^1H NMR (300 MHz, for the major isomer) δ 7.42–7.28 (5H, m), 4.77 and 4.58 (each 1H, 2d, *J*=11.5 Hz), 4.15 (1H, dd, *J*=6.6, 12.5 Hz), 3.76 (1H, ddd, *J*=5.4, 8.8, 12.2 Hz), 3.62 (1H, ddd, *J*=4.0, 8.8, 11.6 Hz), 2.76 (1H, dd, *J*=5.4, 13.9 Hz), 2.46 (1H, ddd, *J*=4.0, 6.6, 12.5 Hz), 2.37 (1H, dd, *J*=12.2, 13.9 Hz), 1.62 (1H, ddd, *J*=11.6, 12.5, 12.5 Hz), 0.90 (9H, s), 0.13 and 0.02 (each 3H, 2s); ^{13}C NMR (75 MHz, for the major isomer) δ 204.6, 137.6, 128.6, 128.1, 127.9, 79.4, 74.1, 72.1, 71.8, 43.5, 35.2, 25.7, 18.4, −4.6, −5.5; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{19}H_{31}O_4Si$, (M+H)$^+$ 351.1992, found 351.1992.

4.4.5. (1*S*,2*S*,4*R*,5*R*)-4-benzyloxy-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-1-methanesulfonyloxy-cyclohexane (47). To a solution of **45** (207 mg, 0.591 mmol) in acetonitrile (4 mL) were added CAN (64.8 mg, 0.118 mmol) and dihydro-2*H*-pyran (0.11 mL, 1.2 mmol), and the reaction mixture was stirred at 0 °C for 50 min. The reaction mixture was diluted with EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 6 g, EtOAc/hexane=1:15) to give *O*-THP ether (227 mg, 89%) as a diastereomer mixture. To a solution of the THP ether (227 mg, 0.522 mmol) in MeOH (5 mL) in the presence of $CeCl_3 \cdot 7H_2O$ (389 mg, 1.04 mmol) was added $NaBH_4$ (29.6 mg, 0.782 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. The reaction mixture was diluted with

EtOAc, and washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a crude **46**, which was dissolved in pyridine (4 mL). To this solution were added MsCl (0.067 mL, 0.86 mmol) and DMAP (15 mg, 0.12 mmol) at 0 °C, and the reaction mixture was stirred at 80 °C for 13 h. The reaction mixture was diluted with 1 mol/L aqueous HCl solution and stirred at room temperature for 1 h. The products were extracted with EtOAc, and the organic layer was washed successively with saturated aqueous $NaHCO_3$ solution and brine, and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 4 g, EtOAc/hexane=1:3) to give **47** (167 mg, 66% from **45**) as a colorless syrup: R_f 0.50 (EtOAc/hexane=1:1); $[\alpha]_D^{22}$ ~0 (*c* 0.92, $CHCl_3$); IR (neat) 3450, 2960, 2930, 2890, 2860, 1255, 1180, 1080, 955, 920, 840 cm^{-1}; ^1H NMR (300 MHz) δ 7.42–7.27 (5H, m), 4.65 and 4.55 (each 1H, 2d, *J*=11.5 Hz), 4.31 (1H, ddd, *J*=4.4, 8.8, 11.7 Hz), 3.68–3.52 (2H, m), 3.22 (1H, ddd, *J*=4.4, 9.0, 11.7 Hz), 3.00 (3H, s), 2.63 (1H, s), 2.52 (1H, ddd, *J*=4.7, 4.9, 12.6 Hz), 2.20 (1H, ddd, *J*=4.4, 4.7, 12.9 Hz), 1.60 (1H, ddd, *J*=11.7, 12.0, 12.6 Hz), 1.35 (1H, ddd, *J*=11.7, 11.7, 12.9 Hz), 0.88 (9H, s), 0.08 and 0.06 (each 3H, 2s); ^{13}C NMR (75 MHz) δ 137.8, 128.6, 128.1, 127.9, 82.2, 71.9, 70.7, 70.6, 38.4, 35.4, 35.0, 25.7, 17.9, −4.4, −4.9; HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{20}H_{35}O_6SSi$, $(M+H)^+$ 431.1924, found 431.1919.

4.4.6. Preparation of enantiomeric cyclohexenone [(−)-6]. A solution of $(COCl)_2$ (2.0 mol/L solution in CH_2Cl_2, 0.11 mL, 0.22 mmol) and DMSO (0.030 mL, 0.423 mmol) in CH_2Cl_2 (0.5 mL) was stirred at −78 °C for 10 min under Ar. To this mixture was added a solution of **47** (15.4 mg, 0.0358 mmol) in CH_2Cl_2 (0.5 mL) at −78 °C. After being stirred at −78 °C for 2 h, the reaction mixture was quenched by addition of Et_3N (0.040 mL, 0.29 mmol) at −78 °C. The resulting suspension was further stirred at 0 °C for 1 h, and then diluted with Et_2O. The organic layer was washed with brine and then dried. Removal of the solvent gave a residue, which was purified by column chromatography (silica gel: 1 g, EtOAc/hexane=1:7) to give (−)-**6** (11.1 mg, 93%) as a white solid: mp 46–47 °C; $[\alpha]_D^{19}$ −22.0 (*c* 0.80, $CHCl_3$); HRMS (FAB$^+$, NBA matrix) *m/z* calcd for $C_{19}H_{29}O_3Si$, $(M+H)^+$ 333.1886, found 333.1889. Anal. Calcd for $C_{21}H_{24}O_5$: C, 68.63; H, 8.49. Found: C, 68.49; H, 8.39. The ^1H and ^{13}C NMR spectra were fully identical with those of (+)-**6**.

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Synthesis of the CD-ring of the anticancer agent streptonigrin: studies of aryl–aryl coupling methodologies

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Abstract—A series of functionalized 4-bromopyridines, representing the C-ring of the anticancer agent streptonigrin have been prepared and their abilities to undergo Pd-catalyzed cross-coupling with streptonigrin D-ring siloxanes were evaluated. The coupling reaction was generally tolerant to the preparation of hindered CD biaryls; however, the electronic effects of both partners play a pivotal role in the success of the coupling process. Analogs of the CD biaryl were prepared by coupling of aryl siloxane derivatives (D-ring component) with highly functionalized 4-bromopyridines (C-ring); however, the CD biaryl of the natural product could not be prepared in high yield by siloxane coupling due to the facile formation of reduced pyridine under the coupling conditions. Alternatively, the fully functionalized CD biaryl of streptonigrin was prepared using a Suzuki coupling of appropriately functionalized C-ring bromide and D-ring aryl boronic acid. The described approach is highly convergent and readily amenable to the synthesis of analogs.

1. Introduction

The formation of carbon–carbon bonds remains the most crucial transformation in synthetic chemistry, and the last 25 years have witnessed the development of a multitude of transition metal-catalyzed reactions that have greatly improved this process.[1] No other metal has been as widely studied for this purpose as palladium, and within this field the Stille,[2] Suzuki–Miyaura,[3] and Negishi[4] couplings have been the most widely explored. However, each of these processes suffers from limitations, including tedious preparation and purification of coupling precursors, reagent toxicity, or lack selectivity exhibited by the coupling partner. Organosilicon-based coupling strategies[5] address these limitations and offer an alternative to existing cross-coupling methodologies. Research from these laboratories has shown that aryltrialkoxysilanes, in the presence of fluoride and catalytic Pd(0), undergo aryl group transfer to a range of aryl halides and triflates.[6–11] Several methods have been developed for the synthesis of siloxane derivatives,[12–14] and the coupling reaction has been found to be of broad utility.

During our continued effort to explore the scope and tolerances of this process, we became interested in the synthesis of the anticancer agent streptonigrin (**1**). This fungal metabolite was isolated over 40 years ago[15] and was found to exhibit potent anticancer and antiviral activity.[16] It has recently been shown that naturally occurring streptonigrin exists as a single atrope isomer (the M configuration has been assigned), with hindered rotation about the CD-ring juncture (Fig. 1).[17]

The desirable biological properties as well as unique structural features of streptonigrin have made this agent a popular target,[18] and three total syntheses have been reported.[19–21] Our interest in the natural product was based on the supposition that the tetracyclic core of streptonigrin could be accessed using two sequential Pd-catalyzed cross-coupling reactions (Scheme 1). This approach offers the advantage of being highly convergent, as the AB, C, and D-rings may be independently prepared and functionalized prior to the coupling steps. In fact, several groups have reported the

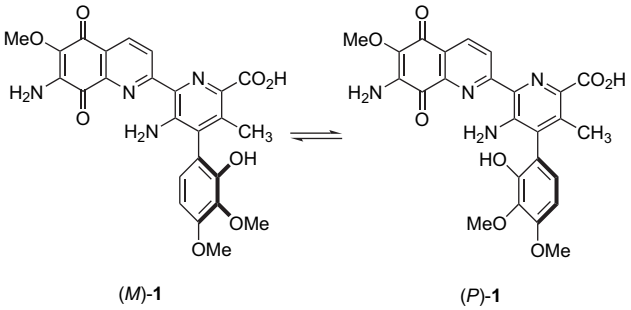

Figure 1. Atrope isomers of streptonigrin.

Keywords: Palladium-catalyzed; Cross-coupling; Organosiloxanes; Streptonigrin.
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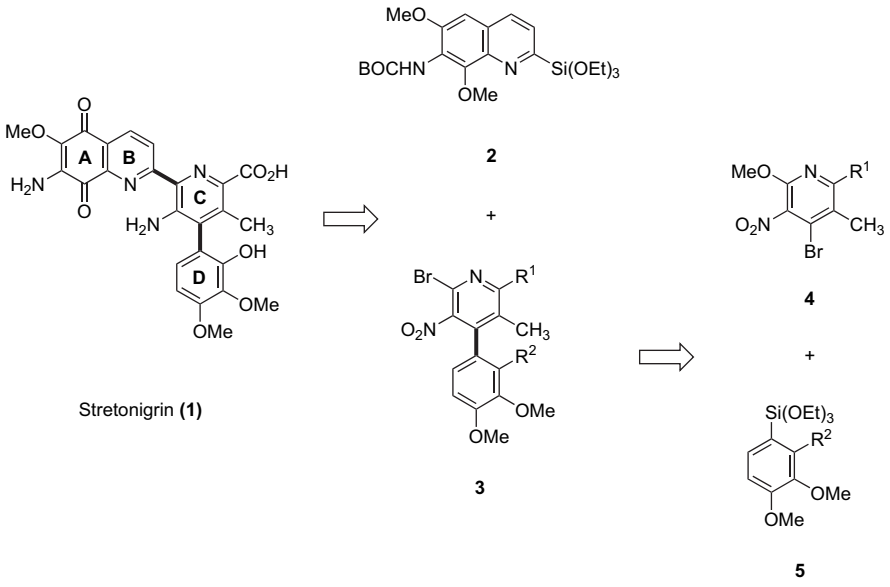


Scheme 1. Retrosynthetic analysis of streptonigrin.

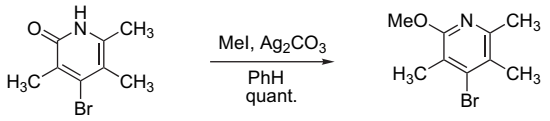
syntheses of streptonigrin model systems based on this approach.[22–30] These studies have demonstrated that the coupling reaction to form the CD biaryl tolerates the use of sterically hindered partners. The electronic effects of the pyridine ring, though, significantly influence the success of this strategy, and at present the natural product has yet to succumb to total synthesis using this approach. We have previously communicated preliminary results regarding siloxane-based couplings to prepare CD model systems of streptonigrin,[31] and report herein our full findings. These studies have culminated in a highly modular synthesis of streptonigrin CD biaryl.

2. Results and discussion

Our retrosynthetic analysis for streptonigrin (**1**) is outlined in Scheme 1. The tetracyclic core was to be established through the Pd-catalyzed coupling of quinoline siloxane **2** with 2-bromopyridine **3**. Conversion to the natural product would then be accomplished upon oxidation of the A-ring to the quinone and global deprotection. Biaryl **3** was envisaged as the product of a Pd-catalyzed coupling of 4-bromopyridine **4** and aryl siloxane **5**. Successful implementation of this strategy would require the development of coupling conditions amenable to the synthesis of highly functionalized (and hindered) biaryl derivatives.

The coupling reaction to form the CD biaryl presented a major synthetic challenge because a pentasubstituted pyridine bearing electron-donating and electron-withdrawing substituents would be one of the components. A siloxane coupling reaction with such a complex aromatic precursor had not been investigated previously and these studies would ultimately define the scope and limitations of the siloxane methodology. We therefore decided to develop a general approach to C-ring coupling precursors that was amenable to the preparation of analogs, in order that the steric and electronic factors that influence the coupling reaction be investigated in a systematic manner.

The starting points for our studies were the known pyridones **6**[32] and **7**.[33] Trimethyl substituted pyridine **8** was readily obtained upon methylation of pyridone **6** (Scheme 2).

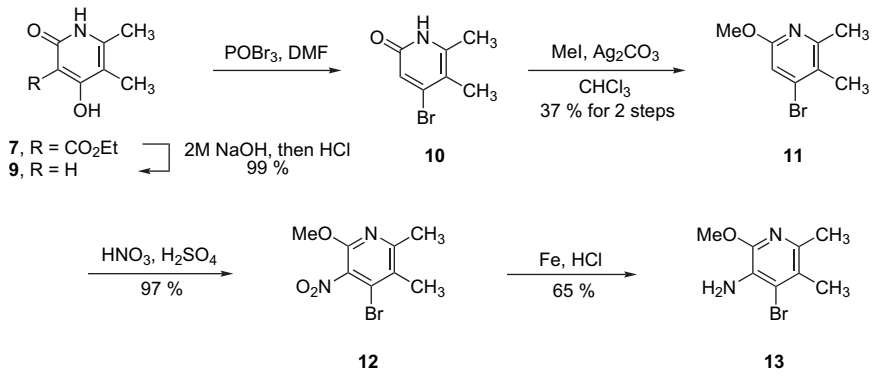


6 8

Scheme 2. Synthesis of bromopyridine **8**.

Pyridone **7** was subjected to ester hydrolysis and acid-promoted decarboxylation to give **9** (Scheme 3). Replacement of the C-4 hydroxyl group of pyridine **9** with a bromine substituent proved to be problematic due to competitive bromination at C-2. After significant experimentation, it was found that reaction of **9** with 0.70 equiv $POBr_3$ in DMF led reproducibly to bromopyridone **10**. Although the bromopyridone could be purified, it was more convenient to carry the crude material through the next step. Thus, methylation of the reaction mixture gave pyridine **11** in 37% isolated yield over two steps. Introduction of a nitrogen substituent at C-3 was accomplished in high yield by nitration of bromide **11** to provide nitropyridine **12**. Reduction of the nitro group occurred uneventfully to provide the amino analog **13**. These fully functionalized pyridine derivatives were now available for preliminary coupling reactions with siloxane derivatives.

Comparison of the cross-coupling reactions of bromopyridines **8** and **11** with an aryl siloxane was expected to provide information regarding the role of steric effects in the coupling process. Similarly, the cross-coupling of pyridines **11**, **12**, and **13**, each with a substituent in the C-3 position, would allow us to examine the influence of electronic factors on the coupling reaction. The results of these coupling reactions are summarized in Table 1. Coupling reactions were performed under identical conditions employing 20 mol %

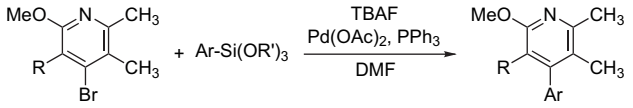


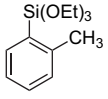
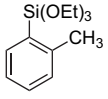
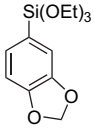
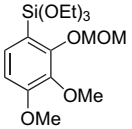
Scheme 3. Synthesis of bromopyridines **11**–**13**.

$Pd(OAc)_2$ and 40 mol % PPh_3, conditions which had proven to be optimal in previous coupling studies: 2 equiv of aryl siloxane and TBAF in DMF at 80 °C.

Coupling of pyridine **11** was investigated first, since this is the least sterically hindered of the bromopyridine series and its behavior should establish the baseline for the coupling reaction. Coupling of **11** and $PhSi(OMe)_3$ gave an excellent yield of the expected adduct (Table 1, entry 1).

Table 1. Coupling reactions of 4-bromopyridine derivatives with various aryl siloxanes



Entry	R	Ar–Si(OR′)$_3$	Yield (%)
1	H, **11**	$PhSi(OMe)_3$	97
2	H, **11**	 **14**	10[a]
3	Me, **8**	$PhSi(OMe)_3$	89
4	Me, **8**	 **14**	10[a]
5	Me, **8**	 **15**	61
6	Me, **8**	 **16**	0[a]
7	NO_2, **12**	$PhSi(OMe)_3$	36[a]

[a] The remainder of the mass balance was reduced bromopyridine.

However, the reaction of **11** with *o*-methyl siloxane **14**[14] gave only a 10% yield of the desired product (entry 2); the major product of the reaction was not the desired biaryl but the reduced (hydrodebrominated) pyridine. This result was surprising in two respects: first, the reduced products had not been observed previously in siloxane-based coupling reactions so their formation in this procedure was unanticipated. Secondly, *o*-tolyl siloxanes such as **14** underwent coupling with a variety of bromobenzene derivatives in excellent yield, demonstrating that steric effects on the siloxane component were not typically important to the success of the coupling reaction.[34] In this case, however, the coupling of the bromopyridine does not follow the typical profile. As it will become apparent in subsequent experiments, the reduction of the bromopyridine under coupling conditions will prove to be a significant complication in this approach (vide infra).

In order to further assess the steric tolerance of the coupling reaction, the coupling of trimethylbromopyridine **8** was examined. The Pd-catalyzed reaction of **8** and $PhSi(OMe)_3$ gave an 89% yield of the coupled adduct, while coupling of **8** and *o*-methyl siloxane **14** provided only 10% of the desired biaryl product (entries 3 and 4, respectively). These results are analogous to those obtained with bromopyridine **11**, and demonstrate that *ortho*-disubstituted bromopyridines are unsuitable coupling partners.

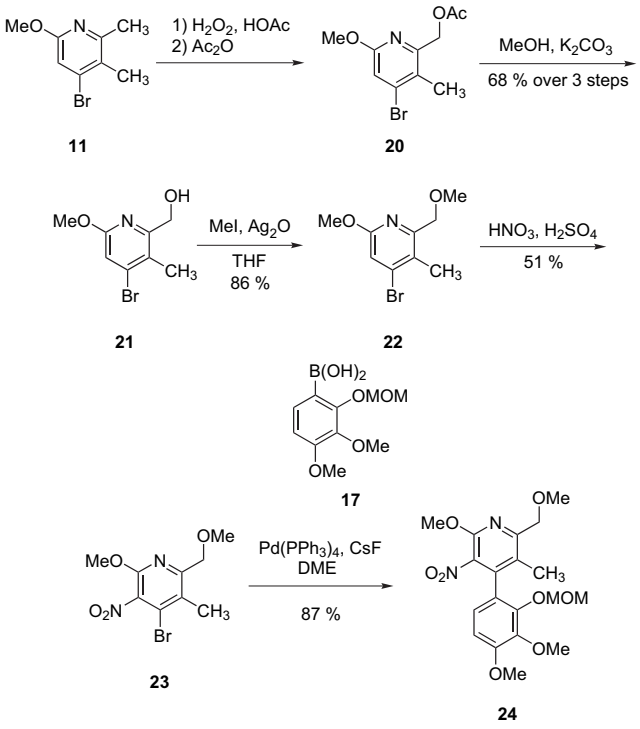
The reaction of bromopyridine **8** with methylenedioxy siloxane **15**[12] gave a 61% yield of the expected biaryl (entry 5). This was a significant finding since it demonstrated that couplings with siloxane derivatives lacking *ortho*-substituents occurred uneventfully. In addition, the substitution pattern of siloxane **15** is similar to the D-ring of streptonigrin, thus demonstrating that this approach would be useful for the synthesis of streptonigrin analogs lacking the *ortho*-phenolic group. It was disappointing that bromopyridine **8** failed to undergo the coupling reaction with siloxane **16** (entry 6), the siloxane that would directly provide the CD-ring system of the natural product. This result was not unexpected, however, based on the coupling results using *o*-tolyl siloxane. In addition, we had previously observed that aryl siloxanes with a heteroatom in the *ortho*-position to silicon underwent rapid protodesilylation under coupling conditions.[13] Even employing a stoichiometric amount of Pd(0) provided no improvement in the coupling outcome.

The couplings of 4-bromopyridine derivatives bearing nitrogen containing substituents at C-3 were also studied. Analogs of bromopyridine **12** with either an amino, azido, and amido functionality at C-3 failed to yield biaryl with $PhSi(OMe)_3$. However, reaction of nitropyridine **12** and $PhSi(OMe)_3$ gave a 36% yield of the corresponding biaryl (entry 7), along with 36% of the reduced (dehalogenated) pyridine. Although the yield of biaryl in this reaction was modest, studies with more complex siloxanes such as those needed for the total synthesis of streptonigrin were undertaken in the hope of improving and optimizing the yield of coupling. Unfortunately, formation of the dehalogenated pyridine was the major product observed from these studies. An extensive study of alternative Pd catalysts, ligands, and activators was performed, but the yield of cross-coupled product never rose above 30% in these reactions. The conclusion that must be drawn from these studies is that the electronic effects on both components played a pivotal role in the coupling process.

The difficulty experienced in preparing the streptonigrin CD skeleton using the siloxane technology led us to wonder if this unit may better be synthesized using organoboron reagents as the coupling reagent (Scheme 4). Reaction of bromopyridine **12** with boronic acid **17** gave a 78% yield of the respective biaryl with no evidence of the dehalogenated pyridine in the reaction products.

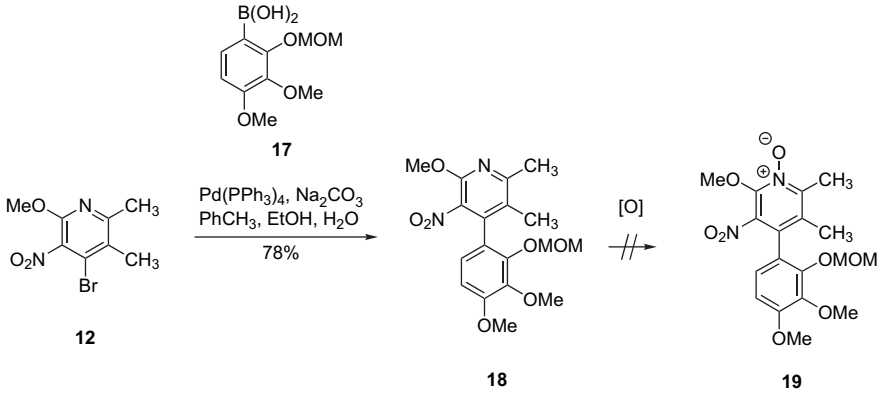
Oxidation of the pyridine C-6 methyl group of **18** to its carboxylic acid (the functional group present at this position in streptonigrin) was explored. The intention was to convert **18** to its *N*-oxide, followed by application of the pyridine *N*-oxide rearrangement[34] to provide a benzylic alcohol at the C-6 position of the pyridine ring. This strategy has been executed previously in an earlier synthesis of streptonigrin.[20] Much to our surprise, conversion of pyridine **18** to *N*-oxide **19** could not be accomplished. Using a wide variety of oxidants, the starting pyridine was recovered. Reduction of the nitro group of **18** and protection of the resulting amino group as its acetamide gave pyridine derivatives that were resistant to oxidation under a variety of conditions.

It was unclear whether the lack of reactivity of pyridine **18** and its derivatives to oxidation was the result of the electron-withdrawing nature of the C-3 substituent. To address this concern, the oxidation of pyridine **11** lacking a substituent at C-3 was investigated (Scheme 5). Treatment of **11** with H_2O_2 in HOAc resulted in complete conversion to the corresponding *N*-oxide, as determined by ^1H NMR. The *N*-oxide was not isolated but was treated immediately with Ac_2O to give rearranged product **20** in 93% yield over two steps. The facile oxidation-rearrangement of pyridine **11** demonstrated that the failure of pyridine **18** to undergo oxidation was due to the presence of the nitrogen substituent(s) at C-3, which must alter the relative basicity of the pyridine lone pair.



Scheme 5. Preparation and cross-coupling of bromopyridine **24**.

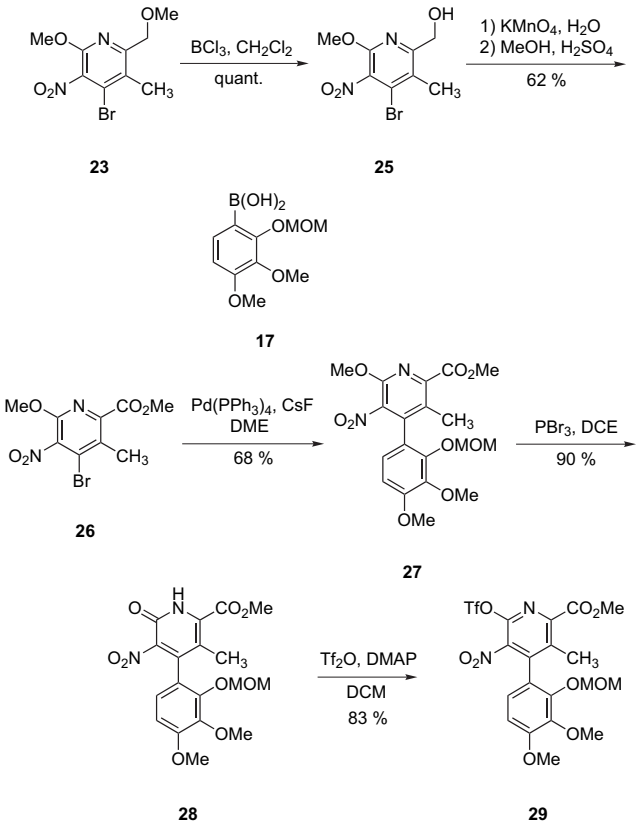
Based on the inability to oxidize the C-6 methyl group of C-ring once the aromatic D-ring had been introduced, the order of C-ring oxidation and D-ring coupling was altered. Treatment of **20** under the previously developed nitrating conditions gave a number of undesired reaction products, which had their origins in cleavage of the acid labile acetate functionality at C-6. It was clear that a robust protecting



Scheme 4. Suzuki coupling of bromopyridine **12** and boronic acid **17**.

group for the 1° alcohol of **21** would be required to effect nitration under the strongly acidic conditions. After extensive investigation, methyl ether **22** was determined to be the most suitable protected derivative. Nitration of methyl ether **22** gave nitropyridine **23** in 51% yield. We attribute the modest yield of the nitration (compared to pyridine **13**, Scheme 4) to be the result of deactivation of the pyridine ring due to protonation of the pyridine nitrogen. Nevertheless, with **23** in hand, the stage was set to explore the key coupling reaction to prepare the CD biaryl. The Pd-catalyzed reaction of bromopyridine **23** with siloxane derivatives was ineffective (vide supra), however, boronic acid **17** coupled with **23** using CsF as the activator in DME[35] provided an 87% yield of biaryl **24**. Unfortunately, oxidation of ether **24** to the corresponding C-6 carboxylic acid could not be achieved. Deprotection of **24** with BCl_3 gave only a 13% yield of the desired product. Analysis of the crude ^1H NMR spectrum indicated that several other reaction products, in which one or more of the D-ring methyl ethers had underwent cleavage, were present in the reaction mixture.

The successful endgame strategy for synthesis of the CD-ring system of streptonigrin is outlined in Scheme 6. Deprotection of the methyl group of bromide **23** with BCl_3 proceeded in quantitative yield to afford alcohol **25**. Subsequent oxidation with $KMnO_4$, followed by acid-catalyzed esterification, gave bromide-ester **26** in 62% yield over two steps. The cross-coupling of bromopyridine **26** with boronic acid **17**, using CsF in DME gave biaryl **27** in 68% yield. Biaryl **27** bears all of the key functionalities found in the CD-portion of streptonigrin. In addition, the C-2 position of the pyridine ring (C-ring) is functionalized



Scheme 6. Synthesis of streptonigrin CD biaryl.

such that the AB-rings of streptonigrin can be introduced via a coupling reaction. The C-2 methyl ether was converted into a suitable coupling substrate by treatment with PBr_3 to give pyridone **28** followed by conversion of the pyridone to triflate **29**. We anticipate that coupling of triflate **29** with appropriate metalloid reagents (siloxanes or boronic acid derivatives) will serve as a vehicle for the introduction of the AB-ring precursors.

3. Conclusion

A series of 4-bromopyridone derivatives have been synthesized and shown to undergo Pd-catalyzed coupling with aryl siloxane and boronic acid derivatives. A fully functionalized streptonigrin CD biaryl was synthesized using a Suzuki coupling of bromopyridine **26** and boronic acid **17**. The biaryl was elaborated to triflate **29**, thus setting the stage for a second coupling with a streptonigrin AB-ring precursor. Studies directed toward the utilization of triflate **29** to complete the total synthesis of streptonigrin (**1**) are underway and will be reported in due course.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer. Chemical shifts are reported in parts per million (ppm). Coupling constants (*J*) are given in hertz (Hz). Spin multiplicities are indicated by standard notation.

Infrared spectra were recorded on a Nicolet 560 FTIR spectrophotometer. Band positions are given in reciprocal centimeters (cm^{-1}) and relative intensities are listed as br (broad), s (strong), m (medium), or w (weak).

Melting points were taken in Kimax soft capillary tubes using a Thomas–Hoover Uni-Melt capillary melting point apparatus equipped with a calibrated thermometer.

Low resolution (LRMS) and high resolution (HRMS) were obtained on a JEOL SX-102A instrument.

Thin layer chromatography (TLC) was performed on 0.25 mm Analtech silica-coated glass plates, with compounds being identified in one or both of the following manners: UV (254 nm) and vanillin/sulfuric acid/ethanol charring. Flash chromatography was performed using glass columns and 'medium pressure' silica gel (Sorbent Technologies, 45–70 μm).

Tetrahydrofuran (THF), diethyl ether, toluene, and 1,4-dioxane were distilled from sodium/benzophenone ketyl. *N*,*N*-Dimethylformamide (DMF) was distilled from calcium hydride and dried over 4 Å molecular sieves. Pyridine, methylene chloride, and 1,2-dichloroethane (DCE) were distilled from calcium hydride. *N*,*N*,*N*,*N*-Tetramethylethylenediamine (TMEDA) and 1,2-dimethoxyethane (DME) were distilled from sodium metal. PBr_3 and $B(OMe)_3$ were distilled prior to use. Triphenylphosphine was recrystallized from hexanes. All other reagents were purchased and used

as received. Glassware used in the reactions was dried overnight in an oven at 120 °C. All reactions were performed under an atmosphere of argon unless otherwise noted. All new compounds were determined to be >95% pure by ^{1}H NMR spectroscopy.

4.1.1. 4-Bromo-2-methoxy-3,5,6-trimethylpyridine (8). To a suspension of 3.45 g (0.0125 mol) of Ag_2CO_3 and 3.58 g (0.0177 mol) of pyridone **6** in 30 mL of benzene was added 1.25 mL (0.0200 mol) of MeI. The resulting solution was heated in the dark at 45 °C for 12 h. The mixture was cooled to 0 °C, filtered, and the filtrate washed with 50 mL of 2% $NaHCO_3$, followed by 50 mL water. The benzene was evaporated under reduced pressure and the remaining aqueous solution was extracted 3× with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo to give 3.84 g (100%) of **8** as a white crystalline solid, mp 44–45 °C, which was used without further purification. IR (CCl_4) 3006 (w), 2953 (w), 2951 (m), 2920 (w), 2893 (w), 2866 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.19 (s, 3H), 2.22 (s, 3H), 2.37 (s, 3H), 3.83 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 14.5, 19.1, 23.8, 53.9, 118.4, 124.0, 139.4, 151.1, 159.9. EIMS *m/z* 231 (100), 229 (87), 216 (29). HRMS for $C_9BrH_{12}NO_2$ calcd 229.0102, found 229.0104.

4.1.2. 4-Hydroxy-5,6-dimethylpyridin-2(1*H*)-one (9). A solution of 61.78 g (0.2925 mol) of pyridone **7** and 111 g of NaOH (2.78 mol) in 1.3 L of water was heated at reflux for 2 h. The solution was cooled to 0 °C, brought to pH=7 with concd HCl, and stirred at room temperature for 12 h. The precipitate thus obtained was filtered and washed with water to yield 40.29 g (99%) of **9** as a white solid, mp>320 °C. IR (KBr) 3421 (w), 3266 (w), 3087 (m), 3002 (m), 2928 (m), 2882 (m), 1662 (s), 1616 (s) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.79 (s, 3H), 2.09 (s, 3H), 5.49 (s, 1H), 10.53 (br s, 1H), 10.91 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 9.8, 16.7, 96.1, 104.2, 142.3, 163.7, 167.3. EIMS *m/z* 139 (100), 111 (76), 110 (80), 69 (72), 44 (84). HRMS for $C_7H_9NO_2$ calcd 139.0633, found 139.0638.

4.1.3. 4-Bromo-6-methoxy-2,3-dimethylpyridine (11). This compound was obtained directly from pyridone **9**. A mixture of 22.06 g (0.1585 mol) of **9** and 30.54 g (0.1068 mol) of $POBr_3$ in 30 mL DMF were heated at 110 °C for 45 min. After cooling, water was added and the resulting solution brought to pH=7 with Na_2CO_3. The precipitate thus obtained was filtered, washed with water and then Et_2O to yield 19.80 g of a yellow solid. Although the intermediate bromopyridone could be purified, it was more convenient to use the crude material in the next step.

To a solution of the crude bromopyridone and 32.2 g (0.117 mol) of Ag_2CO_3 in 110 mL $CHCl_3$ was added 20.0 mL (0.314 mol) MeI and the mixture heated at 50 °C for 24 h in the dark. After cooling, the mixture was filtered and the filtrate concentrated in vacuo. Purification by column chromatography (19:1 hexanes/EtOAc, R_f= 0.38) afforded 12.70 g (37% from **9**) of **11** as a white crystalline solid, mp 38–41 °C. IR (CCl_4) 3014 (w), 2971 (w), 2944 (w), 2897 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 2.44 (s, 3H), 3.85 (s, 3H), 6.80 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 18.0, 24.1, 54.0, 111.6, 124.0, 137.3, 155.4, 161.9. EIMS *m/z* 216 (98), 214 (100), 187 (37), 185 (32).

4.1.4. 4-Bromo-2-methoxy-5,6-dimethyl-3-nitropyridine (12). A solution of 1.32 g (6.11 mmol) of pyridine **11**, 0.60 mL (8.6 mmol) of HNO_3, and 15 mL of H_2SO_4 was stirred at room temperature for 14 h. The solution was diluted with 100 mL of water and neutralized with Na_2CO_3. The solution was extracted 2× with 100 mL Et_2O and the combined organic extracts dried over $MgSO_4$ and concentrated in vacuo to yield 1.55 g (97%) of **12** as a yellow solid, mp 86–89 °C, which was used without further purification. IR (CCl_4) 3025 (w), 2998 (w), 2951 (w), 2924 (w), 2905 (w), 1581 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.51 (s, 3H), 3.97 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 18.5, 24.4, 55.0, 125.2, 127.5, 152.5, 156.9. FABMS *m/z* 263 (66), 261 (61), 155 (57), 152 (59), 119 (68), 103 (48), 85 (100). HRMS for $C_8H_9BrN_2O_3$ calcd 260.9875, found 260.9872.

4.1.5. 4-Bromo-2-methoxy-5,6-dimethylpyridin-3-amine (13). To a solution of 1.39 g (5.32 mmol) of nitropyridine **12**, 28 mL of EtOH, and 7 mL of water were added 3.5 g (63 mmol) of Fe and two drops of concd HCl. The resulting solution was heated at reflux for 2 h. After cooling, the solution was filtered and the filtrate concentrated in vacuo. Purification by column chromatography (9:1 hexanes/EtOAc, R_f=0.40) gave 0.800 g (65%) of **13** as a yellow crystalline solid, mp 52–54 °C. IR (CCl_4) 3487 (s), 3394 (s), 3014 (w), 2983 (w), 2948 (m), 2920 (w), 2858 (w), 1654 (m), 1612 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H), 2.41 (s, 3H), 3.98 (s, 3H), 4.05 (br s, 2H). ^{13}C NMR ($CDCl_3$) δ 18.2, 22.3, 53.3, 119.8, 122.7, 127.1, 140.7, 149.6. EIMS *m/z* 232 (98), 230 (100), 189 (68), 187 (68). HRMS for $C_8H_{11}BrN_2O$ calcd 232.0034, found 232.0049.

4.2. General procedure for the siloxane-based synthesis of biaryls

Coupling reactions were performed under identical conditions using 20 mol % $Pd(OAc)_2$ and 40 mol % PPh_3, 2 equiv of siloxane and 2 equiv of TBAF. The following example is illustrative.

4.2.1. 2-Methoxy-3,5,6-trimethyl-4-phenylpyridine (Table 1, entry 3). To a solution of 261 mg (1.15 mmol) of bromopyridine **8**, 440 mg (2.22 mmol) of phenyltrimethoxysilane, 52 mg (0.21 mmol) of $Pd(OAc)_2$, and 110 mg (0.419 mmol) of PPh_3 in 10 mL DMF was added 2.2 mL (2.2 mmol) of a 1 M solution of TBAF in THF. The solution was degassed via a single freeze-pump-thaw cycle and heated at 80 °C for 12 h. The reaction was quenched with water and the solution was extracted 3× with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (hexanes, R_f=0.14) afforded 230 mg (89%) of the title compound as a colorless oil. IR (CCl_4) 3084 (w), 3056 (w), 2967 (s), 2944 (s), 2924 (m), 2858 (w) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3H), 1.83 (s, 3H), 2.42 (s, 3H), 3.94 (s, 3H), 7.05 (d, *J*=6.8 Hz, 2H), 7.33 (d, *J*=6.8 Hz, 1H), 7.40 (t, *J*=6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 13.5, 16.3, 23.1, 53.6, 115.6, 121.9, 127.5, 128.8, 128.9, 140.9, 120.7, 152.0, 160.0. EIMS *m/z* 227 (70), 226 (100). HRMS for $C_{15}H_{17}NO$ calcd 226.1232, found 226.1228.

4.2.2. 6-Methoxy-2,3-dimethyl-4-phenylpyridine (Table 1, entry 1). Following column chromatography (19:1

hexanes/EtOAc, R_f=0.29), the biaryl was obtained in 97% yield as a white crystalline solid, mp 52–54 °C. IR (CCl_4) 3087 (w), 3060 (w), 2948 (m), 2920 (m), 2850 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.46 (s, 3H), 3.90 (s, 3H), 6.45 (s, 1H), 7.25 (d, J=8.4 Hz, 2H), 7.37 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 14.4, 22.1, 52.3, 106.6, 120.2, 126.5, 127.2, 127.6, 139.2, 151.7, 135.8, 160.3. EIMS m/z 213 (94), 212 (100), 184 (49), 183 (56), 128 (51), 127 (38). HRMS for $C_{14}H_{15}NO$ calcd 213.1154, found 213.1149.

4.2.3. 6-Methoxy-2,3-dimethyl-4-*o*-tolylpyridine (Table 1, entry 2). Following column chromatography (19:1 hexanes/EtOAc, R_f=0.32), the biaryl was obtained in 10% yield as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.86 (s, 3H), 2.04 (s, 3H), 2.46 (s, 3H), 3.90 (s, 3H), 6.36 (s, 1H), 7.02 (d, J=8.0 Hz, 1H), 7.23 (m, 3H), 7.23 (m, 3H).

4.2.4. 2-Methoxy-3,5,6-trimethyl-4-*o*-tolylpyridine (Table 1, entry 4). Following column chromatography (19:1 hexanes/EtOAc, R_f=0.23), the biaryl was obtained in 10% yield as a pale yellow oil. IR (CCl_4) 3072 (w), 3002 (m), 2921 (m), 1581 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.70 (s, 3H), 1.71 (s, 3H), 1.87 (s, 3H), 2.36 (s, 3H), 3.89 (s, 3H), 6.84 (d, J=6.8 Hz, 1H), 7.24 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 13.1, 15.8, 19.8, 23.0, 53.5, 115.5, 121.9, 126.4, 127.9, 128.6, 130.4, 135.6, 139.6, 150.8, 151.5, 160.1. EIMS m/z 241 (100), 240 (84), 226 (94), 216 (65).

4.2.5. 4-(Benzo[*d*][1,3]dioxol-5-yl)-2-methoxy-3,5,6-trimethylpyridine (Table 1, entry 5). Following column chromatography (19:1 hexanes/EtOAc, R_f=0.30), the biaryl was obtained in 61% yield as a colorless oil. IR (CCl_4) 3072 (w), 3002 (m), 2948 (s), 2920 (s), 2829 (s), 1581 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.79 (s, 3H), 1.81 (s, 3H), 2.35 (s, 3H), 3.87 (s, 3H), 5.93 (s, 2H), 6.43 (d, J=7.2 Hz, 1H), 6.44 (s, 1H), 6.79 (d, J=7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.5, 16.4, 23.1, 53.6, 101.5, 108.8, 109.5, 116.0, 122.1, 122.2, 133.7, 147.0, 148.1, 150.1, 151.6, 160.0. EIMS m/z 271 (74), 270 (100). HRMS for $C_{16}H_{17}NO_3$ calcd 270.1130, found 270.1125.

4.2.6. 2-Methoxy-5,6-dimethyl-3-nitro-4-phenylpyridine (Table 1, entry 7). These compounds were prepared according to the general siloxane coupling procedure outlined previously. Following column chromatography (hexanes, R_f=0.19), the biaryl was isolated in 36% yield as a white, crystalline solid, mp 79–82 °C. IR (CCl_4) 3087 (w), 3064 (w), 3025 (w), 2990 (w), 2955 (w), 2920 (w), 2901 (w), 2874 (w) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.50 (s, 3H), 4.00 (s, 3H), 7.16 (m, 2H), 7.40 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 16.0, 23.8, 54.6, 123.2, 128.6, 129.1, 129.3, 133.9, 134.4, 144.3, 152.1, 157.1. EIMS m/z 250 (100), 211 (57). HRMS for $C_{14}H_{14}N_2O_3$ calcd 258.1004, found 258.0997.

The reduced pyridine was obtained in 36% yield as a white crystalline solid, mp 69–72 °C, R_f=0.15 (hexanes). IR (CCl_4) 3025 (m), 2994 (m), 2955 (m), 2928 (m), 2866 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.25 (s, 3H), 2.45 (s, 3H), 4.05 (s, 3H), 8.03 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 18.2, 23.1, 54.9, 124.8, 131.5, 136.4, 154.4, 161.8.

4.3. Siloxane 16 and boronic acid 17

Siloxane **16** and boronic acid **17** were each prepared from 2,3-dimethoxybenzaldehyde as outlined below.

4.3.1. 2,3-Dimethoxyphenol. To a mixture of 9.3 mL (0.066 mol) of 30% H_2O_2 and 9.3 g of boric acid (0.15 mol) in 90 mL of THF was added 3 mL of sulfuric acid. The mixture was stirred at room temperature for 30 min and a solution of 5.0 g (0.030 mol) of 2,3-dimethoxybenaldehyde in 30 mL of THF was added. The mixture was heated at 50 °C for 24 h, quenched with saturated $NaHCO_3$, and filtered. The filtrate was extracted 3× with Et_2O and the combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (3:1 hexanes/EtOAc, R_f=0.29) afforded 3.22 g (70%) of the title compound as a pale yellow oil. Spectral data matched that of the reported compound.[36]

4.3.2. 1,2-Dimethoxy-3-(methoxymethyl)benzene. NaH (60% dispersion in mineral oil, 1.2 g, 30 mmol) was washed 2× with 3 mL of hexanes. To the solid was added 20 mL of DMF and the resulting suspension cooled to 0 °C. A solution of 3.42 g (22.2 mmol) of the phenol in 15 mL of DMF was added and the resulting solution stirred at 0 °C for 30 min. To the solution was added 2.3 mL (30 mmol) of MOM-Cl, causing the immediate evolution of gas. The solution was allowed to warm to room temperature and quenched with 50 mL of water. The solution was extracted 3× with ether and the combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo to afford 4.40 g (100%) of the title compound as a pale yellow oil, which was used without further purification. IR (CCl_4) 2998 (m), 2955 (s), 2932 (s), 2834 (m), 1596 (s) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.47 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 5.18 (s, 2H), 6.58 (d, J=8.2 Hz, 1H), 6.74 (d, J=8.2 Hz, 1H), 6.92 (t, J=8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 56.4, 56.6, 61.3, 95.7, 106.7, 109.9, 124.1, 139.6, 151.4, 154.1.

4.3.3. Triethoxy(3,4-dimethoxy-2-(methoxymethoxy)-phenyl)silane (16). A solution of 1.88 g (9.49 mmol) of the MOM ether and 2.2 mL (1.4 mmol) of TMEDA in 40 mL of THF was cooled to −78 °C. To this solution was added dropwise BuLi (15.3 mL of a 0.80 M solution, 0.014 mol) and the resulting solution stirred at −78 °C for 10 min and then allowed to warm to 0 °C and stirred for an additional 2 h. This solution was added over 30 min to 4.3 mL (1.9 mmol) of $Si(OEt)_4$ dissolved in 40 mL of THF at −78 °C. The resulting solution was allowed to warm to room temperature, quenched with water, and extracted 3× with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (15% EtOAc/hexanes, R_f=0.24) afforded 850 mg (25%) of **16** as a pale yellow oil. IR (CCl_4) 2971 (s), 2924 (s), 2889 (s), 2835 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.22 (t, J=7.0 Hz, 9H), 3.62 (s, 3H), 3.81 (s, 3H), 3.85 (s, 3H), 3.86 (q, J=7.0 Hz, 6H), 5.17 (s, 2H), 6.67 (d, J=8.2 Hz, 1H), 7.32 (d, J=8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 18.6, 56.3, 57.9, 59.0, 61.1, 99.6, 108.0, 117.2, 123.6, 141.9, 155.5, 156.6. EIMS m/z 360 (87), 271 (95), 270 (100), 255 (53), 166 (56), 45 (53). HRMS for $C_{15}H_{28}O_7Si$ calcd 360.1590, found 360.1604.

4.3.4. 3,4-Dimethoxy-2-(methoxymethoxy)phenylboronic acid (17). A solution of 1.08 g (5.45 mmol) of the MOM ether and 0.90 mL (5.4 mmol) TMEDA in 30 mL THF was cooled to −78 °C. To the solution was added dropwise 7.0 mL (5.6 mmol) of a 0.8 M solution of *n*-BuLi in hexanes and the resulting solution allowed to warm to 0 °C. After 1.5 h at 0 °C the solution was cooled to −78 °C and 1.2 mL (11 mmol) of $B(OMe)_3$ in 30 mL THF was added over 10 min. The resulting solution was allowed over 1 h to warm to room temperature and stirred an additional 16 h. HCl (30 mL, 5%) was added and the solution was extracted 3× with Et_2O. The combined organic layers were extracted with 2 M KOH. The aqueous layer was neutralized with concd HCl and extracted 3× with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. An analytical sample was obtained following recrystallization from hexanes/Et_2O as a white crystalline solid, mp 125–129 °C. IR (CCl_4) 3526 (br), 3468 (br), 2998 (m), 2955 (m), 2928 (m), 2854 (m), 2835 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.49 (s, 3H), 3.79 (s, 3H), 3.87 (s, 3H), 5.25 (s, 2H), 6.29 (br s, 2H), 6.72 (d, J=8.4 Hz, 1H), 7.52 (d, J=8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 56.4, 58.6, 61.1, 100.7, 108.5, 131.8, 140.8, 156.5, 156.8.

4.3.5. 2-Methoxy-4-(3,4-dimethoxy-2-(methoxymethoxy)-phenyl)-5,6-dimethyl-3-nitropyridine (18). A mixture of the crude boronic acid, 645 mg (2.47 mmol) 4-bromopyridine **12**, 615 mg (5.32 mmol) $Pd(PPh_3)_4$, and 579 mg (5.46 mmol) Na_2CO_3, 60 mL toluene, 6 mL H_2O, and 6 mL EtOH was heated at reflux for 40 h. After cooling, 60 mL of water was added and the solution was extracted 3× with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (3:1 hexanes/EtOAc, R_f=0.25) afforded a yellow solid, which was recrystallized from hexanes/Et_2O to yield 737 mg (78%) of **18** as a white crystalline solid, mp 128–129 °C. IR (CCl_4) 2990 (m), 2955 (m), 2924 (m), 2874 (m), 2831 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.49 (s, 3H), 3.11 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.99 (s, 3H), 4.80 (d, J=5.6 Hz, 1H), 5.08 (d, J=5.6 Hz, 1H), 6.69 (d, J=8.4 Hz, 1H), 6.76 (d, J=8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 15.9, 23.7, 54.5, 56.4, 57.1, 61.4, 99.4, 108.3, 121.1, 124.2, 124.9, 134.8, 141.4, 142.8, 148.5, 152.2, 155.0, 156.5. EIMS *m*/*z* 378 (100), 332 (37). HRMS for $C_{18}H_{22}N_2O_7$ calcd 378.1427, found 378.1443.

4.3.6. (4-Bromo-6-methoxy-3-methylpyridin-2-yl)methyl acetate (20). This compound was prepared directly from pyridine **11**. A solution of 520 mg (2.41 mmol) pyridine **11** and 1.0 mL (7.2 mmol) of 30% H_2O_2 in 15 mL HOAc were heated at 60 °C for 3 d. After cooling, the solution was concentrated in vacuo and the residue dissolved in 10 mL acetic anhydride. This solution was heated at 120 °C for 24 h and concentrated in vacuo to give 628 mg (93%) of **20** as a pale brown oil, which was used without further purification. IR (CCl_4) 3017 (m), 2983 (m), 2948 (m), 2924 (m), 2905 (m), 2854 (m), 1748 (s) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.11 (s, 3H), 2.28 (s, 3H), 3.85 (s, 3H), 5.14 (s, 2H), 6.93 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 16.9, 21.2, 54.0, 66.3, 114.4, 124.9, 138.1, 151.1, 162.2, 171.1.

4.3.7. (4-Bromo-6-methoxy-3-methylpyridin-2-yl)methanol (21). This compound was obtained directly from pyridine **11**. A solution of 701 mg (3.24 mmol) of pyridine **11**, 0.50 mL (4.4 mmol) 30% H_2O_2, and 22 mL acetic acid was heated at 60 °C for 3 d. After cooling, the solution was concentrated in vacuo and the residue dissolved in 7 mL of acetic anhydride. This solution was heated at 120 °C for 2 h. After cooling, the solution was concentrated in vacuo. To the residue was added 2.28 g (16.5 mmol) K_2CO_3 and 25 mL methanol. The resulting solution was stirred at room temperature for 18 h and concentrated in vacuo. The residue was suspended in water and extracted 3× with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo to yield 509 mg (68%) of **21** as a white crystalline solid, mp 49–52 °C, which was used without further purification. IR (CCl_4) 3456 (br), 3021 (m), 2983 (m), 2951 (m), 2928 (m), 2866 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.15 (s, 3H), 3.82 (br s, 1H), 3.92 (s, 3H), 4.62 (s, 2H), 6.90 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 15.3, 54.2, 62.3, 112.7, 122.0, 138.2, 154.4, 161.9.

4.3.8. 4-Bromo-6-methoxy-2-(methoxymethyl)-3-methylpyridine (22). To a solution of 2.16 g (9.31 mmol) of alcohol **21** and 3.31 g (14.3 mmol) of Ag_2O in 40 mL THF was added 2.0 mL (32 mmol) of iodomethane. The resulting solution was heated in the dark at 65 °C for 4 d. The suspension was filtered through a pad of Celite and the filtrate concentrated in vacuo to yield 1.97 g (86%) of **22** as a white crystalline solid, mp 48–50 °C, which was used without further purification. IR (CCl_4) 3014 (w), 2986 (w), 2951 (m), 2928 (m), 2893 (w), 2918 (w) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H), 3.39 (s, 3H), 3.88 (s, 3H), 4.50 (s, 2H), 6.93 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 17.0, 54.1, 58.9, 75.6, 114.0, 125.9, 138.3, 153.6, 162.0.

4.3.9. 4-Bromo-2-methoxy-6-(methoxymethyl)-5-methyl-3-nitropyridine (23). A solution of 914 mg (3.71 mmol) of pyridine **22** in 1.5 mL of HNO_3 and 8.5 mL of H_2SO_4 was stirred at room temperature for 2 d. The solution was diluted with water, neutralized with Na_2CO_3, and extracted 3× with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (9:1 hexanes/EtOAc, R_f=0.11) afforded 550 mg (51%) of **23** as a white, crystalline solid, mp 47–50 °C. IR (CCl_4) 3025 (m), 2990 (m), 2959 (m), 2928 (m), 2921 (m), 2819 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.40 (s, 3H), 4.00 (s, 3H), 4.53 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 17.5, 55.1, 59.1, 75.1, 111.3, 127.2, 128.7, 152.9, 154.8. EIMS *m*/*z* 292 (6), 290 (8), 262 (91), 260 (100), 247 (30), 245 (28). HRMS for $C_9H_{11}BrN_2O_4$ calcd 291.9882, found 291.9893.

4.3.10. 2-Methoxy-4-(3,4-dimethoxy-2-(methoxymethoxy)-phenyl)-6-(methoxymethyl)-5-methyl-3-nitropyridine (24). Boronic acid **17** was prepared as described above. A solution of 287 mg (1.19 mmol) of the crude boronic acid, 190 mg (0.653 mmol) of bromopyridine **23**, 350 mg (2.30 mmol) CsF, and 138 mg (0.119 mmol) $Pd(PPh_3)_4$ in 6 mL DME was heated at reflux for 20 h. After cooling, the solution was diluted with water and extracted 3× with Et_2O. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column

chromatography (3:1 hexanes/EtOAc, R_f=0.09) yielded 233 mg (87%) of the title compound as a white crystalline solid, mp 71–73 °C. IR (CCl_4) 2994 (m), 2963 (m), 2932 (m), 2893 (m), 2835 (m), 1600 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.07 (s, 3H), 3.09 (s, 3H), 3.43 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 4.02 (s, 3H), 4.52 (d, J=12.0 Hz, 1H), 4.56 (d, J=12.0 Hz, 1H), 4.80 (d, J=5.6 Hz, 1H), 5.08 (d, J=5.6 Hz, 1H), 6.69 (d, J=8.6 Hz, 1H), 6.76 (d, J=8.6 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 14.8, 54.7, 56.4, 57.1, 59.1, 61.4, 74.9, 99.4, 108.3, 120.5, 124.3, 126.8, 136.0, 142.5, 142.8, 148.5, 152.3, 154.3, 155.1. EIMS *m/z* 408 (46), 286 (100). HRMS for $C_{19}H_{24}N_2O_8$ calcd 408.2498, found 408.2484.

4.3.11. (4-Bromo-6-methoxy-3-methyl-5-nitropyridin-2-yl)methanol (25). A solution of 403 mg (1.38 mmol) of methyl ether **23** in 20 mL CH_2Cl_2 was cooled to 0 °C and 3.0 mL (3.0 mmol) of a 1.0 M solution of BCl_3 in CH_2Cl_2 was added dropwise. The resulting solution was stirred 16 h at room temperature and quenched with water. The phases were separated and the aqueous layer was extracted 2× with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo to give 383 mg (100%) of **25** as a white crystalline solid, mp 96–99 °C, which was used without further purification. IR (CCl_4) 3483 (br), 3026 (m), 2991 (m), 2949 (m), 2925 (m), 2898 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H), 3.83 (t, J=4.6 Hz, 1H), 4.05 (s, 3H), 4.70 (d, J=4.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 16.0, 55.4, 62.8, 123.5, 128.9, 153.3, 155.9.

4.3.12. Methyl 4-bromo-6-methoxy-3-methyl-5-nitropyridine-2-carboxylate (26). To a suspension of 397 mg (1.43 mmol) of alcohol **25** and 64 mg (1.6 mmol) NaOH in 30 mL water was added 710 mg (4.49 mmol) $KMnO_4$ and the resulting mixture stirred at room temperature for 24 h. MeOH was added and the suspension stirred 30 min and filtered. The filtrate was acidified with 1 M HCl and concentrated in vacuo. The residue was dissolved in 20 mL MeOH and 4 mL H_2SO_4, and the solution heated at reflux for 16 h. The solution was diluted with water and basicified with K_2CO_3. The MeOH was removed in vacuo and the remaining aqueous solution was extracted 3× with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo to give 269 mg (62%) of **26** as a yellow crystalline solid, mp 90–93 °C, which was used without further purification. IR (CCl_4) 3029 (w), 3002 (w), 2951 (m), 2924 (m), 2850 (w), 1740 (s) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.48 (s, 3H), 3.96 (s, 3H), 4.02 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 18.5, 53.5, 55.6, 127.6, 129.6, 147.0, 153.3, 165.7. EIMS *m/z* 306 (65), 304 (59), 274 (89), 272 (100), 246 (58), 244 (59). HRMS for $C_9H_9BrN_2O_5$ calcd 303.9695, found 303.9683.

4.3.13. Methyl 6-methoxy-4-(3,4-dimethoxy-2-(methoxymethoxy)phenyl)-3-methyl-5-nitropyridine-2-carboxylate (27). A solution of 682 mg (2.24 mmol) of ester **26**, 1.20 g (4.96 mmol) boronic acid **17**, 387 mg (0.335 mmol) $Pd(PPh_3)_4$, and 673 mg (4.43 mmol) CsF in 35 mL DME was heated at 75 °C for 24 h. After cooling, the solution was diluted with water and extracted 3× with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography (4:1 hexanes/EtOAc, R_f=0.20) gave 646 mg (68%) of **27** as a white crystalline solid, mp 90–93 °C. IR (CCl_4) 3002 (m), 2948 (m), 2932 (m), 2897 (m), 2839 (m), 1740 (s) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.16 (s, 3H), 3.11 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 3.96 (s, 3H), 4.04 (s, 3H), 4.84 (d, J=5.6 Hz, 1H), 5.10 (d, J=5.6 Hz, 1H), 6.71 (d, J=8.4 Hz, 1H), 6.76 (d, J=8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 15.9, 53.2, 55.2, 56.5, 57.2, 61.4, 99.5, 108.4, 119.6, 124.2, 127.7, 137.6, 142.8, 143.6, 146.7, 148.5, 152.6, 155.5, 166.7. EIMS *m/z* 422 (100), 300 (32), 272 (36). HRMS for $C_{19}H_{22}N_2O_9$ calcd 422.1325, found, 422.1332.

4.3.14. Methyl 1,6-dihydro-4-(3,4-dimethoxy-2-(methoxymethoxy)phenyl)-3-methyl-5-nitro-6-oxopyridine-2-carboxylate (28). A solution of 81 mg (0.192 mmol) pyridine **27** and 0.12 mL (1.27 mmol) PBr_3 in 4 mL of DCE was heated at reflux for 12 h. After cooling, the reaction was quenched with water and the mixture was extracted 3× with CH_2Cl_2. The combined organic extracts were concentrated in vacuo, the residue was washed with Et_2O to obtain 63 mg (90%) of **28** as a white solid, mp 225–235 °C (decomp.), which was used without further purification. IR ($CHCl_3$) 3515 (br), 3344 (br), 2843 (w), 1750 (w) 1685 (s) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.18 (s, 3H), 3.87 (s, 3H), 3.92 (s, 3H), 3.98 (s, 3H), 6.02 (br s, 1H), 6.51 (d, J=8.8 Hz, 1H), 6.72 (d, J=8.8 Hz, 1H), 10.35 (br s). ^{13}C NMR ($CDCl_3$) δ 15.44, 54.1, 56.3, 61.6, 105.0, 111.6, 122.8, 123.6, 130.6, 135.9, 146.1, 146.4, 146.9, 153.7, 154.0, 161.5. FABMS *m/z* 365 (100). HRMS for $C_{16}H_{16}N_2O_8$ calcd 365.0985, found 365.0979.

4.3.15. 6-(Methoxycarbonyl)-4-(3,4-dimethoxy-2-(methoxymethoxy)phenyl)-5-methyl-3-nitropyridin-2-yl trifluoromethanesulfonate (29). A suspension of 63 mg (0.172 mmol) of pyridone **28** and 23 mg (0.188 mmol) DMAP in 4 mL CH_2Cl_2 was cooled to 0 °C and 35 μL (0.21 mmol) Tf_2O was added. The resulting solution was allowed to warm to room temperature and stirred 12 h. The reaction was quenched with water and the mixture extracted 3× with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. Recrystallization from hexanes/Et_2O gave 71 mg (83%) of **29** as a white crystalline solid, mp 163–165 °C. IR (CCl_4) 3515 (br), 3010, (w), 2955 (w), 2936 (w), 2839 (w), 1740 (m) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H), 3.89 (s, 3H), 3.93 (s, 3H), 3.98 (s, 3H), 6.01 (br s, 1H), 6.55 (d, J=8.4 Hz, 1H), 6.72 (d, J=8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 16.6, 53.7, 56.4, 61.7, 105.1, 110.9, 123.9, 136.1, 137.8, 139.5, 143.4, 146.1, 147.1, 147.5, 154.3, 164.8. EIMS *m/z* 496 (98), 418 (52), 317 (100). HRMS for $C_{17}H_{15}F_3N_2O_{10}S$ calcd 496.0400, found 496.0385.

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Reaction of iodoarenes with potassium peroxodisulfate/trifluoroacetic acid in the presence of aromatics. Direct preparation of diaryliodonium triflates from iodoarenes

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Abstract—Diaryliodonium triflates have been directly prepared by reaction of some iodoarenes with aromatic substrates in good yields by using $K_2S_2O_8/CF_3COOH/CH_2Cl_2$. Treatment of a variety of iodoarenes with iodobenzene under the same conditions resulted in ligand transfer, and (4-iodophenyl)(phenyl)iodonium triflate was obtained. This procedure avoids the use of high temperature and severe reaction conditions.

1. Introduction

The recent demand for highly efficient and environmentally benign syntheses of fine chemicals and pharmaceuticals has encouraged the development of mild, safe, and highly chemoselective oxidizers. Hypervalent iodine reagents have been widely used as green oxidants in organic synthesis due to their low toxicity, ready availability, easy handling, high efficiency, stability to air and moisture, and as an economical alternative to heavy metal reagents such as lead(IV), thallium(III), and mercury(II). Recently, extensive studies on hypervalent iodine compounds such as (diacetoxyiodo)arenes, [bis(trifluoroacetoxy)iodo]arenes, [hydroxy(tosyloxy)iodo]arenes, diaryliodonium salts, etc., have been carried out and their use for organic synthesis has been reported.[1,2] Especially, symmetric and unsymmetrical diaryliodonium salts represent an important class of aromatic iodine(III) derivatives. They are used in organic synthesis mostly as arylating reagents for a large variety of organic and inorganic nucleophiles[2] and, have been applied to the photochemical polymerization process [3] and to chemical amplification in imaging systems.[4] In addition, some of the diaryliodonium salts have also shown biological activity.[5] Diaryliodonium salts are generally solid compounds, mostly stable towards heat, oxygen, and humidity; they are mildly light-sensitive and should be stored in the dark, without refrigeration. Many methods have been described for the preparation of diaryliodonium salts,[6] but very few of these involve the synthesis of diaryliodonium triflate salts. The methods used so far are generally as follows:

(a) One-pot preparation of diaryliodonium triflates through the in situ preparation of a reactive hypervalent iodine(III) reagent from iodosylbenzene and triflic acid and its reaction with aromatic substrates.[7]
(b) A convenient synthesis of diaryliodonium triflates via the reaction of (diacetoxy)iodobenzene, triflic acid with aromatic substrates.[8]
(c) A direct synthesis of diaryliodonium triflates by the reaction of iodosyl triflate with the trimethylsilyl derivatives of aromatic compounds.[9]
(d) Synthesis of unsymmetrical diaryliodonium triflates by the treatment of β-(trifyloxy)vinyl iodonium triflates with aryl lithium reagents.[10]
(e) A generalized synthesis of unsymmetrical functionalized diaryliodonium triflates through the direct reaction of bis(acetoxy)iodoarenes with arenes in a triflic acid or trifluoroacetic acid medium.[11]

Activated aromatic compounds reacted completely with the reagent [PhIO/TfOH] within an hour; weakly deactivated aromatics needed prolonged reaction time to produce reasonable yields of the diaryliodonium triflates.[7] Strongly deactivated aromatics such as nitrobenzene and benzonitrile did not give the corresponding diaryliodonium triflates. However, the reagent [PhIO/TfOH] also gives (*para*-phenylene)bis(aryliodonium)ditriflates as byproducts. Interaction

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of most aromatic substrates with [PhI(OAc)$_2$/TfOH] employed in the present work gave high yields in the 0.5–1 h reaction at room temperature.[8] It is noteworthy that halogenated benzenes require prolonged reaction time (24 h). However, this reagent also was not effective for strongly deactivated aromatics such as nitrobenzene and benzonitrile. The generality of the O=IOTf/ArSiMe$_3$/CH$_2$Cl$_2$ procedure is indicated by the preparation of various aromatic iodonium triflate salts bearing methyl, halogen, and other substituents in the aromatic ring.[9] However, the reaction has limited applicability in case of easily oxidizable aromatic substrates such as hydroxy, methoxy, and amino derivatives. The reaction of β-(trifyloxy)vinyl iodonium triflates with aryl lithium reagents affords unsymmetrical diaryliodonium triflates in good yields, which include synthetically difficult *ortho*- and *meta*-substituted aryliodonium triflates.[10] Reaction of 2 equiv of triflic acid or trifluoroacetic acid with bis(acetoxy)iodoarenes gave an intermediate complex, [ArI(OAc)$_2$/CF$_3$SO$_3$H] or [ArI(OAc)$_2$/CF$_3$CO$_2$H]. These activated complexes were not isolated but were treated in situ with substituted benzene at low temperature (−30 to 0 °C).[11]

Considering the useful properties of salts and other derivatives of triflic acid,[12] the development of a simple and an efficient procedure for the preparation of diaryliodonium triflates is a desirable goal. We have previously reported[13] an easy preparation of [bis(trifluoroacetoxy)iodo]arenes and ArI(OCOCF$_3$)$_2$, from the respective iodoarenes in CF$_3$COOH, using commercial potassium peroxodisulfate, K$_2$S$_2$O$_8$, as the oxidant at 36–38 °C for 20 h. After recrystallization, we obtained the purified products in 36–87% yields. Iodoarenes bearing strong electron-withdrawing groups at the *meta* and *para* positions gave ArI(OCOCF$_3$)$_2$ in good yields. ArI(OCOCF$_3$)$_2$ is a relatively reactive reagent and can undergo arylation to give diaryliodonium salts. Therefore, it was expected that the reaction of iodoarenes with K$_2$S$_2$O$_8$/CF$_3$COOH in the presence of appropriate arenes would give diaryliodonium salts. This preliminary work opened a new way to develop the title method. Herein we wish to report a direct and an effective method for the preparation of diaryliodonium triflates from iodoarenes, which can be used as a strong acid generator.[14]

2. Results and discussion

A simple, easy, and an efficient method for the direct preparation of diaryliodonium triflates, Ar$_2$I$^+$OTf$^-$ from iodoarenes was examined. At first, diaryliodonium trifluoroacetoxylates Ar$_2$I$^+$(OCOCF$_3$)$^-$ were prepared by the reaction of iodoarenes, CF$_3$COOH, and commercial potassium peroxodisulfate, K$_2$S$_2$O$_8$, as the oxidant with aromatic substrates at 36–38 °C. K$_2$S$_2$O$_8$ is used as a strong oxidizing agent in many applications. It has the particular advantage of being almost non-hygroscopic, has particularly good storage stability, and is easy and safe to handle. CH$_2$Cl$_2$ was added for dissolving iodoarenes completely. Next, interaction of Ar$_2$I$^+$(OCOCF$_3$)$^-$ with sodium triflate, NaOTf, solution provided the corresponding diaryliodonium triflates at room temperature in fairly good yields. The results are given in Table 1.

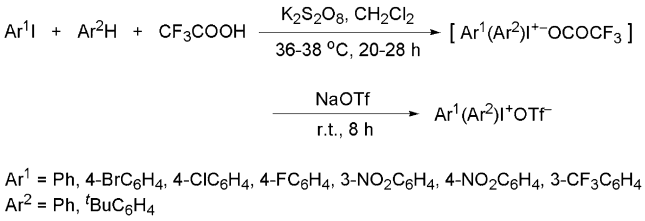
The presence of K$_2$S$_2$O$_8$ (in stoichiometric quantities) in the reaction mixture was indispensable because without its addition the oxidation reactions did not proceed. When K$_2$S$_2$O$_8$ was replaced for sodium peroxodisulfate, Na$_2$S$_2$O$_8$, the starting material was recovered. Deactivated aromatic iodides employed in the present work gave good yields together with the complete absence of effluent or byproduct problems. The essence of our novel method is described in Scheme 1. It is noteworthy that weakly deactivated aromatic substrates such as chlorobenzene and bromobenzene and an activated aromatic compound such as toluene resulted in low yields (20–25%) due to their lower reactivity. This method was not applicable for iodoarenes with strong electron-donating groups and for aromatic substrates with strong electron-donating groups. For example, iodoanisoles, iodonaphthalene, anisoles, naphthalene, etc., were quickly oxidized in the reaction mixtures, but the reaction resulted in decomposition and the formation of tarry products.

Table 1. Preparation of diaryliodonium triflates from iodoarenes[a]

Entry	Iodoarene	Arene	Yield (%)
1	PhI	PhH	78
2	4-BrC$_6$H$_4$I	PhH	72
3	4-ClC$_6$H$_4$I	PhH	71
4	4-FC$_6$H$_4$I	PhH	73
5	4-NO$_2$C$_6$H$_4$I[b]	PhH	67
6	3-CF$_3$C$_6$H$_4$I	PhH	70
7	PhI	^{t}BuPh	69
8	4-BrC$_6$H$_4$I	^{t}BuPh	72
9	4-ClC$_6$H$_4$I	^{t}BuPh	68
10	4-FC$_6$H$_4$I	^{t}BuPh	72
11	4-NO$_2$C$_6$H$_4$I	^{t}BuPh	70
12	3-NO$_2$C$_6$H$_4$I	^{t}BuPh	58
13	3-CF$_3$C$_6$H$_4$I	^{t}BuPh	67

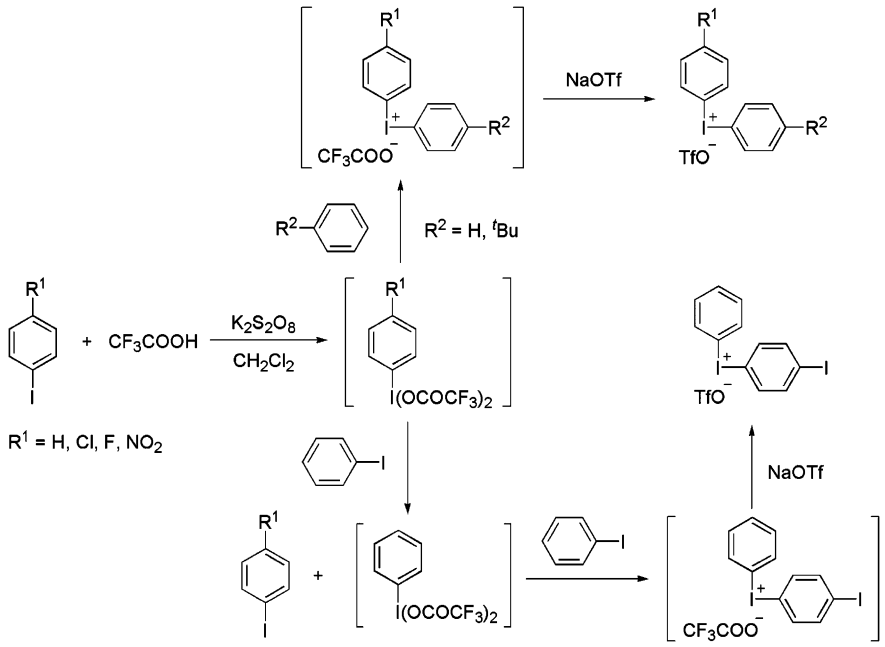
[a] The reaction of an iodoarene (1 mmol) was carried out in ArH (3 mmol), CF$_3$COOH (9 mL), and CH$_2$Cl$_2$ (2 mL) in the presence of K$_2$S$_2$O$_8$ (4 mmol) at 36–38 °C for 20 h. After workup, the NaOTf solution was treated at room temperature for 8 h.

[b] Benzene (3 mmol) was added after 20 h reaction and the reaction was continued for another 8 h.



Scheme 1.

In the reaction of iodoarenes with aromatic substrates, the aromatic substrate was replaced by iodobenzene. Surprisingly, the reaction of 1-chloro-4-iodobenzene, 1-fluoro-4-iodobenzene, and 1-iodo-4-nitrobenzene gave (4-iodophenyl)-(phenyl)iodonium triflate, the same product as from the reaction of iodobenzene. As we reported the formation of [bis(trifluoroacetoxy)iodoarenes in the reaction of iodoarenes with K$_2$S$_2$O$_8$/TFA,[13] it was considered that [bis(trifluoroacetoxy)iodo]arenes were formed in situ and then underwent ligand transfer reactions with iodobenzene to form [bis(trifluoroacetoxy)iodo]benzene. This [bis(trifluoroacetoxy)iodo]benzene reacted with excess iodobenzene to give (4-iodophenyl)(phenyl)iodonium trifluoroacetate, which was treated with the NaOTf solution to yield (4-iodophenyl)(phenyl)iodonium triflate (Scheme 2). Preliminarily,



Scheme 2.

Table 2. Formation of (4-iodophenyl)(phenyl)iodonium triflate from iodoarenes[a]

Entry	Iodoarene	Time (h)	Yield (%)
1	PhI	28	80
2	4-ClC_6H_4I	20	71
3	4-FC_6H_4I	20	73
4	4-$NO_2C_6H_4I$	28	68

[a] The reaction of an iodoarene (1 mmol) was carried out in CF_3COOH (9 mL) and CH_2Cl_2 (2 mL) in the presence of $K_2S_2O_8$ (4 mmol) at 36–38 °C for 20 h. PhI (3 mmol) was added after 20 h and the reaction was continued for another 8 h. After workup, the NaOTf solution was treated at room temperature for 8 h.

we conducted the reaction of 1-[bis(trifluoroacetoxy)iodo]-4-chlorobenzene with iodobenzene in TFA in order to confirm the ligand transfer reaction. We observed the formation of [bis(trifluoroacetoxy)iodo]benzene in the reaction at 36–38 °C for 12 h. Similar-type ligand transfer reactions were observed in the reaction of hydroxy(tosyloxyiodo)benzene with iodoarenes.[15] The results are given in Table 2. The purity of the purified products, diaryliodonium triflates, $Ar_2I^+OTf^-$ was checked by melting points, ^1H NMR and ^{13}C NMR spectra, and elemental analysis.

3. Conclusion

We have found a new (or considerably) improved method for the direct preparation of diaryliodonium triflates, which is easy and cheap. The new method gives diaryliodonium triflates in good yields by the reaction of iodoarenes with $K_2S_2O_8$, CF_3COOH, CH_2Cl_2, and aromatic substrates at 36–38 °C. In the case of an aromatic substrate, iodobenzene, ligand transfer reaction occurred with iodoarenes. Because of its simplicity and convenience we are sure that the present method will continue to attract significant research activity.

4. Experimental

4.1. General

Melting points were determined with a Yanaco micro-melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-AL300 spectrometer and the chemical shifts were expressed in parts per million downfield from tetramethylsilane. Elemental analysis was conducted by the Service Center of the Elemental Analysis of Organic Compounds, Faculty of Science, Kyushu University.

4.2. Optimized procedure for preparing diaryliodonium triflates from iodoarenes

A solution of an appropriate iodoarene (1 mmol) in a mixture of an arene (3 mmol), CF_3COOH (9 mL) and CH_2Cl_2 (2 mL) was heated with stirring to 36–38 °C. Next, potassium peroxodisulfate (4 mmol) was added portionwise during 10 min and the stirring was continued until TLC analysis indicated completion of reaction. Reaction time needed 20 h. After completion of the reaction, water (10 mL) was added. The precipitates were collected by filtration under reduced pressure, washed with CH_2Cl_2 (10 mL), and discarded. The crude product was obtained by extraction of the filtrate with dichloromethane (3×10 mL) followed by drying (anhydrous Na_2SO_4), filtration, and removal of the solvent by evaporation under reduced pressure. The crude product was treated with a NaOTf (10 mL) solution at room temperature for 8 h. The NaOTf solution was prepared by mixing with $NaHCO_3$ (1.0 g), H_2O (10 mL) and TfOH (1.5 mL). The precipitates were collected by filtration under reduced pressure, washed with H_2O (10 mL), and dried in vacuo. Another crop was obtained by extraction of the filtrate with dichloromethane (3×10 mL) followed by drying (anhydrous Na_2SO_4), filtration, and removal of the solvent

by evaporation under reduced pressure. The combined crude product was washed with hexane (10 mL), Et_2O (10 mL), and dried in vacuo. Further purification was conducted by repeating crystallization from CH_2Cl_2/hexane.

4.2.1. Diphenyliodonium triflate. 0.336 g (78%); mp 176–177 °C (lit.[14] mp 178–180 °C); ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (d, J=8 Hz, 4H, ArH), 7.69 (t, J=8 Hz, 2H, ArH), 7.50 (t, J=8 Hz, 4H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 136.42, 133.62, 133.15, 121.80 (q, J_{CF}= 317.7 Hz, SO_2CF_3), 115.89.

4.2.2. (4-Bromophenyl)(phenyl)iodonium triflate. 0.365 g (72%); mp 131–132 °C (lit.[8] mp 129–136 °C); ^{1}H NMR (300 MHz, CD_3OD) δ 8.20 (d, J=8 Hz, 2H, ArH), 8.09 (d, J=8 Hz, 2H, ArH), 7.65–7.71 (m, 3H, ArH), 7.53 (t, J=8 Hz, 2H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 138.08, 136.48, 136.19, 133.76, 133.21, 128.53, 121.77 (q, J_{CF}=317.7 Hz, SO_2CF_3), 116.17, 114.13.

4.2.3. (4-Chlorophenyl)(phenyl)iodonium triflate. 0.331 g (71%); mp 108–110 °C (lit.[7] mp 110–111 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J=8 Hz, 2H, ArH), 7.98 (d, J=8 Hz, 2H, ArH), 7.50 (t, J=8 Hz, 1H, ArH), 7.40 (t, J=8 Hz, 2H, ArH), 7.34 (d, J=8 Hz, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.27, 136.73, 135.38, 132.43, 132.13, 132.07, 120.04 (q, J_{CF}=317.7 Hz, SO_2CF_3), 114.12, 110.86.

4.2.4. (4-Fluorophenyl)(phenyl)iodonium triflate.[16] 0.329 g (73%); mp 133–134 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.25–8.16 (m, 4H, ArH), 7.69 (t, J=8 Hz, 1H, ArH), 7.53 (t, J=8 Hz, 2H, ArH), 7.29 (t, J=8 Hz, 2H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 166.42 (d, J_{CF}=252.2 Hz, CF), 139.32 (d, J_{CF}=8.7 Hz, CCCF), 136.36, 133.73, 133.21, 121.80 (q, J_{CF}=315.8 Hz, SO_2CF_3), 120.54 (d, J_{CF}=23.5 Hz, CCF), 116.39, 109.76.

4.2.5. (4-Nitrophenyl)(phenyl)iodonium triflate. 0.316 g (67%); mp 189–190 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.43 (d, J=9 Hz, 2H, ArH), 8.30 (d, J=9 Hz, 2H, ArH), 8.27 (d, J=8 Hz, 2H, ArH), 7.73 (t, J=8 Hz, 1H, ArH), 7.57 (t, J=8 Hz, 2H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 151.47, 137.67, 136.86, 134.07, 133.42, 127.45, 121.77 (q, J_{CF}=316.5 Hz, SO_2CF_3), 121.56, 116.18. Found: C, 32.91; H, 1.90; N, 2.98%. Calcd for $C_{13}H_9O_5NIF_3S$: C, 32.85; H, 1.89; N, 2.95%.

4.2.6. (3-Trifluoromethylphenyl)(phenyl)iodonium triflate. 0.351 g (70%); mp 107–108 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.59 (s, 1H, ArH), 8.45 (d, J=8 Hz, 1H, ArH), 8.25 (d, J=8 Hz, 2H, ArH), 7.99 (d, J=8 Hz, 1H, ArH), 7.72 (t, J=8 Hz, 2H, ArH), 7.56 (t, J=8 Hz, 2H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 140.10, 136.68, 134.46 (q, J_{CF}=33.4 Hz, CCF$_3$), 133.94, 133.80, 133.30, 133.08 (q, J_{CF}=3.7 Hz, CCCF$_3$), 130.29 (q, J_{CF}=3.7 Hz, CCCF$_3$), 124.13 (q, J_{CF}=270.2 Hz, CCF$_3$), 121.77 (q, J_{CF}= 316.5 Hz, SO_2CF_3), 116.31, 116.05. Found: C, 33.77; H, 1.77%. Calcd for $C_{14}H_9O_3IF_6S$: C, 33.74; H, 1.81%.

4.2.7. (4-*tert*-Butylphenyl)(phenyl)iodonium triflate. 0.344 g (69%); mp 162–163 °C (lit.[8] mp 163–165 °C); ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (d, J=8 Hz, 2H, ArH), 8.09 (d, J=8 Hz, 2H, ArH), 7.66 (t, J=7 Hz, 1H, ArH), 7.58–7.49 (m, 4H, ArH), 1.30 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 157.77, 136.34, 136.25, 133.53, 133.09, 130.48, 121.75 (q, J_{CF}=315.9 Hz, SO_2CF_3), 115.97, 112.39, 36.08, 31.30.

4.2.8. (4-Bromophenyl)(4-*tert*-butylphenyl)iodonium triflate. 0.416 g (72%); mp 199–200 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.11 (d, J=9 Hz, 2H, ArH), 8.07 (d, J=9 Hz, 2H, ArH), 7.65 (d, J=9 Hz, 2H, ArH), 7.58 (d, J=9 Hz, 2H, ArH), 1.31 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 157.97, 137.99, 136.30, 136.15, 130.57, 128.45, 121.78 (q, J_{CF}=316.5 Hz, SO_2CF_3), 114.18, 112.64, 36.10, 31.30. Found: C, 36.15; H, 3.02%. Calcd for $C_{17}H_{17}O_3IBrF_3S$: C, 36.12; H, 3.01%.

4.2.9. (4-*tert*-Butylphenyl)(4-chlorophenyl)iodonium triflate. 0.372 g (68%); mp 178–179 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.13 (d, J=9 Hz, 2H, ArH), 8.08 (d, J=9 Hz, 2H, ArH), 7.58 (d, J=9 Hz, 2H, ArH), 7.53 (d, J=9 Hz, 2H, ArH), 1.31 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 157.89, 140.15, 137.96, 136.30, 133.11, 130.53, 121.76 (q, J_{CF}=316.4 Hz, SO_2CF_3), 113.36, 112.69, 36.06, 31.29. Found: C, 39.22; H, 3.26%. Calcd for $C_{17}H_{17}O_3ICIF_3S$: C, 39.20; H, 3.27%.

4.2.10. (4-*tert*-Butylphenyl)(4-fluorophenyl)iodonium triflate. 0.371 g (72%); mp 137–138 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.23 (dd, J=5, 8 Hz, 2H, ArH), 8.10 (d, J=8 Hz, 2H, ArH), 7.58 (d, J=8 Hz, 2H, ArH), 7.29 (t, J=8 Hz, 2H, ArH), 1.31 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 166.33 (d, J_{CF}=252.2 Hz, CF), 157.90, 139.25 (d, J_{CF}=9.3 Hz, CCCF), 136.18, 130.53, 121.79 (q, J_{CF}=315.8 Hz, SO_2CF_3), 120.46 (d, J_{CF}=22.9 Hz, CCF), 112.88, 109.81, 36.11, 31.31. Found: C, 40.50; H, 3.34%. Calcd for $C_{17}H_{17}O_3IF_4S$: C, 40.48; H, 3.37%.

4.2.11. (4-*tert*-Butylphenyl)(4-nitrophenyl)iodonium triflate. 0.375 g (70%); mp 151–152 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (d, J=9.0 Hz, 2H, ArH), 8.30 (d, J=9.0 Hz, 2H, ArH), 8.17 (d, J=9.0 Hz, 2H, ArH), 7.61 (d, J=9.0 Hz, 2H, ArH), 1.31 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 158.32, 151.42, 137.56, 136.66, 130.76, 127.43, 121.76 (q, J_{CF}=316.42 Hz, SO_2CF_3), 121.65, 112.65, 36.16, 31.29.

4.2.12. (4-*tert*-Butylphenyl)(3-nitrophenyl)iodonium triflate. 0.335 g (58%); mp 159–160 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 9.08 (t, J=2 Hz, 1H, ArH), 8.53 (d, J=8 Hz, 1H, ArH), 8.49 (dd, J=2, 8 Hz, 1H, ArH), 8.18 (d, J=8 Hz, 2H, ArH), 7.78 (t, J=8 Hz, 1H, ArH), 7.61 (d, J=8 Hz, 2H, ArH), 1.31 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 158.37, 150.40, 141.83, 136.58, 133.95, 131.02, 130.76, 128.09, 121.78 (q, J_{CF}=316.5 Hz, SO_2CF_3), 115.63, 112.86, 36.19, 31.30. Found: C, 38.53; H, 3.26; N, 2.64%. Calcd for $C_{17}H_{17}O_5F_3NIS$: C, 38.43; H, 3.20; N, 2.64%.

4.2.13. (4-*tert*-Butylphenyl)(3-trifluoromethylphenyl)-iodonium triflate. 0.375 g (67%); mp 162–163 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.57 (s, 1H, ArH), 8.42 (d, J=8 Hz, 1H, ArH), 8.15 (d, J=8 Hz, 2H, ArH), 7.99 (d, J=8 Hz, 1H,

ArH), 7.73 (t, J=8 Hz, 1H, ArH), 7.60 (d, J=8 Hz, 2H, ArH), 1.32 (s, 9H, Me); ^{13}C NMR (75 MHz, CD_3OD) δ 158.26, 139.99, 136.46, 134.47 (q, J_{CF}=33.4 Hz, CCF_3), 133.81, 133.01 (q, J_{CF}=4.4 Hz, $CCCF_3$), 130.69, 130.29 (q, J_{CF}=3.1 Hz, $CCCF_3$), 124.19 (q, J_{CF}=270.2 Hz, CCF_3), 121.79 (q, J_{CF}=316.5 Hz, SO_2CF_3), 116.12, 112.77, 36.18, 31.30. Found: C, 39.03; H, 3.07%. Calcd for $C_{18}H_{17}O_3IF_6S$: C, 39.00; H, 3.07%.

4.2.14. (4-Iodophenyl)(phenyl)iodonium triflate. 0.476 g (80%); mp 138–140 °C (lit.[7] mp 144–148 °C); ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (d, J=8 Hz, 2H, ArH), 7.91–7.85 (m, 4H, ArH), 7.69 (t, J=8 Hz, 1H, ArH), 7.53 (t, J=8 Hz, 2H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 142.23, 137.75, 136.45, 133.73, 133.20, 121.73 (q, J_{CF}=316.4 Hz, SO_2CF_3), 116.04, 115.12, 100.59.

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An efficient synthesis of the phytoestrogen 8-prenylnaringenin from xanthohumol by a novel demethylation process

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Abstract—8-Prenylnaringenin, a flavonoid, is the strongest known phytoestrogen (plant derived estrogen mimic) used in phytomedicinal applications. Starting from xanthohumol a byproduct of hops-extraction, 8-prenylnaringenin can be synthesized via isoxanthohumol. Of various demethylation procedures tested, the best yield (92%) is obtained by treatment with scandium trifluoromethanesulfonate and potassium iodide without any need of protection. The demethylation with $AlBr_3$/collidine and of the TIPS protected isoxanthohumol provides good results too.

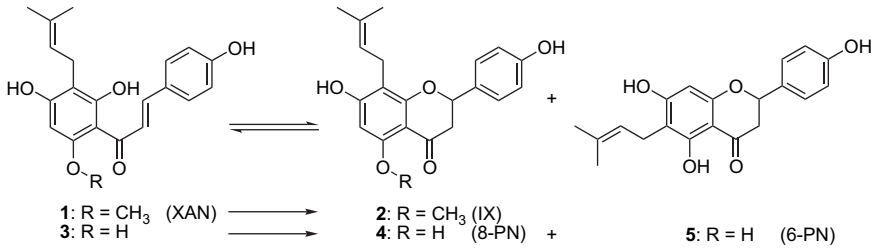
1. Introduction

Phytoestrogens are plant derived compounds with mild estrogenic activity and—compared to steroidal estrogens—often with improved pharmacokinetics and customer acceptance. They are used in treatment for menopause and other, mostly female, health problems. Most phytoestrogens are (iso-)flavonoids, e.g., genestein. The strongest one known is 8-prenylnaringenin, which naturally occurs in minor amounts in different *Wyethia* species[1] and hops.[2] The female inflorescences of the hop plant (*Humulus lupulus*) are used in the brewing industry as a flavoring and preservation agent, and for dietary preparations. Its principal prenylchalkone is xanthohumol (XAN, **1**) together with minor amounts of desmethylxanthohumol (**3**) and other prenylchalkones.[3,4] Both chalkones undergo thermal isomerization in the brew kettle (Scheme 1) to give the isomeric isoxanthohumol (IX, **2**), 8-prenylnaringenin (8-PN, **4**), and mostly 6-prenylnaringenin (6-PN, **5**). Of these compounds, only **4** possesses potent phytoestrogenic properties,[5–9] and recently has become of increasing commercial importance.

The first synthesis of **4** was achieved by direct C-prenylation of commercially available naringenin with prenyl bromide in very low yield.[10–15] The use of prenyl alcohol followed by an europium(III)-catalyzed Claisen rearrangement delivers **4** in four steps and 42–45% overall yield.[16] An alternative route starting from phloroacetophenone was much less successful.[17]

2. Results and discussion

Xanthohumol (**1**) is readily available from CO_2-extracted hops, and is a waste product of the hops industry. Its conversion into the 8-prenylnaringenin (**4**) requires a cyclization and subsequent demethylation. This course of events is crucial, since the reverse order, demethylation to **3** followed by cyclization will predominantly give the isomeric but inactive 6-PN (**5**). Substitution of the random brewing process by defined synthetic methodology thus should provide the high value target 8-PN (**4**) selectively, following the route **1**→**2**→**4**.



Scheme 1. Cyclization of hop chalcones xanthohumol (**1**) and its desmethyl derivative (**3**) in the brewing kettle.

Keywords: 8-Prenylnaringenin; Isoxanthohumol; Xanthohumol; Demethylathion; Phytoestrogen; Ether-cleavage.
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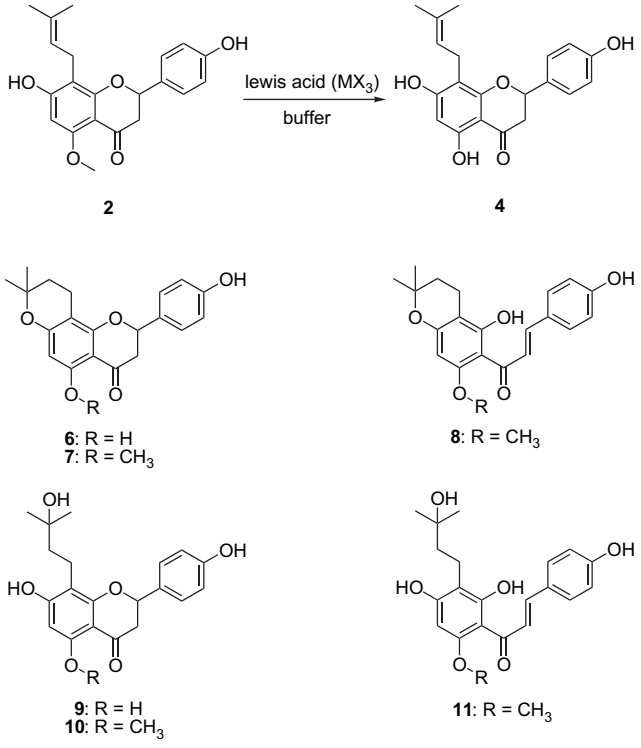
The first step, the isomerization of **1** to give isoxanthohumol (**2**) is known to proceed quantitatively under base catalysis.[18,19] Acid catalysis as well as catalysis with chiral bases is also possible, but is less efficient.[20] In principle, this intramolecular Michael-addition is reversible under the same conditions.

Crucial, however, is the second step, the demethylation of the 5-OMe group. Demethylation of 4′,7-dihydroxy-5-methoxy-6-(1″-geranyl)flavanone with BBr_3 to bonannione A was reported to proceed in high yield.[21] However, in our hands the reaction of isoxanthohumol (**2**) with BBr_3, $AlBr_3$,[22] or $AlCl_3$[23] was not useful and gave only products with cyclized side chain (**6**,[11] **7**,[24] and **8**[25,26]), and/or addition products to the double bond (**9**,[27] **10**,[26] and **11**;[25] Scheme 2). This cyclization reaction of the prenyl group with the neighboring OH-group under acidic conditions is well known.[24–26,28,29] The water addition products result from initial addition of the HBr (formed by reaction of phenol-OH with MBr_3) to the double bond with saponification under basic work-up (aqueous NaOH), or by direct acid catalyzed water addition upon work-up. Also, the application of MX_3-Lewis acids under neutral or basic conditions, or the intermittent protection of the reactive phenol group proved troublesome.



Scheme 2. Demethylation process **2**→**4** and main products of 'classical' demethylation procedures of **2** with MX_3.

The Lewis acid promoted demethylation in the presence of collidine as an acid scavenger[30] gives **4** in maximum 30% yield (Scheme 2, Table 1). Under these conditions **2** mostly remains unreacted. The principal byproduct is **1**, formed by a retro-opening of the chromanone B-ring. The increase of Lewis acid concentration leads to higher amounts of byproducts. The treatment of **2** with chlorotrimethylsilane for protection of the phenol groups and the use of methylaluminium dichloride acting as its own proton scavenger could not improve the yield of **4**.

Non-acidic, nucleophilic demethylation with $NaSCH_2CH_3$[31] in DMF failed. Instead, ringopening occurs to give 98% of **1**. In the reaction of **2** with LiI in pyridine[32] (Scheme 3a) only 8% of **4** could be isolated, accompanied by a new product of MW 486.16, compound **12**, the result of a retro-Friedel–Crafts reaction to split **1**, followed by annelation with **4** to give **12**.

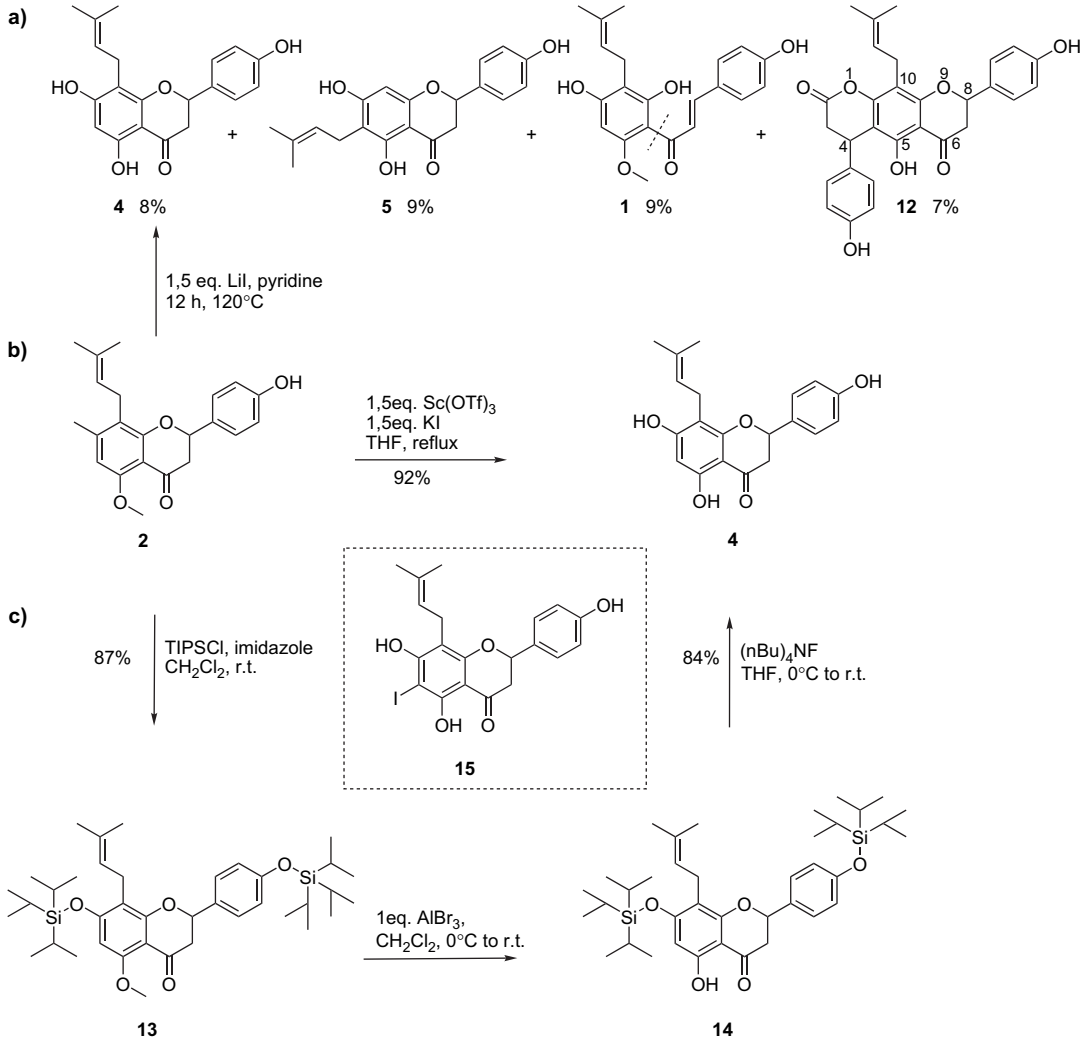
Because neither acidic nor non-acidic demethylation protocols were successful, we decided to protect the phenolic OH-groups with acetate[16] and triisopropylsilyl[33] (TIPS) groups. Acetate protection proved too labile under suitable demethylation conditions (Table 1, entry 1—with 1.2 equiv $AlBr_3$, 0.6 equiv collidine) and gives a mixture of demethylated products **4**, **6**, **9**, and the analogous 6-prenyl compounds. However, TIPS protected **13** reacts quantitatively to demethylation product **14** (Scheme 3c). The ^1H NMR does not show any signals of byproducts. Deprotection of crude **14** with *n*-Bu_4NF cleanly gives **4**. *n*-Bu_4N^+ and Silyl-compounds can be removed on short silica columns.

To improve the yield of **4** further, group IIIb Lewis acids including lanthanide salts were finally tested. They possess a high affinity to oxygen to activate the methyl–O bond for the attack of a nucleophile (e.g., iodide). At the same time, however, overactivation may promote retro-cleavage to the chalcone, which has to be avoided because of subsequent 6-PN formation. For this purpose **2** was treated with various reagent combinations in different solvents, some are shown in Table 2. Only scandium triflate in THF turned out to effect the conversion of **2** to **4** in high yield (Scheme 3b). The other reagents leave IX (**2**) almost unreacted in THF, whereas in $CHCl_3$ the cyclizations to **7** and **8** are favored. The use of pyridine as solvent increases retro-reaction to XAN (**1**). The attempt to reduce the amount of scandium triflate to catalytic quantities decreases the yield of **4** dramatically.

Table 1. Reaction of IX (**2**) with various Lewis acids in the presence of collidine in CH_2Cl_2

Entry	Lewis acid (equiv)	Collidine (equiv)	Conditions	Yield of **4** (%)	Yield of **1** (%)	Recycled **2** (%)	Byproducts (yield)
1	$AlBr_3$ (2)	5.5	5 h/rt	25	1	54	Traces of **8**, **3**, **5**
2[a,b]	$AlBr_3$ (1.5)	6.5	5 h/rt	30	5	35	**3**, **5**
3[b]	BBr_3 (3.5)	5.0	3 h/−80 °C	18	—	18	**6** (10%), **9** (6%), **8** (4%), **10**, **3**, **7**
4[b]	CH_3AlCl_2 (4.5)	4.5	4 h/reflux	~10[c]	~5[c]	~80[c]	**8**
5[b]	CH_3AlCl_2 (3.5)	0	1.5 h/0 °C	—	—	~10[c]	**8** (~70%),[c] **6** (~10%)[c]

[a] TMSCl (2.2 eqiuv).
[b] Longer work up in aqueous NaOH to convert **1** in **2**.
[c] Not isolated, detected by HPLC.



Scheme 3. Synthetic routes to 8-PN (**4**) and structure of 6-iodo-8-PN (**15**).

Table 2. Products formed by the treatment of isoxanthohumol (**2**) with Lewis acids and iodides in various solvents (detected by HPLC, byproducts in brackets)

Lewis acid	THF, 55 °C/24 h	$CHCl_3$, 55 °C/24 h	Pyridine, 80 °C/20 h	Sulfolane, 80 °C/20 h	CH_3OH/H_2O (5:1), 60 °C/6 h
$ZnBr_2$	**2** (**7**, **10**)	**7** (**2**)	**1**, **2** (**7**, **8**)	**8**, **7**, **5**	—
$ZnBr_2$/CuI	**2** (**7**, **10**, **8**, **4**)	**2**, **7** (**10**, **9**, **8**, **1**)	—	—	—
CuI	**2**	Many cpds	—	—	—
$Yb_2(SO_4)_3$/KI	**2** (**1**)	**2** (**7**, **8**)	**1**, **2** (**7**, **8**)	**2**, **1** (**8**, **7**, **5**, **4**)	—
$Yb_2(SO_4)_3$/CuI	**2** (**1**)	**2**, **7** (**8**, **1**)	—	**8** (**1**, **2**, **7**)	—
$Sc(OTf)_3$/KI	**4**	**7** (**8**, **1**)	**1**, **2** (**8**, **9**, **10**)	**1** (**8**)	**2** (**10**, **7**, **1**)
$Sm(OTf)_3$/KI	**2**	—	—	—	—
$CeCl_3$/LiI	**2**	—	—	—	—

But also proportional addition of further scandium triflate up to 1.2 equiv led to an incomplete reaction and higher amounts of byproducts, particularly the oxidation byproduct **15** with iodide in 6-position (Scheme 3—insert).

3. Experimental

3.1. General experimental information

All reactions were performed under an inert atmosphere of argon in dried solvents. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer in acetone-d_6 unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. ESI-MS spectra were taken with an API 150 EX spectrometer. High-resolution ESI mass spectra were recorded on a 7 T FT-ICR-MS—using nitrogen as drying gas at 150 °C. Analytical HPLC was carried out with diode array detector (DAD—was used for detection of UV spectra too) and a LiChrospher100 RP18 5 μm 4×125 mm column. For the preparative separation a HPLC device with a YMC-Pack ODS AA12S05-1520WT column and UV-detection at 210 nm was used. The eluent was acetonitrile/water. For

flash chromatography silica gel 60 (0.040–0.063 mm) was used. Xanthohumol was obtained from hop-industry (special thanks to Dr. Martin Biendl).

3.1.1. Isoxanthohumol (2). Xanthohumol (500 mg, 1.4 mmol) was dissolved in 500 ml 1% NaOH solution and stirred at 0 °C for 2 h. Acidification with 50% H_2SO_4 gives a light yellow precipitate. After filtration and careful washing with water the dried product (100%) can be used for most processes. If further purification is required the material is dissolved in methanol, filtered again, water is added to the solution for precipitation, and evaporated to further drive precipitation. Lyophylization of the filtered product gives **2** as a light yellow powder of at least 95% purity (usually >99.9%). **2**: UV: λ_{max} 288 nm; ^{1}H NMR: δ 1.60 (s, 3H), 1.61 (s, 3H), 2.62 (dd, 1H, J=16.3 Hz, J=2.9 Hz), 2.93 (dd, 1H, J=16.3 Hz, J=12.6 Hz), 3.26 (d, 1H, J=7.1 Hz), 3.73 (s, 3H), 5.20 (t, 1H, J=7.1 Hz), 5.36 (dd, 1H, J=12.6 Hz, J=2.9 Hz), 6.22 (s, 1H), 6.89 (d, 2H, J=8.6 Hz), 7.39 (d, 1H, J=8.6 Hz); ^{13}C NMR: δ 17.87, 22.50, 25.87, 46.12, 55.73, 79.37, 93.49, 106.04, 108.79, 115.85, 123.63, 128.45, 130.83, 131.31, 158.10, 160.84, 161.81, 162.57, 188.49; ESI-MS: 353.3 [M−H$^-$].

3.1.2. Demethylation of isoxanthohumol (2) with $AlBr_3$. To a stirred suspension of isoxanthohumol (**2**) (50 mg, 0.14 mmol) in 4 ml CH_2Cl_2 sym. collidine is slowly added until **2** is completely dissolved (ca. 100 mg, 0.83 mmol). $AlBr_3$ solution (0.28 ml, 1 M in CH_2Br_2) is added dropwise at rt. The mixture is stirred overnight and filtered. The orange precipitate is dried in vacuo and dissolved in 10 ml 0.5 M NaOH. After the solution is stirred for 1.5 h at 0 °C (to convert reformed **1** back to **2**) it is acidified with 50% H_2SO_4. The yellow precipitate is filtered off, washed carefully with water, and dried. The mixture is dissolved in methanol and separated by HPLC with a gradient of 40–70% acetonitrile in water within 20 min: **1**, t_R=21.1 min, **2**, t_R=9.6 min, **4**, t_R=14.4 min. Lyophylization gives **1** (0.5 mg, 1%) as yellow powder, **2** (27 mg, 54%), and **4** (12 mg, 25%, 60% based on recovered starting material) as white powders. 8-Prenylnaringenin (**4**): UV: λ_{max} 293, 335 nm; ^{1}H NMR: δ 1.60 (s, 3H), 1.61 (s, 3H), 2.76 (dd, 1H, J=17.2 Hz, J=3.1 Hz), 3.14 (dd, 1H, J=17.2 Hz, J=12.8 Hz), 3.22 (d, 2H, J=7.3 Hz), 5.19 (t, 1H, J=7.3 Hz), 5.45 (dd, 1H, J=12.8 Hz, J=3.1 Hz), 6.03 (s, 1H), 6.90 (d, 2H, J=8.6 Hz), 7.41 (d, 2H, J=8.6 Hz), 12.14 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 17.93, 21.88, 25.91, 43.17, 78.69, 96.80, 103.11, 106.01, 115.46, 121.42, 127.62, 130.78, 134.96, 155.72, 159.51, 162.03, 163.50, 196.19; ESI-MS: 339.3 [M−H$^-$].

3.1.3. Demethylation of isoxanthohumol (2) with BBr_3. To a stirred suspension of isoxanthohumol (**2**) (50 mg, 0.14 mmol) in 4 ml CH_2Cl_2 sym. collidine is slowly added until **2** is completely dissolved (ca. 100 mg, 0.83 mmol). BBr_3 solution (0.5 ml) (1 M in CH_2Cl_2) is added dropwise at −80 °C. The mixture is stirred for 3 h at −80 °C and filtered. For work up see above. Six fractions are separated by HPLC: **2**, t_R=9.8 min, 9 mg, 18%; **4**, t_R=14.6 min, 9 mg, 18%; 5,4′-dihydroxy-6″,6″-dimethyl-4″,5″-dihydropyrano-[2″,3″:7,8]flavanone (**6**),[11] t_R=20.7 min, 5 mg, 10% as white powder, UV: λ_{max} 218, 295 nm; ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 3H), 1.35 (s, 3H), 1.76 (m, 2H), 2.58 (m, 2H), 2.78 (dd, 1H, J=17.0 Hz, J=3.1 Hz), 3.04 (dd, 1H, J=17.0 Hz, J=13.2 Hz), 5.32 (dd, 1H, J=13.2 Hz, J=3.1 Hz), 5.97 (s, 1H), 6.89 (d, 2H, J=8.6 Hz), 7.34 (d, 2H, J=8.2 Hz), 11.76 (s, 1H); ESI-MS: m/z 339.3 [M−H$^-$]; 4,2′-dihydroxy-6′-methoxy-6″,6″-dimethyl-4″,5″-dihydropyrano-[2″,3″:3′,4′]-chalkone (**8**),[27,28] t_R=26.3 min, 2 mg, 4% as yellow powder, UV: λ_{max} 371 nm; ^{1}H NMR: δ 1.36 (s, 6H), 1.81 (t, 2H, J=6.7 Hz), 2.63 (t, 2H, J=6.7 Hz), 3.88 (s, 3H), 5.88 (s, 1H), 6.86 (d, 2H, J=8.5 Hz), 7.51 (d, 2H, J=8.7 Hz), 7.74 (d, 1H, J=15.6 Hz), 7.83 (d, 1H, J=15.6 Hz), 14.79 (s, 1H); ESI-MS: m/z 353.3 [M−H$^-$]; 8-(3″-hydroxyisoamyl)-naringenin (**9**),[33] t_R=7.6 min, 3 mg, 6% as white powder, UV: λ_{max} 214, 293 nm; ^{1}H NMR: δ 1.18 (s, 6H), 1.64 (m, 2H), 2.64 (t, 2H, J=8.1 Hz), 2.80 (dd, 1H, J=17.0 Hz, J=3.1 Hz), 3.11 (dd, 1H, J=17.0 Hz, J=12.6 Hz), 5.47 (dd, 1H, J=12.6 Hz, J=3.1 Hz), 6.01 (s, 1H), 6.90 (d, 2H, J=8.6 Hz), 7.43 (d, 1H, J=8.4 Hz), 12.11 (s, 1H); ESI-MS: 357.3 [M−H$^-$]; 8-(3″-hydroxyisoamyl)-7,4′-dihydroxy-5-methoxyflavanone (**10**),[28] t_R=4.0 min, 0.5 mg, 1% as light yellow powder, UV: λ_{max} 287 nm; ^{1}H NMR: δ 1.18 (s, 6H), 1.66 (m, 2H), 2.64 (dd, 1H, J=16.3 Hz, J=2.9 Hz), 2.67 (t, 2H, J=8.1 Hz), 2.99 (dd, 1H, J=16.3 Hz, J=12.6 Hz), 3.74 (s, 3H), 5.37 (dd, 1H, J=12.6 Hz, J=2.9 Hz), 6.19 (s, 1H), 6.88 (d, 2H, J=8.6 Hz), 7.41 (d, 1H, J=8.4 Hz); ESI-MS: m/z 371.2 [M−H$^-$].

3.1.4. Demethylation of isoxanthohumol (2) with CH_3AlCl_2. This was conducted as described in procedure 3.1.2 with $AlBr_3$ exchanged for CH_3AlCl_2. Conditions were altered as given in Table 1, entries 4 and 5.

3.1.5. Demethylation of isoxanthohumol (2) with LiI. A solution of **2** (53 mg, 0.150 mmol) in 1.2 ml pyridine is added to LiI (30 mg, 0.224 mmol) and stirred for 12 h at 120 °C. After addition of 10 ml 0.5% HCl the solution is extracted with ethyl acetate (2×30 ml). The combined organic layers are washed with aqueous NH_4Cl solution and water and dried over Na_2SO_4. The solvent is evaporated, and the residue was dissolved in methanol and subjected to HPLC with a gradient of 40–70% acetonitrile in water within 20 min. Lyophylization gives **1**, t_R=20.1 min, 5 mg, 9%; **2**, t_R=9.5 min, 6 mg, 11%; **4**, t_R=14.1 min, 4 mg, 8%; 6-prenylnaringenin (**5**), t_R=18.3 min, 5 mg, 9% as off-white powder, UV: λ_{max} 292, 334 nm; ^{1}H NMR: δ 1.64 (s, 3H), 1.75 (s, 3H), 2.72 (dd, 1H, J=17.0 Hz, J=3.0 Hz), 3.17 (dd, 1H, J=17.0 Hz, J=12.9 Hz), 3.34 (d, 2H, J=7.3 Hz), 5.23 (t, 1H, J=1.4 Hz), 5.42 (dd, 1H, J=13.0 Hz, J=2.9 Hz), 6.03 (s, 1H), 6.89 (d, 2H, J=8.6 Hz), 7.39 (d, 1H, J=8.6 Hz), 12.47 (s, 1H); ESI-MS: m/z 339.3 [M−H$^-$]; 5-hydroxy-10-prenyl-4,8-di-(4-hydroxyphenyl)-3,4,7,8-tetrahydro-pyrano-[3,2-g]chromene-2,6-dione (**12**) mixture of diastereomers, t_R=17.6 min, 5 mg, 7% as off-white powder; ^{1}H NMR: δ 1.63, (s, 6H), 2.92 (m, 2H), 3.26 (m, 2H), 3.31 (dd, 2H), 4.60 (m, 1H), 5.19 (m, 1H), 5.58 (m, 1H), 6.76 (dd, 2H, J=8.6 Hz), 6.92 (d, 2H, J=8.4 Hz), 7.01 (dd, 2H, J=8.4 Hz), 7.44 (dd, 2H, J=8.6 Hz), 12.37 (s, 1H) (most peaks are doubled because of the two diastereomers); ^{13}C NMR (acetone-d_6): δ 17.89, 22.33, 22.36, 25.86, 33.92, 33.97, 37.25, 37.33, 43.31, 43.62, 80.06, 80.17, 105.52, 105.62, 106.99, 107.02, 109.40, 109.43, 116.18, 116.33, 122.61, 122.64, 128.62, 128.94, 128.98, 130.45, 130.52, 132.27, 132.29, 132.99, 133.06, 157.33, 157.35, 157.54, 157.58, 158.40, 158.45, 158.74, 159.88, 159.99, 166.92,

166.98, 199.18, 199.22; HRMS: FTICR: *m/z* 485.161, $C_{29}H_{25}O_7$ (error 9.22e−07) [M−H$^-$].

3.1.6. 7,4′-Di-triisopropylsilyloxy-isoxanthohumol (13). To a stirred suspension of **2** (302 mg, 0.85 mmol) in CH_2Cl_2 (10 ml) imidazole (290 mg, 4.26 mmol) is added, followed by chlorotriisopropylsilane (394 mg, 2.04 mmol) dropwise at 0 °C. The resultant suspension is allowed to warm to rt and stirred overnight. After removal of the solvent, pentane is added, the white precipitate is filtered off and washed with pentane. The organic filtrate solution is quickly washed with 5% HCl (2×10 ml) and H_2O (3×10 ml) and dried with Na_2SO_4. The solvent is removed in vacuo, the residue is dissolved in ethyl acetate, and subjected to flash chromatography (pentane/ethyl acetate = 5:1) to give **13** (495 mg, 87%) as a light yellow solid in the second fraction (R_f=0.16). ^{1}H NMR ($CDCl_3$): δ 1.12 (m, 36H), 1.29 (m, 6H), 1.49 (s, 3H), 1.62 (s, 3H), 2.75 (dd, 1H, *J*=16.5 Hz, *J*=2.9 Hz), 2.99 (dd, 2H, *J*=16.5 Hz, *J*=13.2 Hz), 3.26 (d, 2H, *J*=6.8 Hz), 3.84 (s, 3H), 5.12 (t, 1H, *J*=6.8 Hz), 5.28 (dd, 1H, *J*=13.2 Hz, *J*=2.9 Hz), 6.05 (s, 1H), 6.89 (d, 2H, *J*=8.6 Hz), 7.28 (d, 2H, *J*=8.4 Hz); ^{13}C NMR ($CDCl_3$): δ 12.74, 13.23, 17.91, 17.99, 18.09, 22.55, 25.86, 45.50, 55.95, 78.62, 95.75, 106.23, 112.53, 119.78, 122.58, 127.43, 130.97, 131.49, 156.02, 159.67, 160.28, 162.04, 190.18; ESI-MS: *m/z* 667.3 [M$^+$].

3.1.7. 7,4′-Di-triisopropylsilyloxy-8-prenylnaringenin (14). To a solution of **13** (100 mg, 0.15 mmol) in CH_2Cl_2 (10 ml) cooled to 0 °C is added dropwise a solution of $AlBr_3$ (0.15 ml of 1 M solution in CH_2Br_2). The resultant solution is stirred at 0 °C for 30 min and then allowed to warm to rt. After stirring overnight 0.1 M NaOH (10 ml) is added, and the mixture is stirred vigorously for 15 min. The organic layer is separated after acidification (50% H_2SO_4), washed with aqueous NH_4Cl (2×30 ml) and H_2O (1×30 ml), and dried (Na_2SO_4). Removal of the solvent in vacuo gives crude **14**, which is directly used for deprotection. ^{1}H NMR ($CDCl_3$): δ 1.12 (m, 36H), 1.29 (m, 6H), 1.50 (s, 3H), 1.62 (s, 3H), 2.76 (dd, 1H, *J*=17.1 Hz, *J*=3.0 Hz), 3.06 (dd, 1H, *J*=17.1 Hz, *J*=13.1 Hz), 3.22 (d, 2H, *J*=7.0 Hz), 5.11 (t, 1H, *J*=7.0 Hz), 5.31 (dd, 1H, *J*=13.1 Hz, *J*=3.0 Hz), 6.00 (s, 1H), 6.91 (d, 2H, *J*=8.6 Hz), 7.28 (d, 2H, *J*=8.4 Hz), 11.97 (s, 1H).

3.1.8. Deprotection of 14 to 8-PN (4). To a solution of crude **14** in THF (3 ml) cooled to 0 °C is added dropwise a solution of tetra-*n*-butyl-ammonium fluoride (0.36 ml of 1 M solution in THF). The solution is allowed to warm to rt and stirred for 1 h. After toluene (5 ml) is added, the solvent is removed in vacuo and the resultant residue is redissolved in $CHCl_3$ and subjected to flash chromatography ($CHCl_3$/methanol = 100:1) on a silica gel column to give **4** (42 mg, 84%) as a light yellow solid.

3.1.9. Direct demethylation of isoxanthohumol with scandium trifluoromethanesulfonate. A mixture of **2** (50 mg, 0.14 mmol), KI (36 mg, 0.23 mmol), and scandium triflate (104 mg, 0.21 mmol) is stirred in THF (10 ml) and refluxed for 2.5 h. After stirring overnight at rt the solution is concentrated in vacuo and subjected to filtration though a silica gel pad ($CHCl_3$/methanol = 100:1) to remove the scandium salt. Flash chromatography ($CHCl_3$/methanol = 100:1) on a silica gel column gives **4** (44 mg, 92%) as a light yellow solid.

3.1.10. 6-Iodo-8-prenylnaringenin (15). Another attempt with a smaller amount of scandium triflate (36 mg, 73 μmol, 1.3 equiv) added in three portions within 4 h to 20 mg **2** and 17 mg (0.11 mmol, 1.9 equiv) KI gave 9 mg **4** (47%) and 1.5 mg **15** (6%) as oxidation byproduct: t_R= 20.1, UV: λ_{max} 227, 295, 347 nm; ^{1}H NMR: δ 1.61 (s, 3H), 1.62 (s, 3H), 2.84 (dd, 1H, *J*=17.0 Hz, *J*=3.1 Hz), 3.24 (dd, 1H, *J*=17.0 Hz, *J*=12.6 Hz), 3.34 (d, 2H, *J*=7.3 Hz), 5.15 (t, 1H, *J*=7.3 Hz), 5.52 (dd, 1H, *J*=12.6 Hz, *J*= 3.1 Hz), 6.91 (d, 2H, *J*=8.6 Hz), 7.42 (d, 2H, *J*=8.4 Hz), 13.14 (s, 1H); ESI-MS: *m/z* 465.0 [M−H$^-$].

3.2. General procedure for the treatment isoxanthohumol (2) with Lewis acids and iodides (Table 2)

Isoxanthohumol (**2**) (10 mg, 0.03 mmol), the Lewis acid (1.5 equiv), and the iodide (1.5 equiv) are reacted in the given solvent in a closed glass tube at the temperature and for the time given in Table 2. The solvent is removed in vacuo (except for sulfolane, from which the product is extracted with ethyl acetate vs water). The residue is suspended in acetonitrile, filtered, and the solution is analyzed by HPLC.

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Supplementary data

HPLC-chromatograms and ^{1}H and ^{13}C NMR spectra of principal reactions and selected compounds, respectively. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.060.

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Reaction of 4-methyl, 4-phenyl, and 4-hydrogen substituted 1-lithio-1,3-butadienes with aldehydes: preparation of multiply substituted cyclopentadienes

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Abstract—Reaction of aldehydes with 1-lithio-1,3-butadiene reagents possessing a methyl substituent, a phenyl substituent or a hydrogen at position 4 of the butadienyl skeletons was studied. Polysubstituted cyclopentadiene derivatives were obtained in high yields upon hydrolysis using strong acidic solution. Reaction mechanism study revealed that these cyclopentadienes were formed via an acid-promoted cyclization of conjugated dienols. Thus, stereodefined all-*cis* substituted dienols or a wide diversity of substituted cyclopentadienes can be obtained from the same 1-lithio-1,3-butadiene reagent and aldehyde by adjusting the hydrolysis conditions.

1. Introduction

We have recently demonstrated that 1-lithio-1,3-butadienes **I**–**IV** (Fig. 1) are useful reagents for the preparation of a wide variety of linear and cyclic compounds through their direct addition onto nitriles,[1] CO,[2] CO_2,[3] aldehydes, and ketones.[4,5] Results have shown that the substitution patterns at position 4 of 1-lithio-1,3-butadienes **I** (alkenyl lithium),[1–4] **II** (silyl group),[6] **III** (naphthyl group),[7] and **IV** (hydrogen)[1–4] have remarkable influences on the reactivity of these organolithium reagents. In the case of 1-lithio-1,3-butadienes **IV** (hydrogen), their reaction with aldehydes afforded stereodefined all-*cis* substituted dienols or multiply substituted cyclopentadienes depending on the nature of substituents and work up procedures.[4c] Prompted by the different types of results obtained from 1-lithio-1,3-butadienes **I**–**IV**, we prepared 1-lithio-1,3-butadiene reagent **V** and **VI** possessing a methyl substituent and a phenyl substituent at position 4 of the butadienyl skeletons, respectively, expecting new reaction patterns and synthetically useful methodology. We found that reactions of these organolithium reagents with aldehydes afforded fully substituted cyclopentadienes, via the acid-promoted cyclization of conjugated dienols. This type of reaction provides an alternative method for the preparation of useful cyclopentadiene derivatives[8] and conjugated butadienols.[9,10]

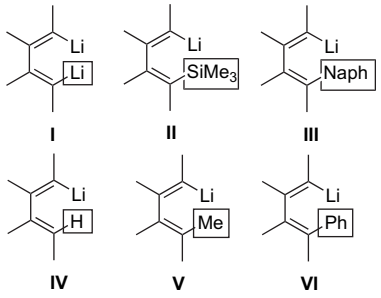

Figure 1. A variety of 1-lithio-1,3-butadienes of different substitution patterns at position 4.

2. Results and discussion

1-Bromo-4-methyl-1,2,3,4-tetraethyl-1,3-butadiene **2a** was successfully obtained in 67% isolated yield via selective lithiation of **1a** followed by treatment with Me_2SO_4 (Eq. 1).[11] Bromotriene **2b** was prepared similarly via selective lithiation of **1a** followed by treatment with LTMP and 1,2-epoxyoctane.[12] 1-Iodo-4-phenyl-1,2,3,4-tetraethyl-1,3-butadiene **2c** and 1-iodo-1,3-butadienes **2d**–**g** were conveniently prepared according to known procedures.[13,14] These halobutadienes and halotrienes are interesting and useful

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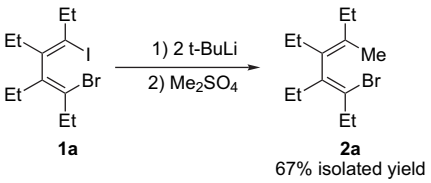
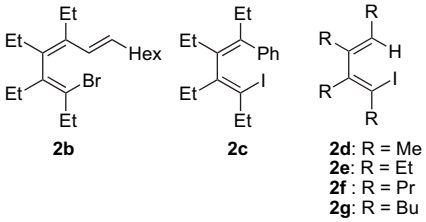
compounds but have not been much utilized in organic synthesis.

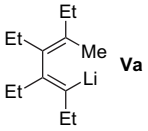
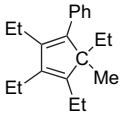
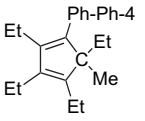
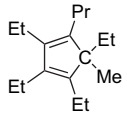
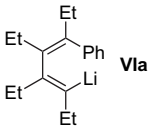
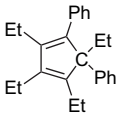
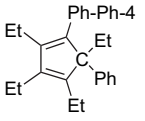
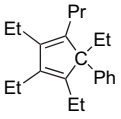
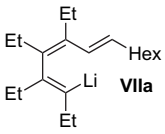
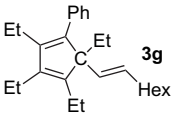


(1)



Lithiation of **2a** with 2 equiv of *t*-BuLi afforded 1-lithio-4-methyl-1,2,3,4-tetraethyl-1,3-butadiene **Va** quantitatively (Eq. 2). Reaction of thus generated **Va** with benzaldehyde went smoothly. However, quenching the reaction mixture with 3 N HCl resulted in two products, which were determined to be the corresponding butadienyl alcohol and a cyclopentadiene derivative. Because the cyclopentadiene derivative might be formed via the known acid-catalyzed cyclization of butadienyl alcohols,[4c,15] we decided to treat the reaction mixture with strong aqueous acidic solution (12 N HCl). Fully substituted cyclopentadiene **3a** was obtained as the sole product in 74% isolated yield. Results are given in Table 1. Both aromatic and aliphatic aldehydes could be applied for this reaction to afford the fully substituted cyclopentadienes in good isolated yields. This type of cyclopentadienes, which are otherwise difficult to prepare, can be expected to have applications as building units for conjugated organic materials. Reaction of the conjugated triene **VIIa** with benzaldehyde generated the cyclopentadiene derivative **3g** in a low yield, probably due to the competitive intramolecular carbo-lithiation of **VIIa**. The structure of product **3e** was determined by single-crystal X-ray structural analysis (Fig. 2).[16]

Table 1. Formation of cyclopentadiene derivatives by acidic quenching of the reaction mixture of **Va**, **VIa**, and **VIIa** with aldehydes[a]

Lithiobutadiene reagent	Aldehyde	Product **3**	Yield of **3** (%)[b]
 **Va**	PhCHO	 **3a**	74
Va	4-Ph–PhCHO	 **3b**	54
Va	PrCHO	 **3c**	56
 **VIa**	PhCHO	 **3d**	65
VIa	4-Ph–PhCHO	 **3e**	77
VIa	PrCHO	 **3f**	62
 **VIIa**	PhCHO	 **3g**	26

[a] Reaction conditions are given in Eq. 2.
[b] Isolated yields.

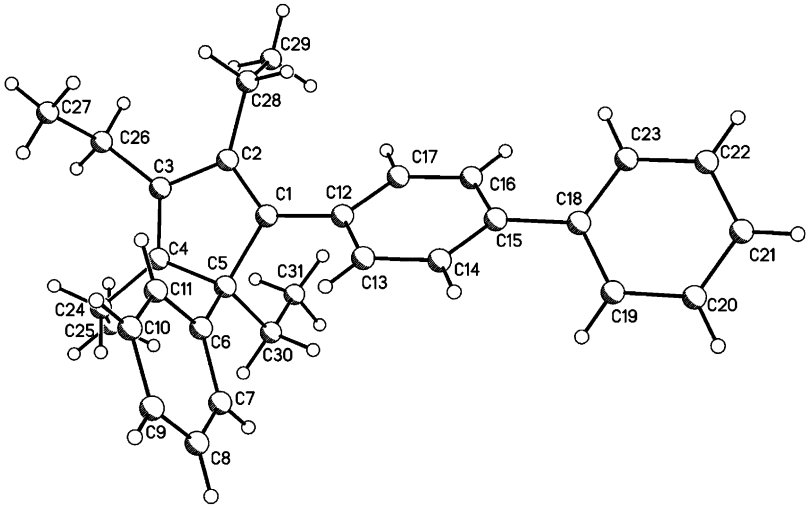
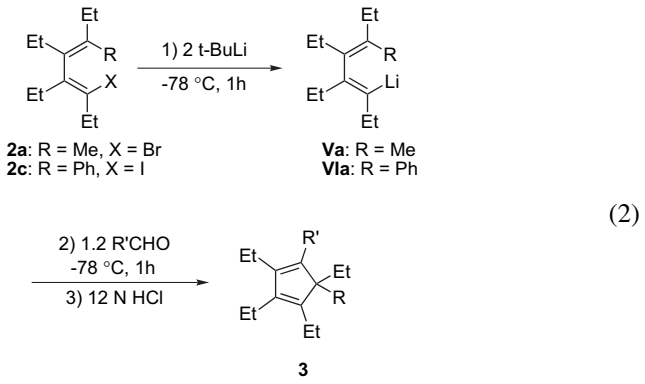
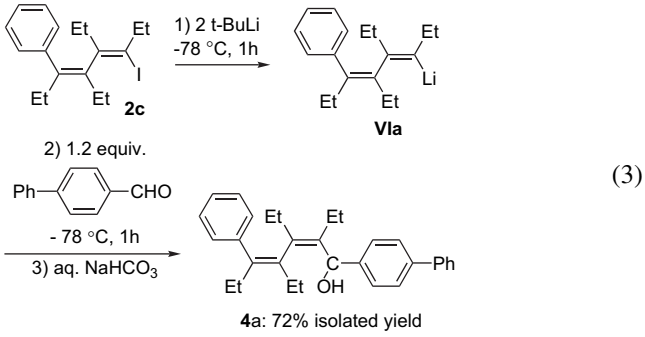


Figure 2. X-ray structure of **3e**.



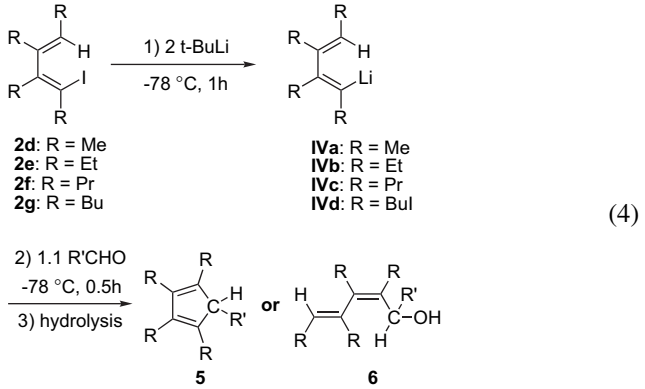
(2)

As discussed above, these cyclopentadienes might be generated via the acid-catalyzed cyclization of their corresponding butadienyl alcohols.[4c,15] In fact, when the reaction mixture of **VIa** with 4-phenylbenzaldehyde was quenched with aqueous $NaHCO_3$ instead of acidic solution, an alcohol **4a** was obtained in 72% isolated yield (Eq. 3). Treatment of the isolated pure butadienyl alcohol **4a** with 12 N HCl afforded the cyclopentadiene derivative **3e** in 77% isolated yield.



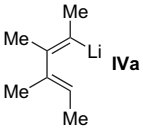
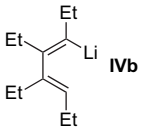
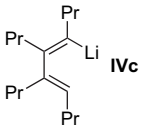
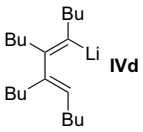
(3)

Similarly, conjugated dienols or cyclopentadiene derivatives could be prepared highly selectively in excellent yields by hydrolysis of the reaction mixtures of 1-lithiobutadienes **IV** (a hydrogen substituent at position 4) with aldehydes (Eq. 4). Results are given in Table 2. As indicated by the hydrolysis conditions shown in Table 1 and Table 2, when there was substituent such as a methyl group (**V**) or a phenyl group (**VI**) at position 4 of the butadienyl skeletons, stronger acid (12 N HCl) was required for the formation of cyclopentadiene derivatives from their corresponding dienols. In the cases of **6a** (run 6 of Table 2) and **6b** (run 8 of Table 2) without substituents at position 4, weaker acid (3 N HCl) was good enough to promote the cyclization. These isolated dienols **6a** and **6b** could be quantitatively transformed to their corresponding cyclopentadiene derivatives when treated with 3 N HCl.



(4)

Table 2. Formation of cyclopentadiene derivatives and dienols by acidic quenching of the reaction mixture of **IV** with aldehydes[a]

Run	Reagent **IV**	Aldehyde	Hydrolysis condition	Product **5** or **6**	Yield (%)[b]
1	 **IVa**	4-OMePhCHO	3 N HCl	**5a**	(53)
2	 **IVb**	PhCHO	3 N HCl	**5b**[c]	(75)
3	**IVb**	2-FurylCHO	3 N HCl	**5c**[c]	(71)
4	**IVb**	4-BrPhCHO	3 N HCl	**5d**[d]	(86)
5	**IVb**	2-ThienylCHO	3 N HCl	**5e**[e]	(78)
6	 **IVc**	PhCHO	Satd aq $NaHCO_3$	**6a**	99 (95)
7	**IVc**	PhCHO	3 N HCl	**5f**[f]	99 (86)
8	**IVc**	PrCHO	Satd aq $NaHCO_3$	**6b**	82 (65)
9	**IVc**	PrCHO	3 N HCl	**5g**[g]	85 (70)
10	 **IVd**	4-OMePhCHO	3 N HCl	**5h**	(81)

[a] Reaction conditions: shown in Eq. 4.
[b] GC yields. Isolated yields are given in parentheses.
[c] Two isomers in 3:1.
[d] Three isomers in 1:1:1.
[e] Three isomers in 2:1:1.
[f] Two isomers in 5:2.
[g] Two isomers in 4:3.

3. Conclusions

We have investigated the preparation and reaction of the useful 1-lithio-1,3-butadiene reagents possessing a phenyl substituent, a methyl substituent, and a hydrogen at position 4 of the butadienyl skeletons. Results of these organolithium reagents with aldehydes show that they are versatile synthetic building units. Cyclopentadiene derivatives thus formed are expected to have useful applications for conjugated organic materials' synthesis.

4. Experimental

4.1. General methods

All reactions were conducted under a slightly positive pressure of dry, pre-purified nitrogen using standard Schlenk line techniques when appropriate. Unless otherwise noted, all starting materials were commercially available and used without further purification. Diethyl ether was refluxed and distilled from sodium benzophenone ketyl under a nitrogen atmosphere. *t*-BuLi was obtained from Acros Organics.

^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, in $CDCl_3$ or C_6D_6 solution on JEOL JNM-AL300 NMR spectrometer.

4.1.1. A typical procedure for the formation of multiply substituted cyclopentadienes. To a solution of **2a** (1.0 mmol) in Et_2O (10 ml) was added *t*-BuLi (1.5 M pentane solution, 2.0 mmol) slowly at −78 °C and the mixture was stirred for 1 h at the same temperature. PhCHO (1.2 mmol) was added and stirring was continued for 1 h at −78 °C. HCl (12 N, 5 ml) was added to the reaction mixture, and it was stirred for 5 h at room temperature. The reaction mixture was extracted with diethyl ether and washed with aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was evaporated in vacuo and the residue was purified by chromatography on silica gel to afford the corresponding product **3a**.

Compound **3a**: colorless liquid, isolated yield 74% (198 mg). ^{1}H NMR ($CDCl_3$): δ 0.53 (t, J=7.2 Hz, 3H), 0.95 (t, J=7.5 Hz, 3H), 1.02 (s, 3H), 1.07–1.14 (m, 6H), 1.46–2.35 (m, 8H), 7.09–7.35 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 8.23, 14.36, 15.19, 15.35, 18.26, 18.88, 19.26, 21.95, 27.53, 58.20, 126.10, 127.86, 129.64, 138.67, 140.66, 143.40, 145.07, 147.04. HRMS calcd for $C_{20}H_{28}$: 268.2191; found: 268.2193.

Compound **3b**: colorless liquid, isolated yield 54% (186 mg). ^{1}H NMR ($CDCl_3$): δ 0.55 (t, J=7.2 Hz, 3H), 0.99 (t, J=7.5 Hz, 3H), 1.07 (s, 3H), 1.09–1.15 (m, 6H), 1.46–2.41 (m, 8H), 7.16–7.64 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 8.26, 14.36, 15.20, 15.41, 18.28, 18.90, 19.34, 22.05, 27.64, 58.32, 126.56, 126.94, 127.00, 128.70, 129.96, 137.77, 138.76, 140.73, 141.09, 143.72, 144.66, 147.29. HRMS calcd for $C_{26}H_{32}$: 344.2504; found: 344.2508.

Compound **3c**: colorless liquid, isolated yield 56% (66 mg). ^{1}H NMR ($CDCl_3$): δ 0.31 (t, J=7.5 Hz, 3H), 0.92–1.07 (m, 15H), 1.40–2.28 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 7.96, 14.58, 15.09, 15.16, 15.25, 18.06, 18.81, 18.90, 22.19, 23.21, 27.94, 28.27, 57.08, 140.58, 140.96, 143.81, 145.25. HRMS calcd for $C_{17}H_{30}$: 234.2348; found: 234.2351.

Compound **3d**: colorless liquid, isolated yield 65% (215 mg). ^{1}H NMR ($CDCl_3$): δ 0.58 (t, J=7.2 Hz, 3H), 0.75 (t, J=7.5 Hz, 3H), 1.09–1.19 (m, 6H), 1.77–2.47 (m, 8H), 6.71–7.24 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 7.34, 13.79, 15.07, 15.16, 18.90, 19.00, 19.45, 22.84, 65.60, 125.75, 125.96, 126.72, 127.64, 127.99, 128.54, 137.32, 141.85, 142.60, 145.56, 145.76, 149.66. HRMS calcd for $C_{25}H_{30}$: 330.2348; found: 330.2344.

Compound **3e**: colorless solid, mp 131–132 °C, isolated yield 77% (313 mg). ^{1}H NMR ($CDCl_3$): δ 0.59 (t, J=7.2 Hz, 3H), 0.75 (t, J=7.8 Hz, 3H), 1.17 (t, J=7.8 Hz, 6H), 1.83–2.54 (m, 8H), 6.80–7.54 (m, 14H). ^{13}C NMR ($CDCl_3$): δ 7.37, 13.83, 15.11, 15.20, 18.89, 18.97, 19.59, 22.99, 65.56, 125.82, 126.32, 126.69, 126.76, 126.90, 128.09, 128.61, 128.74, 136.30, 138.40, 140.88, 141.95, 142.60, 145.20, 145.97, 150.07. HRMS calcd for $C_{31}H_{34}$: 406.2661; found: 406.2658. Anal. Calcd for $C_{31}H_{34}$: C, 91.57; H, 8.43. Found: C, 91.53; H, 8.48.

Compound **3f**: colorless liquid, isolated yield 62% (184 mg). ^{1}H NMR ($CDCl_3$): δ 0.52 (t, J=7.2 Hz, 3H), 0.66–0.74 (m, 6H), 1.06–1.13 (m, 6H), 1.84–2.34 (m, 12H), 7.04–7.16 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 7.38, 14.05, 14.94, 15.07, 15.15, 18.89, 18.95 (2C), 22.53, 22.86, 28.68, 64.54, 125.46, 126.60, 127.64, 142.41, 142.65, 142.83, 145.75, 147.13. HRMS calcd for $C_{22}H_{32}$: 296.2504; found: 296.2505.

Compound **3g**: colorless liquid, isolated yield 26% (47 mg). ^{1}H NMR ($CDCl_3$): δ 0.45 (t, J=7.2 Hz, 3H), 0.89 (t, J=6.9 Hz, 3H), 1.01–1.13 (m, 9H), 1.28–2.38 (m, 18H), 5.02 (d, J=15.9 Hz, 1H), 5.42 (dt, J=15.6, 6.9 Hz, 1H), 7.17–7.27 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 7.42, 14.13, 14.50, 15.17, 15.23, 18.54, 19.00, 19.43, 22.28, 22.69, 28.95, 29.70, 31.82, 33.02, 64.65, 125.87, 127.58, 128.44, 129.01, 133.55, 137.96, 142.56, 143.50, 145.08, 146.46. HRMS calcd for $C_{27}H_{40}$: 364.3130; found: 364.3133.

4.1.2. A typical procedure for the formation of dienol 4a and the acid-promoted cyclization. To a solution of **2c** (0.5 mmol) in Et_2O (10 ml) was added *t*-BuLi (1.5 M pentane solution, 1.0 mmol) slowly at −78 °C and the mixture was stirred for 1 h at the same temperature. 4-Phenylbenzaldehyde (0.6 mmol) was added and stirring was continued for 1 h at −78 °C. The reaction mixture was quenched with aqueous $NaHCO_3$ and extracted with diethyl ether. The extract was washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo and the residue was purified by chromatography on Al_2O_3 to afford the corresponding alcohol **4a**. Colorless liquid, isolated yield 72% (153 mg). ^{1}H NMR (C_6D_6): δ 0.70 (d, J=2.7 Hz, 1H), 0.79 (t, J=7.5 Hz, 3H), 0.88 (t, J=7.2 Hz, 3H), 1.09 (t, J=7.8 Hz, 3H), 1.17 (t, J=7.5 Hz, 3H), 1.84–2.54 (m, 8H), 5.71 (d, J=2.7 Hz, 1H), 6.98–7.67 (m, 14H). ^{13}C NMR (C_6D_6): δ 13.39, 13.85, 13.98, 14.71, 21.27, 25.83, 26.34, 26.84, 74.22, 126.84, 126.95, 127.08, 127.19, 127.34, 128.22, 128.96, 129.32, 137.11, 138.48, 138.90, 139.66, 140.61, 141.56, 142.89, 143.37. HRMS calcd for $C_{31}H_{36}O$: 424.2766; found: 424.2762.

HCl (12 N, 5 ml) was added to the pure alcohol product **4a** with 1 ml Et_2O as solvent, and stirring was continued for 5 h at room temperature. The reaction mixture was extracted with diethyl ether and washed with aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was evaporated in vacuo and the residue was purified by chromatography on silica gel to afford **3e** in 77% isolated yield.

4.1.3. A typical procedure for the preparation of dienols and cyclopentadiene derivatives from reactions of 1-lithio-1,3-butadienes IV with aldehydes. 1-Lithio-1,3-butadiene derivative **IVa** was generated in situ by lithiation of its corresponding 1-iodo-1,3-butadiene **2d**. 4-Methoxybenzaldehyde (1.1 mmol) was then added to **IVa** at −78 °C and the reaction mixture was stirred at the same temperature for 0.5 h. The reaction mixture was then quenched with saturated aqueous 3 N HCl and extracted with ether. The extract was washed with water and brine, and dried over $MgSO_4$. The solvent was evaporated in vacuum to give a brown oil, which was purified by column chromatography (silica gel, hexane/CH_2Cl_2=1:1) to afford **5a** as a colorless liquid in 53% isolated yield.

Compound **5a**: colorless liquid, isolated yield 53% (120 mg). ^{1}H NMR ($CDCl_3$): δ 1.08 (d, J=3.6 Hz, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 2.14 (s, 3H), 3.28 (q, J=11.4 Hz, 1H), 3.96 (s, CH_3, 3H), 7.04 (d, J=4.5 Hz, 2H), 7.31 (d, J=4.5 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 11.07, 11.84, 12.57, 14.87, 50.13, 55.18, 113.59, 129.46, 130.40, 134.91, 135.89, 139.95, 142.35, 157.51. HRMS calcd for $C_{16}H_{20}O$: 228.1514; found: 228.1513.

Compound **5b**: colorless liquid, isolated yield 75% (192 mg), two isomers in 3:1. ^{1}H NMR ($CDCl_3$) of the main product: δ 0.54–0.88 (m, 12H), 1.19–2.35 (m, 8H), 3.05–3.30 (m, 1H), 6.77–7.14 (m, 5H). ^{13}C NMR ($CDCl_3$) of the main product: δ 6.81, 15.21, 15.44, 15.55, 18.53, 19.46, 19.66, 20.74, 52.45, 125.47, 128.09, 128.37, 137.61, 140.63, 141.98, 144.24, 145.04. HRMS calcd for $C_{19}H_{26}$: 254.2034; found: 254.2037.

Compound **5c**: brown liquid, 3:1 mixture of positional double bond isomers, combined isolated yield 71% (175 mg). ^{1}H NMR ($CDCl_3$) of the mixture: δ 0.37 (t, J=7.5 Hz, 3H), 0.85–1.19 (m, 10H), 1.61–2.67 (m, 10H), 3.43 (t, J=4.4 Hz, 1H), 5.46 (q, J=11.1 Hz, 1H), 6.19 (d, J=1.8 Hz, 1H), 3.10 (d, J=1.6 Hz, 0.3H), 6.40–6.46 (m, 1.3H), 7.37 (d, J=0.9 Hz, 1H), 7.42 (d, J=0.9 Hz, 0.3H). ^{13}C NMR ($CDCl_3$) of the mixture: δ 6.68, 11.23, 11.25, 13.30, 14.30, 14.59, 15.14, 15.36, 18.32, 18.74, 19.59, 20.01, 22.01, 27.62, 27.86, 46.06, 50.54, 51.15, 104.74, 107.58, 110.93, 111.02, 113.16, 129.68, 132.14, 140.21, 140.38, 141.29, 142.00, 145.36, 145.49, 150.89, 152.89, 153.23. HRMS calcd for $C_{17}H_{24}O$: 244.1827; found: 244.1822.

Compound **5d**: colorless liquid, three isomers in 1:1:1, isolated yield 86% (284 mg). ^{1}H NMR ($CDCl_3$) of the mixture: δ 0.30 (t, J=7.2 Hz, 3H), 0.81–1.19 (m, 27H), 1.25–1.78 (m, 14H, including 1.43 (d, J=3.6 Hz, 3H) and 1.70 (d, J=3.6 Hz, 3H)), 2.00–2.60 (m, 14H), 3.37 (s, 1H), 3.14 (t, J=4.2 Hz, 1H), 3.52 (s, 1H), 5.29–5.43 (m, 2H), 6.95 (d, J=4.2 Hz, 2H), 7.01 (d, J=4.2 Hz, 2H), 7.11 (d, J=4.2 Hz, 2H), 7.34 (d, J=4.2 Hz, 2H), 7.46 (d, J=4.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) of the mixture: δ 6.76, 10.85, 10.90, 12.79, 12.90, 13.81, 14.17, 14.26, 15.02, 15.18, 15.36, 15.48, 17.79, 17.83, 18.49, 18.68, 19.44, 19.56, 19.66, 19.94, 20.37, 20.68, 26.31, 28.08, 49.81, 50.40, 52.46, 56.08, 56.82, 111.04, 111.77, 118.96, 119.27, 119.50, 128.64, 128.99, 129.90, 131.25, 131.38, 131.41, 136.50, 139.22, 139.39, 140.11, 142.08, 145.05, 145.22, 145.36, 145.53, 146.14, 146.46, 149.77. HRMS calcd for $C_{19}H_{25}Br$: 332.1140; found: 332.1134.

Compound **5e**: colorless liquid, three isomers in 2:1:1, isolated yield 78% (203 mg). ^{1}H NMR ($CDCl_3$) of the mixture: δ 0.35 (t, J=7.2 Hz, 3H), 0.97–1.32 (m, 27H), 1.59 (d, J=3.6 Hz, 3H), 1.71(d, J=3.6 Hz, 3H), 1.76–2.02 (m, 6H), 2.12–2.71 (m, 14H), 3.39 (t, J=4.2 Hz, 1H), 3.74 (s, 0.5H), 3.90 (s, 0.5H), 5.36 (q, J=7.2 Hz, 1H), 6.73 (d, J=2.7 Hz, 1H), 6.78 (d, J=2.7 Hz, 1H), 6.80–6.95 (m, 3H), 7.02–7.08 (m, 3H), 7.18 (d, J=2.7 Hz, 2H). ^{13}C NMR ($CDCl_3$) of the mixture: δ 6.62, 10.84, 11.09, 12.76, 12.82, 13.60, 13.66, 14.17, 14.28, 14.43, 15.16, 15.41, 17.81, 18.44, 19.67, 19.88, 20.00, 20.29, 21.91, 26.16, 27.90, 45.54, 51.06, 51.30, 53.04, 57.20, 111.43, 112.19, 122.25, 122.34, 122.76, 122.85, 123.33, 125.98, 126.46, 126.94, 133.43, 138.17, 139.51, 139.80, 142.18, 145.04, 145.32, 145.40, 145.61, 149.11, 149.50, 150.41, 150.98. HRMS calcd for $C_{17}H_{24}S$: 260.1599; found: 260.1604.

Compound **6a**: quenched with saturated $NaHCO_3$. Colorless liquid, isolated yield 95%, GC yield 99%. ^{1}H NMR ($CDCl_3$, TMS): δ 0.72–0.98 (m, 14H), 1.31–1.43 (m, 8H), 2.03–2.10 (m, 6H), 5.19 (t, J=7.2 Hz, 1H), 5.78 (s, 1H), 7.21–7.39 (m, 5H). ^{13}C NMR ($CDCl_3$, TMS): δ 13.91, 14.22, 14.65, 14.84, 21.54, 21.82, 23.11, 24.41, 29.91, 30.02, 32.15, 32.57, 73.85, 126.06, 126.48, 127.90, 129.22, 135.74, 139.04, 143.47. HRMS calcd for $C_{23}H_{36}O$: 328.2766; found: 328.2757.

Compound **5f**: colorless liquid as a mixture of two double bond positional isomers (5:2) in 99% combined GC yield (combined isolated yield 86%). The NMR data of **5f** were consistent with those reported.[17]

Compound **6b**: colorless liquid, isolated yield 65%, GC yield 82%. ^{1}H NMR ($CDCl_3$, TMS): δ 0.85–0.97 (m, 17H), 1.25–1.59 (m, 10H), 1.93–2.11 (m, 8H), 4.57 (t, J=4.8 Hz, 1H), 4.96 (t, J=7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, TMS): δ 13.97, 14.18, 14.23, 14.60, 15.00, 19.69, 21.57 ($2CH_2$), 23.18, 24.79, 29.18, 29.89, 31.97, 32.46, 37.87, 72.71, 128.73, 136.77, 139.05, 141.78. HRMS calcd for $C_{20}H_{38}O$: 294.2923; found: 294.2929.

Compound **5g**: colorless liquid as a mixture of two double bond positional isomers (4:3) in 85% combined GC yield (combined isolated yield 70%). The NMR data of **5g** were consistent with those reported.[17]

Compound **5h**: colorless liquid, isolated yield 81% (321 mg). ^{1}H NMR ($CDCl_3$): δ 0.65–0.70 (m, 5H), 0.86–1.04 (m, 9H), 1.17–1.60 (m, 14H), 1.99–2.59 (m, 8H), 3.23–3.47 (br, 1H), 3.81 (s, 3H), 6.88 (d, J=4.2 Hz, 2H), 7.14 (d, J=4.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 13.92, 14.00, 14.07, 22.94, 23.07 ($2CH_2$), 23.09, 24.82, 25.46, 26.30, 27.89, 32.68, 33.06, 33.20, 52.52, 55.14, 113.50, 129.45, 130.58, 140.59, 141.08, 141.57, 143.56, 157.52. HRMS calcd for $C_{28}H_{44}O$: 396.3392; found: 396.3386.

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'Two-point'-bound supramolecular complexes from semi-rigidified dipyridine receptors and zinc porphyrins

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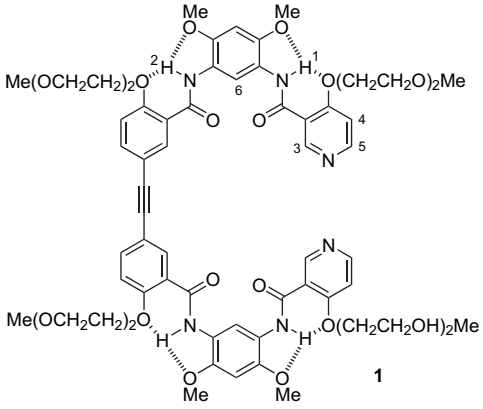
Abstract—Two linear compounds **1** and **2** have been designed and synthesized as new receptors for zinc porphyrins. Both compounds consist of two folded aromatic amide moieties, which are connected with an acetylene linker in **1** or directly in **2**. The rigid conformations of their folded moieties are stabilized by intramolecular tri-centered hydrogen bonding, while the whole molecule adopts a 'S'- or 'C'-styled conformation depending on the relative orientation of the two rigid moieties. Two pyridine units are introduced at the ends of **1** and **2** for the complexation of zinc porphyrin guests. Although the ^{1}H NMR investigation indicated that both compounds can bind two zinc porphyrin guests at high concentrations (≥5 mM) in chloroform, the UV–vis studies revealed that, at low concentration of **1** and **2** (4 μM), both compounds complex one zinc porphyrin guest to form structurally unique 'two-point'-bound 1:1 complexes. The association constants of the 1:1 complexes have been determined with the UV–vis titration experiments.

1. Introduction

The coordination between zinc porphyrin–nitrogen ligand is one of the most used recognition motifs for supramolecular self-assembly.[1,2] Since metal-free and zinc porphyrins have strong absorption bands in the visible light region, their remarkable photoelectronic properties have been utilized to build various supramolecular molecular devices. The coordination bond between zinc porphyrin and nitrogen ligand exchanges rapidly and most supramolecular complexes assembled based on this coordination motif are also dynamic. It is well known that porphyrin zinc is pentacoordinated and therefore can bind only one nitrogen ligand.[3] Theoretically, for a symmetric zinc porphyrin, a nitrogen ligand can equally approach it from both sides of its skeleton macrocycle. However, the feature of pentacoordination implies that the central metal cannot simultaneously bind with two ligands. As a result, there always exists a dynamic equilibrium between the two processes.

Considering the great usefulness of the zinc porphyrin–nitrogen ligand binding motif in molecular recognition and supramolecular self-assembly, it would be of importance to develop suitable models to investigate this equilibrium.



Keywords: Porphyrin; Coordination; Hydrogen bonding; 'Two-point' binding; Molecular recognition.
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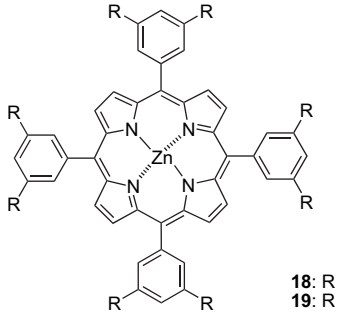
We recently reported the self-assembly of a new series of foldamers,[4] the folded or helical artificial secondary structures by making use of intramolecular hydrogen bonding as driving force.[5] The shape-persistent folded structures have been used as new generation of scaffolds for the construction of nonring synthetic receptors, which can efficiently recognize saccharides,[6] aliphatic ammonium,[7] and fullerenes.[8] In order to further explore the application of folded structures in molecular recognition, we had designed two hydrogen bonded semi-rigidified receptors **1** and **2**. In this paper, we report their synthesis and enhanced complexing properties to zinc porphyrins through an unique dynamic 'two-point' binding motif.

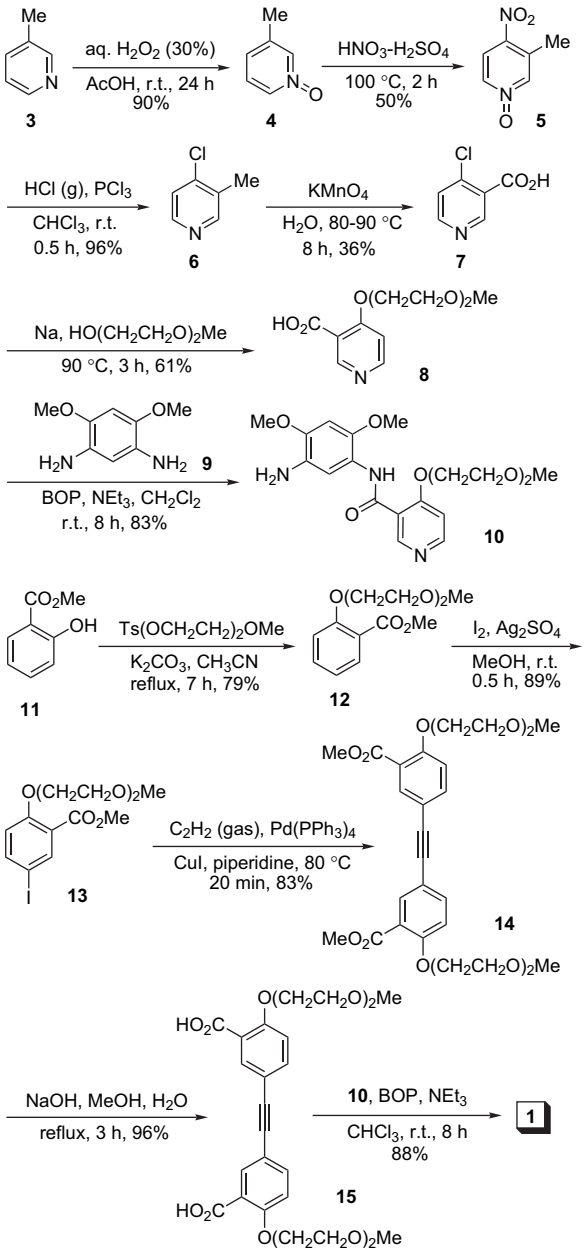
2. Results and discussion

The synthetic route of compound **1** is shown in Scheme 1. Thus, compound **7** was first prepared according to reported methods and then converted into **8** in 61% yield. The latter was coupled with **9** in dichloromethane in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) to give **10** in 83% yield. Then, compound **12** was prepared by alkylation of **11** and followed by treatment with iodine in methanol to afford **13** in 89% yield. The Pd-catalyzed coupling reaction of **13** with acetylene in hot piperidine produced **14** in 83% yield. This diester was hydrolyzed to **15** and then coupled with **10** to give compound **1** in 88% yield.

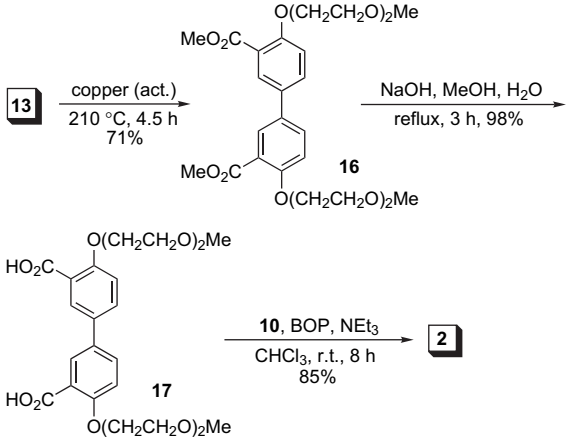
For the synthesis of compound **2** (Scheme 2), compound **16** was first obtained in 71% yield from the Ullman's coupling reaction of **13** and then hydrolyzed with sodium hydroxide to afford diacid **17** in 98% yield. The latter was coupled with **10** in chloroform in the presence of BOP to produce **2** in 85% yield.

The three-centered intramolecular hydrogen bonding motif in compounds **1** and **2** has been established by the X-ray analysis and the ^{1}H NMR and IR spectroscopy.[5b,8a,9] The ^{1}H NMR spectrum of **1** in chloroform-*d* is shown in Figure 1a. The aromatic signals of **1** have been assigned with the 2D NOESY and COSY techniques. It can be found that the two NH protons display two singlets (9.95 and 9.64 ppm, Fig. 1a) in the downfield area. The NH protons of **2** also appear in the downfield area (10.13 and 9.71 ppm, Fig. 1g). These results support that intramolecular hydrogen bonds are also present in both compounds. Due to the rotation of the acetylene unit in **1** and the biphenyl C–C bond in **2**, both compounds can in principle adopt a S- and C-styled conformations in solution. The fact that only one set of signals is observed in the ^{1}H NMR spectra suggests that the two conformations are in rapid exchange.





Scheme 1.



Scheme 2.

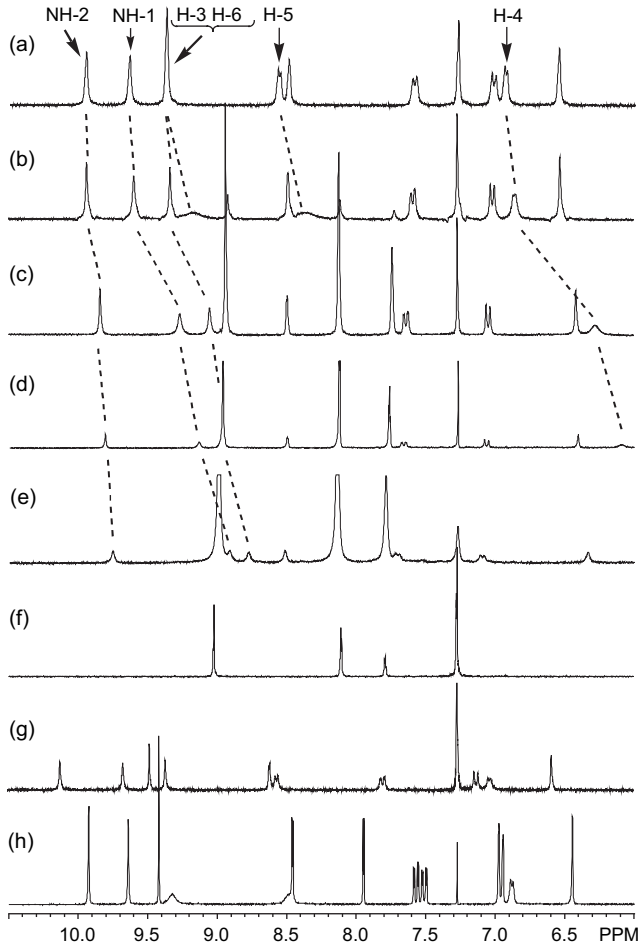


Figure 1. Partial ^{1}H NMR spectrum (400 MHz, 5 mM) of (a) **1**, (b) **1**+**18** (0.1 equiv), (c) **1**+**18** (1 equiv), (d) **1**+**18** (2 equiv), (e) **1**+**18** (4 equiv), (f) **18**, (g) **2**, and (h) **21** in chloroform-*d* at 25 °C.

Adding zinc porphyrin **18** to the solution of **1** in chloroform-*d* caused remarkable upfield shifting of the signals of several aromatic protons of **1** (Fig. 1b–e). For example, upon addition of 1 equiv of **18**, the NH-1 signal moved from 9.64 to 9.27 ppm, and the H-4 signal shifted from 6.93 to 6.28 ppm. In addition, obviously due to the complexation-induced shielding effect of **18**, the signals of H-3 and H-5 of **1** disappeared in the presence of **18** (Fig. 1c–e). Pronounced upfield shifting was also displayed for the pyrrole protons (−0.12 ppm) of **18** as a result of complexation with **1** (Fig. 1c,f). With the addition of more amount of **18**, the above signals of **1** further shifted upfield (Fig. 1d,e). This result may reflect continual approach to complexation saturation or the possibility that both pyridine units of **1** were bound to **18** to form a 1:2 complex. Similar results were also observed for the system of **2** and **18** in chloroform-*d*.

The complexation behavior of **1** and **2** with zinc porphyrin **18** and **19** in chloroform was then investigated by the UV–vis spectroscopy. As examples, the plots of the change of the UV–vis absorbance of **18** and **19** with the incremental addition of **1** are provided in Figures 2 and 3. Remarkable bathochromic effect was exhibited by both porphyrins, indicating strong intermolecular coordination. The Job's plot,

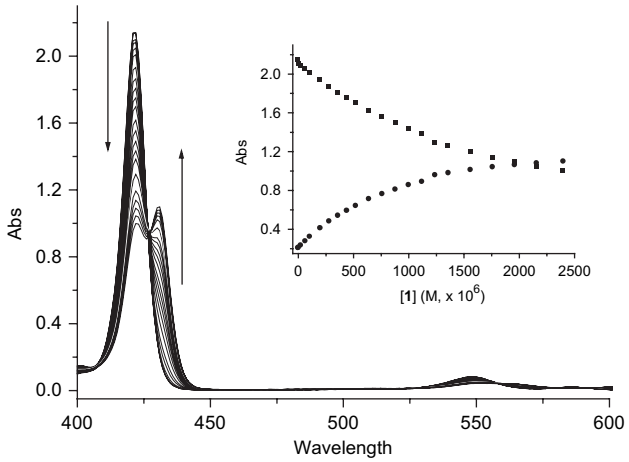


Figure 2. The change of the absorption spectra of **18** (4.0×10^{-6} M) with the addition of **1** (0–600 equiv) in chloroform at 25 °C (inset: plot of the absorption of **18** at 422 [■] and 431 [●] nm vs [**1**]).

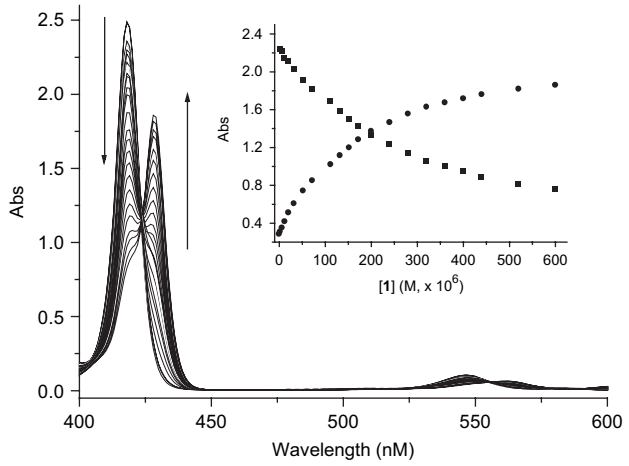


Figure 3. The change of the absorption spectra of **19** (4.0×10^{-6} M) with the addition of **1** (0–520 equiv) in chloroform at 25 °C (inset: plot of the absorption of **19** at 422 [■] and 431 [●] nm vs [**1**]).

based on the UV–vis experiments as shown in Figure 4 with the **1**:**18** system as an example, revealed maximum change in absorbance at [**1**]/([**1**]+[**18**])≈0.5, supporting a 1:1 stoichiometry.[10] In addition, all the UV–vis titration spectra display a clear isobestic point for the Soret band and the Q-band, also suggesting a 1:1 binding mode.[3a] It is obviously unreasonable to assume that only one pyridine unit of **1** or **2** was coordinated with the zinc porphyrin. Therefore, a 'two-point' binding motif should be formed for the complexes of such kind of shape-persistent two-pyridine derivatives with zinc porphyrin receptors. Figure 5 shows such kind of binding mode, with **1** as example of the two-pyridine molecules.

The association constant (K_a) of complexes **1**·**18**, **1**·**19**, **2**·**18**, and **2**·**19** was then evaluated by fitting their UV–vis titration data, obtained in chloroform, to a 1:1 binding mode,[8,11] which gave a value of 9.8 (±0.4)×10^2, 5.3 (±0.2)×10^3, 3.5 (±0.2)×10^2, and 2.8 (±0.1)×10^3 M^{-1}, respectively. Although in principle the coordinating ability of

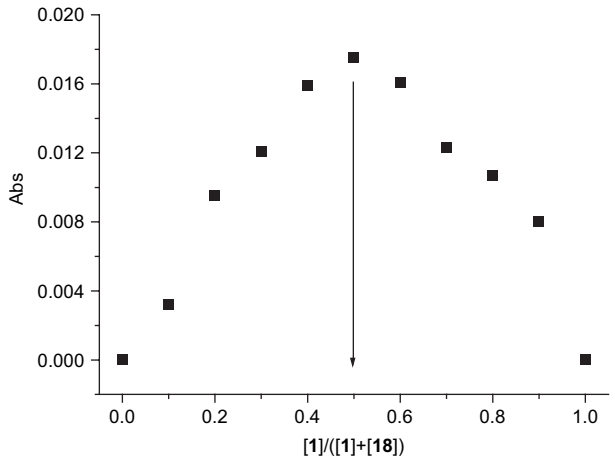


Figure 4. Job's plot of **1** versus **18**, revealing a maximum change of absorbance at 418 nm at **1**:**18**=0.5 ([**1**]+[**18**]=5×10^{-6} M in chloroform at 25 °C).

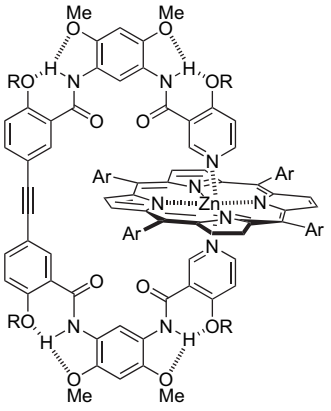
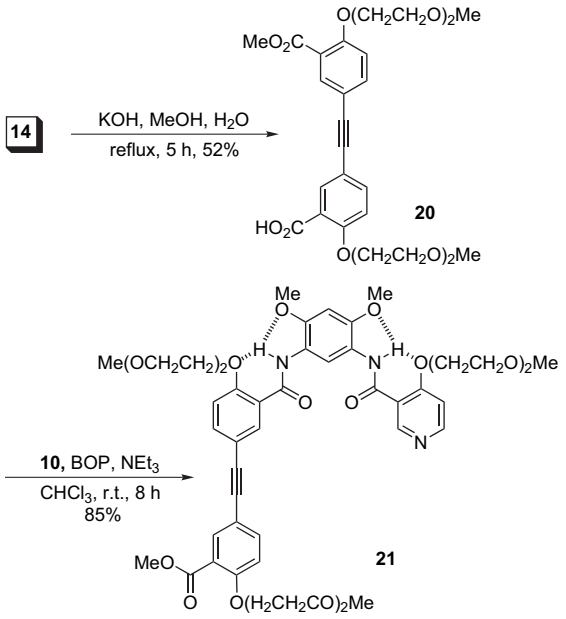


Figure 5. Proposed 'two-point' binding mode for the complex of **1** with a zinc porphyrin receptor.

octa-*tert*-butylated zinc porphyrin **18** is greater than **19** due to the electron-donating ability of the alkyl groups, the K_a of the complexes of **18** is pronouncedly smaller than the corresponding complexes of **19**. This result maybe rationalized by considering the increased steric hindrance of **18** relative to **19**, which reduces its binding affinity.

In order to detect whether this 'two-point' binding mode increases the stability of the corresponding complexes, compound **21** also was prepared (Scheme 3). Thus, compound **14** was first hydrolyzed with potassium hydroxide to give **20** in 52% yield. The acid was then reacted with **10** in chloroform with BOP as a coupling reagent to afford **21** in 85% yield. As expected, the ^{1}H NMR spectrum in chloroform-*d* showed two peaks for the NH protons in the downfield area (Fig. 1h), supporting the existence of intramolecular hydrogen bonding in **21**. On the basis of the UV–vis titration experiments, the K_a of complexes **18**·**21** and **19**·**21** in chloroform was determined to be 4.6 ($\pm$0.1)$\times10^2$ and 1.2 ($\pm$0.1)$\times10^3$ M^{-1}. These values are pronouncedly smaller than those of the corresponding complexes **1**·**18** and **1**·**19**. These results indicate that the existence of the second pyridine unit in **1** promotes the stability of the complexes.



Scheme 3.

Because the UV–vis experiments were performed at very low concentration of porphyrin receptors, the 1:1 binding mode revealed by the UV–vis investigations is not in conflict with the above ^{1}H NMR observation that both pyridine units of **1** and **2** may bind a zinc porphyrin to form a 1:2 complex. The concentration of the samples for the ^{1}H NMR experiments is obviously higher than that necessary for the UV–vis experiments. Therefore, a three-component complex maybe produced. At enough lowered concentration, the percentage of such three-component complexes maybe reduced to be ignorable and, as a result, only the formation of 1:1 complexes can be detected.

Because zinc porphyrin is of pentacoordination, the two-pyridine nitrogen atoms of **1** and **2** cannot bind with the porphyrin moiety simultaneously. Therefore, a dynamic equilibrium should exist for the 'two-point'-bonded 1:1 complexes as shown in Figure 6. Reducing the temperature of the 1:1 solution of **1** or **2** with **18** or **19** in chloroform-*d* to 0 °C caused important shifting of several aromatic signals of both components, but no splitting of the signals was observed. This result indicates that, within the studied temperature range, the exchange process shown in Figure 6 is quick on the ^{1}H NMR time scale. Further experiments at lowered temperature could not be performed due to the reduced solubility of **1** and **2**, therefore at the present stage we cannot determine the activation energy for this dynamic process.

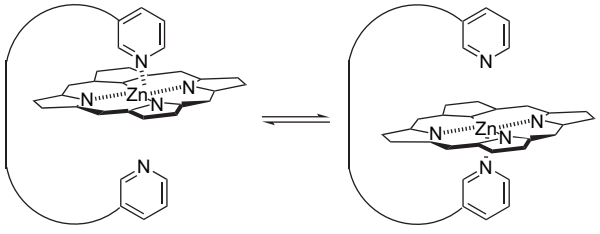


Figure 6. Dynamic process for the proposed 'two-point' binding mode for the complexes of **1** and **2** with zinc porphyrin guest.

3. Conclusion

In summary, we have reported the synthesis of a new series of semi-rigidified bipyridine receptors and their binding behavior toward zinc porphyrins. At high concentration, the new receptors can complex with two zinc porphyrin molecules to form 1:2 complexes, while at sufficiently lowered concentration, only 1:1 complexes are formed. Quantitative UV–vis experiments reveal that, for the 1:1 complexes, the existence of the second pyridine increases the stability of the complexes, which leads to the formation of an unique 'two-point' binding mode. Further work will focus on the design of more rigidified bipyridine receptors to quantitatively investigate the dynamic binding process of the 'two-point'-bound complexes.

4. Experimental

4.1. General methods

The ^{1}H NMR spectra were recorded on 400 or 300 MHz spectrometer in the indicated solvents. Chemical shifts are expressed in parts per million using residual solvent protons as internal standards. Chloroform (7.27 ppm) was used as an internal standard for chloroform-*d*. Elemental analysis was carried out at the SIOC Analytical Center. Unless otherwise indicated, all commercially available materials were used as received. All solvents were dried before use by following standard procedures. All reactions were carried out under an atmosphere of nitrogen. Silica gel (1–4 μ) was used for column chromatography. Compounds **4**,[12] **5**,[13] **6**,[14] **7**,[15] **9**,[16] **18**,[17] and **19**[18] were prepared according to reported methods. The methods for the determination of association constants have been reported in previous papers.[8]

4.1.1. 4-(2-(2-Methoxyethoxy)ethoxy)nicotinic acid (8). A suspension of sodium (0.84 g, 36.0 mmol) in 2-(2-methoxyethoxy)ethanol (10 mL) was stirred at 90 °C until a clear solution was formed. Compound **7** (1.40 g, 9.00 mmol) was then added in one portion. The reaction mixture was stirred at 90 °C for 3 h and then poured into ice water (50 mL). The solution was extracted with dichloromethane (20 mL×3). The aqueous phase was then concentrated under reduced pressure to about 10 mL and the resulting mixture was acidified with hydrochloric acid (5 N) to pH=3. The precipitate formed was filtered, washed with cold water, ether, dried in vacuo, and then recrystallized from ethanol to give compound **8** as a white solid (1.32 g, 61%). ^{1}H NMR (DMSO-d_6): 3.20 (s, 3H), 3.40–3.43 (m, 2H), 3.58–3.61 (m, 2H), 3.79 (t, *J*=4.8 Hz, 2H), 4.45 (t, *J*=4.8 Hz, 2H), 7.62 (d, *J*=6.0 Hz, 1H), 8.78 (d, *J*=6.0 Hz, 1H), 8.92 (s, 1H). ^{13}C NMR (DMSO): 57.99, 68.08, 69.86, 70.72, 71.19, 111.68, 119.65, 144.10, 145.60, 162.88, 168.51. MS (ESI): *m*/*z* 242 $[M+H]^+$. Anal. Calcd for $C_{11}H_{15}NO_5$: C, 54.77; H, 6.27; N, 5.81. Found: C, 54.35; H, 6.14; N, 5.88.

4.1.2. *N*-(5-Amino-2,4-dimethoxyphenyl)-4-(2-(2-methoxyethoxy)ethoxy)nicotinamide (10). To a solution of compound **8** (0.60 g, 2.48 mmol) and **9** (0.82 g, 4.48 mmol) in dichloromethane (50 mL) were added BOP (1.32 g, 2.98 mmol), DMAP (20 mg), and triethylamine (1 mL). The reaction mixture was stirred at room temperature for 8 h and diluted with dichloromethane (50 mL). The solution was washed with 10% aqueous Na_2CO_3 (30 mL×2), water (30 mL), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (CH_2Cl_2/MeOH 50:1) to afford compound **10** as a black powder (0.80 g, 83%). ^{1}H NMR ($CDCl_3$): 3.31 (s, 3H), 3.46–3.49 (m, 2H), 3.66–3.69 (m, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 4.02 (t, *J*=4.5 Hz, 2H), 4.48 (t, *J*=4.5 Hz, 2H), 6.51 (s, 1H), 7.01 (d, *J*=6.0 Hz, 1H), 8.04 (s, 1H), 8.59 (d, *J*=6.0 Hz, 1H), 9.32 (s, 1H), 9.77 (s, 1H). ^{13}C NMR ($CDCl_3$): 56.05, 56.96, 59.00, 68.60, 68.87, 70.83, 71.91, 97.23, 107.56, 109.16, 118.38, 121.55, 129.78, 141.85, 143.62, 153.57, 154.11, 161.20, 162.30. IR (film): 3359, 2925, 1657, 1583, 1538, 1506, 1486, 1456, 1331, 1264, 1199, 1108, 1033 cm^{-1}. MS (EI): *m*/*z* 391 $[M]^+$. Anal. Calcd for $C_{19}H_{25}N_3O_6$: C, 58.30; H, 6.44; N, 10.74. Found: C, 58.42; H, 6.51; N, 10.51.

4.1.3. Methyl 2-(2-(2-methoxyethoxy)ethoxy)benzoate (12). A suspension of compound **11** (4.85 g, 30.0 mmol), anhydrous K_2CO_3 (4.97 g, 36.0 mmol), and 2-(2-methoxyethoxy)ethyl tosylate[3] (8.22 g, 30.0 mmol) in acetonitrile (100 mL) was stirred under reflux for 7 h and then cooled to room temperature. The solid was filtered off and the filtrate was concentrated under reduced pressure. The resulting residue was triturated with ethyl acetate (100 mL) and the solution was washed with hydrochloric acid (1 N, 30 mL×2), aqueous Na_2CO_3 solution (10%, 30 mL×2), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to afford compound **12** as colorless oil (6.00 g, 79%). ^{1}H NMR ($CDCl_3$): 3.39 (s, 3H), 3.55–3.59 (m, 2H), 3.76–3.80 (m, 2H), 4.37–4.41 (m, 5H), 4.22 (t, *J*=4.8 Hz, 2H), 6.96–7.02 (m, 2H), 7.42–7.45 (m, 1H), 7.79 (d, *J*=7.8 Hz, 1H). MS (EI): *m*/*z* 254 $[M]^+$. Anal. Calcd for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Found C, 61.34; H, 7.08.

4.1.4. Methyl 5-iodo-2-(2-(2-methoxyethoxy)ethoxy)-benzoate (13). A suspension of compound **12** (5.90 g, 23.0 mmol), iodine (6.32 g, 25.0 mmol), and silver sulfate (6.98 g, 27.0 mmol) in dry methanol (100 mL) was stirred at room temperature for 0.5 h. After the solid was filtered off, the filtrate was evaporated in vacuo. The resulting residue was dissolved in dichloromethane (100 mL) and the solution was washed with saturated aqueous sodium bicarbonate solution (30 mL×2), water (30 mL), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (petroleum ether/EtOAc 3:1) to afford compound **13** as colorless oil (7.78 g, 89%). ^{1}H NMR ($CDCl_3$): 3.39 (s, 3H), 3.54–3.58 (m, 2H), 3.73–3.76 (m, 2H), 3.87–3.91 (m, 5H), 4.18 (t, *J*=4.8 Hz, 2H), 6.77 (d, *J*=9.0 Hz, 1H), 7.65 (dd, J_1=9.0 Hz, J_2=2.1 Hz, 1H), 8.05 (d, *J*=2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): 51.96, 58.86, 69.00, 69.31, 70.76, 71.82, 82.07, 115.96, 122.68, 139.79, 141.72, 158.07, 164.97. IR (film): 2953, 2923, 1728, 1607, 1487, 1434, 1281, 1225, 1081, 1063, 809, 783 cm^{-1}. MS (EI): *m*/*z* 380 $[M]^+$. Anal. Calcd for $C_{13}H_{17}IO_5$: C, 41.07; H, 4.51. Found: C, 41.57; H, 4.79.

4.1.5. Dimethyl 5,5′-(ethyne-1,2-diyl)bis(2-(2-(2-methoxyethoxy)ethoxy)benzoate) (14). A suspension of **13**

(1.14 g, 3 mmol), $Pd(PPh)_3$ (0.17 g, 0.15 mmol), and CuI (57 mg, 0.30 mmol) in dry piperidine (50 mL) was stirred at 35 °C. When the reaction mixture turned to orange from green, acetylene gas was introduced with a rubber balloon. The mixture was stirred under acetylene atmosphere at 80 °C for 0.2 h. The precipitate was filtered off and the filtrate was evaporated in vacuo. The resulting residue was triturated in ethyl acetate (50 mL) and the solution was washed with hydrochloric acid (1 N, 20 mL×2), water (30 mL), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent under reduced pressure, the crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to afford **14** as yellow oil (0.66 g, 83%). ^{1}H NMR ($CDCl_3$): 3.39 (s, 6H), 3.58–3.54 (m, 4H), 3.79–3.74 (m, 4H), 3.92–3.87 (m, 10H), 4.18 (t, J=4.8 Hz, 4H), 6.77 (d, J=9.0 Hz, 2H), 7.65 (dd, J_1=9.0 Hz, J_2=2.1 Hz, 2H), 8.05 (d, J=2.1 Hz, 2H). ^{13}C NMR ($CDCl_3$): 51.93, 58.92, 68.95, 69.41, 70.87, 71.92, 87.61, 113.64, 115.45, 120.77, 128.36, 128.46, 131.86, 131.95, 132.03, 134.86, 136.15, 158.08, 165.75. IR (film): 2926, 2878, 1732, 1610, 1504, 1451, 1437, 1277, 1244, 1145, 1110, 1081, 1053, 820 cm^{-1}. MS (EI): m/z 531 $[M]^+$. Anal. Calcd for $C_{28}H_{34}O_{10}$: C, 63.39; H, 6.46. Found: C, 63.43; H, 6.21.

4.1.6. 5,5′-(Ethyne-1,2-diyl)bis(2-(2-(2-methoxyethoxy)-ethoxy)benzoic acid) (15). To a solution of **14** (0.60 g, 1.10 mmol) in methanol (15 mL) and water (5 mL) was added sodium hydroxide (0.40 g, 10.0 mmol). The mixture was stirred under reflux for 4 h and then concentrated to about 5 mL. The residue was acidified with hydrochloric acid to pH=3 and the resulting precipitate was filtered, washed with cold water, ether, and dried in vacuo. The crude product was then recrystallized from ethanol to give compound **15** as a white solid (0.54 g, 97%). ^{1}H NMR (DMSO-d_6): 3.22 (s, 6H), 3.44–3.40 (m, 4H), 3.63–3.59 (m, 4H), 3.74 (t, J=4.8 Hz, 4H), 4.18 (t, J=4.8 Hz, 4H), 7.16 (d, J=9.0 Hz, 2H), 7.63 (dd, J_1=9.0 Hz, J_2=2.1 Hz, 2H), 7.74 (d, J=2.1 Hz, 2H). ^{13}C NMR (DMSO-d_6): 58.00, 68.59, 68.64, 69.83, 71.26, 87.58, 114.15, 122.25, 133.33, 135.48, 157.16, 166.36. MS (ESI): m/z 503 $[M+H]^+$. Anal. Calcd for $C_{26}H_{30}O_{10}$: C, 62.14; H, 6.02. Found: C, 62.38; H, 6.03.

4.1.7. *N*-(5-(5-((3-(2,4-Dimethoxy-5-(4-(2-(2-methoxy-ethoxy)ethoxy)nicotinamido)phenylcarbamoyl)-4-(2-(2-methoxyethoxy)ethoxy)phenyl)ethynyl)-2-(2-(2-methoxyethoxy)ethoxy)benzamido)-2,4-dimethoxyphenyl)-*N*-(2-(2-methoxyethoxy)ethoxy)nicotinamide (1). To a solution of **10** (212 mg, 0.54 mmol) and **15** (134 mg, 0.27 mmol) in dichloromethane (30 mL) were added BOP (0.26 g, 0.59 mmol), DMAP (20 mg), and triethylamine (0.1 mL). The mixture was stirred at room temperature for 6 h and then diluted with dichloromethane (50 mL). The solution was then washed with aqueous Na_2CO_3 solution (10%, 30 mL×2), water (30 mL), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (CH_2Cl_2/MeOH 30:1, then 10:1) to afford compound **1** as a pale yellow solid (0.29 g, 86%). ^{1}H NMR ($CDCl_3$): 3.29 (s, 12H), 3.43–3.47 (m, 8H), 3.67–3.71 (m, 8H), 3.94 (s, 12H), 4.00–4.03 (m, 8H), 4.41–4.50 (m, 8H), 6.53 (s, 2H), 7.00–7.02 (m, 4H), 7.58 (d, J=9.0 Hz, 2H), 8.47–8.50 (m, 4H), 9.32–9.37 (m, 4H), 9.64 (s, 2H), 9.95 (s, 2H). ^{13}C NMR ($CDCl_3$): 56.30, 59.03, 68.58, 68.91, 69.18, 70.76, 70.84, 71.96, 88.12, 95.67, 107.61, 113.26, 116.92, 120.45, 120.90, 122.94, 135.66, 135.98, 146.57, 146.83, 153.30, 154.23, 156.28, 161.17, 162.01, 162.43. MS (MALDI-TOF): m/z 1250 $[M]^+$, 1272 $[M+Na-H]^+$. HRMS (MALDI-TOF) Calcd for $C_{64}H_{77}N_6O_{20}$ $[M-H]^+$: 1249.5171. Found: 1249.5187.

4.1.8. Dimethyl 4,4′-bis(2-(2-methoxyethoxy)ethoxy)-biphenyl-3,3′-dicarboxylate (16). An intimate mixture of **13** (1.76 g, 4.60 mmol) and activated copper bronze[4] (3.50 g, 55.0 mmol) was covered with a thin layer of copper bronze and heated at 210–220 °C (internal temperature) for 4.5 h and then cooled to room temperature. The mixture was triturated with hot ethyl acetate (50 mL) and the organic phase was worked up. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (ethyl acetate) to afford **16** as colorless oil (0.83 g, 71%). ^{1}H NMR ($CDCl_3$): 3.40 (s, 6H), 3.57–3.60 (m, 4H), 3.77–3.80 (m, 4H), 3.91–3.95 (m, 10H), 4.26 (t, J=4.8 Hz, 4H), 7.05 (d, J=9.0 Hz, 2H), 7.64 (dd, J_1=9.0 Hz, J_2=2.1 Hz, 2H), 7.99 (d, J=2.1 Hz, 2H). ^{13}C NMR ($CDCl_3$): 51.93, 58.95, 69.12, 69.54, 70.86, 71.94, 114.27, 121.01, 129.66, 131.30, 132.20, 157.59, 166.57. IR (film): 2925, 2878, 1725, 1610, 1490, 1437, 1285, 1238, 1085, 1066, 1029, 924, 814, 786 cm^{-1}. MS (ESI): m/z 507 $[M+H]^+$. Anal. Calcd for $C_{26}H_{34}O_{10}$: C, 61.65; H, 6.77. Found: C, 61.64; H, 7.02.

4.1.9. 4,4′-Bis(2-(2-methoxyethoxy)ethoxy)biphenyl-3,3′-dicarboxylic acid (17). To a solution of **16** (0.70 g, 1.40 mmol) in methanol (20 mL) and water (5 ml) was added sodium hydroxide (0.40 g, 10 mmol). The mixture was stirred under reflux for 4 h and then concentrated to about 5 mL. The resulting residue was acidified with hydrochloric acid to pH=3 and then filtered. The solid was washed with cold water, ether, dried in vacuo, and then recrystallized from ethanol to give **17** as a white solid (0.66 g, 98%). ^{1}H NMR (DMSO-d_6): 3.22 (s, 6H), 3.33–3.44 (m, 4H), 3.59–3.75 (m, 4H), 3.75 (t, J=4.8 Hz, 4H), 4.18 (t, J=4.8 Hz, 4H), 7.16 (d, J=9.0 Hz, 2H), 7.63 (dd, J_1=9.0 Hz, J_2=2.1 Hz, 2H), 7.74 (d, J=2.1 Hz, 2H). ^{13}C NMR (DMSO-d_6): 58.00, 68.64, 68.73, 69.82, 71.26, 114.49, 122.19, 128.03, 130.43, 131.05, 156.51, 167.08. MS (MALDI-TOF): m/z 478 $[M]^+$. Anal. Calcd for $C_{24}H_{30}O_{10}$: C, 60.24; H, 6.32. Found: C, 60.12; H, 6.43.

4.1.10. N^3,$N^{3\prime}$-Bis(2,4-dimethoxy-5-(4-(2-(2-methoxy-ethoxy)ethoxy)nicotinamido)phenyl)-4,4′-bis(2-(2-methoxyethoxy)ethoxy)biphenyl-3,3′-dicarboxamide (2). To a solution of **10** (0.20 g, 0.51 mmol) and **17** (0.12 g, 0.25 mmol) in dichloromethane (30 mL) were added BOP (0.25 g, 0.55 mmol), DMAP (20 mg), and triethylamine (0.1 mL). The reaction mixture was stirred at room temperature for 6 h and then diluted with dichloromethane (50 mL). The solution was washed with aqueous Na_2CO_3 solution (10%, 30 mL×2), water (30 mL), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (CH_2Cl_2/MeOH 30:1, then 10:1) to afford **2** as pale yellow solid (0.26 g, 85%). ^{1}H NMR ($CDCl_3$): 3.28 (s, 6H), 3.30 (s, 6H), 3.46–3.52 (m, 8H), 3.69–3.73 (m, 8H), 3.96 (s, 6H), 3.99 (s, 6H), 4.02–4.07 (m, 8H), 4.46–4.53 (m, 8H),

6.58 (s, 2H), 7.03 (d, J=6.0 Hz, 2H), 7.13 (d, J=8.6 Hz, 2H), 7.80 (dd, J_1=8.6 Hz, J_2=2.4 Hz, 2H), 8.57 (d, J=6.0 Hz, 2H), 8.62 (d, J=2.4 Hz, 2H), 9.37 (s, 2H), 9.48 (s, 2H), 9.67 (s, 2H), 10.13 (s, 2H). ^{13}C NMR ($CDCl_3$): 56.33, 56.35, 59.04, 68.53, 68.91, 69.27, 70.74, 70.84, 71.95, 71.97, 95.79, 107.53, 109.01, 113.74, 116.71, 118.42, 120.56, 121.31, 122.93, 130.58, 130.96, 133.29, 141.64, 146.34, 146.70, 153.43, 154.42, 155.87, 161.20, 162.35, 162.75, 164.21. IR (film): 3358, 2885, 1661, 1615, 1583, 1549, 1484, 1470, 1424, 1337, 1308, 1277, 1243, 1222, 1202, 1109, 1030, 921, 813. MS (MALDI-TOF): m/z 1225 $[M+H]^+$, 1247 $[M+Na]^+$. HRMS (MALDI-TOF) Calcd for $C_{62}H_{77}N_6O_{20}$ $[M+H]^+$: 1225.5165. Found: 1225.5187.

4.1.11. 5-((3-(Methoxycarbonyl)-4-(2-(2-methoxyethoxy)-ethoxy)phenyl)ethynyl)-2-(2-(2-methoxyethoxy)ethoxy)-benzoic acid (20). To a solution of **14** (0.40 g, 0.76 mmol) in methanol (20 mL) was added potassium hydroxide (52 mg, 0.76 mmol). The reaction mixture was stirred at reflux for 6 h. After normal work up, the crude product was purified by column chromatography (CH_2Cl_2/MeOH 30:1) to afford **20** as colorless oil (0.22 g, 56%). ^{1}H NMR ($CDCl_3$): 3.31 (s, 3H), 3.32 (s, 3H), 3.49–3.53 (m, 4H), 3.64–3.71 (m, 4H), 3.82 (s, 3H), 3.82–3.87 (m, 4H), 4.17 (t, J=4.8 Hz, 2H), 4.33 (t, J=4.8 Hz, 2H), 6.89–6.96 (m, 2H), 7.49–7.59 (m, 2H), 7.89 (s, 1H), 8.23 (s, 1H). ^{13}C NMR ($CDCl_3$): 52.10, 59.07, 59.12, 68.63, 69.01, 69.33, 69.50, 70.78, 70.97, 71.88, 72.00, 87.06, 88.59, 113.64, 113.69, 115.18, 117.86, 120.79, 136.08, 136.39, 136.82, 137.32, 156.94, 158.35. IR (film): 2926, 2880, 1732, 1610, 1503, 1453, 1416, 1275, 1244, 1109, 1083, 1047, 917, 821. MS (ESI): m/z 517 $[M+H]^+$. Anal. Calcd for $C_{27}H_{32}O_{10}$: C, 62.78; H, 6.24. Found: C, 62.64; H, 6.02.

4.1.12. Methyl 5-((3-(2,4-dimethoxy-5-(4-(2-(2-methoxy-ethoxy)ethoxy)nicotinamido)phenylcarbamoyl)-4-(2-(2-methoxyethoxy)ethoxy)phenyl)ethynyl)-2-(2-(2-methoxy-ethoxy)ethoxy)benzoate (21). To a solution of **10** (74 mg, 0.19 mmol) and **20** (0.10 g, 0.19 mmol) in dichloromethane (30 mL) were added BOP (0.10 g, 0.23 mmol), DMAP (20 mg), and triethylamine (0.1 mL). The reaction mixture was stirred at room temperature for 6 h and then diluted with dichloromethane (50 mL). The solution was washed with aqueous Na_2CO_3 solution (10%, 30 mL×2), water (30 mL), brine (30 mL), and dried over sodium sulfate. Upon removal of the solvent in vacuo, the crude product was purified by column chromatography (CH_2Cl_2/MeOH 30:1) to afford **21** as pale yellow solid (136 mg, 80%). ^{1}H NMR ($CDCl_3$): 3.27 (s, 6H), 3.37 (s, 3H), 3.42–3.47 (m, 4H), 3.54–3.57 (m, 2H), 3.63–3.68 (m, 4H), 3.73–3.76 (m, 2H), 3.87–3.92 (m, 8H), 3.94–4.00 (m, 4H), 4.22 (t, J= 4.8 Hz, 2H), 4.35–4.42 (m, 4H), 6.44 (s, 1H), 6.88 (d, J=6.0 Hz, 2H), 6.94–6.98 (m, 2H), 7.51 (dd, J_1=8.6 Hz, J_2=2.4 Hz, 1H), 7.57 (dd, J_1=8.6 Hz, J_2=2.4 Hz, 2H), 7.95 (d, J=2.4 Hz, 1H), 8.43–8.54 (m, 2H), 9.33 (br, 1H), 9.42 (s, 1H), 9.64 (s, 1H), 9.92 (s, 1H). ^{13}C NMR ($CDCl_3$): 52.03, 56.30, 59.03, 68.68, 68.88, 69.02, 69.15, 69.50, 70.75, 70.82, 70.96, 71.93, 72.01, 87.80, 88.02, 95.65, 113.17, 113.72, 115.69, 116.82, 116.94, 120.43, 120.77, 122.94, 134.98, 135.44, 136.16, 136.37, 146.55, 146.88, 156.29, 158.14, 161.03, 162.00, 165.84. IR (film): 3359, 2884, 1732, 1666, 1614, 1584, 1544, 1503, 1468, 1272, 1242, 1202, 1108, 1086, 1030, 918, 821 cm^{-1}. MS (MALDI-TOF): m/z 912 $[M+Na]^+$. HRMS (MALDI-TOF) Calcd for $C_{46}H_{55}N_3O_{15}Na$ $[M+Na]^+$: 912.3553. Found: 912.3525.

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New antitumor sesquiterpenoids from *Santalum album* of Indian origin

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Abstract—Three new campherenane-type (**1**, **4**, **7**) and three new santalane-type (**9**, **11**, **12**) sesquiterpenoids, and two aromatic glycosides (**21**, **22**) together with 12 known metabolites including α,β-santalols (**14**, **18**), (*E*)-α,β-santalals (**15**, **19**), α,β-santaldiols (**16**, **20**), α-santalenoic acid (**17**), and vanillic acid 4-*O*-neohesperidoside were isolated from *Santalum album* chips of Indian origin. The structures of the new compounds, including absolute configurations, were elucidated by 1D- and 2D-NMR spectroscopic and chemical methods. The antitumor promoting activity of these isolates along with several neolignans previously isolated from the same source was evaluated for both in vitro Epstein–Barr virus early antigen (EBV-EA) activation and in vivo two-stage carcinogenesis assays. Among them, compound **1** exhibited a potent inhibitory effect on EBV-EA activation, and also strongly suppressed two-stage carcinogenesis on mouse skin.

1. Introduction

The plants belonging to the genus *Santalum* (Santalaceae), which are evergreen parasitic trees and consist of about 25 species, are distributed throughout India, Indonesia, Malaysia, and Australia.[1] Their essential oil (sandalwood oil) is widely used in the cosmetic, perfumery, and aromatherapy industries and has been reported to have various biological properties such as antiviral,[2] anticarcinogenesis,[3] and antitumor effects.[4,5] Among the reported constituents including sesquiterpenoids, triterpenoids,[6–11] and phenylpropanoids,[12] α-santalol, which is one of the major components in most species of the *Santalum* genus, is responsible for most of the activity of the oil. α-Santalol has particularly attracted increasing attention for its neuroleptic property[13–15] and chemopreventive effect[16–17] in in vitro and in vivo bioassay systems. In our continuing study on minor constituents of the heartwood of Indian *S. album*, grown in the Mysore district, which is known as the best tree producing sandalwood,[18] we reported the isolation and characterization of new neolignans[19] and bisabolol-related sesquiterpenoids.[20] Upon further investigation of the same plant, we have isolated and characterized six new sesquiterpenoids and two new phenolic glycosides, together with 12 known compounds that were structurally related to campherenane and santalane skeletons. We report herein the isolation and structure elucidation of the new compounds, and their inhibitory effect on EBV-EA activation in Raji cells, which is used as a convenient in vitro assay for assessing antitumor promoting activity. In vivo antitumor promoting activity was also evaluated by two-stage mouse skin carcinogenesis test.

2. Results and discussion

A methanol extract of chopped heartwood of *S. album* was divided into *n*-hexane-, ethyl acetate-, and water-soluble portions by solvent partition. The combination of chromatographic separation of the *n*-hexane, ethyl acetate, and water-soluble extracts gave eight new compounds (**1**, **4**, **7**, **9**, **11**, **12**, **21**, and **22**) and 12 known metabolites, 2α,12-dihydroxy-10(*Z*)-campherene (**2**),[21] 2β,12-dihydroxy-10(*Z*)-campherene (**5**),[21] 10(*Z*)-sandalnol (**10**),[21] 10(*Z*)-neosandalnol (**13**),[21] α-santalol (**14**),[15,22] (*E*)-α-santalal (**15**),[11] α-santaldiol (**16**),[23] α-santalenoic acid (**17**),[24] β-santalol (**18**),[15,25] (*E*)-β-santalal (**19**),[11] β-santaldiol (**20**),[24] and vanillic acid 4-*O*-neohesperidoside.[26]

Keywords: *Santalum album*; Santalaceae; Cancer chemoprevention; Campherenane-type sesquiterpene; Santalane-type sesquiterpene; Epstein–Barr virus; Mouse skin two-stage carcinogenesis.
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Table 1. ^{1}H NMR data for compounds **1**, **4**, **7**, **9**, and **12** in $CDCl_3$[a]

Position	1	4	7	9	11	12
1	—	—	—	—	—	1.89, m
2	4.06, ddd (9.6, 3 1.8)	3.64, dd (7.8, 3.6)	3.64, dd (7.2, 4.2)	3.29, d (1.8)	3.27, d (1.8)	—
3β	2.17, ddd (13.2, 9.6, 4.8)	1.65, ddd (13.2, 4.2, 3.6)	1.72, ddd (13.2, 4.2, 3.6)	—	—	—
3α	0.99, dd (13.2, 3)	1.75, dd (13.2, 7.8)	1.74, dd (13.2, 7.2)	—		
4	1.79, t (4.8)	1.85, br t (4.2)	1.87, br t (3.6)	1.76, br d (3)	1.84, br d (3.6)	1.82, br d (1.8)
5β	1.68, ddd (13.2, 9.6, 4.8)	1.61, dd (11.4, 3.6)	1.55, m	1.66, dddd (12, 9, 6, 3)	1.66, m	1.55, dddd (12.6, 9.6, 7.2, 1.8)
5α	1.26, ddd (13.2, 8.4, 3)	1.02, ddd (11.4, 9.6, 4.2)	1.05, m	1.66, dddd (12, 9, 6, 3)	1.66, m	1.25, dddd (12.6, 9.6, 6, 3.6)
6β	1.25, dddd (13.2, 9.6, 3, 1.8)	1.51, dt (12, 4.2)	1.54, m	1.04, ddd (11.4, 3, 1.8)	1.46, m	1.42, dddd (12, 9.6, 7.2, 3.6)
6α	1.88, ddd (13.2, 8.4, 4.8)	0.93, ddd (12, 9.6, 3.6)	0.95, m	1.59, ddd (11.4, 9, 6)	1.08, m	1.34, dddd (12, 9.6, 6, 2.4)
7	—	—	—	1.42, m; 1.14, dd (10.2, 1.2)	1.43, m; 1.18, dd (10.2, 1.8)	2.01, m; 1.06, br d (10.2)
8	1.36, dt (13.2, 4.8); 1.13, dt (13.2, 4.8)	1.93, dt (13.2, 4.8); 1.13, dt (13.2, 4.8)	1.30, dt (12.6, 4.8); 1.09, dt (12.6, 4.8)	1.35, ddd (13.8, 10.2, 6); 1.41, m	1.29, m	1.53, m; 1.31, m
9	2.15, ddd (12.6, 7.8, 4.8); 1.97, ddd (12.6, 7.8, 4.8)	2.09, ddd (13.2, 7.8, 4.8); 2.02, ddd (13.2, 7.8, 4.8)	2.22, ddd (12.6, 7.8, 4.8); 1.99, ddd (12.6, 7.8, 4.8)	2.15, m; 2.09, m	2.33, br dd (13.2, 7.2); 2.32, br dd (13.2, 7.2)	2.18, br dd (12.2, 6.6); 2.05, br dd (12.2, 6.6)
10	5.56, br t (7.8)	5.59, br t (7.8)	5.58, br t (7.8)	5.57, br t (7.2)	6.49, ddt (1.2, 2.4, 7.2)	5.59, br t (7.2)
11	—	—	—	—	—	—
12	4.32, br s	4.30, d (12.6); 4.24, d (12.6)	4.34, br s	4.34, d (12); 4.28, d (12)	1.75, m	4.34, d (12); 4.19, d (12)
13	4.21, br s	4.15, br s	4.22, br s	4.20, br s	9.39, s	4.18, s
14	0.85, s	0.90, s	0.91, s	1.08, s	1.10, s	1.21, s
15	0.89, s	0.84, s	1.04, br s	0.85, s	0.90, s	0.89, s

[a] Chemical shifts are shown in δ scale with J values (Hz) in parentheses.

2.1. Structures of new sesquiterpenoids

Compound **1** was isolated as a colorless oil, $[\alpha]_D^{20}$ −9.6 ($CHCl_3$). The molecular formula $C_{15}H_{26}O_3$ for **1** was determined from an ion peak at m/z 236.1767 $[M-H_2O]^+$ in HREIMS and NMR data described below. The ^{1}H NMR spectrum of **1** (Table 1) exhibited characteristic signals for two tertiary methyl groups at δ_H 0.89 (3H, s, H-15), 0.85 (3H, s, H-14), one oxygenated methine proton at δ_H 4.06 (1H, ddd, J=9.6, 3.0, 1.8 Hz, H-2), and a methine proton at δ_H 1.79 (1H, t, J=4.8 Hz, H-4), indicating the presence of a borneol moiety in **1**. The presence of the bicyclo[2.2.1]-heptane-2-ol skeleton was suggested by ^{13}C NMR resonance[27,28] (Table 2), which was further supported by cross peaks (H-3/H-2, -4 and H-5/H-4, -6) in the ^{1}H–^{1}H COSY, and long-range correlation of H-2/C-1, -3 and H-14/C-1, -2, -6 in the HMBC spectrum. The ^{1}H NMR spectrum revealed signals due to one olefinic proton at δ_H 5.56 (1H, br t, J=7.8 Hz, H-10), two hydroxymethylene groups at δ_H 4.32 (2H, br s, H-12), 4.21 (2H, br s, H-13), and four methylene protons at δ_H 2.15 (1H, ddd, J=12.6, 7.8, 4.8 Hz, H-9), 1.97 (1H, ddd, J=12.6, 7.8, 4.8 Hz, H-9), 1.36 (1H, dt, J=13.2, 4.8 Hz, H-8), 1.13 (1H, dt, J=13.2, 4.8 Hz, H-8). The ^{1}H–^{1}H COSY correlations of H-9/H-8, -10 and H-10/H-12, -13 confirmed the presence of a C_6 side chain as a comprising unit. The location of functional groups and the bulky aliphatic residue was further elucidated by key HMBC correlations of H-12/C-10, -11, -13, H-13/C-10, -11, -12, H-10/C-9, -11, -12, 13, and H-8/C-1, -4, -7, -9, -10, -15. The spectral data indicated the attached position of the methylene bridge on the bornane skeleton. The relative stereochemistry at C-7 and C-2 was determined by NOESY correlations of H-15/H-5β, H-2/H-14, -9, -8, and H-3β/H-9, -2. The *endo*-oriented secondary alcohol at C-2 was confirmed by comparison of the NMR chemical shift data of H-2 and C-2 (δ_H 4.06, δ_C 77.3) with those in the literature (for *exo*-OH; ca. δ_H 3.60, δ_C 80.0, for *endo*-OH; ca. δ_H 4.00, δ_C 77.0)[26–29] and significant W-type long-range coupling between H-2 and H-6β (J=1.8 Hz). The absolute configuration at C-2 in **1** was determined using the modified Mosher's method.[30,31] Compound **1** was treated with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride in anhydrous pyridine at room temperature overnight to afford (*R*)- and (*S*)-MTPA ester derivatives (**1a** and **1b**, respectively). A negative value ($\Delta\delta_{S-R}$) was obtained for H-14 and positive difference values for H-3, -4 (Table 3),

Table 2. ^{13}C NMR data for compounds **1**, **4**, **7**, **9**, **11**, and **12** in $CDCl_3$[a]

Position	1	4	7	9	11	12
1	50.4	50.0	49.9	49.0	49.0	52.2
2	77.3	79.7	80.2	83.3	83.9	81.4
3	38.8	39.9	40.1	42.1	42.2	46.7
4	42.1	42.0	42.0	46.8	46.0	47.7
5	28.0	27.1	27.0	26.2	26.2	24.0
6	26.1	34.4	34.1	25.4	25.3	23.2
7	51.3	49.4	49.3	40.9	40.8	34.2
8	32.4	33.2	33.7	42.2	41.1	37.7
9	23.7	23.1	23.1	22.6	24.1	24.5
10	131.7	132.2	132.0	132.1	155.2	131.7
11	136.7	136.6	136.6	136.6	139.1	136.7
12	60.0	59.4	60.1	59.9	14.1	60.1
13	67.6	67.3	67.7	67.8	195.3	67.7
14	13.4	11.4	11.3	19.4	19.4	22.3
15	16.6	16.8	16.5	16.7	16.6	19.8

[a] Chemical shifts are shown in δ scale.

Table 3. Partial ^{1}H NMR data of the (*S*)- and (*R*)-Mosher esters of compounds **1**, **2**, **2c**, **9**, and **10** in $CDCl_3$[a]

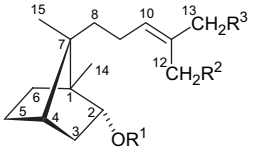
Position	δ_H													
	1a	**1b**	$\Delta\delta_{S-R}$	**2a**	**2b**	$\Delta\delta_{S-R}$	**2d**	**2e**	$\Delta\delta_{S-R}$	**9a**	**9b**	$\Delta\delta_{S-R}$	**10a**	**10b**	$\Delta\delta_{S-R}$
2	5.13	5.10	*R*[b]	5.15	5.12	*R*[b]	5.16	5.11	*R*[b]	4.61	4.62	*R*[b]	4.62	4.63	*R*[b]
3β	2.29	2.28	+0.01	2.34	2.33	+0.01	2.38	2.38	±0	—	—	—	—	—	—
3α	1.14	1.03	+0.11	1.13	1.02	+0.11	1.15	1.05	+0.10	—	—	—	—	—	—
4	1.85	1.81	+0.04	1.88	1.84	+0.04	1.89	1.84	+0.05	1.66	1.68	−0.02	1.66	1.67	−0.01
5β	1.70	1.65	+0.05	1.69	1.66	+0.03	1.68	1.67	+0.01	1.62	1.62	±0	1.65	1.60	+0.05
5α	1.24	1.24	±0	1.23	1.17	+0.06	1.20	1.15	+0.05	1.30	1.33	−0.03	1.33	1.35	−0.02
6β	1.84	1.83	+0.01	1.82	1.82	±0	1.82	1.82	±0	1.57	1.53	+0.04	1.57	1.55	+0.02
6α	1.30	1.30	±0	1.31	1.31	±0	1.33	1.33	±0	1.07	1.00	+0.07	1.06	1.00	+0.06
7	—	—	—	—	—	—	—	—	—	1.48	1.48	±0	1.46	1.47	−0.01
	—	—	—	—	—	—	—	—	—	1.16	1.16	±0	1.15	1.16	−0.01
14	0.80	0.87	−0.07	0.80	0.87	−0.07	0.80	0.88	−0.08	1.06	0.98	+0.08	1.06	0.98	+0.08
15	0.87	0.87	±0	0.88	0.88	±0	0.89	0.89	±0	0.68	0.76	−0.08	0.65	0.74	−0.09

[a] Data were assigned on the basis of the correlation with 2D NMR spectroscopy.
[b] Absolute configuration.

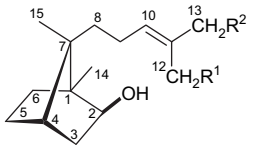
establishing that the absolute configuration of the chiral center at C-2 is *R*. Therefore, the structure of compound **1** was fully assigned to (2*R*,7*R*)-2,12,13-trihydroxy-10-campherene.

The HREIMS of compound **4**, $[\alpha]_D^{20}$ +15.7 ($CHCl_3$), showed a pseudomolecular ion peak at *m/z* 236.1775 $[M-H_2O]^+$, indicating that the molecular formula ($C_{15}H_{26}O_3$) is the same as that of **1**. The ^{1}H and ^{13}C NMR spectral data of **4** (Tables 1 and 2) were also analogous to those of **1** except for H-2 and H-3 signals. Remarkable upfield shift of H-2 (Δδ, 0.42 ppm) and downfield shift of C-2 (Δ 2.40 ppm) were observed in **4** compared with **1**. These spectral characteristics suggested the presence of an *exo*-OH group at C-2.[27–29] In addition, relative stereochemistry was inferred by NOE cross peaks of H-15/H-6β, -5β, and H-2α/H-6α, -3α. We attempted the determination of the absolute configuration at C-2 by modified Mosher's method.[30,31] However, MTPA esters of **4** were not obtained probably because of steric hindrance between the hydroxyl group at C-2 and the side chain. The absolute structure **4** was proposed by comparing its specific optical rotation with that of compound **1**, with reference to synthetic campherenol (**3**) ($[\alpha]_D$ −5.3) and isocampherenol (**6**) ($[\alpha]_D$ +25.0),[32] unless the presence of hydroxyl groups in the side chain influenced the sign of specific rotation. Thus, compound **4** was assigned to (2*S*,7*R*)-2,12,13-trihydroxy-10-campherene, which is the C-2 epimer of **1**.

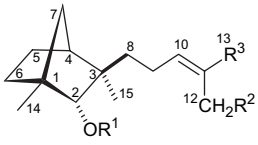
Although compounds **2**, **5**, and **10** were recently reported as constituents of commercial sandalwood,[21] their absolute structures remained unassigned. We have established the absolute stereostructures of **2** and **10** as shown in Figure 1 by a combination of NOESY experiment and modified



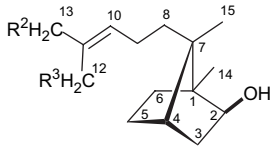
Compd.	R^1	R^2	R^3
1	H	OH	OH
1a	(*S*)-MTPA	*O*-(*S*)-MTPA	*O*-(*S*)-MTPA
1b	(*R*)-MTPA	*O*-(*R*)-MTPA	*O*-(*R*)-MTPA
2	H	OH	H
2a	(*S*)-MTPA	*O*-(*S*)-MTPA	H
2b	(*R*)-MTPA	*O*-(*R*)-MTPA	H
2c	H	*O*-CPh_3	H
2d	(*S*)-MTPA	*O*-CPh_3	H
2e	(*R*)-MTPA	*O*-CPh_3	H
3	H	H	H



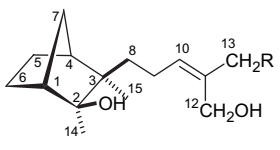
Compd.	R^1	R^2
4	OH	OH
5	OH	H
6	H	H



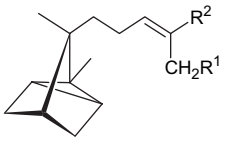
Compd.	R^1	R^2	R^3
9	H	OH	CH_2OH
9a	(*S*)-MTPA	*O*-(*S*)-MTPA	CH_2O-(*S*)-MTPA
9b	(*R*)-MTPA	*O*-(*R*)-MTPA	CH_2O-(*R*)-MTPA
10	H	OH	CH_3
10a	(*S*)-MTPA	*O*-(*S*)-MTPA	CH_3
10b	(*R*)-MTPA	*O*-(*R*)-MTPA	CH_3
11	H	H	CHO



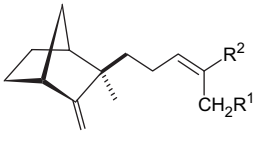
Compd.	R^1	R^2
7	OH	OH
8	H	H



12: R = OH
13: R = H



Compd.	R^1	R^2
14	OH	CH_3
15	H	CHO
16	OH	CH_2OH
17	H	COOH



Compd.	R^1	R^2
18	OH	CH_3
19	H	CHO
20	OH	CH_2OH

Figure 1.

Mosher's method (Table 3). The absolute structure of **5**, whose relative structure was determined by NOESY, was deduced by comparing its specific rotation ($[\alpha]_D^{20}$ −17.6) with that of compound **2** ($[\alpha]_D^{20}$ +17.0) in an argument similar to that between **1** and **4**. Hence, compounds **2**, **5**, and **10** were assigned to (2*R*,7*R*)- and (2*S*,7*R*)-2,12-dihydroxy-10(*Z*)-campherene, and (2*R*,3*R*)-10(*Z*)-sandalnol, respectively.

The molecular formula $C_{15}H_{26}O_3$ of **7** was determined by its HREIMS. Both the ^{1}H and ^{13}C NMR data of compound **7** (Tables 1 and 2) were very similar to those of **4**, however, large chemical shift differences for CH_3-15 and H-8, and the appearance of CH_2-12 as a broad singlet were distinguishing features from **4**. In addition, clear correlations were observed between H-8 and H-6β, -5β in the NOESY experiment of **7**, whereas no correlation was observed between H-15 and H-6β, -5β. These 1D- and 2D-NMR data for **7** suggested that the orientation of the side chain at C-7 was opposite to that of **4**. Thus, compound **7** was characterized to be (2*S**,7*S**)-2,12,13-trihydroxy-10-campherene.

Compound **9** was obtained as a colorless oil, $[\alpha]_D^{20}$ −4.8 ($CHCl_3$). The molecular formula $C_{15}H_{26}O_3$, which was the same as that of **1**, **4**, and **7**, was deduced from NMR data and a pseudomolecular ion peak $[M-H_2O]^+$ at *m*/*z* 236.1773 in HREIMS. In the ^{1}H NMR spectrum of **9** (Table 1), the doublet signal at δ_H 3.29 (1H, d, *J*=1.8 Hz, H-2) attributable to an oxygenated methine proton, clearly differed from the corresponding signals of **1**, **4**, and **7**. One (δ_H 1.08, H-14) of the two tertiary methyl groups also showed downfield shift (ca. 0.2 ppm) relative to those of the above three compounds, suggesting that compound **9** is not a campherene analogue. The presence of an α-fenchol framework as a partial structure was indicated by the close similarity of ^{13}C NMR resonances to the data reported for α-fenchol,[33] as well as by HMBC correlations of H-2/C-1, -3, -14, H-8/C-2, -4, -10, -15, H-14/C-2, -6, -7, and H-15/C-2, -4, -8. Also, the existence of an oxygenated prenyl group as the other constituent unit in **9** was substantiated by spectral resemblance to the corresponding signals in ^{1}H and ^{13}C NMR spectra of **1**, **4**, and **7**. The NOE correlations of H-2/H-7, -8, -14, and H-15/H-5α in **9** indicated the cis relationship of 2-OH and 3-CH_3 groups. The 2*R* configuration was confirmed by the modified Mosher's method (Table 3). Therefore, the structure of **9** was determined to be (2*R*,3*R*)-13-hydroxysandalnol.

The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of compound **11**, $[\alpha]_D^{20}$ −5.1 ($CHCl_3$), were similar to those of **10**, and the only difference was observed in the presence of an aldehyde signal [δ_H 9.39 (1H, s), δ_C 195.3] instead of the methyl signal in **10**. The 2D-NMR analysis and HREIMS data (*m*/*z* 236.1773 $[M]^+$, $C_{15}H_{24}O_2$) showed the structure **11** for this compound. The *E* configuration of the double bond in **11** was assigned by significant NOE of the aldehyde proton (H-13) with H-10 as well as the correlation between H-12 and H-9 in the NOESY spectrum. The chemical shift of H-13 (δ_H 9.39) was also consistent with the trans-oriented α,β-unsaturated aldehyde proton signal generally accepted (δ_H 9.33 for trans, δ_H 10.2 for cis-oriented CHO).[9] Taking the biogenetic pathway into consideration, the stereostructure of **11** was tentatively assigned to (2*R**,3*R**)-10(*E*)-sandalnol-13-al.

Compound **12**, $[\alpha]_D^{20}$ −63.6 ($CHCl_3$), exhibited an ion peak $[M-H_2O]^+$ at *m*/*z* 236.1768 in HREIMS, corresponding to the molecular formula $C_{15}H_{26}O_3$. The ^{1}H NMR spectrum of **12** (Table 1) revealed signals assignable to two tertiary methyl protons at δ_H 0.89 and 1.21 (3H each, s, H-15, -14, respectively), two methine protons at δ_H 1.89 (1H, m, H-1), 1.82 (1H, br d, *J*=1.8 Hz, H-4), a methylene-bridge proton at δ_H 2.01 (1H, m, H-7), 1.06 (1H, br d, *J*=10.2 Hz, H-7), a vinyl proton at δ_H 5.59 (1H, br t, *J*=7.2 Hz, H-10), and two hydroxymethylene protons at δ_H 4.19, 4.34 (1H each, d, *J*=12.0 Hz, H-12), δ_H 4.18 (2H, s, H-13) arising from methylene bicyclo[2.2.1]heptane moiety and C_6 side chain unit. These data resemble to those for β-santaldiol (**20**)[23] except for the presence of 2-OH and 14-CH_3 groups instead of the exomethylene group in **20**. The relative configuration of **12** was determined by key NOESY correlations (H-15/H-14, -5α, H-14/H-15, -6α, and H-8/H-7). Thus, compound **12** was characterized to be (2*S**,3*R**)-13-hydroxy-neosandalnol.

Compound **13**, $[\alpha]_D^{20}$ −26.7 ($CHCl_3$) was previously reported to have a planar structure.[21] The transformation from compound **13** into the dehydrated derivative was observed by NMR measurement of $CDCl_3$ overnight, although the cause for this phenomenon remains uncertain. The dehydrate ($[\alpha]_D^{20}$ −90.3) was identified to be β-santalol (**18**) by NMR and MS analyses, and also by comparison with an authentic specimen. On the basis of this finding, the stereostructure of **13** was assigned to (2*S*,3*R*)-10(*Z*)-neosandalnol.

Campherenane and santalane derivatives have been found in certain species of Illiciaceae,[27] Lauraceae,[28] Rutaceae,[34] Hepaticae,[35] and Santalaceae,[8–11,23,36,37] even though they are very rare classes of sesquiterpenes. These metabolites with diverse structures were presumed to be derived from bisabolol via santalenes including Wagner–Meerwein rearrangement and oxidation steps.

2.2. Structures of new aromatic glycosides

The HRESIMS of compound **21** gave a pseudomolecular ion $[M+NH_4]^+$ at *m*/*z* 490.2280, consistent with the molecular formula of $C_{22}H_{32}O_{11}$. The ^{1}H NMR spectrum of **21** showed signals attributable to 1,3,4-trisubstituted-type aromatic protons at δ_H 7.06 (1H, d, *J*=8.4 Hz, H-5), 6.83 (1H, d, *J*=1.8 Hz, d, H-2), 6.72 (1H, dd, *J*=8.4, 1.8 Hz, H-6), terminal monosubstituted double-bond protons at δ_H 6.00 (1H, m, H-8), 5.01, 5.07 (1H each, m, H-9), a methylene proton at δ_H 3.36 (2H, d, *J*=6.6 Hz, H-7), and a methoxyl group at δ_H 3.85 (3H, s). These signals suggest the presence of a C_6–C_3 moiety, which was substantiated by the HSQC experiment. In addition to aglycone signals, two characteristic anomeric signals at δ_H 5.39 and 5.06, and 11 oxygen-bearing protons at δ_H 4.16–3.38 were observed, along with a doublet methyl signal at δ_H 1.25, indicating the presence of glucose and rhamnose moieties.[26,38] These NMR data in combination with the observed ^{1}H–^{1}H COSY correlations (Fig. 2) suggested that compound **21** is a simple phenolic rhamnoglucoside. The glycosidic linkage of the sugar moiety was determined to be β for glucose and α for rhamnose from the coupling constants of 7.8 and 1.8 Hz for anomeric protons, respectively. The presence of a β-glucosyl and an

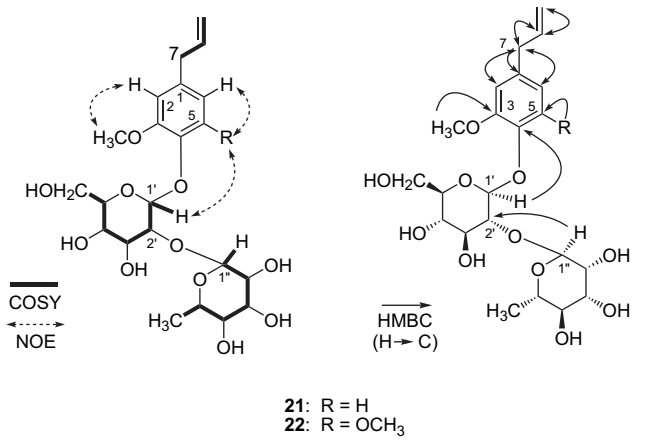


Figure 2. Selected COSY, HMBC, and NOESY correlations of compounds **21** and **22**.

α-rhamnosyl moieties was further evidenced by the ^{13}C NMR spectrum (Table 4).[39–41] These assignments of sugar linkages and the position of a methoxyl group were confirmed unambiguously from HMBC (H-1′/C-4, H-1″/C-2′, and OCH_3/C-3) and NOESY (H-1′/H-5 and OCH_3/H-2) experiments (Fig. 2). Acid hydrolysis of compound **21** gave eugenol, which was confirmed by direct comparison of HPLC and reported NMR data with those of an authentic sample.[42,43] Unfortunately, the limited amount of **21** obtained did not allow elucidation of the absolute configuration of its sugar moiety. Thus, the structure of compound **21** was determined to be a new phenolic glycoside, eugenol 4-*O*-rhamnosyl(1→2)glucoside.

Table 4. NMR spectroscopic data for compounds **21** and **22** in CD_3OD[a]

Position	δ_H		δ_C	
	21	**22**	**21**	**22**
Aglycone moiety				
1			139.1	136.8
2	6.83, d (1.8)	6.54, s	113.8	106.4
3			151.3	153.2
4			145.8	132.9
5	7.06, d (8.4)		118.2	153.2
6	6.72, dd (8.4, 1.8)	6.54, s	121.5	106.4
7	3.36, d (6.6)	3.37, m	40.8	40.1
8	6.00, m	6.00, m	136.7	137.6
9	5.07, m; 5.01, m	5.15, m; 5.08, m	115.7	114.9
OCH_3	3.85, 3H, s	3.86, 6H, s	56.3	55.8
Glucose moeity				
1′	5.06, d (7.8)	5.12, d (7.2)	100.7	101.4
2′	3.75, dd (9.0, 7.2)	3.72, dd (9.0, 7.2)	78.5	78.7
3′	3.63, t (9.0)	3.56, t (9.0)	79.5	77.9
4′	3.41, m	3.49, m	71.5	70.2
5′	3.38, m	3.20, m	78.0	76.7
6′	3.87, dd (12.0, 2.4)	3.77, dd (11.4, 2.4)	62.5	61.4
6′	3.68, m	3.67, dd (11.4, 4.8)		
Rhamnose moiety				
1″	5.39, d (1.8)	5.25, d (1.8)	102.0	101.3
2″	3.98, dd (3.6, 1.8)	4.02, dd (3.6, 1.8)	72.4	71.3
3″	3.70, m	3.81, dd (9.6, 3.6)	72.2	71.5
4″	3.40, m	3.42, t (9.6)	74.0	72.7
5″	4.16, dd (9.6, 6.6)	4.20, dd (9.6, 6.6)	70.0	68.6
6″	1.25, d (6.6)	1.14, d (6.6)	18.1	16.4

[a] Chemical shifts are shown in δ scale with J values (Hz) in parentheses.

Compound **22**, $[\alpha]_D^{20}$ −111.6 (*c* 0.1, MeOH), was obtained as a yellowish syrup. The molecular formula was determined to be $C_{23}H_{34}O_{12}$ by HRESIMS (*m*/*z* 525.1937, $[M+Na]^+$), which was 30 mass units larger than **21**. The ^1H and ^{13}C NMR spectra of **22** (Table 4) were very similar to those of **21**, except for the presence of an extra methoxyl signal [δ_H 3.86 (6H, s), δ_C 55.8, C-5] and a magnetically equivalent 2H-singlet (δ_H 6.54) instead of the ABX aromatic signals in **21**. The structure of **22**, including relative stereochemistry and identity of the aglycone moiety, was determined in a manner similar to that of **21**. The hydrolyzate of **22** obtained upon treatment with acid was identified as methoxyeugenol by comparison with the reported NMR data and direct HPLC comparison with a commercial authentic sample.[44] As a result, the new compound **22** was assigned to be methoxyeugenol 4-*O*-rhamnosyl(1→2)glucoside. Isolation of aromatic neohesperidosides from the genus *Santalum* might thus be of chemotaxonomical significance (Fig. 2).

2.3. Antitumor activity of the isolates from *S. album*

Owing to the immensely rare occurrence of this type of compound in the plant kingdom, the only α-santalol (**14**) has been assessed for biological activities in several bioassays.[13–16] The remarkable antitumor promoting effect of α-santalol (**14**)[17] prompted us to examine the antitumor effects of the purified constituents from *S. album* of Indian origin in vitro and in vivo. The inhibitory effects of the isolates on EBV-EA activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which is a short-term in vitro screening method frequently used to survey possible antitumor promoters in nature, were assessed. As shown in Table 5, compounds **1**, **4**, and **20** among the tested compounds showed a remarkable inhibitory effect on EBV-EA activation of 63.9, 62.3, and 61.8% inhibition, respectively, at a concentration of 500 mol ratio/TPA, preserving high cell viability. These potencies were comparable to that of the positive control, (−)-epigallocatechin gallate (EGCG), which is a well-known antitumor promoting polyphenol from green tea.[45,46] On the basis of the in vitro results, the potent inhibitors **1**, **18**, and **20**, which were well supplied for the in vivo test, were further assessed for the suppression of two-stage mouse skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator and TPA as a promoter. The activity was evaluated in terms of both the rate (%) of papilloma-bearing mice (Fig. 3A) and the average number papillomas per mouse (Fig. 3B) compared with that of the control. The control showed that 100% of the mice bore papillomas after 10 weeks of promotion, whereas treatment with compounds **1**, **18**, and **20** along with the initiator and promoter reduced the percentages of tumor-bearing mice to 33.4–46.7% even at 15 weeks. Among them, compound **1** had the most potent activity, reducing the incidence to 86.6% over 20 weeks. Furthermore, in the treated group, the average number of papillomas per mouse was also reduced to about 50% relative to the control group over 20 weeks. The results of this investigation indicated that compounds **1**, **18**, and **20** might be other potential antitumor promoters of sandalwood and valuable for further study for a possible antitumor promoting mechanism.

Table 5. Relative ratio[a] of EBV-EA activation with respect to positive control (100%) in the presence of isolates from *Santalum album*

Compound	EBV-EA–positive cells % to control (% viability): compounds concentration (mol ratio/32 pmol TPA)			
	1000	500	100	10
Sesquiterpenoids				
1	0.0±0.2 (60)[b]	36.1±1.9	71.3±1.9	88.4±0.5
2	0.0±0.3 (60)	41.5±2.0	74.9±2.2	92.6±0.7
4	0.0±0.2 (60)	37.7±1.9	72.0±2.0	89.9±0.6
14	0.0±0.4 (60)	47.7±2.3	76.8±2.5	93.0±0.9
16	0.0±0.3 (60)	40.8±2.1	73.7±2.2	91.3±0.6
18	0.0±0.3 (60)	44.3±2.1	74.2±2.1	92.1±0.9
20	0.0±0.2 (60)	38.2±2.0	72.7±2.1	90.5±0.6
(+)-α-Nuciferol[c]	0.0±0.4 (70)	49.2±2.3	78.5±2.2	96.8±0.4
Neolignans[c]				
(7*S*,8*S*)-$\Delta^{7'}$-4,5,9,9′-Tetrahydroxy-3,5-dimethoxy-7-*O*-5′,8′-*O*-4′-neolignan	0.0±0.4 (70)	48.0±2.2	77.4±2.1	94.5±0.7
Diethylene glycol monobenzoate	12.7±0.6 (60)	68.5±2.5	87.1±2.6	100±0.3
(−)-Secoisolariciresinol	2.1±0.4 (60)	52.3±2.1	79.6±2.0	96.4±0.4
(7′*S*,8*R*,8′*R*)-Lyoniresinol	8.4±0.5 (60)	55.7±2.4	85.3±2.6	100±0.2
(7*S*,8*S*)-3-Methoxy-3′,7-epoxy-8,4′-oxyneoligna-4,9,9′-triol	0.0±0.4 (70)	49.2±2.3	78.6±2.0	94.9±0.7
Dihydrodehydrodiconiferyl alcohol	3.5±0.4 (70)	53.7±2.2	82.8±2.1	100±0.4
(−)-EGCG[d]	6.4±0.8 (70)	34.9±1.3	68.1±2.1	87.7±0.9

[a] Values represent percentages relative to the positive control value (100%).
[b] Values in parentheses are the viability percentages of Raji cells; unless otherwise stated, the viability percentages of Raji cells were more than 80%.
[c] See Refs. 19 and 20.
[d] Positive control substance.

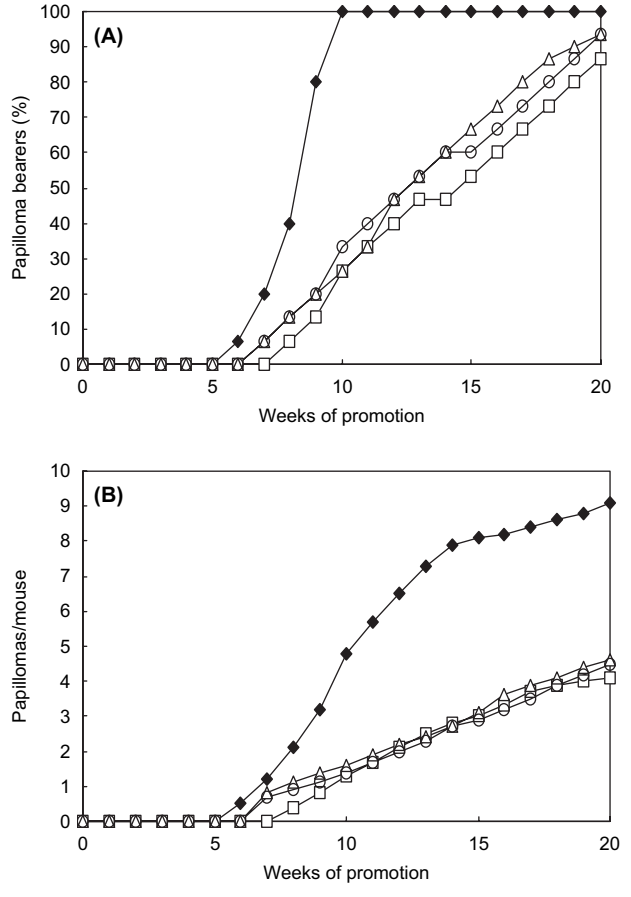


Figure 3. Inhibition of TPA-induced tumor promotion by multiple applications of **1**, **18**, and **20**. All mice were initiated with DMBA (390 nmol) and promoted with 1.7 nmol of TPA given twice weekly starting one week after initiation. (A) Percentage of mice bearing papillomas. (B) Average numbers of papillomas per mouse. ◆, control (TPA alone); □, TPA+**1**; ○, TPA+**18**; △, TPA+**20**.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-4 digital polarimeter. The ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity Inova AS600NB instrument operating at 600 and 150 MHz, respectively. The chemical shifts are given in δ (ppm) values relative to those of the solvents $CDCl_3$ (δ_H 7.26; δ_C 77.0) and CD_3OD (δ_H 3.35; δ_C 49.0) on a tetramethylsilane (TMS) scale. The standard pulse sequences programmed into the instruments were used for each 2D measurement. The J_{CH} value was set at 8 Hz in the HMBC spectra. HRESIMS and ESIMS were obtained on a Micro Mass Auto Spec OA-TOF spectrometer (solvent: 50% MeOH containing 0.1% $AcONH_4$; flow rate: 0.02 ml/min). Normal-phase HPLC was conducted on a YMC-Pack SIL A-003 column (4.6 mm i.d.×250 mm; YMC Co. Ltd) and developed at room temperature with a solvent of *n*-hexane–EtOH (15:1) (flow rate: 1.5 ml/min; detection: UV 205 or 220 nm) or *n*-hexane–EtOH (3:1) (flow rate: 1.5 ml/min; detection: UV 280 nm). Reversed-phase HPLC was carried out on a YMC-Pack ODS A-302 column (4.6 mm i.d.×150 mm; YMC Co., Ltd) and developed at 40 °C with 10 mM H_3PO_4/10 mM KH_2PO_4/CH_3CN (7:3) (flow rate: 1.0 ml/min; detection: UV 205 or 220 nm) or 10 mM H_3PO_4/10 mM KH_2PO_4/CH_3CN (9:1) (flow rate: 1.0 ml/min; detection: UV 280 nm). Column chromatography was carried out on silica gel 60 (Merck, 70–230 mesh), Toyopearl HW-40 (coarse grade; Tosoh Co.), YMC GEL ODS AQ 120-50S (YMC Co., Ltd), MCI GEL CHP-20P (Mitsubishi Kasei Co.), and Sephadex LH-20 (Pharmacia Fine Chemicals Co., Ltd). Preparative TLC was performed on Kieselgel 60 F_{254} plates (0.2 mm layer thickness, Merck).

3.2. Plant material

Chips of *S. album* L. wood collected in Mysore district of India were used. The wood was officially imported from

India under a special treaty between the Indian and Japanese governments to sculpt a Buddhist image in a Japanese temple (Kannonshoji Temple) with a long and distinguished history.

3.3. Extraction and isolation

The heartwood of *S. album* (1.53 kg) was extracted with MeOH at room temperature. The combined crude MeOH extract (73.1 g) was suspended in 20% MeOH (2 l), and then partitioned in turn with *n*-hexane (3×2 l) and EtOAc (3×2 l) to afford dried *n*-hexane- (16.4 g), EtOAc- (27.1 g), and H_2O-soluble (17.5 g) residues. On performing the chromatographic separation, fractions were monitored with normal and reversed-phase HPLC. The *n*-hexane extract (10.0 g) was subjected to silica gel column chromatography (6.0 cm i.d.×42 cm, 70–230 mesh) using *n*-hexane containing increasing amounts of EtOAc in a stepwise gradient to give 12 pools. The eluates of *n*-hexane–EtOAc (9:1) and *n*-hexane–EtOAc (85:15) were subjected to preparative reversed-phase HPLC (YMC-Pack ODS-AM, 20.0 mm i.d.×250 mm) with 70% aqueous CH_3CN, to give α-santalol (**14**) (105.3 mg), (*E*)-α-santalal (**15**) (20.0 mg), (*E*)-β-santalal (**19**) (23.3 mg), and β-santalol (**18**) (111.0 mg). Similarly, the *n*-hexane–EtOAc (1:1) and *n*-hexane–EtOAc (3:2) eluates were purified by preparative reversed-phase HPLC with 40% aqueous CH_3CN to afford pure α-santaldiol (**16**) (98.7 mg), α-santalenoic acid (**17**) (18.0 mg), and β-santaldiol (**20**) (91.4 mg), as well as a crude fraction containing compounds **2**, **5**, **10**, **11**, and **13**. This crude fraction was finally purified by preparative normal-phase HPLC (4.6 mm i.d.×250 mm) developed with *n*-hexane–EtOH (15:1), and yielded pure compounds **2** (23.5 mg, t_R 10.1 min), **5** (7.5 mg, t_R 9.3 min), **10** (3.5 mg, t_R 12.9 min), **11** (2.6 mg, t_R 9.5 min), and **13** (26.6 mg, t_R 4.68 min). A part (7.0 g) of the EtOAc extract was chromatographed on a Toyopearl HW-40 column (coarse grade; 2.2 cm i.d.×65 cm) with H_2O containing increasing amounts of MeOH in a stepwise gradient mode. The 40% MeOH eluate was subjected separately to column chromatography over a YMC GEL ODS AQ 120-50S column (1.1 cm i.d.×41 cm) with aqueous MeOH, and finally purified by preparative normal-phase HPLC (4.6 mm i.d.×250 mm) developed with *n*-hexane–EtOH (15:1), yielding pure compounds **1** (29.0 mg, t_R 27.7 min), **4** (25.3 mg, t_R 22.3 min), **10** (1.8 mg, t_R 33.5 min), **9** (7.4 mg, t_R 18.3 min), and **12** (9.4 mg, t_R 15.6 min). A part (3.0 g) of the H_2O soluble residue was chromatographed over a Diaion HP-20 column (3.2 cm i.d.×35 cm) with H_2O containing increasing amounts of MeOH in a stepwise gradient mode. The 60% MeOH eluate was subjected separately to column chromatography over Sephadex LH-20 (1.1 cm i.d.×38 cm) with MeOH and YMC GEL ODS AQ 120-50S column (1.1 cm i.d.×41 cm) with aqueous MeOH, and finally purified by preparative normal-phase HPLC (4.6 mm i.d.×250 mm) developed with *n*-hexane–EtOH (3:1) to yield pure compounds **21** (2.3 mg, t_R 33.7 min) and **22** (1.3 mg, t_R 27.1 min), and vanillic acid 4-*O*-neohesperidoside (1.7 mg, t_R 5.3 min).

3.3.1. (2*R*,7*R*)-2,12,13-Trihydroxy-10-campherene (1). Colorless oil; $[\alpha]_D^{20}$ −9.6 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 236 $[M-H_2O]^+$ (15), 218 (42), 200 (31), 185 (16), 161 (20), 145 (6), 121 (78), 95 (100); HREIMS *m/z* 236.1767 $[M-H_2O]^+$ (calcd for $C_{15}H_{26}O_3-H_2O$, 236.1776).

3.3.2. (2*R*,7*R*)-2,12-Dihydroxy-10(*Z*)-campherene (2). Colorless oil; $[\alpha]_D^{20}$ −17.6 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 238 $[M]^+$ (13), 220 (35), 202 (41), 187 (18), 159 (32), 145 (37), 121 (51), 91 (82), 58 (100); HREIMS *m/z* 238.1941 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.3. (2*S*,7*R*)-2,12,13-Trihydroxy-10-campherene (4). Colorless oil; $[\alpha]_D^{20}$ +15.7 (*c* 0.5, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 236 $[M-H_2O]^+$ (8), 218 (35), 200 (43), 185 (21), 161 (18), 145 (33), 121 (80), 91 (100); HREIMS *m/z* 236.1775 $[M-H_2O]^+$ (calcd for $C_{15}H_{26}O_3-H_2O$, 236.1776).

3.3.4. (2*S*,7*R*)-2,12-Dihydroxy-10(*Z*)-campherene (5). Colorless oil; $[\alpha]_D^{20}$ +17.9 (*c* 0.1, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 238 $[M]^+$ (10), 220 (17), 202 (21), 187 (13), 159 (18), 145 (17), 121 (35), 91 (53), 58 (100); HREIMS *m/z* 238.1923 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.5. (2*S*,7*S*)-2,12,13-Trihydroxy-10-campherene (7). Colorless oil; $[\alpha]_D^{20}$ −4.6 (*c* 0.5, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 236 $[M-H_2O]^+$ (13), 218 (61), 200 (53), 189 (38), 161 (32), 145 (42), 121 (87), 95 (100); HREIMS *m/z* 236.1771 $[M-H_2O]^+$ (calcd for $C_{15}H_{26}O_3-H_2O$, 236.1776).

3.3.6. (2*R*,3*R*)-13-Hydroxysandalnol (9). Colorless oil; $[\alpha]_D^{20}$ −4.8 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 236 $[M-H_2O]^+$ (8), 218 (21), 200 (37), 185 (20), 157 (43), 121 (72), 91 (78), 58 (100); HREIMS *m/z* 236.1773 $[M-H_2O]^+$ (calcd for $C_{15}H_{26}O_3-H_2O$, 236.1776).

3.3.7. (2*R*,3*R*)-10(*Z*)-Sandalnol (10). Colorless oil; $[\alpha]_D^{20}$ −7.3 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 238 $[M]^+$ (22), 220 (16), 202 (11), 187 (7), 159 (27), 145 (26), 138 (38), 121 (31), 110 (35), 91 (30), 81 (100); HREIMS *m/z* 238.1927 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.8. (2*R,3*R**)-10(*E*)-13-Sandalnol-13-al (11).** Colorless oil; $[\alpha]_D^{20}$ −5.1 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 236 $[M]^+$ (15), 218 (21), 200 (31), 155 (37), 121 (100), 91 (81); HREIMS *m/z* 236.1773 $[M]^+$ (calcd for $C_{15}H_{24}O_2$, 236.1776).

3.3.9. (2*S,3*R**)-13-Hydroxyneosandalnol (12).** Colorless oil; $[\alpha]_D^{20}$ −63.6 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* 236 $[M-H_2O]^+$ (15), 218 (21), 200 (31), 185 (18), 157 (37), 91 (100); HREIMS *m/z* 236.1768 $[M-H_2O]^+$ (calcd for $C_{15}H_{26}O_3-H_2O$, 236.1776).

3.3.10. (2*S*,3*R*)-10(*Z*)-Neosandalnol (13). Colorless oil; $[\alpha]_D^{20}$ −26.7 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR, see Tables 1 and 2; 238 $[M]^+$ (17), 220 (16), 202 (62), 187 (22), 159 (35), 145 (30), 121 (68), 91 (82), 58 (100); HREIMS *m/z* 238.1926 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.11. Eugenol 4-*O*-rhamnosyl(1→2)glucoside (21). Colorless syrup, $[\alpha]_D^{20}$ −118.0 (*c* 0.1, MeOH); ^1H and ^{13}C NMR data, see Table 4; ESIMS *m/z* 490 $[M+NH_4]^+$; HRESIMS *m/z* 490.2280 $[M+NH_4]^+$ (calcd for $C_{22}H_{32}O_{11}+NH_4$, 490.2288).

3.3.12. Methoxyeugenol 4-*O*-rhamnosyl(1→2)glucoside (22). Yellowish syrup, $[\alpha]_D^{20}$ −111.6 (*c* 0.1, MeOH); ^1H and ^{13}C NMR data, see Table 4; ESIMS *m/z* 525 $[M+Na]^+$; HRESIMS *m/z* 525.1937 $[M+Na]^+$ (calcd for $C_{23}H_{34}O_{12}+Na$, 520.1948).

3.4. Tritylation of 2

Compound **2** (5.0 mg) and triphenyl methyl chloride (10.0 mg) were dissolved in 200 μl of dried pyridine, and the mixture was allowed to stand for 48 h at room temperature. Preparative TLC of the crude tritylation product obtained after the usual workup afforded 4.0 mg of tritylated derivative (**2c**) as a colorless oil.

3.5. Preparation of (*S*)- and (*R*)-MTPA ester derivatives of 1, 2, 2c, 9, and 10

Two portions each (each 1–1.5 mg) of compounds **1**, **2**, **2c**, **9**, and **10** were treated with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (10 μl) in anhydrous pyridine (200 μl) at room temperature overnight. The reaction mixtures were purified by preparative TLC with *n*-hexane–acetone (4:1) as developing solvent to afford (*R*)- and (*S*)-MTPA ester derivatives (**1a**, **1b**, **2a**, **2b**, **2d**, **2e**, **9a**, **9b**, **10a**, and **10b**) of **1**, **2**, **2c**, **9**, and **10**. Calculation of the differences of chemical shifts allowed the assignment of absolute stereochemistry of the respective original compound (Table 3).

3.6. Acid hydrolysis of 21 and 22

A solution of **21** (1.0 mg) [or **22** (0.7 mg)] in 1 M HCl (1 ml) was heated for 1 h in a boiling water bath. After cooling, the reaction mixture was separated using Mega Bond Elut C_{18} (Varian, USA) cartridge column to yield aglycone, eugenol (0.5 mg) [or methoxyeugenol (0.3 mg)] from the MeOH eluate. These aglycones were identified by HPLC comparison with authentic samples and spectral data with those reported in the literature.[42–44]

3.7. Assay for inhibition of EBV-EA activation

The inhibition of EBV-EA activation was assayed using Raji cells (virus nonproducer) as described previously.[47,48] The EBV genome carrying lympoblastoid cells was derived from Burkitt's lymphoma, which was cultured in 10% fetal bovine serum (FBS) in RPMI-1640 medium (Nissui, Japan). Spontaneous EBV-EA activation in our Raji cell subline was less than 0.1%. Indicator cells (Raji, 1×10^6/ml) were incubated at 37 °C for 48 h in the medium (1 ml) containing *n*-butyric acid (4 mmol), TPA [20 ng (32 pmol) in DMSO 2 μl] as an inducer, and a known amount of test compound in 5 μl of DMSO. Smears were made from the cell suspension, and the activated cells stained by EBV-EA-positive serum were detected by a conventional indirect immunofluorescence technique.[46] In each assay, at least 500 cells were counted, and the number of stained cells was recorded. Triplicate assays were carried out for each compound. The average EBV-EA induction of the test compound was expressed as the relative ratio to the control experiment (100%), which was carried out with only *n*-butyric acid (4 mmol) plus TPA (32 pmol). EBV-EA induction was typically around 35%. The viability of treated Raji cells was assayed by Trypan Blue staining.

3.8. Assay for antitumor promoting activity in two-stage mouse skin carcinogenesis

Assays were performed according to a previously described method.[47,48] Specific pathogen-free female ICR mice (six weeks old) were obtained from Japan SLC Inc., Shizuoka, Japan. The animals were housed five per polycarbonate cage in a temperature-controlled room at 24±2 °C, and given water and food ad libitum throughout the experiment. The animals were divided into three groups of 15 mice each. The back of each mouse was shaved with surgical clippers one day before initiation, and the mice were topically treated with DMBA (100 μg, 390 nmol) in acetone (0.1 ml) for initiation. One week after initiation, papilloma formation was promoted by applying TPA (1 μg, 1.7 nmol) to the skin twice weekly. One hour before each TPA treatment, the mice were treated with the sample (85 nmol) in acetone (0.1 ml). The incidence of papilloma was examined weekly over 20 weeks.

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Synthesis of short and long chain cardiolipins

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Abstract—A phosphoramidite approach using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite was utilized for the first time to synthesize short chain cardiolipins. The approach was extended to synthesize long chain and their ether analogue. Optically active 1,2-di-*O*-acyl-*sn*-glycerol or 1,2-di-*O*-myristyl-*sn*-glycerol was coupled with phosphoramidite reagent and 2-benzyloxy-1,3-propanediol in presence of 1*H*-tetrazole, followed by in situ oxidation, to give the corresponding protected cardiolipin analogues. The above intermediates were converted into cardiolipin analogues in two steps by deprotection of cyanoethyl and benzyl groups.

1. Introduction

A need for synthetic phospholipids has developed, in part, their use in liposomes, which have become useful carriers of active therapeutic agents, enzymes, antibiotics, antigens, hormones, and anticancer drugs.[1] Cardiolipin (also known as diphosphatidyl glycerol; Fig. 1) constitutes a class of complex anionic phospholipids, typically purified from cell membranes of tissues associated with high metabolic activity, including the mitochondria of heart and skeletal muscles.[2] However, known chromatographic purification techniques cannot resolve cardiolipin into discrete molecular species. As a result, the use of this component in drug formulations has limited potential because the resulting formulations are not homogeneous. In animal tissues cardiolipin contains up to 90% linoleic acid ($C_{18:2}$). Yeast cardiolipin differs in having more oleic ($C_{18:1}$) and palmitoleic ($C_{16:1}$) fatty acids, while the bacterial lipid contains saturated and monoenoic fatty acids with 14–18 carbons. Liposomes containing cardiolipin encapsulate a broad spectrum of therapeutic agents ranging from difficult-to-formulate, water-insoluble drugs to delivery of molecules to intracellular targets. The encapsulation of chemotherapeutic agents[3,4] in a macromolecular carrier, such as liposomes, significantly reduces the volume of distribution in normal tissues and thereby increases the concentration of drug in the tumor. This results in a decrease in toxicities and an increase in therapeutic efficacy. The potential effects of the length and nature of cardiolipin fatty acid chains (i.e., saturated or unsaturated) on liposome aggregation have not been elucidated. However, cardiolipin having short chain fatty acids are unknown till now. Recognizing the need for the development of short chain cardiolipins to improve drug delivery, we undertook a program to design and synthesize a new class of short chain cardiolipins ($C_{8:0}$–$C_{12:0}$), long chain cardiolipin ($C_{14:0}$), and their ether analogue ($C_{14:0}$).

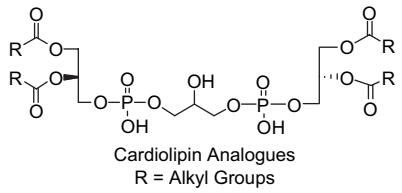

Figure 1. General structure of cardiolipin.

The known methodologies for synthesizing cardiolipin are mainly divided into two groups: (a) coupling the primary hydroxyl groups of a 2-protected glycerol with 1,2-*O*-diacyl-*sn*-glycerol using a phosphorylating agent and (b) condensation at both primary hydroxyl groups of a 2-protected glycerol with phosphatidic acid in the presence of 2,4,6-triisopropylbenzenesulfonylchloride.[5] Cardiolipin has also been generated via a reaction between the silver salt of diacylglycerophosphoric acid benzyl ester[6] with 1,3-diiodopropanol benzyl ether or 1,3-diiodopropanol *t*-butyl ether. Although the schemes were suitable for the preparation of small quantities of cardiolipin, these were unattractive for the routine preparation of larger quantities due to the many steps involved, the requirement for careful purification of intermediates, the use of highly photosensitive silver salt derivatives, and unstable iodo intermediates.

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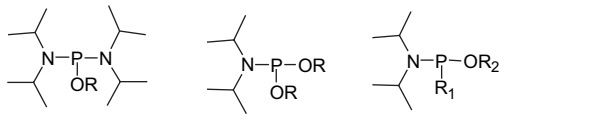


1. R = CH_3
2. R = CH_2Ph
3. R = C_2H_4CN
4. R = $C_4H_8NHCOCF_3$

5. R = CH_3
6. R = CH_2Ph
7. R = t-C_4H_9

8. R_1 = Cl, R_2 = CH_3
9. R_1 = Cl, R_2 = CH_2Ph
10. R_1 = Cl, R_2 = C_2H_4CN

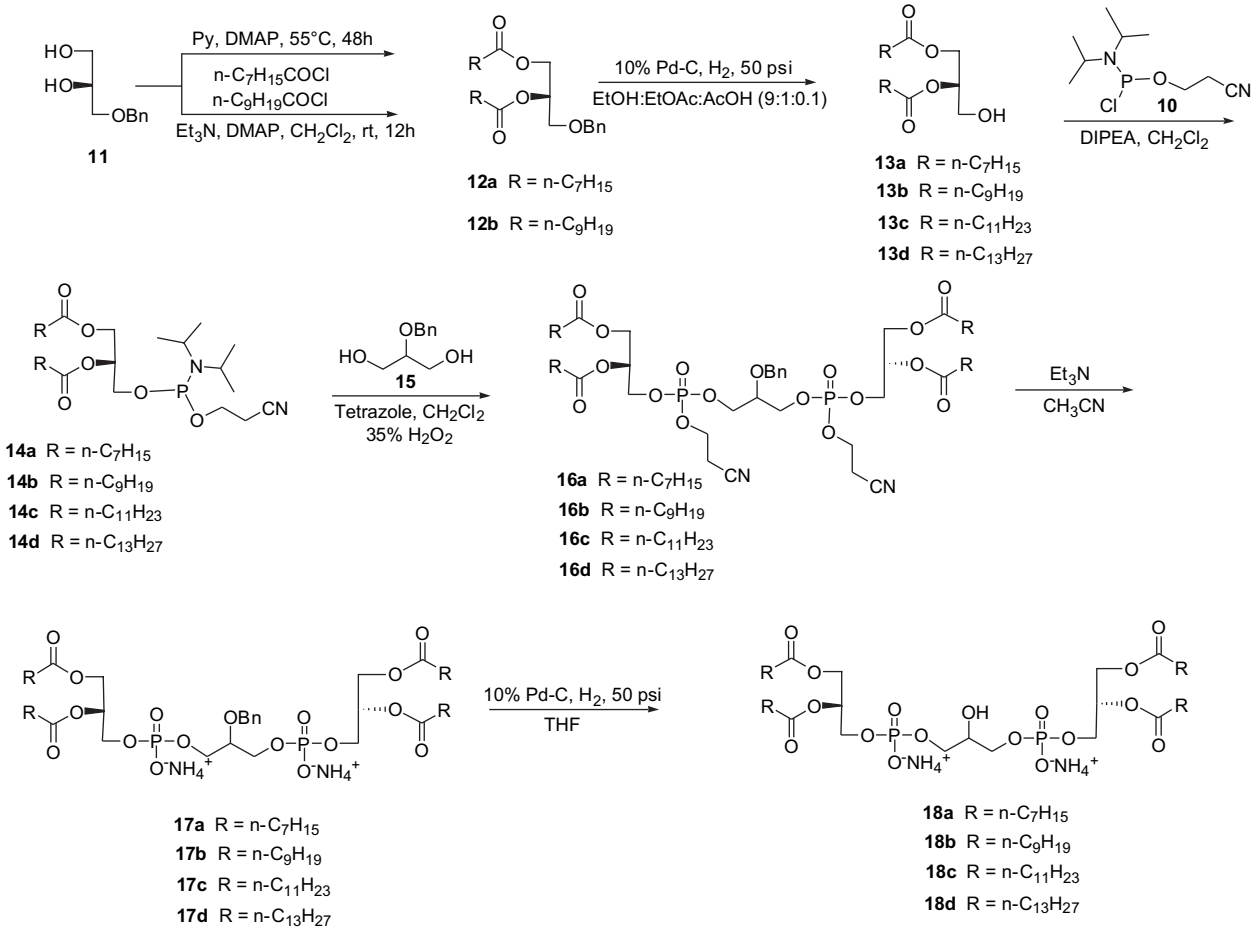
Figure 2. Phosphoramidite reagents.

Several phosphoramidite reagents (Fig. 2) such as methyl-*N*,*N*,*N*,*N*-tetraisopropyl phosphorodiamidite[7] **1**, benzyl-*N*,*N*,*N*,*N*-tetraisopropyl phosphorodiamidite[8] **2**, 2-cyanoethyl *N*,*N*,*N*,*N*-tetraisopropyl phosphorodiamidite[9] **3**, (*N*-trifluoroacetylamino)butyl-*N*,*N*,*N*,*N*-tetraisopropyl phosphoramidite[10] **4**, dimethyl-*N*,*N*-diisopropyl phosphoramidite[11] **5**, dibenzyl-*N*,*N*-diisopropyl phosphoramidite[12] **6**, di-*t*-butyl-*N*,*N*-diisopropyl phosphoramidite[13] **7**, methyl-*N*,*N*-diisopropylchlorophosphoramidite[14] **8**, benzyl-*N*,*N*-diisopropylchlorophosphoramidite[15] **9**, and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite[16] **10** have been used extensively in the synthesis of oligonucleotides,[16] phosphatidylinositols[15] [PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3], and to a lesser extent, in phospholipids' synthesis. Although the use of phosphate triesters and phosphoramidite esters in preparing phospholipids is known,[17] their usage in the synthesis of cardiolipin analogues having varying fatty acid chain lengths is not well established.

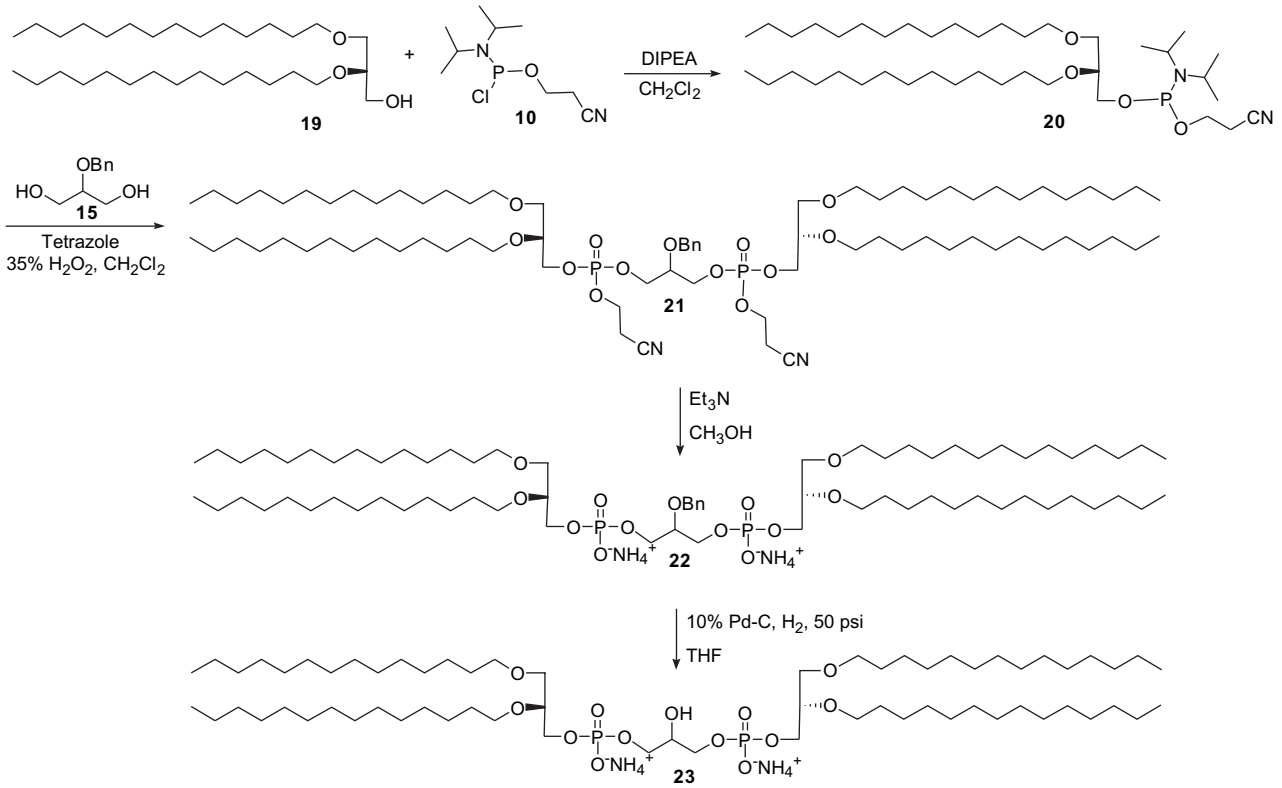
New synthetic methods are needed that can be used to prepare large quantities of cardiolipin analogue species having varying fatty acid chain length, particularly 'short chain cardiolipins'. Such methods would increase the availability of a wider variety of cardiolipin species and would diversify the lipids available for the development of new liposomal formulations containing active agents, which will have more defined compositions than those currently available. The short chain cardiolipin may also be useful for cosmetics and skin care products. As part of ongoing research, we developed new synthetic methods to synthesize cardiolipin and its analogues using the phosphoramidite approach[18,19a] as well as *O*-chlorophenyl dichlorophosphate.[19b] Herein, we report for the first time the use of novel phosphoramidite reagent, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** to prepare short chain cardiolipins, long chain cardiolipin, and cardiolipin ether analogues.

2. Synthesis

The synthesis of cardiolipin analogues **18a**, **18b**, **18c**, and **18d** are outlined in Scheme 1. The synthetic methodology that we have developed involves the application of



Scheme 1. Synthesis of cardiolipin-ester analogues.



Scheme 2. Synthesis of cardiolipin-ether analogues.

chlorophosphoramidite **10** as the phosphitylating agent to build a cardiolipin core structure through a phosphotriester approach. The method is attractive because phosphorus(III) reagents are generally more reactive than phosphorus(V) reagents. Commercially available (*R*)-(+)-3-*O*-benzyl-1,2-propanediol **11** was treated with octanoyl chloride in pyridine at 55 °C to obtain 1,2-di-*O*-octanoyl-3-*O*-benzyl-*sn*-glycerol **12a**. The glycerol **11** on esterification with decanoyl chloride and triethylamine in dichloromethane afforded 1,2-di-*O*-decanoyl-3-*O*-benzyl-*sn*-glycerol **12b**. Debenzylation of **12a** and **12b** via hydrogenation over 10% Pd–C catalyst in ethanol/ethyl acetate/acetic acid (9:1:0.1) gave the corresponding glycerols **13a** and **13b**, respectively. 1,2-Di-*O*-octanoyl-*sn*-glycerol **13a** and 1,2-di-*O*-decanoyl-*sn*-glycerol **13b** were reacted with the bifunctional phosphitylating reagent 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** in dichloromethane in the presence of diisopropylethylamine for 1 h to afford 1,2-diacyl-*sn*-glycerol-*N*,*N*-diisopropylaminophosphoramidite intermediates **14a** and **14b**, respectively. Similarly commercially available 1,2-di-*O*-lauroyl-*sn*-glycerol **13c** and 1,2-di-*O*-myristoyl-*sn*-glycerol **13d** were reacted with the bifunctional phosphitylating reagent **10**, in dichloromethane in the presence of diisopropylamine, for 1 h to afford 1,2-di-*O*-acyl-*sn*-glycerol-*N*,*N*-diisopropylaminophosphoramidite intermediates **14c** and **14d**, respectively. The crude products were used as such for the next reaction without any purification. The phosphoramidite intermediates **14a–d** on coupling with 2-benzyloxy-1,3-propanediol **15** in the presence of 1*H*-tetrazole gave phosphite triester intermediates, which was oxidized in situ with 35% H_2O_2 to afford protected cardiolipin analogues **16a–d**, respectively. Deprotection of the cyanoethyl group in the presence of triethylamine by β-elimination yields benzyl protected cardiolipin analogues **17a–d**. Finally, **17a–d** subjected to hydrogenolysis with Pd–C, H_2, at 50 psi in tetrahydrofuran at room temperature for 6 h furnished short chain cardiolipins **18a**, **18b**, **18c**, and long chain cardiolipin **18d**, respectively.

The synthesis of cardiolipin ether analogue **23** is outlined in Scheme 2. Commercially available (*R*)-(+)-3-*O*-benzyl-1,2-propanediol was converted in two steps into 1,2-di-*O*-myristyl-*sn*-glycerol[20] **19**. The bifunctional phosphitylating reagent 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** mixed with **19** in dichloromethane in the presence of diisopropylethylamine for 2 h afforded 1,2-bis-tetradecyloxy-*sn*-glycerol-*N*,*N*-diisopropylaminophosphoramidite intermediate **20**, which was used as such for the next reaction without any purification. The phosphoramidite intermediate **20**, on coupling with 2-benzyloxy-1,3-propanediol **15** in the presence of 1*H*-tetrazole gave phosphite triester intermediate, which was oxidized in situ with hydrogen peroxide to produce the desired protected cardiolipin ether analogue **21**. Deprotection of the cyanoethyl group of 2-*O*-benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn-glycero*-3)-phosphoryl] glycerol dicyanoethyl ester **21** followed by hydrogenolysis gave cardiolipin ether analogue **23**.

3. Conclusions

We used commercially available, low cost, and efficient phosphoramidite reagent, such as 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, to produce cardiolipin analogues in higher yield. The 2-cyanoethyl group provided three advantages.[17c] First, the high reactivity of the

2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite ensured that a bulky primary alcohol could form efficient new O–P bonds. Second, it has been known that the 2-cyanoethyl phosphite derivatives were more stable to chromatography steps than the corresponding benzyl phosphites.[15c] Third, not only could the 2-cyanoethyl group serve as the source of the 3-amino-propyl linker, but also it could be removed by β-elimination using a base to provide high yield of the phosphodiesters. The deprotection can be accomplished by mild basic conditions and catalytic hydrogenolysis. The routes are short and proceed in good overall yield.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a Varian Inova NMR spectrometer at 500 MHz. ^{1}H chemical shifts are reported in parts per million from internal tetramethylsilane. Mass spectral analyses [electron spray ionization (ESI)] were carried out on a Triple Quadruple LC/MS/MS mass spectrometer API 4000 (Applied Biosystems). Accurate mass measurements (HRMS–ESI) were done at University of Minnesota on a Bruker Daltonics BioTOF II spectrometer. Melting points were determined at atmospheric pressure and uncorrected. Infrared (IR) spectra were recorded on a Nicolet Nexus 470 FTIR. Samples were prepared by ATR method. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates (250 μm) and developed with the appropriate solvents. The TLC spots were visualized either by UV light or by heating the plates sprayed with a solution of phosphomolybdic acid (5% ethanolic solution). Flash column chromatography was carried out on silica gel (230–400 mesh). All chemicals and anhydrous solvents purchased from Aldrich Chemical Co. (Milwaukee, WI), except 1,2-di-*O*-lauroyl-*sn*-glycerol and 1,2-di-*O*-myristoyl-*sn*-glycerol (Genzyme Pharmaceutical, Cambridge, MA). All of the extracts were dried over anhydrous Na_2SO_4. Anhydrous dichloromethane, acetonitrile, ethanol, and tetrahydrofuran were used as such without further drying.

4.1.1. 1,2-Di-*O*-octanoyl-3-*O*-benzyl-*sn*-glycerol (12a). To an oven dried, 100 mL three-neck round bottom flask equipped with a stir bar, condenser, heating mantle, addition funnel, and a temperature probe under an argon atmosphere were added (*R*)-(+)-3-*O*-benzyl-1,2-propanediol **11** (4.5 g, 24.7 mmol) and anhydrous pyridine (45 mL). The solution was mixed moderately while octanoyl chloride (10.0 g, 61.5 mmol) was added dropwise via an addition funnel over 15 min maintaining the reaction temperature below 40 °C. To this reaction mixture was added 4-(dimethylamino) pyridine (0.320 g, 2.47 mmol) all at once. The reaction mixture was stirred vigorously for 48 h while refluxing at 55 °C. Then pyridine was removed under reduced pressure to afford a deep red oil. To this oil was added ethyl acetate (300 mL) and extracted with water (3×100 mL), 0.5 N HCl (3×100 mL), and brine solution (3×100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford 13.5 g of oil. The oil was purified as such using flash chromatography (SiO_2) and eluted (3–10% ethyl acetate/hexane) to afford **12a** (5.4 g, 50%) as a colorless oil. TLC (SiO_2) R_f=0.34 (hexane/ethyl acetate, 9:1). ^{1}H NMR δ ($CDCl_3$, 500 MHz) 0.87 (t, *J*=7.0 Hz, 6H), 1.22–1.34 (m, 16H), 1.52–1.66 (m, 4H), 2.22–2.34 (m, 4H), 3.58 (d, *J*=4.2 Hz, 2H), 4.18 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.34 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.51 (d, *J*=12.2 Hz, 1H), 4.57 (d, *J*=12.2 Hz, 1H), 5.21–5.28 (m, 1H), 7.28–7.36 (m, 5H, Ar–*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.2, 172.1, 137.2, 137.2, 128.5, 128.1, 128.1, 128.1, 128.0, 75.7, 75.8, 70.4, 67.4, 34.3, 34.1, 31.7, 31.6, 29.1, 29.0, 28.9, 28.9, 24.9, 22.6, 22.6, 14.0. HRMS (ESI) Calcd for $C_{26}H_{42}O_5$ ($M+Na^+$) 457.2924; found 457.2947.

4.1.2. 1,2-Di-*O*-decanoyl-3-*O*-benzyl-*sn*-glycerol (12b). To an oven dried, 250 mL three-neck round bottom flask equipped with rubber septum, argon inlet, and ice bath were added (*R*)-(+)-3-*O*-benzyl-1,2-propanediol **11** (7.38 g, 40.51 mmol), triethylamine (18.4 g, 182.39 mmol, 24.5 mL), and anhydrous CH_2Cl_2 (110 mL) with mixing. To the cooled solution was added dropwise decanoyl chloride (10.0 g, 91.15 mmol) over 15 min, followed by the addition of 4-(dimethylamino) pyridine (0.495 g, 4.05 mmol) with mixing. The reaction mixture was stirred at room temperature for 12 h, diluted with CH_2Cl_2 (200 mL), washed successively with water (3×100 mL), and (3×100 mL) brine, dried over anhydrous Na_2SO_4, and concentrated down to deep red oil. The oil was purified as such using flash chromatography (SiO_2) and eluted with step gradient from 3 to 10%, ethyl acetate/hexane to give **12b** (6.4 g, 32%) as an oil. TLC (SiO_2) R_f=0.34 (hexane/ethyl acetate, 9:1). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, *J*=6.8 Hz, 6H), 1.22–1.34 (m, 24H), 1.52–1.66 (m, 4H), 2.22–2.34 (m, 4H), 3.58 (d, *J*=4.2 Hz, 2H), 4.18 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.34 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.51 (d, *J*=12.2 Hz, 1H), 4.57 (d, *J*=12.2 Hz, 1H), 5.21–5.28 (m, 1H), 7.28–7.36 (m, 5H, Ar–*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9, 172.5, 137.2, 128.4, 128.3, 127.8, 127.7, 127.6, 75.9, 75.7, 71.1, 67.3, 33.1, 33.1, 35.5, 31.0, 30.0, 30.0, 29.7, 25.4, 23.1, 14.1. HRMS (ESI) Calcd for $C_{30}H_{50}O_5$ ($M+Na^+$) 513.3555; found 513.3571.

4.1.3. 1,2-Di-*O*-octanoyl-*sn*-glycerol (13a). To a solution of 1,2-dioctanoyl-3-*O*-benzyl-*sn*-glycerol **12a** (5.4 g, 12.42 mmol) in EtOH/EtOAc/AcOH (9:1:0.1, 24 mL) in a pressure vessel containing a stir bar was added palladium catalyst (0.7 g). The reaction mixture was stirred for 18 h at 40 psi of hydrogen before filtering the reaction over Celite to remove the catalyst. The solvent was removed under reduced pressure to afford an oil. The oil was purified as such using flash chromatography (SiO_2) and eluted with a step gradient of ethyl acetate/hexane (1:1 to 4:1). The solvents were removed and the resulting oil was kept under high vacuo for 4 h to afford **13a** (4.0 g, 94%) as pure oil. The compound was used as such for further reactions. TLC (SiO_2) R_f=0.51 (ethyl acetate/hexane, 1:1). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (*J*=7.0 Hz, 6H), 1.22–1.34 (m, 16H), 1.52–1.66 (m, 4H), 2.12 (t, *J*=6.2 Hz, 1H, –OH), 2.32 (t, *J*=7.6 Hz, 2H), 2.35 (t, *J*=7.6 Hz, 2H), 3.73 (t, *J*=6.0 Hz, 2H), 4.22 (dd, *J*=5.8 and 11.9 Hz, 1H), 4.33 (dd, *J*=5.8 and 11.9 Hz, 1H), 5.08 (quintet, *J*=5.1 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 173.3, 77.9, 67.2, 63.3, 34.2, 34.1, 31.7, 31.7, 29.1, 29.0, 28.9, 28.9, 24.9, 24.8, 22.6, 22.6, 14.0. HRMS (ESI) Calcd for $C_{19}H_{35}O_5$ ($M+Na^+$) 367.2455; found 367.2442.

4.1.4. 1,2-Di-*O*-decanoyl-*sn*-glycerol (13b). To a solution of 1,2-di-*O*-decanoyl-3-*O*-benzyl-*sn*-glycerol **12b** (6.4 g, 13.04 mmol) in EtOH/EtOAc/AcOH (9:1:0.1, 55 mL) was added palladium on carbon catalyst (1.92 g). The reaction mixture was stirred vigorously for 18 h under 40 psi of hydrogen. The catalyst was filtered over a Celite bed, and the solvent was removed to afford oil. The oil was purified as such using flash chromatography (SiO_2) with a step gradient of ethyl acetate/hexane (1:1 to 4:1), concentrated, and kept under high vacuo to yield **13b** (4.9 g, 94%) as a oil. TLC (SiO_2) R_f=0.39 (hexane/ethyl acetate, 3:2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, J=7.0 Hz, 6H), 1.22–1.34 (m, 24H), 1.52–1.66 (m, 4H), 2.03 (t, J=6.2 Hz, 1H, –OH), 2.32 (t, J=7.6 Hz, 2H), 2.35 (t, J=7.6 Hz, 2H), 3.73 (t, J=6.0 Hz, 2H), 4.22 (dd, J=5.8 and 11.9 Hz, 1H), 4.33 (dd, J=5.8 and 11.9 Hz, 1H), 5.08 (quintet, J=5.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.4, 172.3, 78.1, 67.0, 63.5, 33.9, 33.6, 32.5, 32.4, 30.3, 30.3, 30.1, 30.1, 30.0, 30.0, 29.8, 29.7, 25.4, 25.4, 23.4, 23.1, 14.0. HRMS (ESI) Calcd for $C_{23}H_{44}O_5$ (M+Na$^+$) 423.3081; found 423.3093.

4.1.5. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-octanoyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16a). To a solution of 1,2-di-*O*-octanoyl-*sn*-glycerol **13a** (4.0 g, 11.6 mmol) in anhydrous dichloromethane (30 mL) were added diisopropylethylamine (1.65 g, 12.76 mmol) and dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** (3.02 g, 12.76 mmol) under a steady stream of dry argon. The reaction mixture was stirred moderately at room temperature under an argon atmosphere for 1 h. To this stirring solution were added 1*H*-tetrazole (0.97 g, 13.91 mmol, 30.9 mL of 0.45 M solution in acetonitrile) and dropwise a solution of 2-benzyloxy-1,3-propanediol **15** (0.95 g, 5.23 mmol) in anhydrous dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 3 h and cooled to −40 °C while hydrogen peroxide (0.51 g, 15.11 mmol, 1.34 mL of 35 wt % H_2O_2) was added dropwise. After stirring the reaction at −40 °C for 15 min, the reaction was allowed to come to room temperature over 2 h, diluted with CH_2Cl_2 (200 mL), and washed with 10% sodium thiosulfate solution (50 mL). The organic layer was extracted with water (2×50 mL), brine (2×50 mL), then dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to yield 6.7 g as a colorless syrup. The residue was purified on a (SiO_2) column using a step gradient from 50% ethyl acetate/hexane to 100% ethyl acetate, which gave **16a** (5.6 g, 85%). TLC (SiO_2) R_f=0.35 (EtOAc/CH_2Cl_2, 1:3, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J=7.0 Hz, 12H, –CH_3), 1.26–1.29 (m, 32H, –CH_2), 1.58–1.62 (m, 8H), 2.28–2.34 (m, 8H), 2.66–2.73 (m, 4H), 3.85–3.88 (m, 1H), 4.07–4.32 (m, 16H), 4.67 (s, 2H), 5.22–5.25 (m, 2H), 7.35–7.36 (m, 5H, Ar–H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.8, 137.2, 137.1, 128.5, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 116.3, 77.2, 77.0, 76.7, 75.4, 75.4, 75.3, 72.3, 69.2, 69.2, 66.0, 65.9, 65.9, 62.2, 62.1, 61.5, 34.1, 33.9, 31.6, 29.0, 28.9, 28.8, 24.7, 22.5, 19.5, 19.4, 14.0. HRMS (ESI) Calcd for $C_{54}H_{90}N_2O_{17}P_2$ (M+Na$^+$) 1123.5607; found 1123.5610.

4.1.6. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-decanoyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16b). Compound **16b** was synthesized from compound **13b** in an 86% yield as a colorless syrup following the procedure used for the synthesis of **16a**. TLC (SiO_2) R_f=0.38 (EtOAc/CH_2Cl_2, 1:3). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.26–1.28 (m, J=10.5 Hz, 48H), 1.58–1.61 (m, J=6.0 Hz, 8H), 2.28–2.34 (m, J=2.0 Hz, 8H), 2.70–2.72 (m, J=6.0 Hz, 4H), 3.83–3.88 (m, 1H), 4.13–4.31 (m, 16H), 4.67–4.68 (s, 2H), 5.22–5.24 (m, 2H), 7.35–7.37 (m, 5H, Ar–*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.8, 172.7, 137.2, 137.2, 128.5, 128.1, 128.0, 127.9, 116.3, 77.2, 77.0, 76.7, 75.4, 75.3, 72.3, 69.2, 69.1, 66.0, 66.0, 65.9, 65.8, 62.2, 62.2, 62.1, 61.5, 34.1, 33.9, 31.8, 29.4, 29.2, 29.2, 29.1, 29.0, 24.8, 22.6, 19.6, 19.5, 19.4, 14.0. HRMS (ESI) Calcd for $C_{62}H_{106}N_2O_{17}P_2$ (M+Na$^+$) 1235.6879; found 1235.6893.

4.1.7. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-lauroyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16c). Compound **16c** was synthesized from **13c** in an 87% yield as a colorless syrup by following the procedure used for the synthesis of **16a**. TLC (SiO_2) R_f=0.48 (EtOAc/CH_2Cl_2, 1:3). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J=7.0 Hz, 12H), 1.25–1.28 (m, 64H), 1.58–1.60 (m, 8H), 2.28–2.34 (m, 8H), 2.66–2.72 (m, 4H), 3.83–3.88 (m, 1H), 4.05–4.34 (m, 16H), 4.66–4.67 (s, 2H), 5.22–5.24 (m, 2H), 7.35–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.8, 137.2, 130.8, 128.8, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.9, 127.7, 116.3, 77.2, 77.0, 76.7, 75.4, 75.3, 70.6, 70.5, 69.2, 68.1, 66.0, 65.9, 62.2, 62.2, 62.1, 61.6, 61.5, 38.7, 34.1, 33.9, 31.9, 30.3, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 29.1, 28.9, 24.8, 23.7, 22.9, 22.6, 19.6, 19.5, 14.1, 14.0. HRMS (ESI) Calcd for $C_{64}H_{122}N_2O_{17}P_2$ (M+Na$^+$) 1241.7585; found 1241.7566.

4.1.8. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-myristoyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16d). Compound **16d** was synthesized from compound **13d** in an 87% yield as a colorless oil following the procedure used for the synthesis of **16a**. TLC (SiO_2) R_f=0.26 (EtOAc/CH_2Cl_2, 1:3). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25 (m, 80H), 1.58–1.59 (m, 8H), 2.27–2.33 (m, 8H), 2.64–2.75 (m, 4H), 3.90 (m, 1H), 4.13–4.17 (m, 16H), 4.67 (s, 2H), 5.19–5.28 (m, 2H), 7.28–7.38 (m, 5H, Ar–H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 173.2, 138.0, 128.4, 127.7, 127.6, 77.2, 77.0, 76.7, 70.3, 70.3, 66.7, 63.6, 62.7, 34.3, 34.1, 31.9, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.1, 29.1, 24.9, 24.9, 22.7, 14.0. HRMS (ESI) Calcd for $C_{78}H_{138}N_2O_{17}P_2$ (M+Na$^+$) 1459.9363; found 1459.9394.

4.1.9. 1,3-Bis[(1,2-di-*O*-octanoyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17a). A solution of the protected cardiolipin analogue **16a** (1.95 g, 1.77 mmol), Et_3N (1.36 g, 13.48 mmol), and water (0.1 mL) in acetonitrile (10 mL) was stirred overnight. The reaction mixture was evaporated to dryness. The residue was converted into ammonium salt by adding 2 mL NH_4OH and purified on SiO_2 column (15% MeOH in CH_2Cl_2 containing 1% NH_4OH) to give **17a** (606 mg, 65%) as a colorless syrup that slowly solidified. TLC (SiO_2) R_f~0.46 ($CHCl_3$/MeOH/NH_4OH, 6.5:2.5:0.5). ^{1}H NMR δ ($CDCl_3$, 500 MHz) 0.88 (t, J=7.0 Hz, 12H), 1.22–1.39 (m, 32H), 1.56–1.63 (m, 8H), 2.22–2.34 (m, 8H), 3.66–3.76 (m, 1H), 3.82–4.06 (m, 8H), 4.08–4.18 (m, 2H), 4.26–4.37 (m, 2H), 4.60 (s,

2H), 5.14–5.26 (m, 2H), 7.22–7.36 (m, 5H), 7.49 (br, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.8, 172.7, 128.4, 127.7, 127.6, 77.2, 77.0, 76.7, 71.4, 70.4, 63.7, 62.7, 45.8, 34.3, 34.1, 31.7, 29.1, 29.0, 28.9, 24.9, 24.8, 22.6, 14.0. HRMS (ESI) Calcd for $C_{48}H_{90}N_2O_{17}P_2$ $(M+2NH_4^+)^{2-}$ 496.2519; found 496.2519.

4.1.10. 1,3-Bis[(1,2-di-*O*-decanoyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17b). Compound **17b** was synthesized from **16b** in an 88% yield as a colorless syrup following the procedure used for the synthesis of **17a**. TLC (SiO_2) R_f=0.47 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25–1.28 (m, J=16 Hz, 48H), 1.56 (m, 8H), 2.24–2.30 (m, J=3.0 Hz, 8H), 3.72 (m, 1H), 3.89–4.00 (m, J=6.5 Hz, 8H), 4.10–4.14 (m, J=4.0 Hz, 2H), 4.32–4.34 (m, 2H), 4.61 (s, 2H), 5.19 (m, 2H), 7.24–7.33 (m, 5H, Ar–H), 7.55 (br, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 138.1, 128.3, 127.6, 77.2, 77.0, 76.7, 71.5, 70.4, 70.3, 63.5, 63.4, 62.7, 45.7, 34.2, 34.0, 31.8, 29.5, 29.4, 29.3, 29.1, 24.9, 24.8, 22.6, 14.0. HRMS (ESI) Calcd for $C_{56}H_{106}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 552.3145; found 552.3167.

4.1.11. 1,3-Bis[(1,2-di-*O*-lauroyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17c). Compound **17c** was synthesized from **16c** in an 89% yield as a colorless syrup by following the procedure used for the synthesis of **17a**. TLC (SiO_2) R_f=0.34 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25–1.30 (m, J=7.5 Hz, 64H), 1.56 (m, 8H), 2.24–2.29 (m, J=4.0 Hz, 8H), 3.70–3.72 (m, 1H), 3.89–3.91 (m, 8H), 4.09–4.14 (m, 2H), 4.32–4.34 (m, 2H), 4.61 (s, 2H), 5.17–5.20 (m, 2H), 7.23–7.33 (m, 5H, Ar–H), 7.25 (br s, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 138.0, 128.3, 127.7, 127.6, 77.2, 77.0, 76.7, 76.2, 76.1, 71.5, 70.4, 70.3, 63.6, 63.5, 62.7, 34.3, 35.0, 31.9, 29.7, 29.6, 29.5, 29.3, 29.3, 29.2, 29.1, 24.9, 24.8, 22.6, 14.0. HRMS (ESI) Calcd for $C_{64}H_{122}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 609.3821; found 609.3831.

4.1.12. 1,3-Bis[(1,2-di-*O*-myristoyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17d). Compound **17d** was synthesized from compound **16d** in an 85% yield as a colorless oil following the procedure used for the synthesis of **17a**. TLC (SiO_2) R_f=0.54 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25–1.31 (m, 80H), 1.56 (m, 8H), 2.23–2.30 (m, 8H), 3.71 (m, 1H), 3.90–3.98 (m, 8H), 4.10–4.14 (m, 2H), 4.31–4.34 (m, 2H), 4.60 (s, 2H), 5.18–5.20 (m, 2H), 7.24–7.32 (m, 5H, Ar–H), 7.51 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 138.0, 128.4, 127.7, 127.6, 77.2, 77.0, 76.7, 71.5, 70.4, 70.3, 63.5, 62.7, 34.3, 34.0, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 29.3, 29.2, 29.1, 24.0, 24.8, 22.7, 22.6, 14.0. HRMS (ESI) Calcd for $C_{72}H_{138}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 665.4447; found 665.4443.

4.1.13. 1,3-Bis[(1,2-di-*O*-octanoyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18a, tetraoctanoyl cardiolipin diammonium salt). A solution of benzyl protected cardiolipin analogue **17a** (1.33 g, 1.29 mmol) in tetrahydrofuran (15 mL) was hydrogenated at 50 psi over 10% Pd–C (665 mg) for 10 h. The catalyst was filtered off over Celite bed and concentrated. The residue was dissolved in $CHCl_3$, filtered through a 0.25 μ filter, and precipitated with acetone to give tetraoctanoyl ($C_{8:0}$) cardiolipin **18a** (0.99 g, 85%) as a white semi solid. TLC (SiO_2) R_f~0.43 ($CHCl_3$/MeOH/NH_4OH, 6.5:2.5:0.5). ^{1}H NMR δ ($CDCl_3$, 500 MHz) 0.88 (t, J=7.0 Hz, 12H), 1.22–1.34 (br s, 32H), 1.52–1.64 (m, 8H), 2.26–2.34 (m, 8H), 3.82–3.98 (m, 9H), 4.12–4.18 (m, 2H), 4.35–4.42 (m, 2H), 5.14–5.24 (m, 2H), 7.41 (br, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.3, 77.2, 77.0, 76.7, 62.6, 34.2, 34.1, 31.7, 31.6, 29.1, 29.0, 28.9, 24.8, 22.6, 22.5, 14.0. FTIR (ATR) 3250, 3015, 2945, 2922, 2840, 1746, 1473, 1371, 1198, 1082, 1069 cm^{-1}. HRMS (ESI) Calcd for $C_{41}H_{84}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 451.2324; found 451.2266.

4.1.14. 1,3-Bis[(1,2-di-*O*-decanoyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18b, tetradecanoyl cardiolipin diammonium salt). Compound **18b** was synthesized from **17b** in a 97% yield as a white solid by following the procedure used for the synthesis of **18a**. Mp 177–179 °C. TLC (SiO_2) R_f=0.38 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J=7.0 Hz, 12H), 1.26 (br s, 48H), 1.58–1.59 (m, J=6.0 Hz, 8H), 2.27–2.33 (m, J=7.0 Hz, 8H), 3.62 (br, 1H), 3.90–3.91 (m, J=4.5 Hz, 9H), 4.13–4.17 (m, J=7.0 Hz, 2H), 4.35–4.37 (m, J=10 Hz, 2H), 5.19–5.20 (m, J=2.0 Hz, 2H), 7.44 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.3, 77.2, 77.0, 76.7, 62.6, 34.3, 34.1, 31.9, 31.8, 29.5, 29.3, 29.2, 29.1, 24.2, 24.8, 22.6, 14.0. FTIR (ATR) 3228, 3022, 2946, 2914, 2850, 1744, 1466, 1387, 1212, 1095, 1070 cm^{-1}. HRMS (ESI) Calcd for $C_{49}H_{100}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 507.2962; found 507.2953.

4.1.15. Synthesis of 1,3-bis[(1,2-di-*O*-lauroyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18c, tetralauroyl cardiolipin diammonium salt). Compound **18c** was synthesized from **17c** in a 98% yield as a white solid by following the procedure used for the synthesis of **18a**. Mp 156–158 °C. TLC (SiO_2) R_f=0.31 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.26 (m, 64H), 1.58–1.59 (m, 8H), 2.27–2.33 (m, 8H), 3.90–3.92 (m, 9H), 4.13–4.17 (m, 2H), 4.36–4.38 (m, 2H), 5.20–5.21 (m, 2H), 7.41 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.3, 167.7, 132.4, 130.8, 128.8, 70.3, 70.3, 69.5, 68.1, 66.6, 63.6, 63.5, 62.7, 38.7, 34.3, 34.1, 31.9, 30.3, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 24.9, 24.8, 23.7, 22.9, 22.6, 14.0. FTIR (ATR) 3207, 3035, 2956, 2918, 2850, 1737, 1467, 1378, 1206, 1092, 1067 cm^{-1}. HRMS (ESI) Calcd for $C_{57}H_{116}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 563.3553; found 563.3540.

4.1.16. Synthesis of 1,3-bis[(1,2-di-*O*-myristoyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18d, tetramyristoyl cardiolipin diammonium salt). Compound **18d** was synthesized from compound **17d** in a 98% yield as a white solid following the procedure used for the synthesis of **18a**. Mp 181–182 °C. TLC (SiO_2) R_f=0.29 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H, –CH_3), 1.25 (br, 80H, –CH_2–), 1.58–1.59 (m, 8H), 2.27–2.33 (m, 8H), 3.06 (br, 1H), 3.90 (m, 9H), 4.13–4.17 (m, 2H), 4.36–4.38 (m, 2H), 5.20–5.21 (m, 2H), 7.41 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$,

125 MHz) δ 173.6, 173.3, 77.2, 77.0, 76.7, 70.34, 70.3, 66.7, 63.6, 62.7, 34.3, 34.1, 31.9, 29.7, 29.6, 29.4, 29.2, 29.1, 24.9, 24.8, 22.7, 14.0. FTIR (ATR) 3207, 3035, 2956, 2918, 1737, 1461, 1378, 1206, 1092, 1067 cm^{-1}. HRMS (ESI) Calcd for $C_{65}H_{132}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 619.4199; found 619.4173.

4.1.17. Synthesis of 2-*O*-benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn*-*glycero*-3)-phosphoryl] glycerol dicyanoethyl ester (21). To a 250-mL three-neck round bottom flask equipped with stir bar, rubber septum, and argon inlet was added 1,2-di-*O*-myristyl-*sn*-glycerol[19] **19** (9.2 g, 19.01 mmol) in anhydrous dichloromethane (150 mL). To the mixture was added diisopropylethylamine (5.46 g, 42.26 mmol) and stirred for 10 min. To this mixture was added dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** (5.0 g, 21.13 mmol), and the reaction mixture was stirred moderately at room temperature. The reaction mixture was stirred for 2 h before adding 1*H*-tetrazole (1.59 g, 22.77 mmol of 0.45 M solution in acetonitrile) and dropwise a solution of 2-benzyloxy-1,3-propanediol **15** (1.55 g, 8.54 mmol) in anhydrous dichloromethane (20 mL). The reaction mixture was stirred for 3 h, and then cooled to −20 °C while 35 wt % H_2O_2 (0.42 g, 12.38 mmol, 1.09 mL) was added dropwise. After stirring the reaction mixture at −20 °C for 15 min, the reaction was allowed to come to room temperature over 2 h, diluted with CH_2Cl_2 (200 mL), and washed with 10% sodium thiosulfate solution (50 mL). The organic layer was extracted with water (2×50 mL), brine (2× 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to afford a colorless syrup. The residue was purified as such by flash chromatography on a (SiO_2) column using a step gradient from 50 to 70% ethyl acetate/petroleum ether to yield **21** (7.1 g, 61%). TLC (SiO_2) R_f=0.27 (ethyl acetate/hexane, 3:1). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, *J*=7.0 Hz, 12H), 1.25–1.31 (br, *J*=7.5, 4.5, and 9.5 Hz, 80H), 1.53–1.54 (m, 8H), 2.23–2.27 (m, 8H), 2.70–2.71 (m, 4H), 3.01 (m, 1H), 4.05–4.11 (m, 4H), 4.16–4.23 (m, 4H), 4.24–4.30 (m, 4H), 4.61–4.63 (d, 2H), 4.67–4.69 (m, 2H), 7.34–7.36 (m, 5H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 128.5, 128.4, 128.0, 127.9, 127.8, 77.5, 77.2, 77.0, 76.7, 72.1, 71.8, 70.7, 70.6, 69.3, 69.3, 67.7, 61.9, 61.8, 60.8, 31.9, 30.0, 29.9, 29.6, 29.5, 29.4, 29.3, 26.0, 22.6, 19.5, 19.45, 14.0. HRMS (ESI) Calcd for $C_{78}H_{146}N_2O_{13}P_2$ $(M+Na^+)$ 1404.0197; found 1404.0234.

4.1.18. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn*-glycerol-3)-phosphoryl] glycerol diammonium salt (22). To a (250 mL) round bottom flask were added 2-*O*-benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn*-*glycero*-3)-phosphoryl] glycerol dicyanoethyl ester **21** (3.48 g, 2.51 mmol) and anhydrous acetonitrile (50 mL). The solution was mixed vigorously while triethylamine (2.18 g, 21.52 mmol, 3.0 mL) was added. The reaction mixture was stirred vigorously for 24 h at room temperature. The solvents were removed under reduced pressure, and the crude oil was kept under high vacuo for 4 h. The residue was purified by flash chromatography on a (SiO_2) column using a step gradient of ($CHCl_3$/MeOH/NH_4OH, 100:15:1, v/v) and ($CHCl_3$/MeOH/NH_4OH, 65:15:1, v/v) to yield **22** (2.8 g, 88%). TLC (SiO_2) R_f=0.43 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, *J*=7.0 Hz, 12H), 1.25 (br, 80H), 1.52 (m, 8H), 3.00–3.02 (d, 1H), 3.39–3.44 (m, 4H), 3.49–3.51 (d, 4H), 3.56 (m, 4H), 3.83 (m, 6H), 3.98 (br, 4H), 4.60 (s, 2H), 7.28–7.31 (m, 5H, Ar–H), 7.56 (br s, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 128.3, 127.6, 77.2, 77.0, 76.7, 71.8, 71.4, 70.6, 31.9, 30.10, 29.8, 29.7, 29.7, 29.6, 29.4, 26.1, 22.7, 14.1. HRMS (ESI) Calcd for $C_{72}H_{146}N_2O_{13}P_2$ $(M-2NH4+)^{2-}$ 637.4901; found 637.4901.

4.1.19. 1,3-Bis[(1,2-di-*O*-myristyl-*sn*-glycerol-3)-phosphoryl] glycerol diammonium salt (23, tetradecyloxy cardiolipin diammonium salt). To a solution of 2-*O*-benzyl-1,3-bis-[(1,2-di-*O*-myristyl-*sn*-*glycero*-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt **22** (1.2 g, 0.91 mmol) in tetrahydrofuran (30 mL) in a pressure vessel, was added palladium on carbon catalyst (600 mg). The reaction mixture was stirred under 50 psi of hydrogen at room temperature for 6 h. The palladium catalyst was filtered over Celite, washed with chloroform/methanol (1:1, 100 mL), and concentrated under reduced pressure to afford white solids. The product was purified on a flash chromatography (SiO_2) column using a step gradient of ($CHCl_3$/MeOH/NH_4OH, 100:15:1, v/v) and ($CHCl_3$/MeOH/NH_4OH, 65:15:1, v/v) to yield **23** (1.1 g, 98%). Mp 174–176 °C. TLC (SiO_2) R_f=0.34 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, *J*=7.0 Hz, 12H), 1.25–1.27 (br, 80H), 1.31 (br, 8H), 1.54–1.55 (m, 8H), 3.42–3.43 (m, 4H), 3.53–3.54 (m, 4H), 3.57–3.58 (m, 4H), 3.85 (br, 4H), 7.43 (br s, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 77.2, 77.0, 76.7, 71.8, 31.9, 30.0, 29.8, 29.8, 29.7, 29.6, 29.4, 26.2, 26.1, 22.7, 14.1. HRMS (ESI) Calcd for $C_{65}H_{140}N_2O_{13}P_2$ $(M-2NH_4^+)^{2-}$ 591.4613; found 591.4597.

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New diterpenes of the pseudopterane class from two closely related *Pseudopterogorgia* species: isolation, structural elucidation, and biological evaluation

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Abstract—Parallel chemical investigations of the hexane and chloroform extracts of the sea plumes *Pseudopterogorgia bipinnata* and *Pseudopterogorgia kallos* has led to the discovery of seven new diterpenoids of the pseudopterane class, namely, kallolide D (**2**), kallolide C acetate (**3**), kallolide E (**4**), kallolide F (**5**), kallolide G (**6**), kallolide H (**7**), and kallolide I (**8**), in addition to nine previously described compounds of the pseudopterane and gersolane families of diterpenes. The chemical structures of the new metabolites were established by 1D and 2D NMR, IR, UV, HRMS, and, in some instances, by single-crystal X-ray crystallographic analyses. Biological screening of these metabolites revealed significant anti-parasitic activity albeit marginal anti-tubercular activity.

1. Introduction

When extracted exhaustively with organic solvent mixtures such as $CHCl_3$–MeOH or CH_2Cl_2–MeOH, the majority of the Caribbean gorgonian species belonging to the genus *Pseudopterogorgia* ooze diterpenoids of very unusual structure types.[1] *Pseudopterogorgia* species are among the most common of the Caribbean species with more than 15 species having been taxonomically described.[2] As part of our investigations of new anti-infective agents derived from marine sources, we now report the isolation and structure characterization of seven new related pseudopteranes from two closely related *Pseudopterogorgia* species, namely, *P. bipinnata*[3] and *P. kallos*.[4] Based on biogenetic grounds, the new metabolites **2**, **3**, **5**, **6**, **7**, and **8** could arise from regioselective oxidation of known kallolide A (**1**), the major compound isolated during this investigation.[4] On the other hand, novel metabolite **4** must arise through a distinct biogenetic pathway.[5] Crude extracts of *P. bipinnata* and *P. kallos* collected near Providencia (Old Providence) Island, Colombia in March of 2002 exhibited in vitro inhibition of *Plasmodium falciparum* and *Mycobacterium tuberculosis*. Bioassay-guided purification led to the isolation of 16 compounds, the structures of which were determined through interpretation of the NMR, IR, UV, mass spectral, and X-ray crystallographic data. Seven compounds were found to be novel, which we have named kallolide D (**2**), kallolide C acetate (**3**), kallolide E (**4**), kallolide F (**5**), kallolide G (**6**), kallolide H (**7**), and kallolide I (**8**);[6] and nine were identified as previously known metabolites kallolide A (**1**),[4] kallolide A acetate (**9**),[4] 2-*O*-ethylkallolide A (**10**),[7] gersemolide (**11**),[8] kallolide C (**12**),[4] bipinnapterolide A (**13**),[9] pinnatin B (**14**),[10] gersolide (**15**),[11] and pinnatin D (**16**).[10] The hexane extracts, prepared from 0.11 kg (dry weight) of *P. bipinnata* and 1.07 kg (dry weight) of *P. kallos*, were each chromatographed on a Bio-Beads SX-3 stationary phase using toluene as eluent. The $CHCl_3$ extracts, on the other hand, were chromatographed either isocratically, under flash column chromatography conditions on a silica gel stationary phase with 1% MeOH in $CHCl_3$ (*P. bipinnata*), or using a step gradient of hexane–EtOAc (*P. kallos*) as eluent. Active fractions were purified further by successive silica gel column chromatography and HPLC to yield pure compounds **1**–**16**. While almost all of these metabolites exhibited marginal anti-tubercular activity, a significant number of them displayed moderate to strong anti-parasitic properties.

Initial inspection of the ^1H and ^{13}C NMR spectra of **2**–**8** (Tables 1 and 2), suggested that the molecular structures

Keywords: Pseudopterane diterpenes; *Pseudopterogorgia kallos*; *Pseudopterogorgia bipinnata*; Malaria; Tuberculosis; X-ray crystal structure analysis.
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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **2–5**[a]

Atom	Kallolide D (**2**)		Kallolide C acetate (**3**)		Kallolide E (**4**)		Kallolide F (**5**)	
	δ_H, mult, J in Hz	δ_C, mult	δ_H, mult, J in Hz	δ_C, mult	δ_H, mult, J in Hz	δ_C, mult	δ_H, mult, J in Hz	δ_C, mult
1	2.61, ddd, 10.5, 6.5, 1.3	53.5 (CH)	2.64, ddd, 11.9, 7.2, 5.0	44.1 (CH)	2.26, dt, 11.1, 3.3	50.2 (CH)	2.75, br d, 7.3	45.2 (CH)
2	4.02, d, 10.5	72.1 (CH)	5.38, d, 11.8	68.2 (CH)	4.36, d, 11.1	67.1 (CH)	4.25, d, 2.6	81.3 (CH)
3		184.0 (C)		180.9 (C)		177.8 (C)		212.3 (C)
4		112.4 (C)		111.0 (C)		113.4 (C)	2.55, q, 7.0	50.2 (CH)
5		200.4 (C)		99.9 (C)		200.4 (C)		101.3 (C)
6		100.2 (C)		201.6 (C)		99.5 (C)		199.7 (C)
7	3.51, d, 4.2	53.1 (CH)	3.41, d, 5.5	51.0 (CH)	3.32, br s	55.7 (CH)		129.0 (C)
8	4.94, br d, 3.2	78.9 (CH)	5.08, dd, 5.5, 1.3	78.9 (CH)	5.08, br s	80.4 (CH)	5.86, br s	77.6 (CH)
9	7.22, br s	146.4 (CH)	6.68, br s	145.0 (CH)	7.16, br s	149.4 (CH)	6.88, br s	143.3 (CH)
10		132.9 (C)		135.6 (C)		130.2 (C)		137.6 (C)
11α	2.40, m	23.6 (CH_2)	2.50, dd, 13.7, 4.6	21.8 (CH_2)	2.50, m	24.9 (CH_2)	2.42, m	23.8 (CH_2)
11β	2.40, m		2.24, m		2.05, m		2.42, m	
12α	2.07, m	32.3 (CH_2)	1.24, m	28.8 (CH_2)	1.77, m	26.7 (CH_2)	1.62, m	30.4 (CH_2)[b]
12β	1.64, m		1.70, m		1.28, m		2.13, m	
13		145.7 (C)		143.3 (C)		144.7 (C)		145.1 (C)
14α	4.98, br s	115.6 (CH_2)	4.87, br s	114.6 (CH_2)	4.95, br s	115.5 (CH_2)	4.88, br s	112.9 (CH_2)
14β	5.01, br s		5.02, br s		4.97, br s		4.77, br s	
15	1.77, s	17.9 (CH_3)	1.71, s	18.2 (CH_3)	1.81, s	17.7 (CH_3)	1.80, s	23.1 (CH_3)
16	1.79, s	6.0 (CH_3)	1.84, s	6.4 (CH_3)	1.67, s	6.3 (CH_3)	1.03, d, 7.0	8.5 (CH_3)
17		138.6 (C)		139.4 (C)		142.0 (C)		140.5 (C)
18α	5.42, br s	118.9 (CH_2)	5.30, br s	119.7 (CH_2)	5.21, br s	119.4 (CH_2)	1.73, s	23.1 (CH_3)
18β	5.47, br s		5.39, br s		5.22, br s			
19	2.03, s	24.5 (CH_3)	2.07, s	23.9 (CH_3)	2.13, s	23.4 (CH_3)	1.93, s	20.4 (CH_3)
20		172.5 (C)		174.3 (C)		172.7 (C)		174.4 (C)
21				170.3 (C)				
22			2.02, s	20.4 (CH_3)				

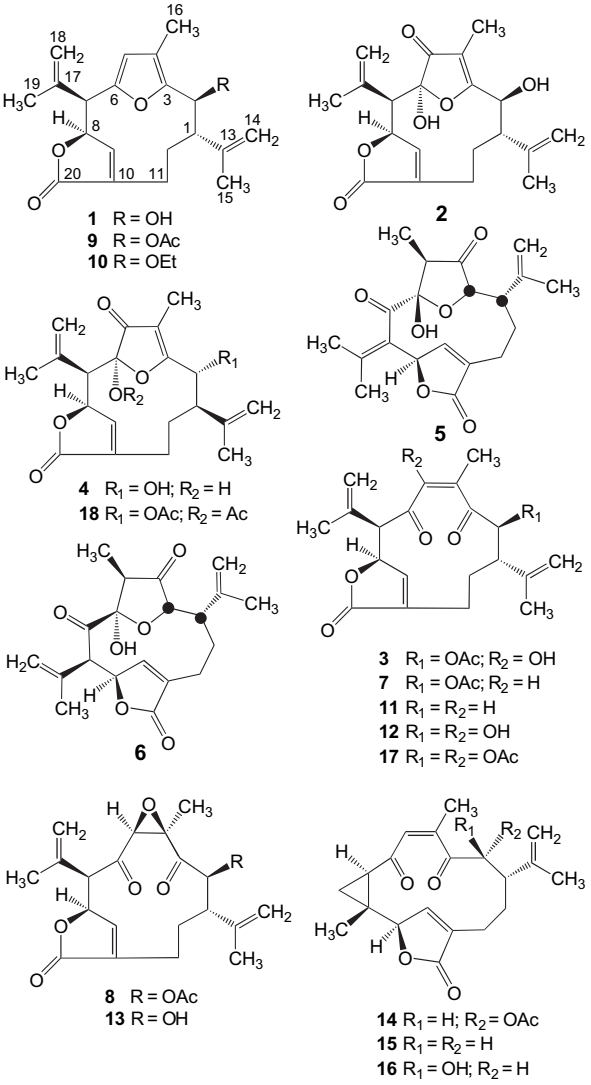
[a] Data recorded in $CDCl_3$ at 25 °C. Assignments were aided by ^{1}H–^{1}H COSY, DEPT, HMBC, HMQC, and NOESY NMR experiments.
[b] Due to the broad low-intensity nature of this resonance line the chemical shift value shown has been estimated.

of these compounds are closely related. The ^{1}H and ^{13}C NMR spectra of kallolide I (**8**) contain a number of broadened resonances, which led to low sensitivity in 2D hetero-correlated NMR experiments. However, the NMR spectra of compounds **2–7** contained mostly sharp resonances and therefore, the majority of the structural characterization was carried out on the latter metabolites.

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **6–8**[a]

Atom	Kallolide G (**6**)		Kallolide H (**7**)		Kallolide I (**8**)	
	δ_H, mult, J in Hz	δ_C, mult	δ_H, mult, J in Hz	δ_C, mult	δ_H, mult, J in Hz	δ_C, mult
1	2.50, br dt, 4.0, 11.8	44.3 (CH)	2.55, m	45.3 (CH)	2.18, m	42.6 (CH)[b]
2	3.00, d, 3.2	79.7 (CH)	5.57, br s	77.6 (CH)	4.67, br d, 11.7	78.3 (CH)
3		209.3 (C)		202.0 (C)		212.7 (C)[b]
4	2.21, q, 7.0	52.2 (CH)		154.0 (C)		68.3 (C)[b]
5		102.0 (C)	6.46, br s	125.6 (CH)	3.49, br s	63.9 (CH)
6		196.5 (C)		197.6 (C)		198.3 (C)[b]
7	4.50, br s	50.8 (CH)	3.45, br s	62.1 (CH)	3.60, br d, 1.6	56.8 (CH)[b]
8	5.30, t, 1.4	81.2 (CH)	5.45, br s	79.5 (CH)	5.07, br s	79.5 (CH)
9	7.41, br s	148.9 (CH)	7.39, br s	151.6 (CH)	6.64, br s	145.7 (CH)[b]
10		130.5 (C)		132.7 (C)		137.4 (C)
11α	2.43, m	21.9 (CH_2)	2.59, m	22.1 (CH_2)	2.40, m	20.0 (CH_2)[b]
11β	2.28, m		2.59, m		2.20, m	
12α	2.03, m	28.5 (CH_2)	2.17, m	25.6 (CH_2)	2.12, m,	26.2 (CH_2)[b]
12β	1.97, m		1.56, m		1.57, m	
13		144.4 (C)		143.7 (C)		140.8 (C)[b]
14α	4.91, br s	113.7 (CH_2)	4.83, br s	115.0 (CH_2)	5.00, br s	116.3 (CH_2)[b]
14β	4.81, br s		4.98, br s		4.95, br s	
15	1.74, s	23.8 (CH_3)	1.74, s	22.1 (CH_3)	1.60, s	17.5 (CH_3)[b]
16	1.13, d, 7.0	7.7 (CH_3)	1.94, d, 1.4	21.6 (CH_3)	1.54, s	21.7 (CH_3)[b]
17		139.7 (C)		138.0 (C)		137.7 (C)[b]
18α	5.09, br s	116.0 (CH_2)	5.26, br s	116.1 (CH_2)	5.20, br s	118.4 (CH_2)
18β	5.27, br s		5.54, br s		5.09, br s	
19	1.87, s	22.4 (CH_3)	1.76, s	23.1 (CH_3)	1.97, s	21.8 (CH_3)[b]
20		173.7 (C)		173.6 (C)		173.7 (C)
21				170.0 (C)		172.2 (C)[b]
22			2.23, s	20.8 (CH_3)	2.19, s	20.5 (CH_3)

[a] Data recorded in $CDCl_3$ at 25 °C. Assignments were aided by ^{1}H–^{1}H COSY, DEPT, HMBC, HMQC, and NOESY NMR experiments.
[b] Approximate chemical shift value due to the broad low-intensity nature of the resonance line.

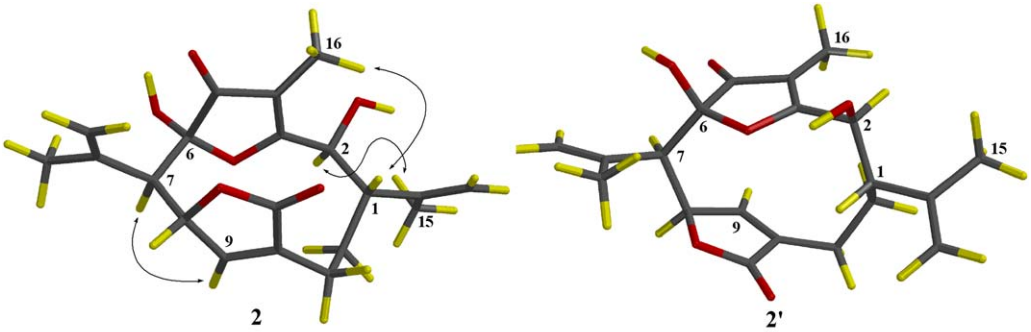


2. Results and discussion

Chemical analysis of the hexane extract of *P. bipinnata* led to the isolation of known compounds kallolide A acetate (**9**),[4] gersemolide (**11**),[8] and pinnatin B (**14**),[10] whereas the analysis of its $CHCl_3$ extract afforded known kallolide C (**12**)[4] and bipinnapterolide A (**13**),[9] along with new pseudopterane metabolites **2**–**6**. Mass spectral analysis of kallolide D (**2**) confirmed a formula of $C_{20}H_{24}O_6$, which requires nine degrees of unsaturation. The ^{13}C NMR spectra suggested the presence of two carbonyl groups. A carbonyl absorption in the IR spectrum of **2** at 1752 cm^{-1}, together with a one-proton resonance at δ 7.22 in the ^1H NMR spectrum and ^{13}C NMR signals at δ 172.5 (C), 146.4 (CH), 132.9 (C), and 78.9 (CH), suggested the presence of an α-substituted α,β-unsaturated γ-lactone functionality. This structural feature was assigned confidently by comparison of the spectral data with a number of natural products, including kallolide A (**1**), which also contains this functional group.[4] Further examination of the NMR data showed that two isopropenyl groups, as found in kallolide A, were present in the molecule. Resonances in the ^{13}C NMR spectrum at δ 145.7 (C), 138.6 (C), 115.6 (CH_2), and 118.9 (CH_2), along with four ^1H NMR signals at δ 5.47 (br s, 1H), 5.42 (br s, 1H), 5.01 (br s, 1H), and 4.98 (br s, 1H), confirmed the presence of two terminal olefins in **2**. ^1H–^1H COSY experiments more precisely defined each terminal olefin as methyl substituted. A broad IR absorption at 3398 cm^{-1}, coupled with a resonance in the ^{13}C NMR spectrum at δ 72.1 (CH) and a ^1H NMR signal (Table 1) at δ 4.02 (d, 1H, J=10.5 Hz), suggested the presence of a secondary alcohol as found in kallolide A. Resonances in the ^{13}C NMR spectrum of **2** also compared favorably to **1** at carbons 1, 2, 7–15, and 17–20. The planar structure of kallolide D, therefore, was proposed to be identical to **1** at all carbons except for those of the furan constellation that was obviously not present. The basic skeleton of a pseudopterane diterpene for **2** was supported by key HMBC correlations (H-1/C-2, C-3, C-11, C-12, C-13, C-14; H-2/C-1, C-3, C-4, C-12; H-7/C-5, C-6, C-8, C-18, C-19; H-8/C-6, C-7; H-9/C-8, C-20; H-11$\alpha\beta$/C-9, C-10, C-20; H-12$\alpha\beta$/C-2, C-10; H_3-16/C-3, C-4, C-5) and from straightforward connections of the different proton spin systems around the carbon skeleton through ^1H–^1H COSY experiments.

The major difference between the two molecules was most apparent from consideration of other NMR data. The familiar furan proton was not present in the spectrum of **2**, and furthermore, analysis of the ^{13}C NMR data showed that, in place of the resonances for a furan, four new signals were present in the spectrum of kallolide D (**2**) at δ 200.4 (C), 184.0 (C), 112.4 (C), and 100.2 (C). Also, a ^{13}C NMR resonance at δ 6.0 (CH_3) was assigned to an unusually shielded methyl group in the molecule (C-16).

These data suggested that the furan had been replaced by an oxidation product possessing both a conjugated ketone [δ 200.4 (C)] and a hemiketal functionality [δ 100.2 (C)]. The UV absorption at 282 nm (ε 2800) compared favorably to UV data [λ_{max} 278–284 nm (ε 7000–11,000)] for a series of macrocyclic diterpenes that possessed the same 4-methyl-3(2*H*)-furanone chromophore.[12] Proof for the presence of the C-6 hemiketal hydroxy group in **2** came from key HMBC correlations: H-7/C-5, C-6 and H-8/C-6, C-7. On the basis of this comparison, and considering the remaining functional groups, a structure for **2** was formulated as shown. Since kallolide D very likely was formed by the oxidation of kallolide A (**1**), it must have one of the two diastereoisomeric structures **2** or **2′**. A distinction between these structures was achieved by a combination of NOESY and molecular modeling studies. The McSpartan '04 molecular modeling program was used to calculate the conformer distribution of each possible structure; key inter-proton distances were then measured on the lowest energy conformer. These distances are given in Figure 1 together with selected observed NOESY correlations. From Figure 1 it is evident that the observed NOESY correlations are consistent only with structure **2**. Thus a NOESY correlation was observed for H-1/H_3-16, and structure **2** is the only structure for which this internuclear distance is calculated to be less than 3 Å. Conversely, a NOESY correlation was not observed for H-2/H_3-16; such correlation would have been expected for structure **2′**. On the basis of the above spectral evidence, the structure of kallolide D including its relative stereochemistry was confidently assigned as **2**.



Energy and bond distances (Å) of selected NOESY correlations for compounds **2** and **2'**[a]

compound	Energy (kcal/mol)	H-1/H_3-16	H-2/H_3-16	H-7/H-9	H2-/H_3-15
2	102	2.7 (Y)	4.0 (N)	2.6 (Y)	2.4 (Y)
2'	104	4.6	2.3	3.1	3.8

[a]Calculated using the MacSpartan '04 program
(Y) = NOE correlations observed (N) = NOE correlations not observed

Figure 1. Energy-minimized molecular model of kallolide D (**2**) with diagnostic NOESY correlations observed. Also shown for comparison purposes is a stereo-view of C-6 epimer **2'** generated from computer modeling along with relevant inter-proton distances.

Kallolide C acetate (**3**) was isolated as a colorless oil with a molecular formula of $C_{22}H_{26}O_7$ established by HR-FABMS of the $[M+H]^+$ ion. The difference of 42 mass units in the molecular formula of **3** in relation to that of known kallolide C (**12**) suggested that the C-2 hydroxy group in **12** must be acetylated in **3**.[4] This was corroborated by the spectral data (^1H and ^{13}C NMR) of **3**, which were generally very similar to those of **12** (Table 1) but showed the presence of an additional acetate methyl signal at δ 2.02 (s, 3H) as well as a noticeable downfield shift for H-2 (from δ 4.48 in **12** to δ 5.38 in **3**).[13] Because similar NOEs were observed in both compounds, it was concluded that these compounds have the same relative stereochemistry at all the chiral centers. Difficulties arose in confirming these conclusions through X-ray crystallographic analysis as kallolide C acetate (**3**) failed to suitably crystallize under a variety of conditions. Notwithstanding, the structural relationship of compounds **3** and **12** was decisively demonstrated when both metabolites, upon acetylation, yielded the same diacetate, **17**, previously prepared by Fenical et al. from **12**.[4]

Kallolide E (**4**), a colorless oil, showed a pseudomolecular ion peak at *m/z* 361.1650 $[M+H]^+$ in the HR-FABMS (calcd *m/z* 361.1651), corresponding to a molecular formula of $C_{20}H_{24}O_6$. Thus, the elemental composition of **4** was identical to that of kallolide D (**2**), indicating that both compounds were isomers. Moreover, the UV and IR spectra of **4** exhibited the same patterns as those of **2**.

HMBC data provided evidence for connecting the smaller molecular segments deduced from vicinal coupling; and long-range ^1H–^1H couplings verified many of the connectivities between protonated carbons and quaternary carbons. After the ^1H and ^{13}C NMR data of **4** had been assigned by analysis of its 2D NMR spectra, it was obvious that the gross structure of kallolide E (**4**) was the same as that of kallolide D (**2**). Thus kallolide E (**4**) was believed to differ from **2** only in relative stereochemistry at C-1 and C-2. This contention was supported by the strong NOESY correlations of H-2 (β-orientation in planar conformation) and H_3-16 and also of H-1 (α-orientation) and H-9, both of which were notably absent in **2**. Conversely, NOESY correlations were not observed in **4** for H-1/H_3-16; such correlations, as mentioned earlier, are indeed present in structure **2**. The relative configuration of **4** was firmly established by X-ray crystallographic analysis of diacetate **18**, obtained in excellent yield, after acetylation of kallolide E (Fig. 2).

The positive-ion high-resolution ESIMS of kallolide F (**5**) showed an accurate $[M+Na]^+$ ion peak at *m/z* 383.1479, in accordance with a molecular formula of $C_{20}H_{24}O_6$, implying nine degrees of unsaturation, which was supported by the ^{13}C NMR spectrum and DEPT data. The IR exhibited an absorption band at 3504 cm^{-1} supporting the presence of a hydroxy group in **5**, and an intense absorption at 1753 cm^{-1} due to two overlapped bands, as well as a smaller band at 1696 cm^{-1}, assignable to three carbonyl groups. The ^1H NMR spectrum of **5** showed a characteristic signal in the olefinic region [δ 6.88 (1H, br s)] assigned to H-9 indicating the presence of an α-substituted α,β-unsaturated γ-lactone functionality. The olefinic signals at δ 4.88 (1H, br s) and 4.77 (1H, br s), which long-range coupled to a methyl singlet at δ 1.80 assigned to H_3-15, revealed the presence of one isopropenyl group. The carbonyl absorption at 1696 cm^{-1}, the UV absorption maximum at 206 nm, together with two methyl resonances at δ 1.93 and 1.73 in the ^1H NMR

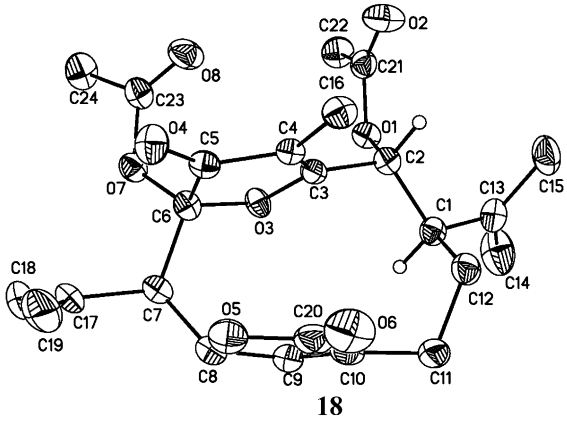


Figure 2. ORTEP drawing of kallolide E diacetate (**18**).

spectrum and ^{13}C NMR signals at δ 199.7 (C), 129.0 (C), 140.5 (C), 23.1 (CH_3), and 20.4 (CH_3), suggested the presence of an α-substituted β,β-dimethyl-α,β-unsaturated ketone functionality in **5**. Further examination of the NMR data, including data from ^1H–^1H COSY, HMQC, and HMBC experiments, showed that a 5-hydroxy-4-methyl-dihydro-3-furanone moiety was present in the molecule. Resonances in the ^{13}C NMR spectrum at δ 212.3 (C), 101.3 (C), 81.3 (CH), 50.2 (CH), and 8.5 (CH_3), along with three ^1H NMR signals at δ 4.25 (d, 1H, J=2.6 Hz), 2.55 (q, 1H, J=7.0 Hz), and 1.03 (d, 3H, J=7.0 Hz), ascribable to H-2, H-4, and H_3-16, respectively, confirmed the presence of this uncommon functionality.[14] From these data, eight of the nine degrees of unsaturation could be accounted for demonstrating that **5** was monocarbocyclic. Furthermore, the ring size of **5** was concluded to be 12-membered from consideration of the observed functional groups. Overall, these structural features suggested that compound **5** has the same framework of **1**–**4**, but has differences in functionalities and oxygen substitution. The HMBC spectra of kallolide F (**5**) showed similar correlations, except for C-2 and C-5, confirming the same skeleton. The oxygenated proton doublet resonating at δ 4.25 showed correlations to the methine carbon at δ 45.2 (C-1) and the carbonyl carbon at δ 212.3 (C-3), and thus was assigned to H-2. This proton showed correlations to a quaternary carbon (C-5, δ 101.3) and a methine carbon (C-4, δ 50.2). Moreover, a proton quartet resonating at δ 2.55 (H-4) showed correlations to the ketone carbonyls at δ 212.3 (C-3) and 199.7 (C-6), the hemiketal carbon at δ 101.3 (C-5), and the methyl carbon at δ 8.5 (C-16). The downfield shift of C-2 (CH), the upfield shift of C-5 (C), and the appearance of C-4 as a CH signal in DEPT, indicated that the new ether bridge in **5** was in between C-2 and C-5. Furthermore, the significant downfield shift of H-8 (δ 5.86) and the absence of a typical proton signal ascribable to H-7, indicated than an isopropylidene group was now installed at C-7. Data from ^1H–^1H COSY, HMQC, and HMBC provided additional support to justify the gross structure shown for **5**.

The relative stereochemistry of kallolide F (**5**) was deduced by scalar couplings, NOESY experiment, and molecular modeling studies. The methine protons H-1 and H-2 exhibited a J value of 2.6 Hz, establishing a cis-relationship of H-1 and H-2. This assignment was further supported by the strong NOESY correlations of H-1 (β-orientation in planar conformation) and H-2, and weak correlations of H-2 and H_3-16. The H-4 proton exhibited stronger NOESY correlations to the terminal methylene protons at C-14, placing the isopropenyl side chain in the same α-plane as H-4. After calculating the conformer distribution of each possible stereoisomeric structure of kallolide F at C-5 and C-8, the observed NOESY correlations of H_3-16/H_3-18 and H-8/H_3-19 were consistent only with structure **5**. Only in this stereoisomer, which also possessed the lowest calculated energy (64 kcal mol^{-1}), the internuclear distances for the latter protons were calculated to be 2.7 and 2.2 Å, respectively. Conversely, NOESY correlations were not observed for H-2/H-9; such correlation would have been expected had the C-5 hydroxy group in **5** protruded in the α-orientation as found in kallolide G (**6**). These analyses confidently established the overall relative stereochemistry of kallolide F as 1*S**, 2*S**, 4*S**, 5*S**, and 8*R**.

The lipophilic extracts of *P. kallos* afforded the known kallolide A (**1**),[4] kallolide A acetate (**9**),[4] 2-*O*-ethylkallolide A (**10**),[7] gersemolide (**11**),[8] kallolide C (**12**),[4] bipinnapterolide A (**13**),[9] pinnatin B (**14**),[10] gersolide (**15**),[11] and pinnatin D (**16**),[10] along with the new pseudopterane analogs kallolide C acetate (**3**), kallolide E (**4**), and kallolides G–I (**6**–**8**). In the present study, we also provide for the first time a complete proton and carbon atom assignment for gersolide (**15**), a confirmation of the proposed structure for 2-*O*-ethylkallolide A (**10**) by X-ray diffraction analysis (Fig. 3), a revised proton and carbon atom assignments for gersemolide (**11**) (see Section 4), and a revised ^{13}C atom assignment for kallolide C (**12**).[13]

Kallolide G (**6**) was obtained as a white crystalline solid. Its molecular formula, $C_{20}H_{24}O_6$, was established via HR-FABMS, ^{13}C NMR, and DEPT spectral data. As in kallolide F (**5**), the ^1H NMR spectrum of **6** showed characteristic signals assignable to a 5-hydroxy-4-methyl-dihydro-3-furanone moiety at δ 3.00 (1H, d, J=3.2 Hz, H-2), 2.21 (1H, q, J=7.0 Hz, H-4), and 1.13 (3H, d, J=7.0 Hz, H_3-16), while the HMBC spectrum demonstrated correlations for H-2 with C-1, C-3, C-12 and C-13, H-4 with C-2, C-3, C-5, C-6 and C-16, and H_3-16 with C-3, C-4 and C-5. The olefinic signal at δ 7.41 (1H, br s, H-9) in combination with the oxymethine resonance at δ 5.30 (1H, t, J=1.4 Hz, H-8) revealed the presence of an α-substituted α,β-unsaturated γ-lactone functionality. This conclusion was supported by the UV absorption maxima and the strong absorption band in the IR spectrum at 1764 cm^{-1}. Two distinct sets of terminal methylenes at δ 4.91 (1H, br s)/4.81 (1H, br s) and 5.27 (1H, br s)/5.09 (1H, br s), each displaying long-range coupling to the methyl singlets at δ 1.74 and 1.87, respectively, revealed the presence of two isopropenyl groups instead of one as found in kallolide F (**5**). Consequently, the tetrasubstituted double bond in **5** must have shifted to a terminal position in **6** since the telltale methine signal (δ 4.50, H-7) was now visible in the spectrum of **6**. The downfield shift experienced by H-7 in **6** is compatible with it being flanked by carbonyl and olefin groups. Thus, the ^1H and ^{13}C NMR spectra of **6** (Table 2) suggested close structural homology with that of kallolide F (**5**) with one additional isopropylene side chain. Curiously, the chemical shift of the C-6 carbonyl (δ 196.5) in kallolide G (**6**) is consistent with a ketone functionality as observed in **5**, thus suggesting that there must exist some distortion in the planarity of the tetrasubstituted α,β-unsaturated ketone of **5**. The relative configuration of kallolide G (1*S**, 2*S**, 4*S**, 5*R**, 7*R**, and 8*R**) was deduced from X-ray crystallographic analysis (Fig. 3) and was supported by the following key NOESY correlations: H-1/H-2, H-1/H-11β, H-2/H-11β, H-7/H-8, H-7/H-9, and H-9/H-12α.[15] Interestingly, the oxymethine proton H-2 in **6** is unusually shielded to δ 3.00 (the corresponding proton in **5** appears at δ 4.25). As seen in Figure 3 the geometry of kallolide G is such that H-2, which lies close to the C-6 and C-20 C=O bonds, is in the shielding portion of the induced magnetic fields.

Kallolide H (**7**) was obtained as a white crystalline solid, $[\alpha]_D^{20}$ +22.5 (*c* 1.2, $CHCl_3$). The HR-EIMS of kallolide H (**7**) exhibited its molecular ion at *m*/*z* 386.1733, appropriate for a molecular formula of $C_{22}H_{26}O_6$. The IR and UV spectra of **7** were similar to those of known gersemolide (**11**).[8]

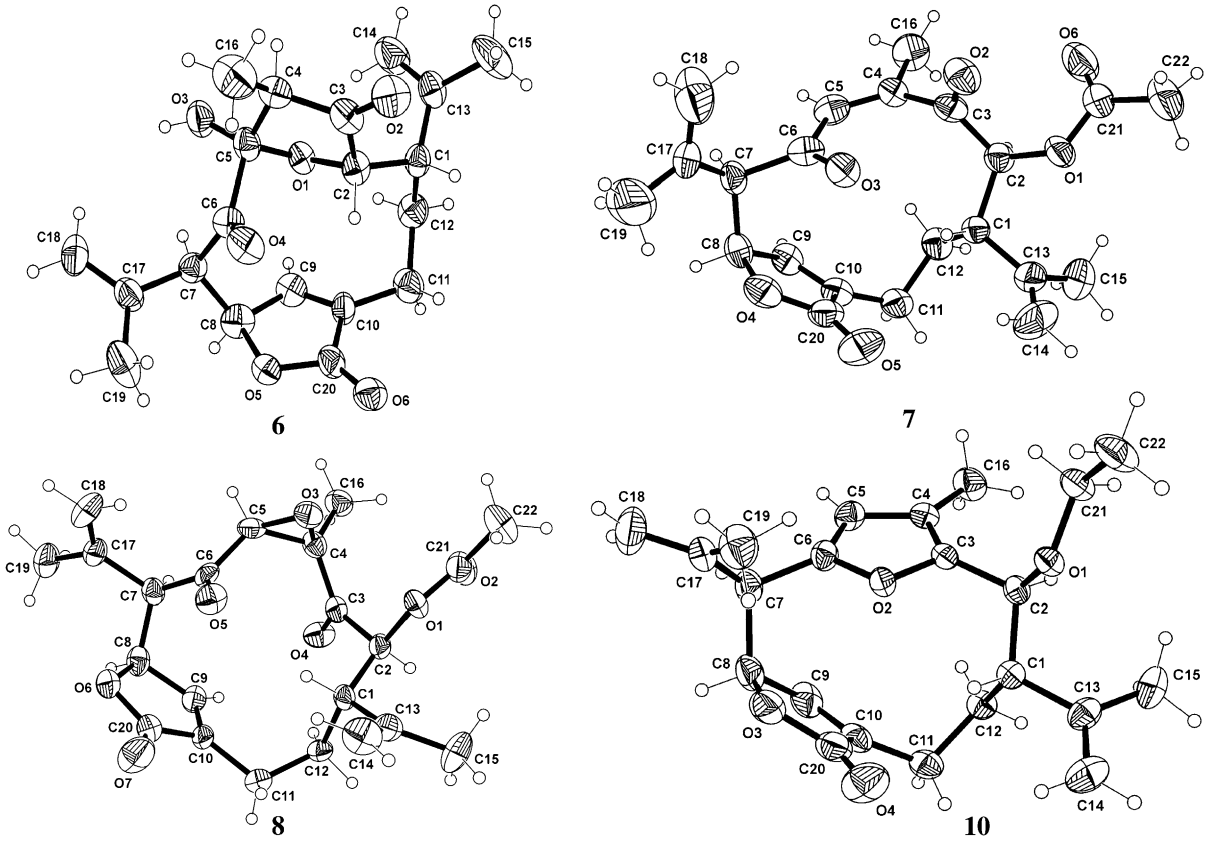


Figure 3. Perspective views of kallolide G (**6**), kallolide H (**7**), kallolide I (**8**), and 2-*O*-ethylkallolide A (**10**) with the atomic numbering scheme. The carbon and oxygen atoms are drawn as 30% thermal ellipsoids; hydrogen atoms are depicted as spheres with arbitrary radii. The X-ray experiments did not define the absolute configuration, so the enantiomers shown are an arbitrary choice.

However, **7** showed an additional absorption band at 1736 cm^{-1} that was not present in the IR spectrum of **11**. Except for the presence of additional signals ascribable to an acetoxy group, the ^{1}H and ^{13}C NMR data (Table 2) closely resembled those of **11**, and ^{1}H–^{1}H COSY, HMQC, and HMBC spectra of **7** revealed that the two compounds have the same skeletal connectivities. Consequently, kallolide H (**7**) was thought to be the oxyacetylated analog of **11**. The fact that the new functionality appears located in the C-2 position of the molecule was evident from ^{1}H–^{1}H COSY, NOESY, and HMBC experiments.[16] Thus kallolide H was assigned structure **7**, and its structure, including relative stereochemistry, was subsequently confirmed by single-crystal X-ray diffraction (Fig. 3).

Kallolide I (**8**) was also isolated as a white crystalline solid, whose molecular formula $C_{22}H_{26}O_7$ was confirmed by HR-EIMS (*m*/*z* 402.1679, calcd 402.1679). Its IR spectrum showed the presence of two ester carbonyls (1759 and 1736 cm^{-1}) and two ketone carbonyls (1716 and 1706 cm^{-1}). The NMR spectral data of **8** were very similar to those of known bipinnapterolide A (**13**) except for the presence of an acetyl group at C-2.[9] Interestingly, kallolide I, like **13**, gave rise to NMR spectra characterized by an abundance of broad signals of very low intensity, suggesting rapid intramolecular mobility near the NMR probe temperature. The downfield shift of H-2 from δ 3.86 (1H, d, J=11.0 Hz) in **13** to δ 4.67 (1H, br d, J=11.7 Hz) in **8** confirmed that the acetoxy group at C-2 in **8** replaced the hydroxy group at C-2 in **13**. The relative stereochemistry of kallolide I (**8**) was established by its NOESY spectrum.[17] The structure for this compound was confirmed by chemical correlation upon acetylation of **13** and by X-ray crystal diffraction (Fig. 3), which showed that kallolide I (**8**) was indeed the acetylated analog of bipinnapterolide A (**13**). Alternatively, kallolide I (**8**) can be thought of as the β-epoxidized version of kallolide H (**7**) at the Δ^4 olefin.

The potent in vivo anti-inflammatory properties of kallolide A (**1**) have been documented.[4] In the present account the in vitro activity of compounds **1**–**16** against *M. tuberculosis* $H_{37}Rv$, the principal causative agent for tuberculosis, and the malaria parasite *P. falciparum*, is reported in Table 3. All of the pseudopterane (**1**–**13**) and gersolane (**14**–**16**) diterpenes described were found to be marginally active against *M. tuberculosis* at the two concentrations tested (128 and 64 μg mL^{-1}). On the other hand, over half of the compounds tested showed significant anti-plasmodial activity using a novel DNA-based microfluorimetric method.[18] The significant difference in biological activity of compounds **1**–**16** indicate that neither the furan ring, the C-2 hydroxy, nor the γ-butenolide moiety alone appear to play a pivotal role in the anti-parasitic activity. Nevertheless, these results provide valuable data to suggest that the conformational nature and size of the carbocyclic ring is essential for anti-plasmodial activity.

Table 3. In vitro anti-mycobacterial and anti-parasitic activities of compounds **1–16**

Compound	*M. tuberculosis*[a]		*P. falciparum*[b]
	% inhibition at 128 μg mL^{-1}	% inhibition at 64 μg mL^{-1}	IC_{50} (μM)[c]
Kallolide A (**1**)	29.0	12.0	39.6
Kallolide D (**2**)	30.1	6.6	30.6
Kallolide C acetate (**3**)	48.2	27.6	>100[d]
Kallolide E (**4**)	35.0	29.0	>100[d]
Kallolide F (**5**)	49.2	33.6	52.8
Kallolide G (**6**)	18.0	15.6	83.3
Kallolide H (**7**)	6.6	9.2	41.5
Kallolide I (**8**)	16.2	18.3	57.2
Kallolide A acetate (**9**)	7.5	8.7	>100[d]
2-*O*-Ethylkallolide A (**10**)	20.9	30.0	>100[d]
Gersemolide (**11**)	60.8	41.7	21.3
Kallolide C (**12**)	7.0	8.4	>100[d]
Bipinnapterolide A (**13**)	16.2	18.3	>100[d]
Pinnatin B (**14**)	31.6	17.8	23.3
Gersolide (**15**)	NT[e]	NT[e]	45.7
Pinnatin D (**16**)	30.2	15.3	43.6

[a] Rifampin was used as a positive control during the anti-TB assays.
[b] Chloroquine-resistant clone Indochina W2.
[c] Chloroquine was used as a positive control during the anti-malaria assays.
[d] Not active.
[e] NT=Not tested.

3. Conclusions

The striking structural similarity of the secondary metabolites isolated from *P. bipinnata* and *P. kallos* attests to their close taxonomic relationship. The presence of the same metabolites within these organisms supports the theory that *P. bipinnata* and *P. kallos* are intimately related taxonomically.[2] The diversity of pseudopterane and gersolane diterpenoids isolated from these gorgonians is indeed astonishing, and quickly raises the question of the true origin of these metabolites. One likely scenario that should not be discounted is that kallolide A (**1**) is indeed a true gorgonian metabolite, but that the microbes inherently associated with the octocoral species are ultimately responsible for the posterior oxidation of **1** thus affording a large array of minor secondary metabolites.[19,20] On the other hand, kallolide E (**4**), the only pseudopterane diterpene reported thus far in which the C-1 isopropyl group is β (pointing upward) must originate through ring contraction of a cembrane-type precursor that belongs to the β series.[10,21]

4. Experimental

4.1. General

Infrared spectra were recorded with a FTIR spectrophotometer and optical rotations were measured with an automatic polarimeter. ^{1}H NMR spectral data were generated with a 500 or 300 MHz FT-NMR spectrometer and the ^{13}C NMR spectral data with a 125 or 75 MHz FT-NMR spectrometer. ^{1}H–^{1}H COSY, NOESY, APT, HMQC, and HMBC experiments were measured with a 300 MHz FT-NMR spectrometer. Normal-phase HPLC separations were carried out either on a 10 mm×25 cm Ultrasphere-Cyano Polar-Bonded column or a 10 mm×25 cm Ultrasphere-Silica Gel column eluted isocratically with hexane–2-isopropanol mixtures at 1.0–2.0 mL min^{-1}, with UV detection at 220 nm. Lowest energy conformers were searched using MMFF force field implemented in the McSpartan '04 Program (Wavefunction, Inc.). Column chromatography was performed on silica gel (35–75 mesh). TLC analyses were carried out using glass silica gel plates and spots were visualized by exposure to I_2 vapors or heating silica gel plates sprayed with 5% H_2SO_4 in EtOH. All solvents used were of spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the crude organic extract. The identities of the known compounds isolated during this investigation were established by comprehensive spectral data comparisons with data previously reported in the literature.

4.2. Animal material

Large and healthy specimens of the sea plumes *P. bipinnata* and *P. kallos* were collected by hand using SCUBA at depths of 83–91 ft near Providencia (Old Providence) Island, Colombia on March 15, 2002. The taxonomic identification of these gorgonian species was conducted by Dr. Juan A. Sánchez (Universidad de Los Andes, Bogotá). Voucher specimens have been stored at the Chemistry Department of University of Puerto Rico, Río Piedras.

4.3. Extraction and isolation

The partially air-dried specimens of *P. bipinnata* were frozen, freeze-dried (0.11 kg), cut in small pieces, and blended with 1:1 MeOH/$CHCl_3$ (10×1 L). The combined organic extracts were filtered and concentrated to give a brown residue (25.0 g) that was suspended in H_2O and extracted with hexane, $CHCl_3$, and EtOAc. The hexane extract (12 g) was purified subsequently by size-exclusion chromatography on a Bio-Beads SX-3 column eluted with toluene. The last fraction (949 mg) was purified by flash chromatography over silica gel (30 g) with mixtures of hexane/EtOAc of increasing polarity (10–100%) to yield, after consecutive column chromatography and normal-phase HPLC (Ultrasphere-Silica Gel), known kallolide A acetate (**9**) (27 mg, 0.11%),[4] gersemolide (**11**) (1.0 mg, 0.004%),[8] and pinnatin B (**14**) (5 mg, 0.020%).[10]

Rotoevaporation of the $CHCl_3$ extract of *P. bipinnata* produced 4.1 g of a greenish oil that was loaded onto a column of silica gel (150 g) and eluted with a 99:1 mixture of $CHCl_3$/MeOH. Several of the least polar fractions obtained were further purified by column chromatography over silica gel followed by normal-phase HPLC to afford known compounds kallolide C (**12**) (2.0 mg, 0.008%)[4] and bipinnapterolide A (**13**) (37.2 mg, 0.15%),[9] and the new metabolites kallolide D (**2**) (2.5 mg, 0.01%), kallolide C acetate (**3**) (14.3 mg, 0.06%), kallolide E (**4**) (4.0 mg, 0.02%), kallolide F (**5**) (3 mg, 0.01%), and kallolide G (**6**) (2.0 mg, 0.01%).

P. kallos was sun-dried, frozen, and lyophilized prior the its extraction. The dry organism (1.07 kg dry weight) was blended and filtered exhaustively using a mixture of 1:1 CH_2Cl_2/MeOH (20×1 L). After filtration the crude extract was evaporated and stored under vacuum to yield a green gum (166.3 g). After the crude extract was partitioned between hexane and H_2O, the aqueous suspension was extracted with $CHCl_3$ (3×2 L). The resulting $CHCl_3$ extract

was concentrated in vacuo to yield 39.3 g of a brown amorphous solid that was chromatographed over silica gel (673 g) using a step gradient of EtOAc/hexane as eluent and separated into 32 fractions (A-FF) on the basis of TLC and ^{1}H NMR analyses. Spontaneous crystallization of fraction J (312 mg) upon standing followed by recrystallization from diethyl ether gave pure kallolide A acetate (**9**) (12.4 mg, 0.01%).[4] Consecutive purifications of fraction L (1.3 g) by column chromatography on silica gel and normal-phase HPLC (Ultrasphere-CN) gave the known pseudopterane diterpenes gersemolide (**11**) (27.1 mg, 0.02%),[8] 2-*O*-ethylkallolide A (**10**) (11.3 mg, 0.01%),[7] kallolide A (**1**) (359.2 mg, 0.22%),[4] and kallolide C (**12**) (56 mg, 0.03%),[4] as well as the new pseudopterane kallolide G (**6**) (6.1 mg, 0.004%). Purification of fraction M (510 mg) by successive column chromatography on silica gel and normal-phase HPLC (Nucleosil Silica gel and Ultrasphere-CN) gave new metabolites kallolide H (**7**) (12.1 mg, 0.01%) and kallolide I (**8**) (5.1 mg, 0.003%) as well as the known gersolane-type diterpene pinnatin B (**14**) (29.4 mg, 0.02%).[10] Purification of fraction O (1.59 g) by silica gel column chromatography with 1% acetone in $CHCl_3$ followed by normal-phase HPLC (Ultrasphere-CN) led to known pinnatin D (**16**) (8.6 mg, 0.005%).[10] Subsequent purification of fraction V (1.2 g) by silica gel (65 g) column chromatography using 3% EtOAc in $CHCl_3$ followed by normal-phase HPLC (Ultrasphere-CN) afforded pure kallolide E (**4**) (4.2 mg, 0.003%).

The hexane soluble extract (71.9 g) of *P. kallos* was dissolved in a small volume of toluene, filtered through two layers of sand and Celite, concentrated, and passed through a large Bio-Beads SX-3 column eluted with toluene. The last fraction (2.4 g) was further purified by column chromatography on silica gel (140 g) using 20% EtOAc in hexane to afford, after successive column chromatography and HPLC, kallolide C acetate (**3**) (26.4 mg, 0.02%) and the known compounds kallolide A acetate (**9**) (201 mg, 0.12%),[4] kallolide A (**1**) (343 mg, 0.21%),[4] pinnatin B (**14**) (25.9 mg, 0.02%),[10] gersolide (**15**) (5.3 mg, 0.003%),[11] and bipinnapterolide A (**13**) (12.2 mg, 0.007%).[9]

4.3.1. Kallolide D (2). Colorless oil; $[\alpha]_D^{20}$ +52.7 (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ε 4400), 282 nm (ε 2800); IR (neat) ν_{max} 3398 (br), 3076, 3013, 2927, 2864, 1752, 1710, 1616, 1445, 1379, 1177, 1158, 1073, 1018, 899 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HR-FABMS (magic bullet) *m/z* [M+H]$^+$ calcd for $C_{20}H_{25}O_6$ 361.1651, found 361.1650.

4.3.2. Kallolide C acetate (3). Colorless oil; $[\alpha]_D^{20}$ −40.4 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} 203 nm (ε 12,300), 282 nm (ε 5800); IR (neat) ν_{max} 3367 (br), 3076, 3019, 2930, 2861, 1753, 1720, 1633, 1441, 1376, 1226, 1030, 901 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HR-FABMS (magic bullet) *m/z* [M+H]$^+$ calcd for $C_{22}H_{27}O_7$ 403.1757, found 403.1757.

4.3.3. Kallolide E (4). Colorless oil; $[\alpha]_D^{20}$ +75.0 (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ε 19,900), 282 nm (ε 11,100); IR (neat) ν_{max} 3404 (br), 3082, 3021, 2932, 2869, 1749, 1718, 1629, 1449, 1379, 1179, 1126, 1093, 1069, 1051, 894 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HR-FABMS (magic bullet) *m/z* [M+H]$^+$ calcd for $C_{20}H_{25}O_6$ 361.1651, found 361.1650.

4.3.4. Kallolide F (5). Colorless oil; $[\alpha]_D^{20}$ −35.0 (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ε 4500); IR (neat) ν_{max} 3504, 3081, 2926, 2853, 1753, 1696, 1648, 1454, 1382, 1157, 1085, 1054, 1025, 984 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HR-ESIMS *m/z* [M+Na]$^+$ calcd for $C_{20}H_{24}O_6Na$ 383.1471, found 383.1479.

4.3.5. Kallolide G (6). White solid; $[\alpha]_D^{20}$ −101.6 (*c* 1.13, $CHCl_3$); UV (MeOH) λ_{max} 202 nm (ε 11,900), 247 nm (ε 6200); IR (neat) ν_{max} 3275, 2976, 2938, 1764, 1724, 1641, 1448, 965, 899 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HR-FABMS (magic bullet) *m/z* [M+H]$^+$ calcd for $C_{20}H_{25}O_6$ 361.1651, found 361.1650.

4.3.6. Kallolide H (7). White solid; $[\alpha]_D^{20}$ +22.5 (*c* 1.2, $CHCl_3$); UV (MeOH) λ_{max} 204 nm (ε 33,800), 284 nm (ε 5100); IR (diamond cell) ν_{max} 3090, 2972, 2947, 1754, 1736, 1713, 1683, 1649, 1605, 1439, 1376, 1243, 899 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); LR-EIMS *m/z* [M]$^+$ 386 (2), 345 (2), 326 (5), 194 (48), 151 (100); HR-EIMS *m/z* [M]$^+$ calcd for $C_{22}H_{26}O_6$ 386.1729, found 386.1733.

4.3.7. Kallolide I (8). White solid; $[\alpha]_D^{20}$ +21.2 (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 202 nm (ε 10,400), 209 nm (ε 10,500); IR (diamond cell) ν_{max} 3092, 3073, 2986, 2960, 2930, 2918, 2861, 1759, 1736, 1716, 1706, 1644, 1468, 1441, 1381, 1322, 1245, 1232, 1114, 1067, 903 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); LR-EIMS *m/z* [M]$^+$ 402 (3), 360 (9), 343 (18), 259 (34), 248 (51), 230 (35), 194 (89), 164 (66), 150 (52), 139 (100), 119 (56), 95 (76), 85 (82); HR-EIMS *m/z* [M]$^+$ calcd for $C_{22}H_{26}O_7$ 402.1679, found 402.1679.

4.3.8. Gersemolide (11). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.32 (m, 1H, H-1), 2.58 (dd, 1H, *J*=15.2, 4.3 Hz, H-2), 2.46 (dd, 1H, *J*=15.2, 9.4 Hz, H-2′), 6.37 (d, 1H, *J*=1.4 Hz, H-5), 3.36 (br s, 1H, H-7), 5.42 (br s, 1H, H-8), 7.16 (br s, 1H, H-9), 2.52 (m, 1H, H-11), 2.40 (m, 1H, H-11′), 2.08 (m, 1H, H-12), 1.35 (m, 1H, H-12′), 4.82 (br s, 1H, H_2-14), 4.80 (br s, 1H, H_2-14′), 1.71 (s, 3H, H_3-15), 1.89 (br d, 3H, *J*=1.4 Hz, H_3-16), 5.52 (br s, 1H, H_2-18), 5.26 (br s, 1H, H_2-18′), 1.79 (s, 3H, H_3-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 41.2 (CH, C-1), 44.9 (CH_2, C-2), 208.3 (C, C-3), 156.2 (C, C-4), 123.8 (CH, C-5), 197.6 (C, C-6), 62.4 (CH, C-7), 79.0 (CH, C-8), 150.0 (CH, C-9), 134.1 (C, C-10), 21.9 (CH_2, C-11), 31.4 (CH_2, C-12), 146.4 (C, C-13), 112.2 (CH_2, C-14), 19.9 (CH_3, C-15), 22.0 (CH_3, C-16), 138.1 (C, C-17), 116.1 (CH_2, C-18), 23.1 (CH_3, C-19), 173.7 (C, C-20).

4.3.9. Gersolide (15). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.43 (m, 1H, H-1), 2.80 (d, 1H, *J*=12.5 Hz, H-2), 2.20 (dd, 1H, *J*=12.5, 9.6 Hz, H-2′), 5.95 (br d, 1H, *J*=1.4 Hz, H-5), 1.83 (dd, 1H, *J*=8.2, 6.5 Hz, H-7), 1.43 (t, 1H, *J*=6.0 Hz, H-9), 0.97 (dd, 1H, *J*=8.3, 5.6 Hz, H-9′), 5.13 (br s, 1H,

H-10), 6.75 (br s, 1H, H-11), 2.39 (dd, 1H, J=13.6, 12.7 Hz, H-13), 2.28 (dd, 1H, J=13.6, 5.4 Hz, H-13′), 2.02 (m, 1H, H-14), 1.32 (m, 1H, H-14′), 5.22 (br s, 1H, H_2-16), 4.96 (br s, 1H, H_2-16′), 1.72 (s, 3H, H_3-17), 2.06 (d, 3H, J=1.4 Hz, H_3-18), 1.29 (s, 3H, H_3-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 40.0 (CH, C-1), 45.7 (CH_2, C-2), 202.7 (C, C-3), 143.3 (C, C-4), 138.0 (CH, C-5), 199.4 (C, C-6), 28.8 (CH, C-7), 32.9 (C, C-8), 17.0 (CH_2, C-9), 83.4 (CH, C-10), 146.6 (CH, C-11), 140.0 (C, C-12), 21.1 (CH_2, C-13), 35.7 (CH_2, C-14), 144.7 (C, C-15), 114.4 (CH_2, C-16), 17.8 (CH_3, C-17), 21.3 (CH_3, C-18), 15.9 (CH_3, C-19), 173.6 (C, C-20).

5. Synthetic transformations

5.1. Acetylation of kallolide C acetate (3)

A solution of kallolide C acetate (1 mg) in 1:1 acetic anhydride/pyridine (2 mL) was stirred at 25 °C for 24 h. The reaction mixture was concentrated in vacuo and the oily residue obtained was purified by silica gel column chromatography with 100% $CHCl_3$ to afford pure kallolide C diacetate (**17**) (0.90 mg, 82% yield).

5.1.1. Data for kallolide C diacetate (17). Most of the data for diacetate **17** has been previously reported:[4] ^{13}C NMR ($CDCl_3$, 125 MHz) δ 43.4 (CH, C-1), 67.4 (CH, C-2), 177.5 (C, C-3), 114.1 (C, C-4)[a], 99.7 (CH, C-5), 201.6 (C, C-6), 51.9 (CH, C-7), 82.0 (CH, C-8), 146.3 (CH, C-9), 135.5 (C, C-10), 21.9 (CH_2, C-11)[b], 30.2 (CH_2, C-12), 143.0 (C, C-13), 114.6 (CH_2, C-14)[a], 18.0 (CH_3, C-15), 6.2 (CH_3, C-16), 141.2 (C, C-17), 117.2 (CH_2, C-18), 21.7 (CH_3, C-19)[b], 174.4 (C, C-20), 169.7 (C, C-21), 20.6 (CH_3, C-22), 166.6 (C, C-23), 20.3 (CH_3, C-24) (values with the same superscripts are interchangeable).

5.2. Acetylation of kallolide C (12)

A solution of kallolide C (5.0 mg) in 1:1 acetic anhydride/pyridine (2 mL) was stirred at 25 °C for 24 h. The reaction mixture was concentrated in vacuo and the oily residue obtained was purified by silica gel column chromatography with 100% $CHCl_3$ to afford pure kallolide C diacetate (**17**) (4.5 mg, 73% yield).

5.3. Acetylation of kallolide E (4)

A solution of kallolide E (**3**) (2.5 mg) in 1:1 acetic anhydride/pyridine (4 mL) was stirred at 25 °C for 18 h. The reaction mixture was concentrated in vacuo and the oily residue obtained was purified by silica gel column chromatography with 1% acetone in $CHCl_3$ to afford pure kallolide E diacetate (**18**) (2.8 mg, 91% yield).

5.3.1. Data for kallolide E diacetate (18). Colorless needles; $[\alpha]_D^{20}$ +29.8 (c 0.8, $CHCl_3$); IR (thin film) ν_{max} 3074, 2946, 1757, 1731, 1654, 1228 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.47 (ddd, 1H, J=12.0, 7.0, 3.5 Hz, H-1), 5.50 (d, 1H, J=12.0 Hz, H-2), 3.47 (br s, 1H, H-7), 5.09 (br s, 1H, H-8), 7.22 (br s, 1H, H-9), 2.53 (br d, 1H, J=13.2 Hz, H-11a), 2.16 (m, 1H, H-11b), 1.84 (m, 1H, H-12a), 1.40 (ddd, 1H, J=11.0, 6.0, 3.0 Hz, H-12b), 4.87 (s, 1H, H_2-14a), 4.82 (q, 1H, J=1.5 Hz, H_2-14b), 1.69 (br s, 3H, H_3-15), 1.79 (s, 3H, H_3-16), 5.26 (s, 1H, H_2-18a), 5.22 (br s, 1H, H_2-18b), 2.10 (br s, 3H, H_3-19), 1.95 (s, 3H, H_3-22), 1.99 (s, 3H, H_3-24); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 46.7 (CH, C-1), 67.9 (CH, C-2), 172.3 (C, C-3), 117.0 (C, C-4), 196.8 (C, C-5), 99.6 (C, C-6), 53.8 (CH, C-7), 81.1 (CH, C-8), 149.6 (CH, C-9), 130.6 (C, C-10), 25.3 (CH_2, C-11), 27.8 (CH_2, C-12), 144.6 (C, C-13), 113.7 (CH_2, C-14), 18.2 (CH_3, C-15), 6.2 (CH_3, C-16), 140.1 (C, C-17), 119.7 (CH_2, C-18), 24.6 (CH_3, C-19), 172.8 (C, C-20), 169.4 (C, C-21), 20.5 (CH_3, C-22), 167.3 (C, C-23), 21.0 (CH_3, C-24); LR-EIMS m/z [M]$^+$ 444 (24), 403 (6), 384 (8), 342 (9), 279 (23), 246 (8), 167 (46), 149 (100), 113 (16), 83 (23); HR-EIMS m/z calcd for $C_{24}H_{28}O_8$ 444.1784, found 444.1779.

5.4. Acetylation of bipinnapterolide A (13)

A solution of bipinnapterolide A (**13**) (5 mg) in 1:1 acetic anhydride/pyridine (4 mL) was stirred at 25 °C for 28 h. The reaction mixture was concentrated in vacuo and the oily residue obtained was purified by silica gel column chromatography with 2% acetone in $CHCl_3$ to afford pure kallolide I (**8**) (3.4 mg, 61%).

6. X-ray single crystallographic analyses

The X-ray data were collected at 298 K with a diffractometer equipped with a graphite monochromator and Mo Kα radiation (λ=0.71073 Å) using the SMART software. Crystallographic data for **6**, **7**, **8**, **10**, and **18** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under the reference numbers CCDC 275343–275346 and 297500, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

6.1. Crystal data for kallolide G

Kallolide G (**6**) was recrystallized by slow evaporation from a 9:1 MeOH/$CHCl_3$ mixture. $C_{20}H_{24}O_6$, M_r=360.39; orthorhombic; space group $P2_12_12_1$ (No.19); a=9.752(2), b=11.644(3), c=16.827(4) Å, V=1910.8(8) Å^3; Z=4; ρ_{calcd}=1.253 Mg m^{-3}; F_{000}=768; λ(Mo Kα)=0.71073 Å; μ=0.092 mm^{-1}. *Data collection and reduction*: crystal size, 0.19×0.02×0.01 mm^3; θ range, 2.13–25.60°; 10,151 reflections collected, 3593 independent reflections (R_{int}=0.1347), final R indices ($I>2\sigma(I)$): R_1=0.0667, wR_2=0.1296 for 240 variable parameters; GOF=0.842.

6.2. Crystal data for kallolide H

Kallolide H (**7**) was recrystallized by slow evaporation from a 9:1 MeOH/$CHCl_3$ mixture. $C_{22}H_{26}O_6$, M_r=386.43; orthorhombic; space group $P2_12_12_1$ (No.19); a=8.389(2), b=10.674(2), c=23.143(5) Å, V=2073.3(7) Å^3; Z=4; ρ_{calcd}=1.239 Mg m^{-3}; F_{000}=824; λ(Mo Kα)=0.71073 Å; μ=0.090 mm^{-1}. *Data collection and reduction*: crystal size, 0.17×0.08×0.06 mm^3; θ range, 1.76–28.06°; 14,497 reflections collected, 4994 independent reflections (R_{int}=0.1066), final R indices ($I>2\sigma(I)$): R_1=0.0453, wR_2=0.0870 for 257 variable parameters; GOF=0.712.

6.3. Crystal data for kallolide I

Kallolide I (**8**) was recrystallized by slow evaporation from a 9:1 MeOH/$CHCl_3$ mixture. $C_{22}H_{26}O_7$, M_r=402.43; orthorhombic; space group $P2_12_12_1$ (No.19); a=8.763(2), b=10.798(3), c=22.621(6) Å, V=2140.4(10) Å^3; Z=4; ρ_{calcd}=1.249 Mg m^{-3}; F_{000}=856; λ(Mo Kα)=0.71073 Å; μ=0.093 mm^{-1}. *Data collection and reduction*: crystal size, 0.05×0.04×0.02 mm^3; θ range, 1.80–25.09°; 8993 reflections collected, 3760 independent reflections (R_{int}=0.1305), final R indices ($I>2\sigma$(I)): R_1=0.0503, wR_2=0.07001 for 266 variable parameters; GOF=0.800.

6.4. Crystal data for 2-*O*-ethylkallolide A

Compound **10** was recrystallized by slow evaporation from diethyl ether. $C_{22}H_{28}O_4$, M_r=356.44; orthorhombic; space group $P2_12_12_1$ (No.19); a=9.690(2), b=12.498(2), c=16.566(2) Å, V=2006.3(5) Å^3; Z=4; ρ_{calcd}=1.180 Mg m^{-3}; F_{000}=768; λ(Mo Kα)=0.71073 Å; μ=0.080 mm^{-1}. *Data collection and reduction*: crystal size, 0.30×0.22×0.20 mm^3; θ range, 2.04–27.52°; 11,423 reflections collected, 4456 independent reflections (R_{int}=0.0304), final R indices ($I>2\sigma$(I)): R_1=0.0383, wR_2=0.0948 for 240 variable parameters; GOF=1.033.

6.5. Crystal data for kallolide E diacetate (18)

Compound **18** was recrystallized by slow evaporation from a 3:1 CH_3CN/H_2O mixture. $C_{24}H_{28}O_8$, M_r=444.46; orthorhombic; space group $P2_12_12_1$ (No.19); a=7.4417(17), b=13.246(4), c=24.041(5) Å, V=2369.7(10) Å^3; Z=4; ρ_{calcd}=1.246 Mg m^{-3}; F_{000}=944; λ(Mo Kα)=0.71073 Å; μ=0.093 mm^{-1}. *Data collection and reduction*: crystal size, 0.38×0.05×0.02 mm^3; θ range, 2.29–23.27°; 11,537 reflections collected, 3405 independent reflections (R_{int}=0.0680), final R indices ($I>2\sigma(I)$): R_1=0.0404, wR_2=0.0620 for 294 variable parameters; GOF=0.842.

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16. NOESY correlations for kallolide H (**7**): H-1/H-14β, H_3-15; H-2/H-9, H-12αβ; H-5/H_3-16, H_3-19; H-7/H-8, H-9, H_3-19; H-8/H-9; H-9/H-11; H-11/H-12αβ; H-12α/H-12β, H_3-15; H-14α/H-14β, H_3-15; H-18α/H-18β, H_3-19.
17. NOESY correlations for kallolide I (**8**): H-1/H-2, H-12α, H-14α; H-2/H-12αβ, H_3-15; H-5/H-7, H_3-16; H-7/H-9, H-18α, H_3-19; H-8/H-9, H_3-19; H-9/H-12α; H-11α/H-11β, H-12α; H-11β/H-12β, H-14β; H-12α/H-12β; H-14β/H_3-15; H-18α/H-18β, H_3-19; H-18β/H_3-19.
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C-4′ Truncated carbocyclic formycin derivatives

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Abstract—Formycin is a naturally occurring C-glycoside (C-nucleoside) that possesses antitumor, antibacterial, antifungal, and antiviral activity. In connection with our ongoing interest in the design and syntheses of C-nucleoside derived antiviral agents this report describes the preparation of carbocyclic formycin and its 7-hydroxy (oxo) analog lacking the C-4′ hydroxylmethylene moiety in racemic form (**4** and **6**, respectively). An antiviral analysis of (±)-**4** did not disclose any activity.

1. Introduction

Aristeromycin (**1**) and formycin A (**2**) (Fig. 1) are two examples of naturally occurring nucleosides that possess significant antiviral activity.[1] Aristeromycin belongs to the carbocyclic nucleoside class and formycin A and formycin B (**3**) (Fig. 1) are representatives of C-nucleosides. In the former case, the ribofuranose oxygen of the more common nucleosides is replaced by a methylene while, with C-nucleosides, the ribofuranosyl moiety is linked to the heterocyclic base by a carbon–carbon bond at the anomeric center. Both carbocyclic and C-nucleosides are stable to purine nucleoside phosphorylase (PNP),[1a,2] an enzyme that renders typical nucleosides with limited potential as therapeutic agents.[2] However, the high toxicity associated with these compounds has restricted their development.

In our ongoing search for new antiviral agents, we sought compounds that combined the structural features of **1**–**3** that could improve their antiviral profiles and show less cytotoxicity. Compound **4** was chosen as a target because

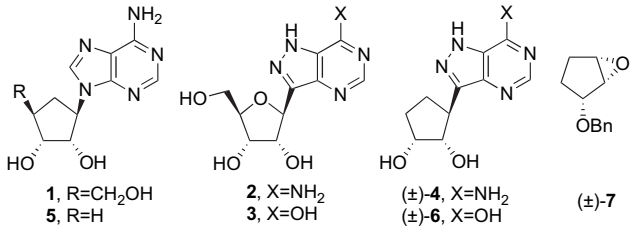

Figure 1.

of its isomeric relationship to **5**,[3a] which is an antiviral candidate with activity towards the orthopoxviruses[3b,c] and human cytomegalovirus[3d] by apparent virtue of its inhibition of *S*-adenosylhomocysteine hydrolase.[3a]

While the synthesis of carbocyclic C-nucleosides has been challenging[4,5] our laboratory previously communicated a model synthesis for a formycin representative.[6] Herein, we report further progress in this endeavor, leading to a novel and practical synthesis of (±)-**4** and its companion (±)-**6** (Fig. 1).

2. Chemistry

The syntheses began with ring opening of the readily available (±)-*cis*-3-(benzyloxy)-1,2-epoxycyclopentane (**7**)[7] by the lithiated derivative of 3,3-diethoxy-1-propyne (**8**, commercially available) in the presence of boron trifluoride etherate[8] to provide (±)-**9** (21%, 71% based on recovered starting material) (Scheme 1). Attempts to improve the yield by using either excess **8** or alternative solvents such as toluene, hexamethylphosphoramide, methylene chloride or co-solvents (such as tetrahydrofuran/hexamethylphosphoramide) were unsuccessful.

Hydrolysis of (±)-**9** with a mixture of acetic acid and 10% aqueous hydrochloric acid followed by treatment of the resulting acetylenic aldehyde with hydrazine monohydrate gave a pyrazole derivative[2,9] that was acetylated to provide the key synthetic intermediate (±)-**10** (Scheme 1). To introduce the fused pyrimidine N-4, nitration of (±)-**10** with ammonium nitrate and trifluoroacetic anhydride in trifluoroacetic acid following literature conditions[10] was attempted. However, the major component in the product mixture (by ^{1}H NMR) possessed a nitrated benzyl unit. Thus, replacing the benzyl protecting group of (±)-**10** with an acetyl to give (±)-**11** led to (±)-**12** that was, in turn, converted into (±)-**13** by a *cine*-substitution reaction with potassium

Keywords: Formycin; Carbocyclic C-nucleosides; Epoxide ring opening; Antiviral testing.
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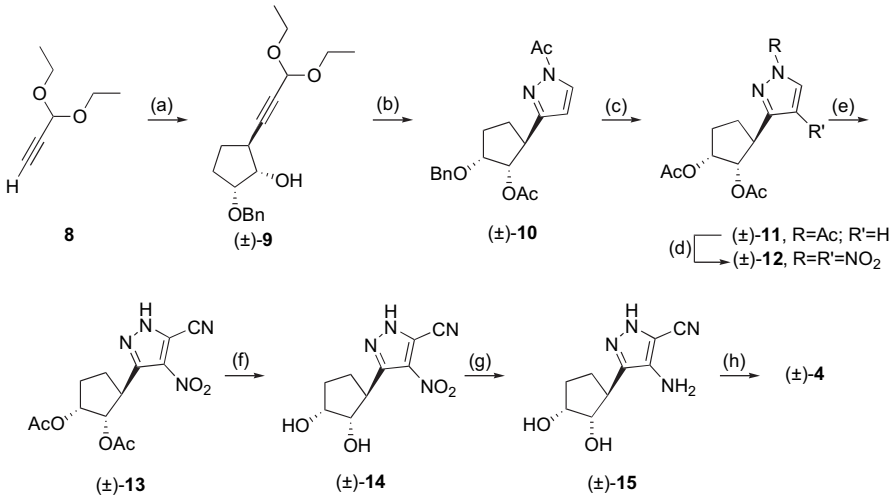
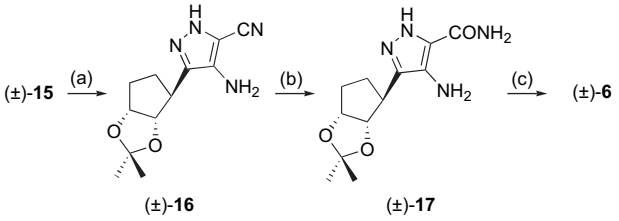


Scheme 1. Reagents and conditions: (a) i. *n*-BuLi/hexanes; ii. (±)-(**7**), $BF_3 \cdot Et_2O$, 71% (based on recovered starting material); (b) i. 10% HCl, AcOH; ii. $N_2H_4 \cdot H_2O$, AcOH; iii. Ac_2O, pyridine, DMAP, 65%; (c) i. 50 psi H_2, 10% Pd/C, MeOH, ii. Ac_2O, Et_3N, DMAP, CH_2Cl_2, 96%; (d) NH_4NO_3, TFA, TFAA; (e) using **12**, KCN, EtOH, EtOAc, 89%; (f) NH_3, MeOH, 95%; (g) 30 psi H_2, 10% Pd/C, MeOH, 92%; (h) $HC(=NH)NH_2 \cdot AcOH$, EtOH, 71%.

cyanide.[11] Deacetylation to (±)-**14** followed by hydrogenation in the presence of palladium/carbon afforded a quantitative amount of (±)-**15**. Treatment of (±)-**15** with formamidine acetate in refluxing ethanol proceeded with ring annulation to (±)-**4**.

Steps towards (±)-**6** (Scheme 2) began with unsuccessful attempts to carry out hydration of (±)-**15** using hydrogen peroxide in methanol to afford a requisite amide. Success was achieved, however, by protecting the hydroxyl groups of (±)-**15** with 2,2-dimethoxypropane (to (±)-**16**) (Scheme 2). Subsequent hydration of (±)-**16** provided amide (±)-**17**. Cyclization of (±)-**17** with formamidine acetate and deprotection yielded (±)-**6**.



Scheme 2. Reagents and conditions: (a) concd HCl, acetone, $Me_2C(OMe)_2$, 98%; (b) H_2O_2, NH_4OH, MeOH, 50%; (c) i. $HC(=NH)NH_2 \cdot AcOH$, EtOH; ii. 1 N HCl, 56% for two steps.

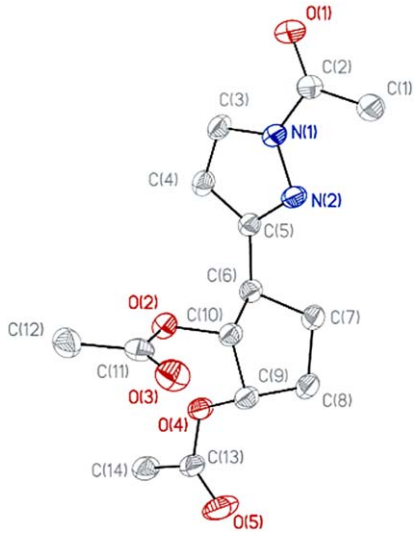


Figure 2. X-ray structure for compound (±)-**11**.[14]

To confirm the stereochemical orientations of the cyclopentyl ring substituents in these reactions an X-ray structure determination of (±)-**11** was obtained (Fig. 2).

3. Antiviral results

Compound (±)-**4** was subjected to broad antiviral analysis and was found to be inactive.[12,13] Weak cellular cytotoxicity was observed towards four of the host cell lines: MA-104, respiratory syncytial virus assay (IC_{50} 54 μg/mL); HeLa Ohio-1, rhinovirus type 2 assay (IC_{50} 80 μg/mL); BS-C-1, Pichinde assay (IC_{50} 53 μg/mL); and CV-1, measles assay (IC_{50} 76 μg/mL).

4. Conclusion

The antiviral potential[3b,c,d] of previously reported chiral **5**[3a] does not extend to isomer **4** in its racemic form. At this time, such an observation does not encourage us to seek an enantiospecific synthesis of the enantiomers represented by (±)-**4**. However, the synthetic process[6] elaborated upon herein opens an accessible means to carbocyclic C-nucleosides of potential usefulness in biochemical studies requiring novel adenosine isomers.

5. Experimental

5.1. General

Melting points were recorded on a Meltemp II melting point apparatus and the values are uncorrected. The combustion analyses were performed at Atlantic Microlab, Norcross,

GA. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AC 250 spectrometer (250 MHz for proton and 62.5 MHz for carbon) or a Bruker AV 400 spectrometer (400 MHz for proton and 100 MHz for carbon), referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The X-ray analysis was conducted by using a Bruker APEX CCD single crystal X-ray diffractometer. The electrospray ionization mass spectral data were obtained using a Waters Micromass QTOF mass spectrometer. The reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp. Column chromatography was performed on Whatman silica, 230–400 mesh, and 60 Å using elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

5.1.1. (±)-(1α,2α,3β)-1-Benzyloxy-2-acetoxy-3-(1-acetyl-1*H*-pyrazol-3-yl)cyclopentane (10). To a solution of 3,3-diethoxy-1-propyne (97%) (6.3 g, 48 mmol) in anhydrous toluene (130 mL) was added *n*-butyllithium (2.5 M solution in hexanes, 20 mL, 50.0 mmol) at −78 °C under an atmosphere of N_2. After the reaction mixture was stirred for ca. 30 min, a solution of epoxide (±)-**7** (7.3 g, 38 mmol) in anhydrous toluene (10 mL) was added via syringe, followed by $BF_3\cdot OEt_2$ (6.3 mL, 50 mmol) over 5 min. The reaction mixture was stirred at −78 °C for 2 h and then at 0–5 °C for additional ca. 5 h. The reaction mixture was quenched by addition of saturated aqueous NH_4Cl solution and partitioned between EtOAc and H_2O. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and the filtrate evaporated in vacuo to afford a liquid residue. Purification of the residue by flash column chromatography (hexanes/EtOAc, 4:1 to 3:1) led to recovered starting material (5.1 g, 27 mmol) and (±)-(1α,2α,3β)-1-benzyloxy-2-hydroxy-3-(3,3-diethoxy-prop-1-ynyl)cyclopentane (**9**) (2.6 g, 8.2 mmol, 71% based on recovered starting material) as a light yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (m, 6H), 1.64 (m, 1H), 1.78 (m, 1H), 1.96 (m, 1H), 2.16 (m, 1H), 2.83 (m, 2H), 3.56 (m, 2H), 3.72 (m, 2H), 3.95 (m, 1H), 4.03 (m, 1H), 4.50 (d, 1H, *J*=11.7 Hz), 4.60 (d, 1H, *J*=11.7 Hz), 5.28 (d, 1H, *J*=1.5 Hz), 7.29–7.35 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5, 27.9, 36.2, 59.1, 61.4, 63.4, 72.0, 78.3, 80.1, 88.0, 91.9, 128.1, 128.3, 128.9, 138.3.

To a solution of **9** (2.3 g, 7.2 mmol) in glacial AcOH (72 mL) was added 10% HCl (18 mL), and this mixture was stirred at room temperature for 1 h. To this a solution of hydrazine monohydrate (4.5 g) in glacial AcOH (36 mL) was added dropwise over 20 min. The resulting solution was heated at reflux overnight and concentrated in vacuo to afford dark brown oil. The crude product was dissolved in pyridine (100 mL) and Ac_2O (8 mL) and DMAP were added. The resulting solution was stirred for 16 h at room temperature. The solvent was removed in vacuo, and the crude residue dissolved in EtOAc (800 mL), washed with 10% HCl and brine, dried (Na_2SO_4), concentrated, and the residue chromatographed to afford **10** (1.6 g, 4.7 mmol, 65%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (m, 1H), 1.94 (m, 1H), 2.04 (m, 1H), 2.09 (s, 3H), 2.25 (m, 1H), 2.64 (s, 3H), 3.50 (ddd, 1H, *J*=8.4 Hz), 4.16 (ddd, 1H, *J*=4.9 Hz), 4.55 (s, 2H), 5.20 (dd, 1H, *J*=4.9, 7.6 Hz), 6.30 (d, 1H, *J*=2.9 Hz), 7.26 (m, 1H), 7.30 (m, 4H), 8.16 (d, 1H, *J*=2.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 21.8, 25.9, 28.7, 41.1, 71.9, 78.4, 78.8, 108.8, 127.6, 127.7, 128.5, 129.0, 138.5, 158.5, 169.5, 170.8. Anal. calcd for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.73; H, 6.56; N, 7.96.

5.1.2. (±)-(1α,2α,3β)-1,2-Diacetoxy-3-(1-acetyl-1*H*-pyrazol-3-yl)cyclopentane (11). To a solution of **10** (5.0 g, 12 mmol) in MeOH (60 mL) was added catalytic amount of 10% Pd/C. The mixture was shaken under 50 psi of H_2 at room temperature overnight. After this period, the mixture was filtered, and the filtrate concentrated in vacuo to afford a colorless oil. This crude product was dissolved in CH_2Cl_2 (100 mL) and to this triethylamine (1.4 mL), Ac_2O (2.3 mL), and catalytic amount of DMAP were added. The resulting solution was stirred overnight and then washed with brine, dried (Na_2SO_4), concentrated, and chromatographed (EtOAc/hexanes, 1:3) to afford **11** (3.3 g, 11 mmol, 96%) as a white solid, mp 79–81 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (m, 2H), 2.03 (s, 3H), 2.09 (s, 3H), 2.31 (m, 2H), 2.66 (s, 3H), 3.48 (ddd, 1H, *J*=8.9 Hz), 5.26 (m, 1H), 5.40 (m, 1H), 6.32 (d, 1H, *J*=2.8 Hz), 8.17 (d, 1H, *J*=2.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.0, 21.2, 21.9, 26.1, 27.0, 28.3, 40.7, 73.4, 77.8, 108.6, 129.3, 157.8, 169.6, 170.5. Anal. calcd for $C_{14}H_{18}N_2O_5$: C, 57.13; H, 6.16; N, 9.52. Found: C, 57.41; H, 6.24; N, 9.50.

5.1.3. (±)-(1α,2α,3β)-1,2-Diacetoxy-3-(5-cyano-4-nitro-1*H*-pyrazol-3-yl)cyclopentane (13). Trifluoroacetic anhydride (15.5 mL) was added dropwise to a stirred solution of **11** (3.3 g, 11 mmol) containing ammonium nitrate (8.9 g) in trifluoroacetic acid (130 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and then stirred overnight. The solvent was evaporated by means of a rotavapor. The residue was placed in CH_2Cl_2 and then washed with H_2O, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated in vacuo to give the **12** (3.8 g) as a white solid that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (m, 2H), 1.99 (s, 3H), 2.11 (s, 3H), 2.27 (m, 1H), 2.49 (m, 1H), 4.09 (ddd, 1H, *J*=8.5 Hz), 5.42 (m, 1H), 5.52 (m, 1H), 9.12 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 21.0, 26.3, 28.3, 39.6, 73.3, 76.5, 125.3, 134.3, 149.1, 170.2 (2C).

A solution of the **12** (3.8 g) prepared above in EtOH (36 mL) and EtOAc (36 mL) was added dropwise to a stirred solution of KCN (5.2 g, 80 mmol) in EtOH (95 mL) and H_2O (23 mL). Following an additional 5 min at room temperature, the reaction mixture was neutralized with HOAc (8.5 mL). After evaporation of the solvent, the residue was diluted with EtOAc (500 mL), washed with H_2O and brine, dried (Na_2SO_4), concentrated in vacuo, and chromatographed (CH_2Cl_2/MeOH, 20:1) to afford **13** (3.2 g, 10 mmol, 89% based on **11**) as a white solid, mp 129–131 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (m, 1H), 2.03 (m, 1H), 2.05 (s, 3H), 2.14 (m, 1H), 2.16 (s, 3H), 2.32 (m, 1H), 2.59 (m, 1H), 4.23 (m, 1H), 5.48 (m, 1H), 5.53 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 21.2, 26.7, 28.2, 38.6, 73.0, 75.8, 110.8, 123.0, 134.1, 144.9, 171.2, 172.1. Anal. calcd for $C_{13}H_{14}N_4O_6$: C, 48.45; H, 4.38; N, 17.38. Found: C, 48.16; H, 4.31; N, 17.42.

5.1.4. (±)-(1α,2α,3β)-1,2-Dihydroxy-3-(5-cyano-4-nitro-1*H*-pyrazol-3-yl)cyclopentane (14). Ammonia gas was introduced to a solution of compound **13** (2.0 g, 6.2 mmol) in MeOH (100 mL). This reaction mixture was allowed to stir at room temperature until TLC analysis indicated starting material was no longer present. The solvent was then removed in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH, 3:1) to afford **14** (1.4 g, 5.9 mmol, 95%) as a light yellow solid, mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.63 (m, 2H), 1.97 (m, 1H), 2.15 (m, 1H), 3.80 (m, 1H), 3.96 (m, 1H), 4.10 (m, 1H), 4.68 (br s, 1H), 4.91 (br s, 1H), 14.81 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.0, 29.8, 39.2, 71.6, 77.9, 112.0, 121.8, 133.7, 147.7. Anal. calcd for $C_9H_{10}N_4O_4$: C, 45.38; H, 4.23; N, 23.52. Found: C, 45.43; H, 4.32; N, 23.43.

5.1.5. (±)-(1α,2α,3β)-1,2-Dihydroxy-3-(4-amino-5-cyano-1*H*-pyrazol-3-yl)cyclopentane (15). A catalytic amount of 10% Pd/C was added to a solution of **12** (1.23 g, 5.20 mmol) in MeOH (100 mL). The resulting mixture was shaken under 30 psi of H_2 overnight. The solvent was then evaporated in vacuo and the product purified by chromatography (CH_2Cl_2/EtOAc/MeOH, 8:1:0.5) to afford **15** as a white solid (1.0 g, 4.8 mmol, 92%), mp 190–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56 (m, 2H), 1.97 (m, 2H), 3.08 (dd, 1H, J=8.9, 17.8 Hz), 3.79 (m, 1H), 3.88 (br s, 1H), 4.36 (br s, 2H), 4.52 (br s, 1H), 4.69 (d, 1H, J=6.3 Hz), 12.97 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.9, 30.1, 38.2, 71.4, 78.2, 114.1, 115.3, 130.9, 133.1. Anal. calcd for $C_9H_{12}N_4O_2$: C, 51.92; H, 5.81; N, 26.91. Found: C, 52.10; H, 5.93; N, 27.09.

5.1.6. (±)-(1α,2α,3β)-1,2-Dihydroxy-3-[(7-amino-1*H*-pyrazolo[4,3-*d*]pyrimid-3-yl)]cyclopentane (4). A solution of **15** (0.15 g, 0.72 mmol) in EtOH (30 mL) was stirred with formamidine acetate (0.10 g, 0.96 mmol) under reflux for 30 min. The resulting white precipitate was isolated by filtration, washed with EtOH, and dried to afford **4** (0.12 g, 0.51 mmol, 71%) as a white solid, mp 261–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66 (m, 1H), 1.90 (m, 1H), 1.99 (m, 1H), 2.09 (m, 1H), 4.02 (br s, 1H), 4.23 (br s, 1H), 4.40 (m, 2H), 4.91 (br s, 1H), 7.25 (br s, 2H), 8.15 (br s, 1H), 12.4 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.2, 30.3, 41.4, 66.4, 72.0, 77.9, 121.9, 139.5, 147.2, 150.8. HRMS calcd for $C_{10}H_{13}N_5O_2$ $[M+H]^+$ 236.1147, found 236.1141.

5.1.7. (±)-(1α,2α,3β)-1,2-*O*-Isopropylidene-3-(4-amino-5-cyano-1*H*-pyrazol-3-yl)cyclopentane (16). Concentrated HCl (0.21 mL) was added to a stirred suspension of **15** (590 mg, 2.83 mmol) in dry acetone (60 mL) and 2,2-dimethoxypropane (60 mL) causing immediate clarification. After 4 h at room temperature, the solution was neutralized by addition of concentrated NH_4OH and evaporated to dryness. The residue was purified by chromatography (CH_2Cl_2/MeOH, 19:1) to give 690 mg (2.78 mmol, 98%) of **16** as a white solid, mp 148.5–149.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (s, 3H), 1.39 (s, 3H), 1.61 (m, 2H), 1.75 (m, 1H), 2.02 (m, 1H), 3.24 (m, 1H), 4.69 (m, 4H), 12.9 (br s, 1H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 15.2, 24.2, 26.6, 28.1, 30.9, 40.7, 65.0, 80.3, 83.9, 109.6, 115.0, 132.0. Anal. calcd for $C_{12}H_{16}N_4O_2$: C, 58.05; H, 6.50; N, 22.57. Found: C, 58.32; H, 6.85; N, 22.89.

5.1.8. (±)-4-Amino-5-[(1β,2α,3α)-2,3-*O*-isopropylidene-cyclopent-1-yl]-2*H*-pyrazole-3-carboxylic acid amide (17). Hydrogen peroxide (50%, 0.19 mL) was added to a rapidly stirred mixture of **16** (570 mg, 2.30 mmol) in concentrated NH_4OH (10 mL) and MeOH (20 mL) at 0 °C. The solution was allowed to stir at room temperature overnight. After the solvent was evaporated, the residue was purified by chromatography (EtOAc/MeOH, 9:1) to afford **17** (310 mg, 1.16 mmol, 50%) as an off-white solid, mp 191.6–192.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26 (s, 3H), 1.41 (s, 3H), 1.65 (m, 1H), 1.76 (m, 2H), 2.02 (m, 1H), 3.23 (m, 1H), 4.43 (br s, 1H), 4.62 (m, 1H), 4.71 (m, 2H), 7.22 (br s 2H), 12.03 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.0, 26.4, 27.4, 31.0, 41.1, 80.6, 84.0, 108.7, 120.4, 131.3, 141.4, 161.9. Anal. calcd for $C_{12}H_{18}N_4O_3$: C, 54.12; H, 6.81; N, 21.04. Found: C, 53.98; H, 6.98; N, 20.81.

5.1.9. (±)-(1α,2α,3β)-1,2-Dihydroxy-3-[(7-hydroxy-1*H*-pyrazolo[4,3-*d*]pyrimid-3-yl)]cyclopentane (6). A solution of **17** (0.26 g, 0.98 mmol) in EtOH (50 mL) was stirred with formamidine acetate (0.14 g, 1.3 mmol) under reflux for 40 min. The resulting white precipitate was isolated by filtration and washed with EtOH. This white solid was dissolved in 1 N HCl (5 mL). The reaction mixture was allowed to stir at room temperature for 3 h. The quantity of solvent was reduced and the mixture was then neutralized with NH_4OH to afford **6**, upon filtration, as a white solid (0.13 g, 0.55 mmol, 56%), mp 245–246 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62 (m, 1H), 1.80 (m, 1H), 1.97 (m, 1H), 2.07 (m, 1H), 3.99 (s, 1H), 4.17 (s, 1H), 4.43 (s, 1H), 4.62 (m, 1H), 7.81 (s, 1H), 12.18 (br s, 2H), 13.77 (s, 1H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 26.7, 30.3, 41.2, 72.0, 77.9, 127.3, 137.1, 142.0, 148.2, 153.2. HRMS calcd for $C_{10}H_{12}N_4O_3$ (M^++H) 237.0987, found 237.0980.

5.1.10. X-ray data for compound (±)-11. Crystallographic data (excluding structure factors) for (±)-**11** has been deposited with Cambridge Crystallographic Data Centre as supplementary number CCDC 284037. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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14. To simplify viewing the ORTEP representation in Figure 2 the hydrogen atoms are omitted. However, the hydrogen atom on C(6) is oriented down while hydrogens on C(9) and C(10) are up.

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Theoretical study of the regioselectivity of the cycloaddition reaction between cyclopentadiene and methyleneketene

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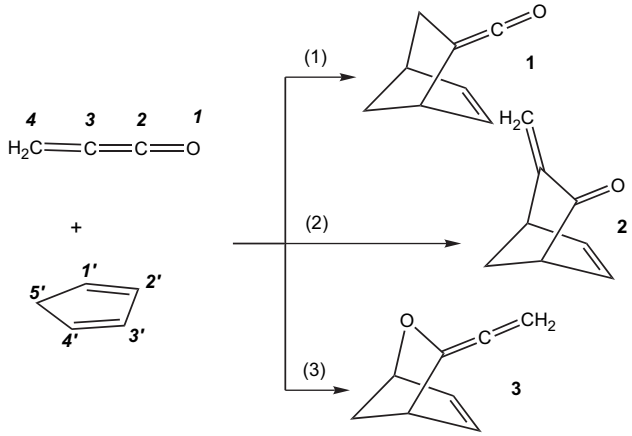
Abstract—Three possible reaction schemes for the cycloaddition reaction between methyleneketene and cyclopentadiene were studied by DFT (density functional theory) and ab initio calculations. All of these cycloaddition reactions are exothermic, concerted but nonsynchronous processes. The computed activation energies indicate that the norbornene product yielded from a 1,2-addition of methyleneketene with cyclopentadiene (reaction (1)) is the primary product. The performance of various computational methodologies, MP2, MP4, and CCSD(T), in conjunction with a wide array of basis sets, 6-31G(d), 6-311+G(d,p), aug-cc-pVDZ, and aug-cc-pVTZ, in obtaining reliable activation and reaction energies of the reactions under investigation has been critically analyzed.

1. Introduction

Heterocumulenes are highly versatile synthetic intermediates, and unlike most unsaturated compounds, they undergo either thermal [2+2] or [2+4] cycloadditions readily. This notion generates a great interest in the industrial[1] and pharmaceutical applications.[2,3] A special class of heterocumulenes, based on parent methyleneketene ($H_2C{=}C{=}C{=}O$) and its substituted derivatives, might exhibit different reaction preferences than the other previously studied ketenes.[4,5] It is known that ketenes react with cyclopentadiene in a [2+2] process forming cyclobutanones (the Staudinger reaction) rather than in a [4+2] reaction leading to norbornenones.[6–9] So far based on the studied cycloaddition of methyleneketene one assumes that their behavior is analogous to that of ketene.[10] However, a single example of the parent methyleneketene undergoing a [4+2] cycloaddition had been reported, and the only cycloaddition product, which takes place on the interior C=C double bond of methyleneketene with 5-methylene-1,3-dioxan-4,6-dione, had been experimentally and theoretically studied.[11]

The possibility of another [4+2] cycloaddition for methyleneketene, i.e., the reaction between methyleneketene and cyclopentadiene, was revealed during our research devoted to the study of the cycloaddition of cumulene.[11b,12] The cycloaddition between methyleneketene and cyclopentadiene is expected to yield a norbornene product, rather than norbornanone or 5-oxobicyclo[2.2.1]hept-2-ene. In spite of the synthetic interest in these products,[13] no theoretical study regarding this reaction has been reported. Therefore the mechanisms of these types of reactions require detailed investigation. In this paper, the possible reaction mechanisms of the [4+2] cycloaddition between methyleneketene and cyclopentadiene were theoretically studied in the gas phase and polar solvents by means of ab initio methods and density functional theory.

Due to its specific structure, methyleneketene provides three double bonds on which three possible [4+2] reactions with cyclopentadiene may take place (Scheme 1), i.e., cycloaddition with cyclopentadiene may involve either the C=C or the C=O bonds of methyleneketene: addition to the C=C double bond to give either norbornene derivative **1**



Scheme 1.

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(bicyclo[2.2.1]hept-5-en-2-ylidene-methanone) or norbornanone derivative **2** (3-methylene-bicyclo[2.2.1]hept-5-en-2-one), or less likely addition to the C═O double bond to give 5-oxobicyclo[2.2.1]hept-2-ene derivative **3** (3-vinylidene-2-oxa-bicyclo[2.2.1]hept-5-ene).[14–16] All these possible products may have potential industrial and pharmaceutical applications.[2,17] Thus, the investigation of the regioselectivity of cycloaddition between methyleneketene and cyclopentadiene is of great interest.

2. Computational method

The geometry of the reactants, products, and transition states were optimized at the B3LYP level of theory using the 6-31G(d) basis set by means of the Berny approach, a modified Schlegel method.[18] Harmonic vibration frequency calculations were performed to confirm whether the obtained geometry represents a transition or minimum energy structure.

Inclusion of the correlation energy is necessary for a reasonable prediction of the activation energy because these contributions are often larger for the transition structures than for the minimum energy structures. In this study the energies were evaluated at the MP2/6-31G(d) level of theory using the B3LYP/6-31G(d) optimized structures. The geometry of the reactants, products, and transition states were also re-optimized at the MP2/6-31G(d) level. Entropies and enthalpies of activation were obtained at the B3LYP/6-31G(d) and MP2/6-31G(d) levels.

To better account for the effects of electron correlation, the activation and reaction energies were also evaluated at MP4, CCSD, and CCSD(T) using the geometries optimized at the MP2/6-31G(d) level.

The 6-31G(d) basis set was used for the geometrical optimization. To assess the basis set effect on the activation and reaction energies, a wide array of basis sets, namely, 6-31G(d), 6-311+G(d,p), aug-cc-pVDZ, and aug-cc-pVTZ, was used at the B3LYP and MP2 levels of theory.

The reaction and activation energies in the solvents were also evaluated at the B3LYP/6-31G(d) level by the polarized continuum model applying the integral equation formalism[19] using the geometries optimized in the gas phase. In this model, the liquid is represented by a dielectric continuum, characterized by its dielectric constant ε. The solute is placed in a cavity created in the continuum. The dielectric constants of ε=8.93 and 20.7 (that correspond to dichloromethane and acetone) were used. The specific interactions, such as hydrogen bonding are not covered by this model.

All calculations were performed using GAUSSIAN03.[20]

3. Results and discussion

3.1. Reactants and products

The qualitative picture obtained from the valence-bond diagrams and the quantitative information from the molecular orbital calculations can provide guidance for the interpretation of the behavior of methyleneketene. One of the first studies of such species were performed by Radom who calculated the π-electron distributions perpendicular to the molecular plane for the various atoms in methyleneketene using ab initio molecular orbital theory.[21]

It is well-known that methyleneketene is not linear, unlike the cumulene $H_2C{=}C{=}C{=}CH_2$. The B3LYP/6-31G(d)- and MP2/6-31G(d)-optimized geometrical parameters as well as the experimental data obtained from the microwave spectra of several isotopic species of methyleneketene[22] are shown in Figure 1. One can see that the geometrical parameters predicted at the B3LYP/6-31G(d) level are in excellent agreement with the previous microwave measurement and theoretical studies.[23] Interestingly, the B3LYP/6-31G(d) parameters are in better agreement with the experiments than those obtained from the MP2 level calculations.

The structures of three products of the Diels–Alder reaction between methyleneketene and cyclopentadiene are depicted in Figure 1. The reaction energies at the different levels of theory are summarized in Table 1. All of the reactions are exothermic. The reaction energies at the B3LYP/6-31G(d) level amount to 35.1 and 42.0 kcal/mol for reactions (1) and (2), respectively. The reaction energy for reaction (3) at the B3LYP/6-31G(d) level is predicted to be 7.7 kcal/mol; this suggests that the addition to the C═O double bond is very unfavorable compared to the additions to the C═C double bonds of methyleneketene.

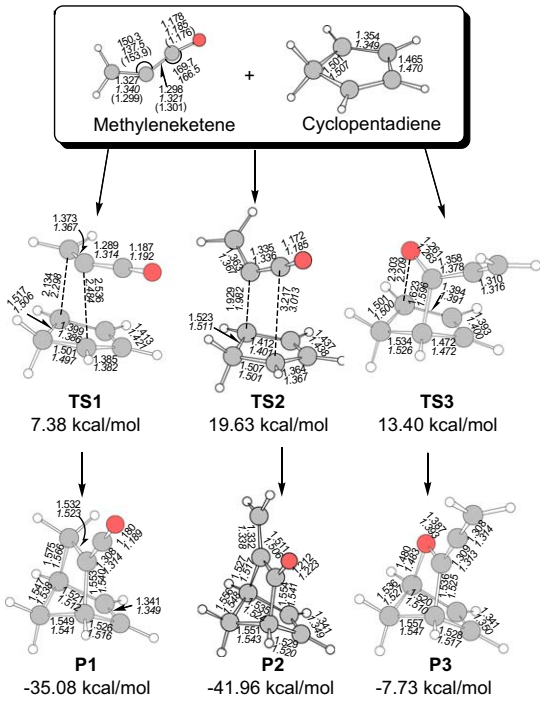

Figure 1. The B3LYP/6-31G(d)-optimized geometrical parameters for the reactants, transition structures, and products. For comparison, the MP2/6-31G(d)-optimized geometrical parameters are listed in italic. The experimental determined geometrical parameters for methyleneketene are shown in the parenthesis. The relative energies were obtained at the B3LYP/6-31G(d) level.

Table 1. The activation and reaction energies at the different levels of theory for the stationary points located on the [4+2] cycloaddition between methyleneketene and cyclopentadiene

	B3LYP[a]		MP2[b]		MP4(SDTQ)[c]	CCSD[c]	CCSD(T)[c]
	$\Delta E(\Delta E_0)/\Delta E(\Delta E_0)$	ΔH	$\Delta E(\Delta E_0)$	ΔH	ΔE	ΔE	ΔE
TS1	7.38(9.8)/*−2.21(0.2)*	8.8	−2.39(−0.2)	−1.1	2.99	10.26	6.34
TS2	19.63(20.9)/*12.89(14.1)*	20.2	12.92(13.9)	13.3	16.35	24.57	19.45
TS3	13.40(16.0)/*10.24(12.8)*	14.6	9.36(12.1)	10.7	10.44	16.35	11.70
P1	−35.08(−29.4)/*−50.08(−44.4)*	−31.1	−49.71(−43.8)	−45.4	−44.61	−45.66	−43.77
P2	−41.96(−36.6)/*−55.92(−50.6)*	−38.3	−55.51(−50.2)	−51.8	−51.14	−54.11	−51.98
P3	−7.73(−2.9)/*−15.73(−10.9)*	−4.5	−15.23(−10.4)	−11.9	−11.88	−16.35	−14.32

The energies are in kcal/mol.
[a] B3LYP/6-31G(d) energy at the B3LYP/6-31G(d) optimized structure. MP2/6-31G(d)//B3LYP/6-31G(d) energy is in italic, the zero-energy corrected energy are in the parenthesis.
[b] MP2/6-31G(d) energy at the MP2/6-31G(d) optimized structure.
[c] Single point energy using 6-31G(d) basis set at the MP2/6-31G(d) optimized structure.

The reaction energies predicted at the MP2, MP4(SDTQ), and CCSD(T) levels of theory are also listed in Table 1. From Table 1, one can see that the reaction energies at the MP2, MP4(SDTQ), and CCSD levels of theory are larger than the CCSD(T) results.

Compared to the CCSD(T) results, B3LYP underestimates the reaction energies. The differences amount to 8.7, 10.0, and 6.6 kcal/mol for reactions (1), (2), and (3), respectively. On the contrary, MP2 and CCSD overestimate the reaction energies by about 2–6 kcal/mol. The substantial differences in reaction energies between the MP2, CCSD, and the CCSD(T) values indicate that the triple substitution makes a measurable contribution to accurate description of the reaction energy.

The MP4-predicted reaction energies are in most cases in agreement with the CCSD(T) reaction energies, and the differences are smaller than 1 kcal/mol, except for the reaction energy for the C═O addition, which differs by 2.4 kcal/mol. These differences can be related to the high-order electron correlation contributions. The reason that MP4 is in the closest agreement with CCSD(T) is due to the close connection between the two methods. The SDQ components of MP4 approximate those of CCSD, while the triple components of MP4 are similar in structure to the CCSD(T) correction. Therefore, MP4 level calculations with single, double, triple, and quadruple substitutions are expected to provide reliable reaction energies.

The reaction energies were also evaluated at the MP2 level using different basis sets. As one can see from Table 2, at the MP2/6-31G(d) level, the reaction energies for the 2,3-C═C, 1,2-C═C, and C═O addition reactions are −49.7, −55.5, and −15.2 kcal/mol, respectively. The further expansion of the basis set to 6-311+G(d,p), and aug-cc-pVTZ has less impact on the accuracy of the reaction energies. The differences in the reaction energies are around 1 kcal/mol when the basis set changes from 6-31G(d) to aug-cc-pVTZ. All data are in good agreement with the CCSD(T) method.

However, the values of reaction energy were significantly affected by the size of the basis set at the B3LYP level. The differences in the reaction energies amount up to 4 kcal/mol at the B3LYP/6-31G(d) level when different basis sets were used. This indicates that B3LYP, unlike the MP2 method, is inadequate for quantitatively describing the reaction energies.

3.2. Transition states

The geometries of three transition states were first optimized at the B3LYP/6-31G(d) level, followed by the MP2/6-31G(d) single point energy evaluation. The activation energies for reactions (1), (2), and (3) are listed in Table 1. There are big differences in the activation energies between the two levels of theory. For instance, the MP2/6-31G(d)-computed activation energy for TS1 is −2.2 kcal/mol, while the B3LYP/6-31G(d)-predicted activation energy is 7.4 kcal/mol. The difference amounts to 9.5 kcal/mol!

Since the different activation values are predicted by B3LYP/6-31G(d) and MP2/6-31G(d) calculations, it is necessary to benchmark the study of the reaction under investigation in order to find a reliable approach to predict the reaction

Table 2. The basis set effect on the activation and reaction energies at the B3LYP and MP2 levels of theory

	B3LYP[a]				MP2[b]			
	6-31G(d)	6-311+G(d,p)	Aug-cc-pVDZ	Aug-cc-pVTZ	6-31G(d)	6-311+G(d,p)	Aug-cc-pVDZ	Aug-cc-pVTZ
TS1	7.4	10.0	8.4	10.9	−2.4	−4.6	−9.1	−7.4
TS2	19.6	22.4	20.2	23.1	12.9	10.5	6.9	9.1
TS3	13.4	17.9	14.3	18.5	9.4	9.9	4.7	6.8
P1	−35.1	−29.5	−32.7	−27.8	−49.7	−49.3	−51.0	−49.3
P2	−42.0	−35.6	−39.5	−34.1	−55.5	−54.1	−57.0	−54.6
P3	−7.7	−1.2	−5.0	−1.0	−15.2	−13.3	−16.5	−16.3

The energies are in kcal/mol.
[a] At the B3LYP/6-31G(d) optimized structure.
[b] At the MP2/6-31G(d) optimized structure.

activation barriers and the structural and electronic features of these kinds of reactions.

3.2.1. Consideration of electron correlation. It is well known that the Hartree–Fock-based methods significantly overestimate the energy barrier, and increasing the basis set quality even worsens the results.[24] In order to obtain the correct energetics, it is very important to include dynamic correlation. Therefore the single point energies at the MP4, CCSD, and CCSD(T) levels using the MP2/6-31G(d) optimized structures were computed and listed in Table 1. The T1 diagnostic values[25] for the transition structures were also calculated at the CCSD level, and small values (<0.018) T1 diagnostic indicates that the coupled cluster method provides reliable estimate of the reaction barrier height.

B3LYP and MP2 take into account the electron correlation in quite different ways. Comparing with the CCSD(T) computed energies, MP2 underestimates the activation barrier for reactions (1) and (2) by about 8.7 and 6.6 kcal/mol, respectively. Thus MP2 is clearly unsuitable for quantitative prediction. This is in agreement with a recent study of regioselectivity, stereoselectivity, and asynchronicity in a series of hetero-Diels–Alder reactions.[26] When full MP4 method with single, double, triple, and quadruple substitutions is considered, the activation energy is slightly improved; however, it becomes underestimated compared to the CCSD(T) results. The MP4 level activation energies are about 3.0 kcal/mol lower than the CCSD(T)-predicted results.

It is interesting to notice that the gradient-corrected hybrid density functional method, B3LYP, has shown excellent agreement with the CCSD(T) results. By comparing the activation energies listed in Table 1, the activation energies obtained at the B3LYP/6-31G(d) level differ from the CCSD(T) energies by only about 1.0, 0.2, and 1.7 kcal/mol for reactions (1), (2), and (3), respectively.

3.2.2. Basis set effect on transition structures. The 6-31G(d) basis set was used throughout for the geometry optimization of the transition states. The further expansion of the basis set has less impact on the accuracy of the molecular parameters.[27,28] Only minor changes of the geometrical parameters were observed in selected organic systems when the basis set was extended in the B3LYP calculations. The 6-31G(d) basis set is a good compromise between efficiency and accuracy.[29] We have also used the 6-311+G(d) and aug-cc-pVDZ basis sets for some of the studied species, and the geometrical parameters are essentially the same.[30] Therefore we will only discuss the geometries obtained from the 6-31G(d) basis set, unless otherwise mentioned. The transition states were also optimized using the MP2/6-31G(d) method; the geometries obtained at the B3LYP/6-31G(d) and MP2/6-31(d) levels are close to each other.

3.2.3. Basis set effect on activation energy. The activation energies computed at the B3LYP and MP2 levels using different basis sets are listed in Table 2. The results demonstrate that the activation energies at the B3LYP level had less variation as a function of the basis set than the MP2 results. B3LYP/6-31G(d) is expected to yield reliable activation energies, while the MP2 method underestimates the activation energy.

In brief, B3LYP/6-31G(d) method predicts reliable geometrical parameters for the reactants, products, and transition structures. Though the level of electron correlation is of importance for quantitative prediction of reaction and activation energies, B3LYP/6-31G(d) method can be still used for qualitative description of the energetics of the reactions under investigation. Therefore, only the B3LYP/6-31G(d) results will be used for further discussion of the reaction mechanism, unless otherwise mentioned.

3.2.4. Discussion. We started with reaction (1) between methyleneketene and cyclopentadiene, which takes place on the C3═C4 bond of methyleneketene. A transition state TS1 (see Fig. 1) was located and characterized. The main geometrical parameters of the stationary point, optimized at the B3LYP/6-31G(d) and MP2/6-31G(d) levels, are shown in Figure 1.

The distances of C4–C1′ and C3–C4′ are 2.134 and 2.536 Å, respectively, at the B3LYP/6-31G(d) level. The C4–C1′ distance obtained at the MP2/6-31G(d) level is slightly longer (2.298 Å), while the C3–C4′ distance is slightly shorter (2.484 Å). The results of both methods indicate that the reaction appears to be concerted but nonsynchronous.

Due to the attack of methyleneketene, the five carbon atoms of cyclopentadiene are no longer on the same plane, the dihedral angle C2′–C3′–C4′–C5′ amounts to −18.1° at the B3LYP/6-31G(d) level. The methyleneketene moiety is almost parallel to the cyclopentadiene moiety plane. The C2–C3–C4 angle amounts to 149.7°, slightly smaller than that in methyleneketene.

Mulliken population shows that methyleneketene donates 0.19 units of negative charge to cyclopentadiene in TS1. The activation barrier for TS1 is predicted to be 7.4 kcal/mol at the B3LYP/6-31G(d) level.

A transition state (TS2) was located and characterized for the cycloaddition reaction with cyclopentadiene taking place on C2═C3 double bond of methyleneketene. The selected geometrical parameters for the optimized transition structure at both the B3LYP/6-31G(d) and MP2/6-31G(d) levels are shown in Figure 2. Reaction (2) is also a concerted but nonsynchronous. The B3LYP/6-31G(d)-predicted C3–C1′ distance amounts to 1.929 Å, much shorter than the C2–C4′ distance (3.217 Å at the B3LYP/6-31G(d) level). Only a 0.03 e charge was transferred from methyleneketene to cyclopentadiene. The activation barrier of this reaction is 19.6 kcal/mol, much higher than reaction (1).

In addition to the two approaching modes considered earlier, the possibility of a cycloaddition reaction with cyclopentadiene taking place on the C═O bond of methyleneketene was also studied. A transition state (TS3) was located. As one can see from Figure 1, the C2–C4′ distance in the transition structure TS3 is predicted to be 1.623 Å at the B3LYP/6-31G(d) level. This indicates that the C2–C4′ bond is almost formed in transition structure TS3. The O1–C1′ bond is not yet formed; the distance between O1 and C1′ is 2.303 Å at the B3LYP/6-31G(d) level. The formation of the C2–C4′ bond in TS3 raises the question of whether reaction (3) is a step-wise reaction. Attempts have been made,

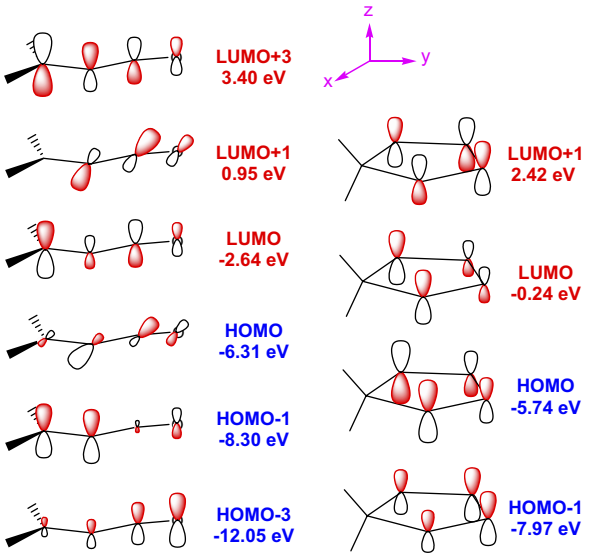

Figure 2. The energy levels of the frontier orbitals at the B3LYP/6-31G(d) level. The sizes of orbital coefficients on each atom of methyleneketene and cyclopentadiene are represented by the sizes of lobes on the orbitals.

however, no other possible transition state or intermediate on the reaction potential surface was located. Moreover, the IRC analysis shows that TS3 is directly connected with the reactants and the final [4+2] cycloaddition product. Therefore, we concluded that it is a concerted but nonsynchronous reaction. Mulliken population analysis shows that there is 0.24 e charge transfer from methyleneketene to cyclopentadiene in TS3. The activation barrier for TS3 is predicted to be 13.4 kcal/mol at the B3LYP/6-31G(d) level.

3.3. Frontier orbital analysis

FMO (frontier molecular orbital) theory has been useful in rationalizing the regioselectivity preferences of these cycloadditions. The cycloaddition can be either $HOMO_{(diene)}$–$LUMO_{(dienophile)}$ or $HOMO_{(dienophile)}$–$LUMO_{(diene)}$, depends on the direction of electron transfer during the reaction. The interaction between the frontier orbitals is expected to be stronger if the difference of the orbital energy levels is smaller and as a result the reaction will proceed easier. The greatest overlap of these orbitals occurs when the nucleophile interacts at the site of the compound that has the largest LUMO coefficient. Similarly, the electrophilic addition takes place most likely at the most nucleophilic terminus of a diene with the largest HOMO coefficient, and this terminus should become attached to the site of dienophile with the largest LUMO coefficient. Therefore, the regiochemical preference can be understood by examining the orbital coefficients on the individual atom.

By means of an analysis of the frontier molecular orbitals of the reactants (see Fig. 2) and the direction of electron transfer described in the preceding section, one can recognize that, for reaction (1), TS1 is mostly stabilized by the interaction of the HOMO-1 of methyleneketene with the LUMO of cyclopentadiene. The coefficients on C3 and C4 of methyleneketene are of approximately the same size, with the coefficient on C4 being slightly larger than that on C3; therefore, the slightly stronger orbital overlap between C4 and C1′ results in a shorter C4–C1′ distance in TS1. The energy gap of this FMO interaction amounts to 8.06 eV at the B3LYP/6-31G(d) level.

In transition state TS2, 0.03 e charge was transferred from methyleneketene to cyclopentadiene. By analyzing the frontier orbitals of the reactants shown in Figure 2, one can draw the conclusion that it is the interaction of HOMO of methyleneketene with LUMO of cyclopentadiene that stabilizes TS2. The larger orbital coefficients for the bonding atoms are localized on C3 of methyleneketene and C1′ of cyclopentadiene. This leads to a much shorter distance between the C3 and C1′ atoms than that between the C2 and C4′ atoms in TS2. The energy gap of this FMO interaction is 6.07 eV at the B3LYP/6-31G(d) level.

As for reaction (3), only the interaction of LUMO of cyclopentadiene with HOMO-3 of methyleneketene stabilizes TS3. However, this energy gap of FMO interaction is 11.08 eV at the B3LYP/6-31G(d) level, much higher than the energy gaps of the FMO interactions in TS1 and TS2. It is also known that the C═O π bond is stronger than the C═C π bond, therefore attack on the C═O bond is less probable. All these factors lead to the fact that reaction (3) has the highest energy barrier.

It is also interesting to notice that the energy gap of the FMO interaction in TS2 is lower by about 2.0 kcal/mol than that of TS1. However, TS2 possesses a higher activation barrier than TS1. The reason why TS1 is characterized by a lower activation energy can be interpreted by analyzing the orientation of the FMO interactions in TS1 and TS2. From Figure 1, one can see that the approaching modes of methyleneketene to cyclopentadiene are different in the structures TS1 and TS2. In TS1, the p_z orbital of methyleneketene interacts with the LUMO of cyclopentadiene, while the overlap between the p_x orbital of methyleneketene and the p_z orbital of cyclopentadiene stabilizes TS2. The latter approaching mode induces a strong steric repulsion between the CH_2 group of methyleneketene and cyclopentadiene in TS2 and thus destabilizes TS2. As a result, a higher activation barrier is observed. Therefore the cycloaddition at the terminal C═C bond of methyleneketene is most likely to be observed. This is different than in the case of the cycloaddition between methyleneketene and 5-methylene-1,3-dioxan-4,6-dione in which the addition takes place on the interior C═C double bond of methyleneketene.[11]

To investigate the electrostatic rationale for the intrinsic preference of the regioselectivity of addition of cyclopentadiene to methyleneketene, the solvent effects and their influence on the stabilities of transition structures were predicted with the polarized continuum model using the integral equation formalism.[19] The reference geometries optimized in the gas phase were used. In the gas phase, larger charge transfer in TS1 and TS3 than in TS2 was predicted. The values of the charge transfer are further enhanced in the polar medium, and the activation energies of TS1 and TS3 are lowered in the polar solvents. As one can see from Table 3, the activation energies for TS1 and TS3 decrease from 7.4 and 13.4 kcal/mol in the gas phase to 5.8 and 10.7 kcal/mol in dichloromethane and 5.6 and 10.4 kcal/mol in acetone,

Table 3. The B3LYP/6-31G(d) level activation energies in the gas phase and in the solutions

	Gas phase	Dichloromethane (ε=8.93)	Acetone (ε=20.7)
Reaction (1)	7.4	5.8	5.6
Reaction (2)	19.6	21.0	21.2
Reaction (3)	13.4	10.7	10.4

respectively. However, the cycloaddition taking place on the interior C═C bond of methyleneketene is hampered by a polar solvent. This suggests that a polar medium facilitates reaction (1).

4. Conclusions

The reaction mechanisms of the [4+2] cycloaddition between methyleneketene and cyclopentadiene have been investigated by ab initio and density functional theory calculations. According to the obtained results we draw the following conclusions.

(1) There are three possible reaction mechanisms for the cycloaddition between methyleneketene and cyclopentadiene, as shown in Scheme 1. The cycloadditions at different double bonds of methyleneketene are all concerted but nonsynchronous processes. An analysis based on frontier molecular orbitals shows that the reaction mechanisms correspond to the [4+2] description.
(2) All the reactions are exothermic; however, the small exothermicity of the addition to the C═O double bond suggests that reaction (3) is less favorable compared to additions to the C═C double bonds of methyleneketene (reactions (1) and (2)).
(3) The activation barriers for the cycloaddition of methyleneketene with cyclopentadiene are 7.4, 19.6, and 13.4 kcal/mol at the B3LYP/6-31G(d) level for reactions (1), (2), and (3), respectively. These computational results show that the energy barrier for the reaction leading to the 1,2-adduct norbornene (reaction (1)) is the lowest one. The predicted solvent effect indicates that reaction (1) is facilitated in a polar solvent.
(4) The performance of various computational methodologies in obtaining activation as well as reaction energies of the reactions under investigation has been critically analyzed. The hybrid density functional B3LYP method shows excellent agreement with the CCSD(T) method in obtaining activation energies, and it shows less variation as a function of basis set. On the contrary, the activation energies obtained at the MP2 level are underestimated, and thus the MP2 method is inadequate for the study of the Diels–Alder reactions. It should be noted that B3LYP, which works well for the activation energy, yet significantly underestimates the reaction energies and is sensitive to the size of basis set. For a quantitative description of the reaction energy, full MP4 level method with single, double, triple, and quadruple substitutions is necessary.

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Supplementary data

The Cartesian coordinates of the optimized stationary point structures (in Å) for the cycloadditions between methyleneketene and cyclopentadiene at the B3LYP/6-31G(d) and MP2/6-31G(d) levels of theory (PDF). The geometrical parameters of optimized transition structures TS1 and TS3 at the B3LYP/6-31G(d), MP2/6-31G(d), B3LYP/6-311+G(d,p), and B3LYP/aug-cc-pVTZ levels. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.067.

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Structure of pyrimidinocyclophanes in solution by NMR

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Abstract—The conformations and self-associative properties of novel pyrimidinocyclophanes with a substituted nitrogen atom in a spacer have been studied using several independent NMR methods (NOE, aromatic shielding effect, 2D DOSY, GIAO DFT chemical shift calculations, DNMR) in a variety of solvents, in acidic and basic media. At room temperature the title compounds in neutral solution are in slow exchange on the NMR time-scale and exist in a folded conformation. In the acidic medium protonation occurs at the bridge nitrogen, and that leads to dramatic modifications of the structure and dynamics of the compound. The protonated form exists at the equilibrium of 'folded' and 'extended' conformers. Moreover, the protonated form is prone to association and can be effectively described as a dimer at room temperature.

1. Introduction

Nucleotide bases and their derivatives play a crucial role in different processes in biochemistry, pharmacology, and medicinal chemistry. Therefore, there have been constant efforts to investigate such systems and their properties by different experimental and theoretical methods[1]. These efforts attest to the great interest currently focused on understanding and controlling nucleotide base interactions.[1c,2] However, in spite of the remarkable progress in this area there is still a lot to be understood. There are no clear ideas about the forces that control and stabilize primary, secondary, and supramolecular structure of the synthetic and biological systems containing nucleotide bases, particularly nucleic acids and their complexes with proteins.[3] It is well known that hydrogen bonding is crucial in the determination of the conformational and supramolecular structures of the systems with nucleotide bases. However, such strong stabilization is controlled not only by hydrogen bonding, the mechanism of the interactions seems to be more complicated, and new hypotheses have been developed.[2i,4] Most of the investigations are theoretical, thus experimental studies have become a necessity.

To promote these investigations some simpler model systems are needed, which can offer an access to control and subtle structural variations the effects of which can be assessed and comprehended at the molecular level.[5] To develop such systems, modeling interactions between nucleic acids and proteins or intercalating agents, a variety of new compounds has been synthesized. Almost all of them consist of nucleic acids or their derivatives linked by a polymethylene spacer with another nucleic bases (pyrimidines or purines or their combination) or with aromatic or heteroaromatic system.[5c–i,6] The analysis of hypochromic and in very few cases of ^{1}H NMR effects can provide insights into geometry, which is controlled by weak noncovalent interactions (hydrogen bonding (HB), van der Waals (vdW)). However, the very flexible nature of the proposed acyclic models allows one to establish only the fact of folded/unfolded conformation or complexed/not complexed structure and no exact 3D solution structure can be obtained in most cases.

The use of macrocyclic molecules containing nucleic bases and their derivatives as models seems to be more promising because even with the same flexible spacers, NMR and UV effects are stronger, probably due to the higher rigidity of macrocycles versus acyclic analogues. For example, in the case of purinophanes and pyrimidinophanes high field shifts for purine and pyrimidine protons and remarkably increasing hypochromic effects were observed in comparison with their acyclic counterparts both in $CDCl_3$ and aqueous solution.[7] At the same time, although these data are an indirect indication of stacking between various nucleic bases in these macrocycles, there has been little success in the determination of the 3D geometry of macrocycles in solution by NMR.

NMR techniques have proven to be powerful tools in the conformational analysis of biologically important macrocycles such as peptides and proteins.[8] However, in the case of macrocycles containing nucleic acid bases, the problem is more complicated: due to the diversity of weak interactions of similar energy (weak HB, vdW interactions) these

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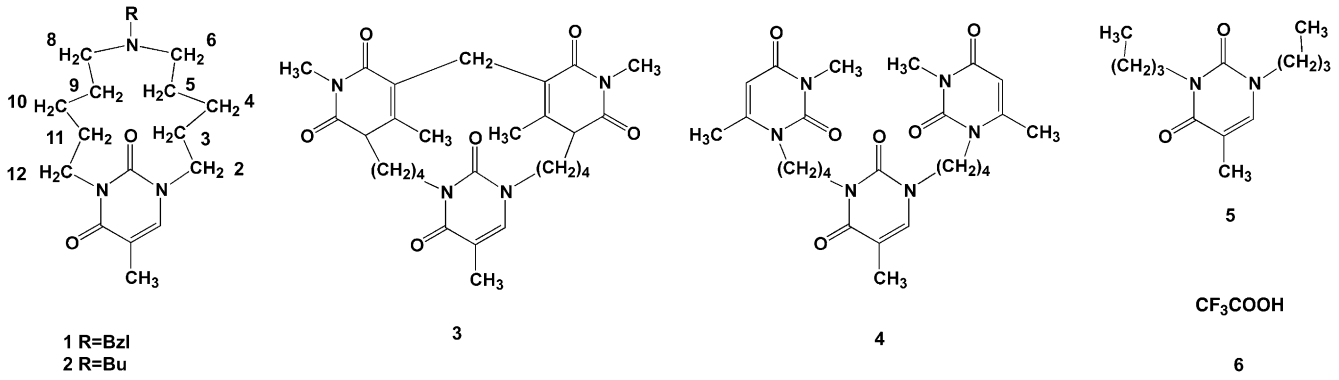


Scheme 1.

compounds in solution are in equilibrium in a variety of conformations of close energy, and no folded-structure-specific NOEs were seen.[7b,9]

Moreover, the equilibrium between different tautomeric forms and the contribution of protonated forms is very probable.[10] Thus nucleotide macrocycles have to be essentially flexible and a variety of different structural forms can be expected. Moreover, association or self-association processes may take place, thus additionally complicating the structure determination problem.[11] In addition, the dispersion of the signals in ^{1}H NMR is worse than in the spectra of peptides. Therefore, it is clear that macrocycles containing pyrimidine bases are difficult objects for NMR investigation.

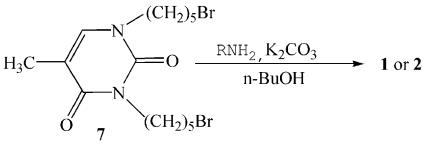
We faced such problems when we started a new project concerning macrocycles with three pyrimidine fragments.[†] Our attempts to make use of variable temperature NMR experiments were of little success: at low temperatures extensive broadening of most signals in proton NMR was observed, and it was not possible to derive conclusions about conformational and/or supramolecular structure from those experiments.

Only recently we have obtained new macrocycles (**1**, **2**) (Scheme 1), which can be considered as cyclophanes containing pyrimidine rings in place of aromatic unit, called pyrimidinocyclophanes.[12] These macrocyclic compounds allowed us insight into their 3D structures in solution and, perhaps, to propose an explanation why the line shape evolution for previous compounds was so dramatic and difficult to analyze. Here we report our recent results on the structure and association properties of new pyrimidinocyclophanes in solution.

2. Results

2.1. Synthesis

Pyrimidinocyclophanes **1** and **2** were prepared by amination of 1,3-bis(bromopentyl)thymine **7** with 2–3-fold excess of appropriate amine in *n*-BuOH in the presence of K_2CO_3 under heating (Scheme 2). The yields of the macrocycles are poor (17 and 19%), and attempts to increase them were unsuccessful. In particular, the reactions were carried out under high-dilution conditions, *n*-BuOH was substituted by other solvents, and salts of transition metals as catalysts were introduced into reaction mixtures. However, the yields of the obtained pyrimidinocyclophanes didn't exceed 20%. It seems that the reactions are subjected mainly to statistic factors.



Scheme 2.

Compounds **3**, **4**, and **5** were prepared by procedures reported earlier.[7b]

2.2. Conformational structure

The full assignment of signals in proton and carbon spectra of **1** was carried out by 2D COSY, HSQC, HMBC, and NOESY methods.[‡13] The analysis started from the thymine fragment, for which signals can be easily assigned,[14] e.g., starting from $C(6)_{thy}H$ by COSY, HSQC, and HMBC correlation techniques. The key correlations to distinguish H2 and H12 of the aliphatic spacers are between $C(6)_{thy}$/H2 and $C(4)_{thy}$/H12 in HMBC, and $C(6)_{thy}H$/H2 in NOESY. The –N–CH_2–Ph fragment was also unequivocally assigned in the same manner starting from the aromatic fragment. The H6/H8 and the C6/C8 show correlations with NCH_2–Ph carbons and protons, respectively. Thus, the fully assigned ^{1}H NMR spectrum of **1** is shown in Figure 1.The most important and interesting resonances are those of the H2 and H12 protons: it is quite unexpected that geminal CH_2 protons at C2 (and C12) are nonequivalent. One could expect rapid flipping of the long aliphatic chain around a thymine fragment and therefore the geminal protons at the benzylic positions should be chemically equivalent due to the fast exchange on the NMR time-scale and only one signal would be

[†] Results of low temperature NMR investigation of macrocycles containing one 6-methyluracil and two 2-thio-4-amino-6-methylpyrimidine units are in preparation and will be published elsewhere.

[‡] All details can be found in Supplementary data.

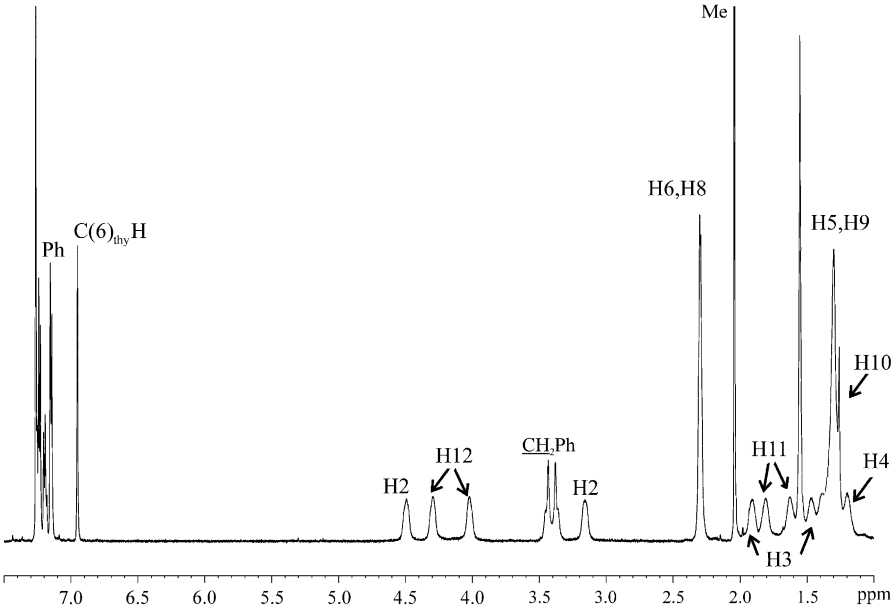


Figure 1. ^{1}H NMR spectrum of **1** in $CDCl_3$ at T=303 K.

observed for H2 and H12 protons. For example, for macrocycles possessing thymine and two 3,6-dimethyluracil moieties linked by similar aliphatic spacers (**3**) one observes ^{1}H NMR spectra for these protons that correspond to fast exchange when $n\geq4$ and these spectra are very similar to those observed for acyclic analogues (**4**).[§]

These results imply that this compound is conformationally rigid, at least in the neighborhood of the thymine fragment: the chemical shifts of the geminal H12a/H12b and the H2a/H2b protons reflect their position with respect to the plane of the thymine ring.

At the same time, there is broadening in the spectrum at T=303 K that might be due to some exchange processes. In order to explore this process, low temperature experiments were carried out. These experiments in $CDCl_3$ in the range of temperatures from 303 to 213 K did not show any remarkable modification of the NMR parameters, particularly for H2 and H12. Only some low field shifts for $C(6)_{thy}H$ and 5-Me were detected, and minimal modification of the line shape for CH_2Ph protons was observed.

Such line shape evolution can be interpreted as if the title compounds (macrocycles) exist basically in one form. Only some minor process near to the bridging nitrogen (N-inversion and/or rotation around N–CH_2) might be the reason why the N–CH_2 proton spectrum is slightly modified at lower temperature.

The conformational structure of **1** has been established by three NMR techniques. First of all, there are NOEs at T=213 K between the phenyl and 5-Me protons (Fig. 2) that are due to the close proximity of these protons. In addition, there are deshielding effects of phenyl ring on $C(6)_{thy}H$ and 5-Me protons (Fig. 2b). As one can see, there are low field shifts for these protons in comparison with the spectrum for the model compound **5** (Fig. 2b) where there is no such effect. Finally, comparison of the calculated and experimental chemical shifts for the H2 and H12 protons in this conformation is in good agreement with this conclusion.[7b] In fact, the thymine ring is magnetically anisotropic, and chemical shifts of vicinal protons depend strongly on their exact position in respect to the thymine plane (angle around N–C2 and N–C12 bonds). Therefore, we searched for the stable conformers of **1** by the MM method (program ChemOffice)[15] and found that the 'folded' structure (Fig. 3a) corresponds to energy minima, and nonempirical ^{1}H chemical shift calculation in the frame of GIAO DFT approach[16] shows quite good correspondence between the experimental and theoretical chemical shifts for this geometry (Fig. 3b). This additionally supports our conclusion about the conformational structure of **1** because even a small discrepancy in geometry would have been reflected in chemical shifts of the vicinal protons.

Thus, we have concluded that compound **1** in $CDCl_3$ predominantly exists in a folded conformation (Fig. 3a).

In addition, ^{1}H NMR experiments at different concentrations were carried out in order to see whether this molecule is prone to association. In proton spectra only minimal changes were observed when concentration changed from 1 to 50 mmol/l. Thus, we concluded that the molecule in $CDCl_3$ solution exists as a monomer in folded form.

In general, the fact that this molecule exists in a folded conformation and exchange is slow in NMR time-scale is quite an unexpected result. For example, for pyrimidinophane with one thymine and two 3,6-dimethyluracil fragments (**3**, **4**) the exchange is fast even for aliphatic spacer $(–CH_2–)_n$ with n=4, moreover, for n=5 the spectrum is very similar to that observed for acyclic analogue **4**. This suggests that there may be some extra interactions, which stabilize this folded conformation of **1**.

[§] Results of NMR investigation of pyrimidinophane **3** and acyclic analogue **4** are under preparation and will be published elsewhere.

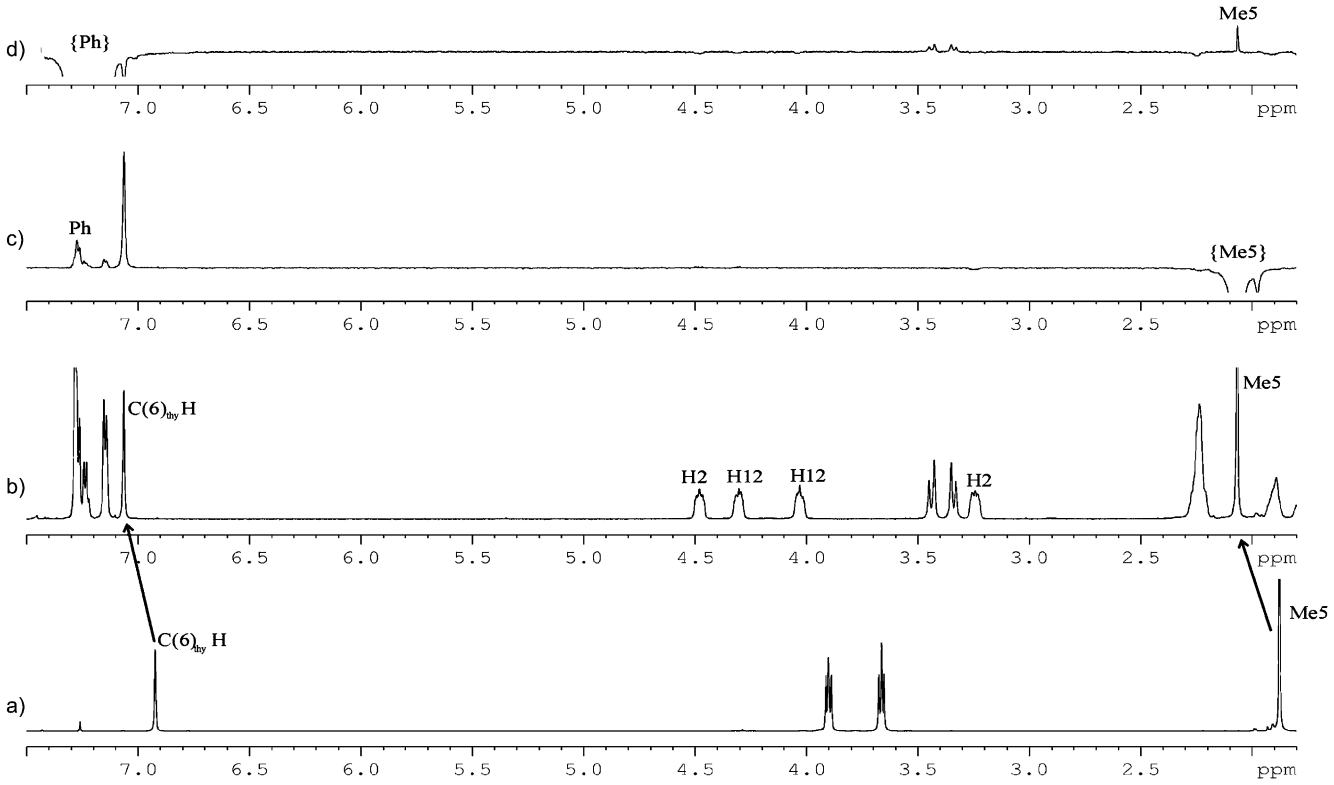


Figure 2. ^{1}H NMR (a, b) and NOE spectra (c, d) (τ_m=0.6 s) of compounds **1** and **5** in $CDCl_3$: (a) **5** at T=303 K; (b–d) **1** at T=213 K (irradiated atoms shown in braces).

In this case several noncovalent interactions may be considered as a contribution to stabilization. There are a number of publications concerning this subject but there are still many uncertainties in the energy gain due to these interactions. In fact, most of these reports are based on theoretical results and there are only little experimental data. Moreover, even amongst the theoretical estimations there is dispersion in values of energies depending on the method used to calculate particular interaction, level of theory, choice of the model system, and the model to account for the solvent effects.[3a,b,17]

In fact, from the variety of possible interactions in this system the HB is the strongest one. There are several polar atoms and groups (C═O, N, $C(5)_{thy}H$) that may be proton donors or acceptors. If this assumption is correct then polar solvents should disrupt such intramolecular interactions and destabilize the folded conformation.[1a,18] To verify this idea the ^{1}H NMR spectra in different polar solvents at room temperature were obtained, and it was found that replacement of $CDCl_3$ by acetone, acetonitrile, and methanol only has insignificant effect on the spectra, particularly on indicative H2 and H12 resonances. Therefore, we concluded that dynamics and equilibrium of **1** in these solvents are essentially the same. Thus, HB can probably be excluded from the interactions that stabilize this conformation.

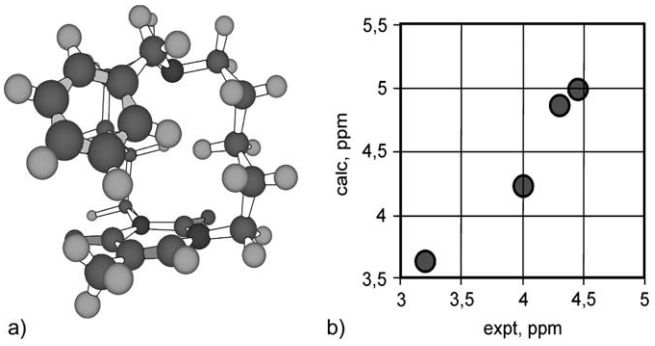
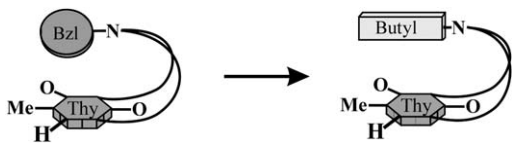


Figure 3. (a) 3D structure (left) and (b) correlation of experimental and calculated (GIAO B3LYP/6-31G(d)//RHF/6-31G) ^{1}H chemical shifts for **1** (right).

It is possible that aromatic π–π (e.g., face-to-edge) interactions may take place in this case.[3a,b] Recently, the role of different aromatic–aromatic and aromatic–aliphatic interactions has been reviewed and, according to calculations, stabilization of up to 3.2 kcal/mol could be expected.[3b] To check if these interactions work in this case the model compound with aliphatic substituent instead of phenyl ring on bridged nitrogen (**2**) was synthesized and ^{1}H NMR experiments were carried out.



The ^{1}H NMR spectrum of **2** is shown in Figure 4. For comparison the spectrum of **1** is also given. As one can see, the H2 and H12 proton signals are very similar. Taking into

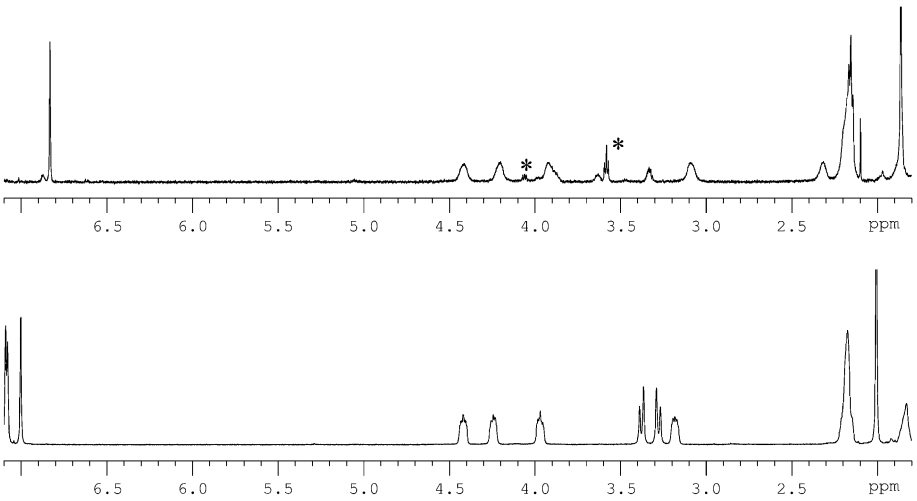


Figure 4. ^{1}H NMR spectra of **2** (top, R=butyl, C=12 mM) and **1** (bottom, R=Bzl, C=7 mM) in $CDCl_3$ at T=303 K. *Undetermined admixture.

account the strong anisotropy of the thymine ring and almost equal chemical shifts of the H2 and H12 protons in **1** and **2**, we can conclude that geometries of these molecules are effectively the same.

Low temperature ^{1}H NMR experiments were also in good agreement with this conclusion. In addition, NOE between 5-Me and the aliphatic substituent at N unambiguously proves that **2** in solution exists also in folded conformation.

Thus both compounds are in an identical folded conformation irrespective to the nature of the substituent at bridge nitrogen, and no indication of π–π 'edge-to-face' interactions was obtained for the title compounds. Most probably, the realization of the folded conformation for such systems is explained by the structure of polymethylene spacers and the number of methylene groups.

2.3. Protonation and association

Another aspect of our efforts was to explain why the ^{1}H NMR spectra of **1** still remain well resolved at lower temperature and correspond to one conformation, with no indications of association even at temperatures close to the solvent's m.p. For macrocycles containing other pyrimidine bases, however, extensive broadening of signals was seen even at moderately low temperature.

We supposed that other macrocycles are prone to association due to the existence of protonated or charged forms.[10] Therefore, we decided to try to provoke protonation of compound **1** too.

^{1}H NMR experiments in $CDCl_3$ with titration by CF_3COOH (**6**) at room temperature have demonstrated marked modification of the spectra (Fig. 5). Some changes could be expected due to protonation of the bridge nitrogen, and therefore vicinal CH_2 protons (H6, H8, CH_2Ph) should reflect this process. However, the most important is the observed line shape evolution of the H2 and H12 protons: as the concentration of acid is increased the signals of these protons start to broaden and then finally they coalesce. It is particularly apparent for the H2 protons, while in pure $CDCl_3$ the geminal CH_2 protons were not equivalent and resonate at 4.5 and 3.2 ppm. At intermediate concentrations of acid their signals broadened extensively, and then close to full protonation, one exchange averaged signal was observed for each of these protons.

Such line shape evolution can be explained either by exchange between two symmetric forms that becomes fast in NMR time-scale or/and stabilization of the additional form in which the chemical shifts of these protons differ from those in the folded conformation.

In order to determine the structure of the protonated form(s), NMR experiments with variation of the temperature were carried out (Fig. 6). As the temperature decreases, the signals of the H2 and H12 protons start to collapse and finally at T=223 K the spectrum corresponds to slow exchange of several forms. The lines, in addition, are extensively broadened, perhaps due to self-association.

Unfortunately, due to ambiguous line shape evolution and extensive broadening it is difficult to determine the number of components in equilibrium and to assign the signals in the low temperature ^{1}H spectra to corresponding groups in order to establish their conformational structure.

Therefore to the association of the title compound in solution under protonation and particularly at low temperature (corresponding extensive broadening of the lines in ^{1}H spectra) we tried to estimate effective volume of the molecule by the measurement of self-diffusion coefficient.[10a,19] Translation self-diffusion coefficients were measured by 2D DOSY method with bipolar gradients. Every value was averaged over two–three measurements. The results are shown in Figure 7. It was established that at room temperature the coefficient is approximately 1.5 times larger in pure $CDCl_3$ than in a solution of the 1:1 mixture of compounds **1** and **6**. Then according to Stock's model[19] the effective radius of the protonated form is about 1.5 of the radius of noncharged molecule. Therefore, the effective volume of the charged form is twice as large as the noncharged one. Thus if in neutral solution **1** exists as monomer, protonation leads to association of the molecule into dimer.

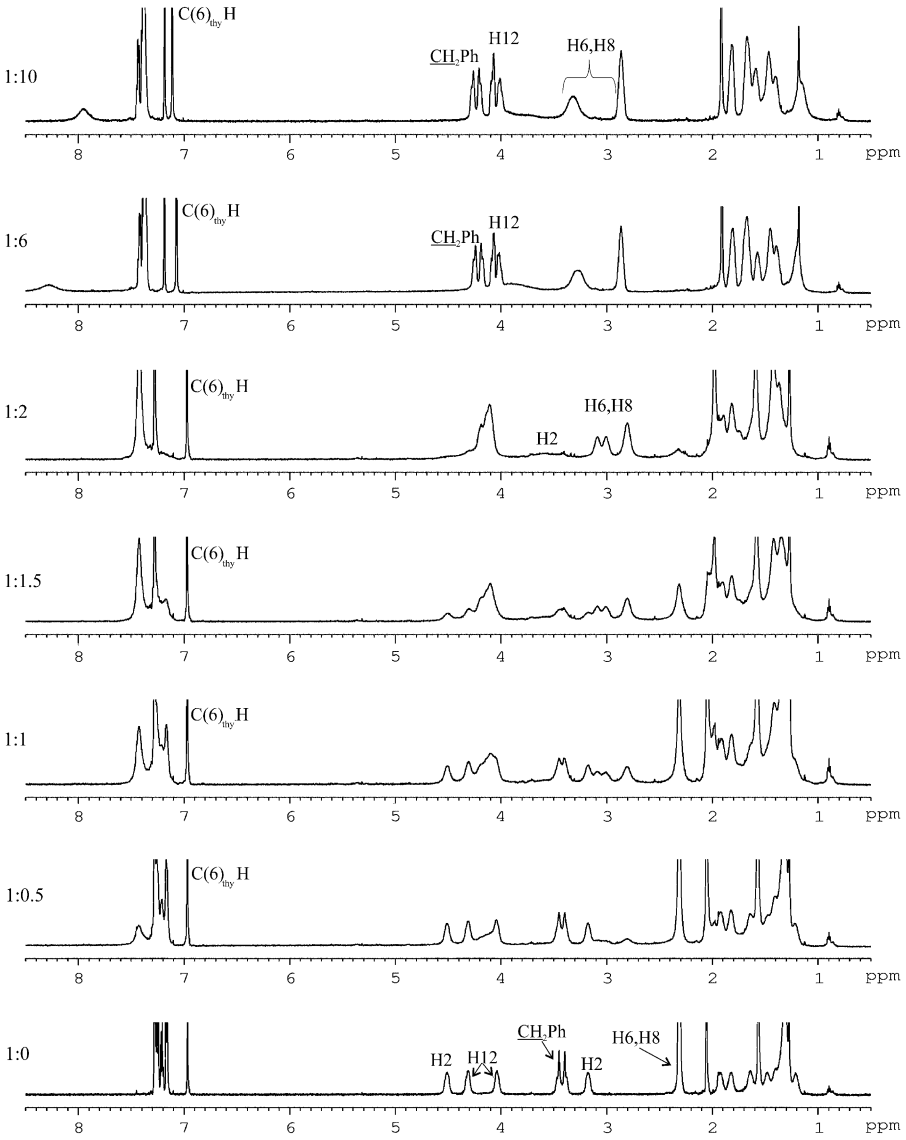


Figure 5. Dependence of ^{1}H NMR spectra of mixture **1+6** on the **1**:**6** ratio in $CDCl_3$ at T=303 K.

In addition, the self-diffusion coefficient was measured at lower temperatures (Fig. 7). The temperature dependence of the self-diffusion coefficient for neutral molecule can be well explained by its dependence directly on temperature and indirectly on viscosity. At the same time for the protonated form this dependence reflects also the fact that at low temperature higher associates become more stable and low temperature self-diffusion coefficient corresponds to four molecular associates. Thus association is thermodynamically more favorable than the monomeric form in acidic media.

In the next step we tried to get insight into the conformations of the protonated form. Room temperature ^{1}H NMR spectra of the protonated form in solution are much broadened and cannot be used for spectra-structure correlations. The ^{13}C NMR spectrum of **1** at room temperature also cannot be used to determine the conformational structure. However, a comparison of ^{13}C spectra with those for the nonprotonated form shows (Fig. 8) that they are different and this difference may be due to the contribution of some additional conformers. As can be seen from Figure 8, most spectacular differences were observed for $C(2)_{thy}$, $C(4)_{thy}$, $C(5)_{thy}$, and C6/8. None of them were seen in the acidic medium due to extensive broadening. Therefore, we concluded that this could be due to equilibrium of the forms where these carbons have large chemical shift differences.

To check this hypothesis we carried out several experiments with variation of temperature. As mentioned above, the evolution of the proton NMR spectra was dramatic but it was very difficult to follow these changes and to ascribe it either to slowing down of exchange between the conformers and/or to association effect. Therefore, we tried to run similar ^{13}C experiments at lower temperature, but due to low concentration a regular spectrum could not be accumulated, and information on ^{13}C chemical shifts of carbons directly bonded to protons was obtained only from 2D HSQC spectra (Fig. 9).

As can be seen in Figure 9, the number of carbon signals in the low temperature spectrum is twice that at room temperature (for carbons C2, C12, C6, C8, CH_2Ph). Therefore,

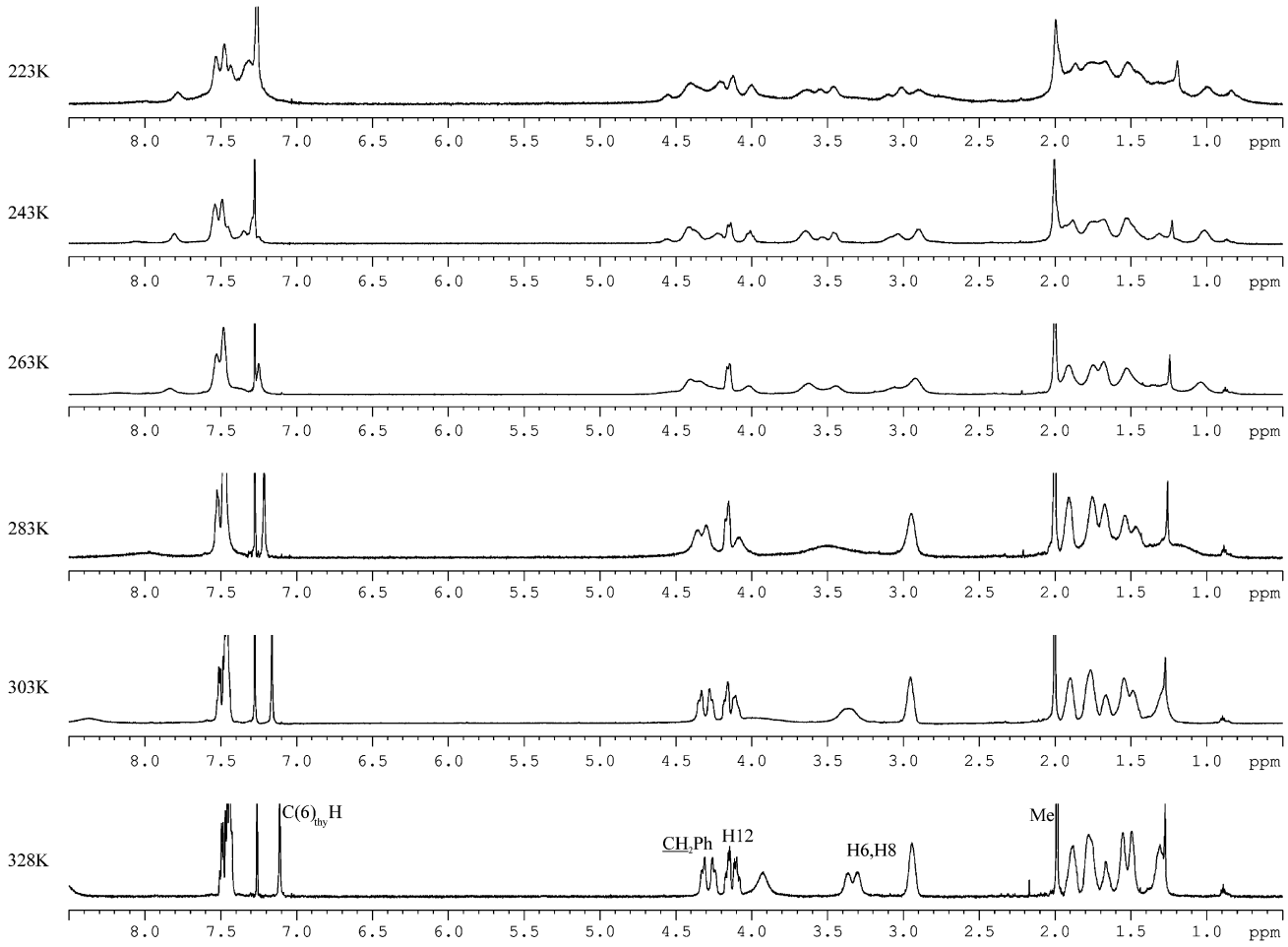


Figure 6. Temperature-dependent ^{1}H NMR spectra for a mixture of **1+6** (1:6) in $CDCl_3$.

we concluded that this spectrum corresponds to a two-component equilibrium. Moreover, comparison of room temperature chemical shifts with low temperature data and analysis of CH correlation data allowed us to assign signals as shown in Figure 9.

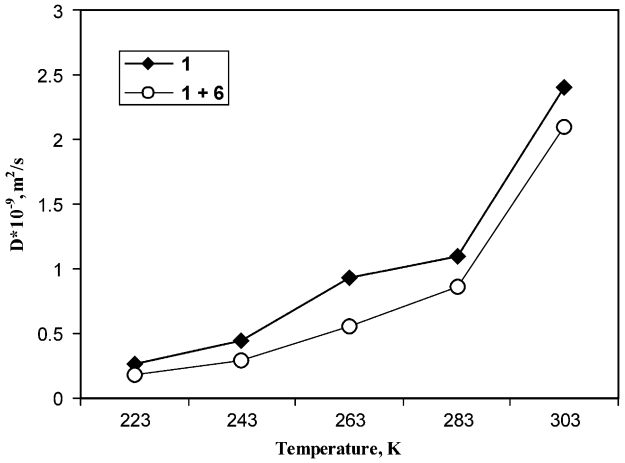


Figure 7. Temperature dependences of self-diffusion coefficients for **1** and **1+6** (1:1) in $CDCl_3$.

As can be seen, chemical shifts for the carbons in one form (except for the C6/8 and C2) are very similar to those observed in the nonprotonated form in $CDCl_3$ at room temperature, while for another conformer they are different. Particularly important is the difference for the C6/8 and C12 carbons of the spacer: $\Delta\delta$ are ca. 8 and 4 ppm, respectively.

Thus, we can conclude that the first form has a conformation similar to nonprotonated form, i.e. folded conformation. Some differences in chemical shifts of C6, C8, and C2 can be explained by the change of the local structure upon N-protonation. The analysis of the chemical shift difference for the second form allowed us to conclude that the observed low field shifts with respect to the folded form's shifts for C6, C8 carbons can be attributed to γ-effects on these carbons due to a change of orientation around C4–C5 and C10–C9 bonds that leads to extended conformation shown schematically in Figure 10. Thus, the title compound in solution upon protonation exists in equilibrium of two forms, one being folded and the second being extended.

In addition, the extended form is very prone to association and its structure can be accessed by high field shifts for H4, H10 protons at low temperature.[‡] Namely, in such dimers (trimer, tetramer, and higher associates) the protons

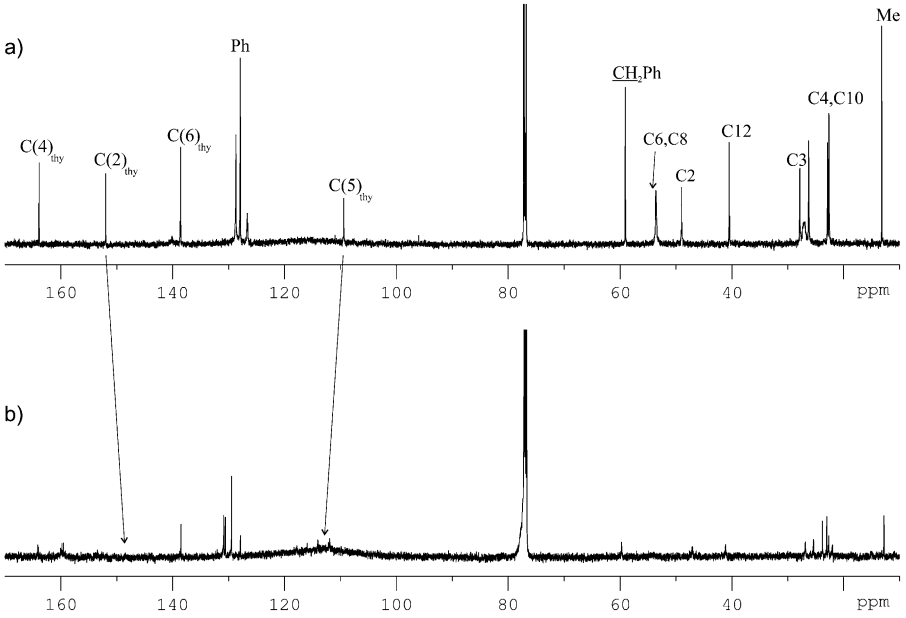


Figure 8. ^{13}C NMR spectra of **1** (a) and **1+6** (1:6) (b) at room temperature in $CDCl_3$.

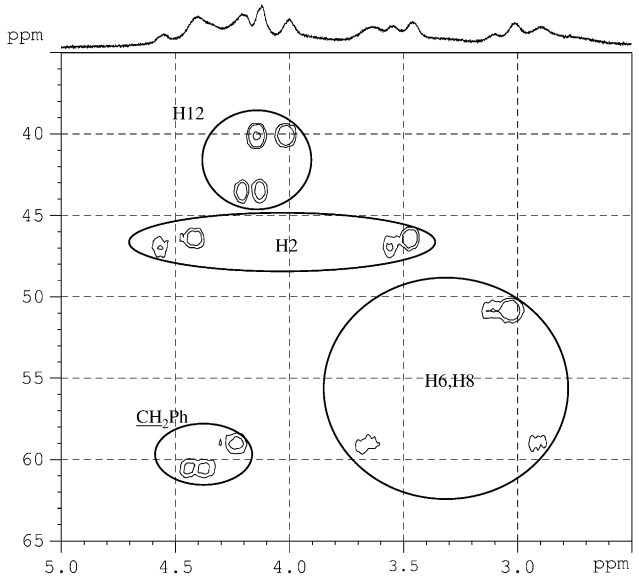


Figure 9. Fragment of 2D HSQC (^1H–^{13}C) spectrum of **1+6** (1:6) at T=223 K in $CDCl_3$.

at C4 and C10 of spacer of one molecule are located in the shielding cone of the phenyl ring of another molecule (Fig. 11). Therefore, at lower temperature the contribution of the higher associates, which have similar NMR parameters, increases and this leads to high field shift of H4, H10 protons.

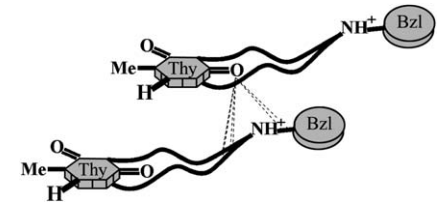


Figure 11. Schematic presentation of dimer of **1**.

3. Discussion

Besides the particular data related to this type of compounds (the protonation site and conformational structure in solution and in protonated form) there are two questions that have general interest: (1) the mechanism of stabilization of such strained forms and (2) the mechanism of stabilization of associates and their structure. (1) Folded conformations appear to be stabilized by the 'edge-to-face' π–π interactions. Different authors estimate such interactions in the range of 0.9–2.6 kcal/mol.[3b] At this point, however, its magnitude and its value in solution remain unclear. In particular there are almost no experimental data.

According to our experiments there is no indication of such interactions in the ground state in the title compounds. In the case of such interactions, the energy barrier of interconversion between symmetric conformers would increase for **1** versus **2** (when butyl is changed for Bzl) and it should affect

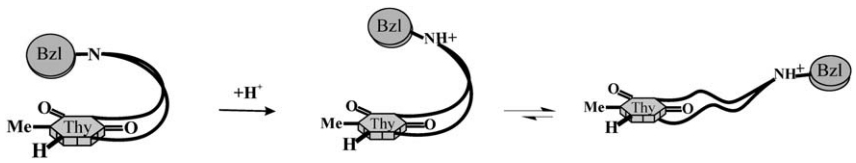


Figure 10. Schematic presentation of the equilibrium of folded and extended conformations of **1**.

NMR line shape (broadening or coalescence temperature). Some broadening at room temperature is very similar in both compounds (**1** and **2**) and this proves that exchange rate is very much the same in these compounds irrespective of the substituent at the bridge nitrogen atom.

(2) To explain the stabilization of such associates under protonation and also the mechanism an additional argument in favor of the structure depicted in Figure 11 is the chemical shift of $C(2)_{thy}$=O. It is likely that this oxygen atom participates in binding when dimer and higher associates are formed. There is no signal for this carbon in the room temperature ^{13}C spectra although other signals are present. (Unfortunately due to low *S*/*N* at low temperature we were not able to obtain the ^{13}C NMR spectra.) Based on this observation we concluded that its signal is much broadened at room temperature, which might take place if the chemical shift of this carbon in above-mentioned conformers (forms) is substantially different. This difference may originate from the bonding by the C=O group if, e.g., HB are formed in one of the forms. Indeed, GIAO DFT calculations of chemical shifts (with RHF/6-31G optimized geometry) for **1** with $C(2)_{thy}$=O involved in HB with three ethyl amine cation as example of proton donor group and for **1** which is not involved in HB predict maximal differences just for $C(2)_{thy}$ (+4.4 ppm) and $C(5)_{thy}$ (+5.4 ppm, positive signs mean low field shifts in HB complex).

Which group could donate proton to this group to form HB? There are different options: first of all, N^+H could participate in such bonding. Such bonds can be excluded as dominant, however, because the chemical shift of this proton does not depend remarkably on temperature. Second, vicinal to protonated nitrogen the N^+CH_2 protons may give HB, which are in total even stronger than classical HB, as it was recently proposed theoretically.[10b,17c] Three such groups can interact with C=O and in doing so stabilize such associates. This can explain the low field shift of these protons when the temperature decreases. In this structure high field shifts have to be observed for the protons at C4 and C10 of the bridges due to the shielding effect of the phenyl ring.

According to theory, enormous stabilization (90–95 kcal/mol in the gas phase were calculated for the [N^+–C–H$\cdots$O=C] model)[17c] can be expected due to these hydrogen bonds although solvent effects have to diminish such interactions to some extent (energy drops to 20 kcal/mol in $CHCl_3$). In order to verify this hypothesis we carried out additional ^1H NMR experiments for the protonated form in $CDCl_3$ with titration by DMSO as HB disrupting solvent. Indeed, the change of the spectra was observed and it can be ascribed to the increase of the monomeric form.

Such dependence of the conformational structure and association on acid concentration models a real situation where properties change with the solution pH.[20] In physiological systems pH in aqueous solution may vary from 1.9 up to 10.4 and this can effectively produce changes in the structure, which are responsible for definite properties.

Unfortunately, this compound dissolves poorly in water and we could not demonstrate the above statement directly in 'physiological' solvents. However, we were able to see these effects in the mixture of solvents: alcohol/water (methanol/water, 2/1). NMR experiments at room temperature with titration by HCl showed similar evolution of the line shape as observed in $CDCl_3$ under titration with CF_3COOH.[‡] The most spectacular were the changes of the H2/H12 protons signals: they start to broaden extensively upon the addition of ca. 1 equiv of HCl. Moreover, these changes are reversible: when equimolar NaOH was added the reverse evolution of the line shape was seen.

This fact demonstrates that being regulated by the solution pH, 3D and supramolecular association may be a means of recognition and modulation of that recognition.

4. Conclusion

The title macrocycles in solution exist in a folded conformation with bridge N substituents proximal to the thymine Me and H_5. The barrier of interconversion is very high.

A small amount of acid in solution may protonate the compounds and change dramatically not only the conformation but also the supramolecular structure. Interactions (HB) of protons vicinal to protonated nitrogen with carbonyl oxygen ($N^+CH\cdots O$=C) are strong when compared to other noncovalent ones, and this result strongly supports the theoretical prediction. The role of such interactions may be important if one takes into account that protonation in physiological solutions occurs very often. The supramolecular association of designed macrocycles and closely related natural systems can be regulated by the solution pH.

5. Experimental

5.1. General

NMR experiments were recorded with a Bruker AVANCE-600 spectrometer (14.1 T) equipped with a pulsed gradient unit capable of producing magnetic field pulse gradients in the *z*-direction of 56 G/cm. All spectra were acquired in a 5-mm inverse probehead in 5-mm tubes. Chemical shifts (ppm) are internally referenced to the TMS signal (0 ppm) in all cases. Complete assignments of the ^1H and ^{13}C NMR spectra of the title compounds were accomplished by 2D COSY, TOCSY, HSQC, HMBC, and NOESY experiments. In some cases 1D DPFGNOE method in rotating frame was used to measure NOEs.[21] A Hermite-shaped pulses were used for selective irradiation.

In order to minimize convection effects, 2D DOSY experiments were performed using the bipolar pulse longitudinal eddy current delay (BPPLED) pulse sequence. The duration of the magnetic field pulse gradients (δ) was optimized for each diffusion time (t) in order to obtain 1–5% residual signal with maximum gradient strength. The pulse gradients (g) were incremented from 2 to 95% of the maximum gradient strength in a linear ramp. All diffusion coefficients reported are means of at least three measurements.

For DNMR spectroscopy, a standard unit calibrated using a methanol reference controlled the probe temperature; the

samples were allowed to equilibrate for 15 min at each temperature before recording spectra.

Most of the solvents and all the reagents were commercial and used without further purification. All dry solvents were prepared according to the standard procedures and stored over molecular sieves.

Molecular mechanics (employing the MM2 force field)[22] were performed with CS Chem3D Ultra 6.0 (CambridgeSoft Corp.) on a AuthenticAMD Athlon (Im)computer. Chemical shifts were determined within the DFT framework using a hybrid exchange-correlation functional, B3LYP, at the 6-31G(d) level as implemented in Gaussian 98.[23] Full geometry optimizations were done at the ab initio RHF/6-31G level. All data were referred to TMS (^{1}H and ^{13}C) chemical shifts that were calculated at the same conditions.

The mass spectra (EI) were obtained on a Finnigan MAT-212 mass spectrometer (resolution was 1000, direct inlet of the sample into the ion source, energy of ionizing electrons was 70 eV, electron emission current was 1 mA). Melting points were measured on a Boetius hot-stage apparatus and are uncorrected. Thin-layer chromatography was performed on Silufol-254 plates; visualization was carried out with UV light. For column chromatography neutral Al_2O_3 (activity II) was used. Chemicals and reagents were purchased from Lancaster or Aldrich Chemical companies.

Thyimidinocyclophanes **1** and **2** were prepared by the reaction of 1,3-bis(5-bromopentyl)thymine (**7**) with benzylamine or butylamine.

5.1.1. 7-Benzyl-15-methyl-1,7,13-triazabicyclo[11.3.1]-heptadeca-15-en-14,17-dione (1). At 90 °C to a stirred mixture of benzylamine (2.14 g, 20 mmol) and K_2CO_3 (2.95 g, 21 mmol) in *n*-BuOH (250 mL) compound **7** (4.1 g, 9.7 mmol) in *n*-BuOH solution (100 mL) was added and stirring was continued for 7 h at 100–110 °C. The solvent was distilled off and the residue was treated by $CHCl_3$, filtered, concentrated, and transferred to a column with Al_2O_3. Elution with ether gave thyimidinocyclophane **1** in a yield of 0.6 g (17%); mp 130–131 °C; HRMS found *m/z* 369.2420, $C_{22}H_{31}N_3O_2$ required 369.2416; MS (EI) *m/z* 369 (M+, 94), 368 (M−1, 42), 340 (M−29, 33), 278 (M−91, 95), 250 (M−119, 29), 91 (100). ^{1}H NMR (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 1.16–1.44 (8H, m, $(CH_2)_{4,5,9,10}$), 1.48 (1H, m, H_3), 1.64 (1H, m, H_{11}), 1.8 (1H, m, H_{11}), 1.9 (1H, m, H_3), 2.04 (3H, s, $C(5)_{thy}$Me), 2.32 (4H, m, $H_{6,8}$), 3.18 (1H, m, H_2), 3.38 and 3.46 (2H, AB, J=13.0 Hz, CH_2Ph), 4.03 (1H, m, H_{12}), 4.28 (1H, m, H_{12}), 4.48 (1H, m, H_2), 6.97 (1H, s, $C(6)_{thy}$H), 7.16 (2H, d, J=6.7 Hz, Ar_{ortho}), 7.21 (1H, t, J=6.7 Hz, Ar_{para}), 7.26 (2H, t, J=7.4 Hz, Ar_{meta}); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.2 ($C(5)_{thy}$*Me*), 22.6 (C_9), 22.9 (C_5), 26.3 (C_{11}), 27.1 (C_{10}), 27.3 (C_4), 27.9 (C_3), 40.5 (C_{12}), 49.0 (C_2), 53.5 ($C_{6,8}$), 59.0 (CH_2Ph), 109.4 ($C(5)_{thy}$), 126.4 (Ar_{para}), 127.8 (Ar_{meta}), 128.4 (Ar_{ortho}), 138.6 (Ar_{ipso}), 152.0 ($C(2)_{thy}$), 163.9 ($C(4)_{thy}$).

5.1.2. 7-Butyl-15-methyl-1,7,13-triazabicyclo[11.3.1]heptadeca-15-en-14,17-dione (2). At 70 °C to a stirred mixture of *n*-butylamine (2.07 g, 28.4 mmol), K_2CO_3 (4.00 g, 29.0 mmol), and catalytic amount TBA·HSO_4 in *n*-BuOH (250 mL) compound **7** (3.0 g, 7.08 mmol) in *n*-BuOH solution (100 mL) was added and stirring was continued for 11.5 h at 70–75 °C. After evaporating the solvent, treating by $CHCl_3$, filtering, and concentration of $CHCl_3$ solution residue was eluated through column with Al_2O_3 by 2:1 ether/petroleum ether mixture. From the fractions of the eluent, thyimidinocyclophane **2** was obtained in a yield of 0.45 g (19%); mp 44–45 °C; HRMS found *m/z* 335.2570, $C_{19}H_{33}N_3O_2$ required 335.2573, *m/z* 292.2020, $C_{16}H_{26}N_3O_2$ required 292.2025; MS (EI) *m/z* 335 (M+, 13), 292 (M−43, 100), 278 (M−57, 8). ^{1}H NMR (600.0 MHz, $CDCl_3$) δ_H (ppm) 0.85 (3H, m, $(CH_2)_3CH_3$), 1.12 (2H, m, J=7 Hz, $(CH_2)_2CH_2CH_3$), 1.15–1.28 (9H, m, $H_{3,4,5,9,10}$), 1.34 (2H, m, J=7.4 Hz, $CH_2CH_2C_2H_5$), 1.42 (1H, m, H_{11}), 1.57 (1H, m, H_{11}), 1.75 (1H, m, H_3), 1.86 (3H, s, $C(5)_{thy}$Me), 2.11–2.25 (6H, m, $H_{6,8}$), 2.32 (2H, m, $CH_2C_3H_7$), 3.09 (1H, m, H_2), 3.92 (1H, m, H_{12}), 4.21 (1H, m, H_{12}), 4.42 (1H, m, H_2), 6.83 (1H, s, $C(6)_{thy}$H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ_C (ppm) 13.2 ($C(5)_{thy}$*Me*), 14.0 ($(CH_2)_3CH_3$), 20.4 ($(CH_2)_2CH_2CH_3$), 22.4 ($C_{5,9}$), 27.4 (C_{10}), 27.8 (C_3), 28.0 (C_4), 30.1 ($(CH_2)_2CH_2CH_3$), 40.9 (C_{12}), 49.0 (C_2), 52.8 ($C_{6,8}$), 55.0 ($CH_2C_3H_7$), 110.0 ($C(5)_{thy}$), 138.7 ($C(6)_{thy}$), 153.1 ($C(2)_{thy}$), 163.5 ($C(4)_{thy}$).

8,14,23,26,27-Pentamethyl-1,6,8,14,16,21-hexaazatetracyclo-[19,3,1,1^{6,10},1^{12,16}]heptacosa-10(27),12(26),23(24)-triene-7,9,13,15,22,25-hexaone (**3**), 1,3-bis[4-(3,6-dimethyluracil-1-yl)butyl-1-]thymine (**4**), 1,3-dibutylthymine (**5**) were prepared by known procedures.[7b]

5.1.3. 8,14,23,26,27-Pentamethyl-1,6,8,14,16,21-hexaazatetracyclo-[19.3.1.1^{6,10}.1^{12,16}]heptacosa-10(26),12(27),23(24)-triene-7,9,13,15,22,25-hexaone (3). HRMS found *m/z* 526.2530 $C_{26}H_{34}N_6O_6$ required 526.2540; MS (EI) *m/z* 527 $(M+1)^+$ (23), 526 $(M)^+$ (78), 511 $(M-15)^+$ (100), 373 (38), 333 (18), 292 (37), 235 (17), 206 (31), 193 (48), 181 (63), 166 (46), 153 (52), 141 (21), 127 (22), 122 (19). ^{1}H NMR[¶] (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 1.49–1.57 (4H, m, $N(1)_{thy}(CH_2)_2CH_2CH_2$; $N(3)_{thy}(CH_2)_2CH_2CH_2$), 1.62–1.69 (4H, m, $N(1)_{thy}CH_2CH_2(CH_2)_2$; $N(1)_{thy}CH_2CH_2(CH_2)_2$), 1.92 (3H, s, $C(6)_{ur1}CH_3$), 2.06 (3H, s, $C(5)_{thy}$Me), 2.11 (3H, s, $C(6)_{ur2}CH_3$) 3.39 (3H, s, $N(3)_{ur1}CH_3$), 3.40 (3H, s, $N(3)_{ur2}CH_3$), 3.75 (2H, m, $N(1)_{thy}CH_2(CH_2)$), 3.87 (2H, s, $C(5)_{ur1}CH_2C(5)ur_2$), 3.89–4.01 (6H, m, $N(3)_{thy}CH_2(CH_2)_3$; $N(1)_{thy}(CH_2)_3CH_2$; $N(3)_{thy}(CH_2)_3CH_2$), 6.91 (1H, s, $C(6)_{thy}$H); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.0 ($C(5)_{thy}$*Me*), 16.5 ($C(6)_{ur1}CH_3$), 16.7 ($C(6)_{ur2}CH_3$), 22.1 ($C(5)_{ur1}CH_2C(5)_{ur2}$), 23.2 ($N(1)_{thy}CH_2CH_2C_2H_4$), 23.7 ($N(3)_{thy}CH_2CH_2C_2H_4$), 27.1 ($N(1)_{thy}(CH_2)_2CH_2CH_2$), 27.8 ($N(3)_{thy}(CH_2)_2CH_2CH_2$), 28.0 ($N(3)_{ur1}CH_3$), 28.5 ($N(3)_{ur2}CH_3$), 40.7 ($N(3)_{thy}CH_2(CH_2)_3$), 44.6 ($N(1)_{thy}(CH_2)_3CH_2$), 44.9 ($N(3)_{thy}(CH_2)_3CH_2$), 48.9 ($N(1)_{thy}CH_2(CH_2)_3$), 109.6 ($C(5)_{thy}$), 110.5 ($C(5)_{ur1}$), 111.0 ($C(5)_{ur2}$), 138.4 ($C(6)_{thy}$), 148.6 ($C(6)_{ur1}$), 149.0 ($C(6)_{ur2}$), 151.2 ($C(2)_{thy}$), 151.8 ($C(2)_{ur1}$), 151.9 ($C(2)_{ur2}$), 162.8 ($C(4)_{ur1}$), 163.0 ($C(4)_{ur2}$), 163.7 ($C(4)_{thy}$).

5.1.4. 1,3-Bis[4-(3,6-dimethyluracil-1-yl)butyl-1-]thymine (4). Found (%): C, 58.41; H, 6.74; N, 16.37.

¶ thy—thymine unit, and ur2—3,6-dimethyluracil attached to N(1)thy and N(3)thy, respectively.

$C_{25}H_{34}N_6O_6$ required (%): C, 58.35; H, 6.66; N, 16.33. ^{1}H NMR[‖] (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 1.66–1.76 (8H, m, $N(1)_{thy}CH_2(CH_2)_2CH_2$; $N(3)_{thy}CH_2(CH_2)_2CH_2$), 2.26 (6H, s, $C(6)_{ur}CH_3$), 3.30 (6H, s, $2N(3)_{ur}CH_3$), 3.33 (3H, s, $C(5)_{thy}Me$), 3.84 (4H, t, J=7 Hz, $N(1)_{thy}(CH_2)_3CH_2$; $N(3)_{thy}(CH_2)_3CH_2$), 3.92 (4H, t, J=7 Hz, $N(1)_{thy}CH_2(CH_2)_3$; $N(3)_{thy}CH_2(CH_2)_3$), 5.59 (2H, s, 2 $C(5)_{ur}H$), 7.06 (1H, s, $C(6)_{thy}H$); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.0 ($C(5)_{thy}Me$), 19.7 ($C(6)_{ur}CH_3$), 25.0 ($N(1)_{thy}CH_2CH_2C_2H_4$), 25.8 ($N(3)_{thy}CH_2CH_2C_2H_4$), 26.1 ($N(1)_{thy}(CH_2)_2CH_2CH_2$), 26.3 ($N(3)_{thy}(CH_2)_2CH_2CH_2$), 27.8 ($N(3)_{ur}CH_3$), 40.7 ($N(3)_{thy}CH_2(CH_2)_3$), 44.3 ($N(1)_{thy}(CH_2)_3CH_2$), 44.9 ($N(3)_{thy}(CH_2)_3CH_2$), 48.7 ($N(1)_{thy}CH_2(CH_2)_3$), 101.6 ($C(5)_{ur}$), 110.0 ($C(5)_{thy}$), 138.5 ($C(6)_{thy}$), 151.0 ($C(6)_{ur}$), 151.3 ($C(2)_{thy}$), 152.2 ($C(2)_{ur}$), 162.3 ($C(4)_{ur}$), 163.7 ($C(4)_{thy}$).

5.1.5. 1,3-Dibutylthymine (5). Found (%): C, 65.44; H, 9.37; N, 11.85. $C_{13}H_{22}N_2O_2$ required (%): C, 65.51; H, 9.30; N, 11.75. ^{1}H NMR (600.0 MHz, $CDCl_3$, 303 K) δ_H (ppm) 0.87–0.94 (6H, m, $N(1)_{thy}(CH_2)_3CH_3$; $N(3)_{thy}(CH_2)_3CH_3$), 1.28–1.36 (4H, m, $N(1)_{thy}(CH_2)_2CH_2CH_3$; $N(3)_{thy}(CH_2)_2CH_2CH_3$), 1.56 (2H, m, J=7.8 Hz, $N(1)_{thy}CH_2CH_2C_2H_5$), 1.62 (2H, m, J=7.8 Hz, $N(3)_{thy}CH_2CH_2C_2H_5$), 1.88 (3H, s, $C(5)_{thy}Me$), 3.66 (2H, t, J=7.3 Hz, $N(1)_{thy}CH_2(CH_2)_2CH_3$), 3.9 (2H, t, J=7.3 Hz, $N(3)_{thy}CH_2(CH_2)_2CH_3$), 6.92 (1H, s, $C(6)_{thy}H$); ^{13}C NMR (150.9 MHz, $CDCl_3$, 303 K) δ_C (ppm) 13.0 ($C(5)_{thy}Me$), 13.7 ($N(1)_{thy}(CH_2)_3CH_3$), 13.8 ($N(3)_{thy}(CH_2)_3CH_3$), 19.8 ($N(1)_{thy}(CH_2)_2CH_2CH_3$), 20.3 ($N(3)_{thy}(CH_2)_2CH_2CH_3$), 29.7 ($N(3)_{thy}CH_2CH_2C_2H_5$), 31.2 ($N(1)_{thy}CH_2CH_2C_2H_5$), 41.3 ($N(3)_{thy}(CH_2)_3CH_3$), 49.2 ($N(1)_{thy}(CH_2)_3CH_3$), 109.6 ($C(5)_{thy}$), 138.3 ($C(6)_{thy}$), 151.4 ($C(2)_{thy}$), 163.8 ($C(4)_{thy}$).

5.1.6. 1,3-Bis(5-bromopentyl)thymine (7). A solution of 1,5-dibromopentane (155.9 g, 677.8 mmol) in DMF (90 mL) was added dropwise with stirring to a suspension of 14.4 g (84.7 mmol) of disodium salt of thymine in DMF (150 mL). The mixture was stirred for 5 h at 50–60 °C, after which it was evaporated in a vacuum and the residue was treated with 150 mL of $CHCl_3$. The precipitate that formed was filtered off. The solution was concentrated and submitted to chromatography over Al_2O_3. The column was successively washed with petroleum ether and a 2:1 ether/petroleum ether mixture. From ether/petroleum ether mixture fractions compound **6** was obtained as oil in a yield of 15.9 g (45%): MS (EI) m/z 426 (M+, 9), 424 (M+, 22), 422 (M+, 10), 346 (28), 345 (88), 344 (28), 343 (88), 275 (75), 195 (86), 140 (100). Anal. Calcd for $C_{15}H_{24}Br_2N_2O_2$: C, 42.47; H, 5.70; N, 6.60; Br, 37.67. Found: C, 42.48; H, 5.81; N, 6.53; Br, 37.75. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.01 (s, 1H), 3.95 (2H, t, J=7 Hz), 3.73 (2H, t, J=7 Hz), 3.42 (4H, m), 1.93 (3H, s), 1.89 (4H, m), 1.76–1.58 (4H, m), 1.50 (4H, m).

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[‖] thy—thymine unit, ur—two 3,6-dimethyluracil units.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.064

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Formation of benzylamines from triazene compounds via a 1,2-proton shift

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Abstract—A new approach to benzylamines using triazene compounds has been developed that is facilitated by the lithiation of aryltriazenes followed by treatment with an electrophile. The regioselectivity of the reaction can be controlled by means of the substituents in the aryl group. The reaction contains the following steps: intramolecular carbon–carbon bond formation involving lithiation of an alkyl group on a 3-nitrogen atom; a 1,2-proton shift; and the subsequent release of nitrogen gas. Through the use of a deuterated triazene, we were able to determine that the reaction proceeds through a 1,2-proton shift.

1. Introduction

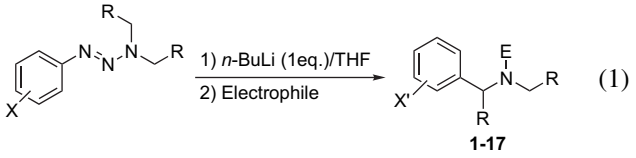
Benzylamines are very important intermediates of fine chemical derivatives such as biologically active compounds and material chemicals. The methods of forming benzylamines are well-known, for example, the condensation of benzyl halide with amine derivatives and the reduction of the benzonitriles etc. In the case of phenols and arylamines as substrates, functionalized benzylamines were created using the bimolecular aromatic Mannich reaction with iminium salt.[1] 1-Aryl-3,3-dialkyltriazenes are also used in many ways in organic syntheses, including the following:[2] as protective groups for aniline derivatives;[3] as substrates of benzyne generation;[4] in diazo coupling;[5] in the Sandmeyer–Gattermann reaction;[6] and in the hydroxylation of positive ion exchange resins.[7] Nicolaou et al. showed that aryltriazenes are used in the construction of aryl ethers and applied these results for the synthesis of vancomycin.[8] In combinatorial chemistry, triazenes are used as linkers in solid-phase synthesis[9] and as alkylating polymers in solution-phase synthesis.[10] Recently, Haley's group has reported the cyclization of 2-alkynylphenyl triazenes.[11]

We have previously reported the preliminary results of a unique transformation of 3,3-dialkyl-1-aryltriazenes into benzylamines.[12] We now report the results of our further investigations into the transformation of triazenes to benzylamines. In fact, that reaction proceeded via a 1,2-proton shift. Since a 1,2-proton shift on an aromatic ring has never been reported, we demonstrated the phenomenon using NMR, mass spectrometry, and computational chemistry.

2. Results and discussion

2.1. Formation of benzylamines

The alkyltriazenes were prepared from arylamines in good yields according to the standard conditions ($NaNO_2$–HCl, then the addition of respective amines).[13] And trimethylsilylphenyltriazenes were synthesized by way of the halogen–metal exchange reaction from the corresponding bromophenyltriazenes according to Welch's procedure.[3a] The general procedure for the transformation of triazenes into benzylamines is as follows: the alkyltriazenes were treated with *n*-BuLi (1 equiv) in THF at 0 °C for 1 h, followed by the addition of electrophiles to produce the corresponding benzylamine derivatives (Eq. 1).



1-17 (1)

The aminoalkyl group of the benzylamines connected to *ortho* position of the original triazenyl group was derived

Keywords: C–C bond forming; Dearomatization; Nucleophilic addition; 1,2-Proton shift; Triazene.
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Table 1. Results of transformation reaction of triazenes into benzylamines

Entry	Substrates			Products			Yield (%)
	X	R	Electrophile	X′[a]		E	
1	H	H	H_2O	**1**	H	H	57
2	H	H	*n*-BuBr	**2**	H	*n*-Bu	78
3	H	H	$(Boc)_2O$	**3**	H	Boc	81
4	H	Me	$(Boc)_2O$	**4**	H	Boc	47
5	H	$-(CH_2)_2-$	$(Boc)_2O$	**5**	H	Boc	69
6	*o*-Me	H	H_2O	**6**	*m*-Me	H	74
7	*m*-Me	H	H_2O	**7**	*o*,*p*-Me (2:1)	H	71
8	*m*-Me	H	$(Boc)_2O$	**8**	*o*,*p*-Me (2:1)	Boc	90
9	*p*-Me	H	H_2O	**6**	*m*-Me	H	95
10	*p*-Me	H	$(Boc)_2O$	**9**	*m*-Me	Boc	95
11	*m*-MeO	H	$(Boc)_2O$	**10**	*o*-MeO	Boc	70
12	*p*-MeO	H	$(Boc)_2O$	**11**	*m*-MeO	Boc	65
13	*o*-F	H	$(Boc)_2O$	**12**	*m*-F	Boc	Trace
14	*m*-F	H	$(Boc)_2O$	**13**	*o*-F	Boc	41
15	*p*-F	H	$(Boc)_2O$	**12**	*m*-F	Boc	89
16	*o*-Cl	H	$(Boc)_2O$	**14**	*m*-Cl	Boc	29
17	*m*-Cl	H	$(Boc)_2O$	**15**	*o*,*p*-Cl (1:1)	Boc	45
18	*p*-Cl	H	$(Boc)_2O$	**14**	*m*-Cl	Boc	54
19	*o*-TMS	H	$(Boc)_2O$	**16**	*m*-TMS	Boc	68
20	*m*-TMS	H	$(Boc)_2O$	**17**	*p*-TMS	Boc	81
21	*p*-TMS	H	$(Boc)_2O$	**16**	*m*-TMS	Boc	70

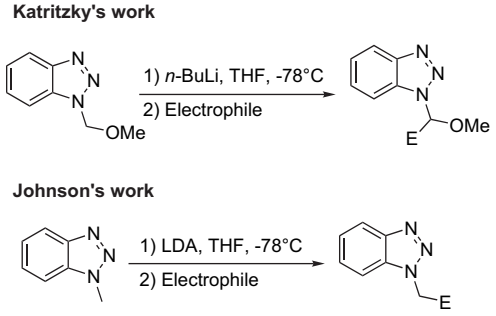
[a] The ratio of diastereomers was determined by ^1H NMR.

from the alkyl group on 3-nitrogen of the triazenyl group. The results of transformation of the triazenes to benzylamines are summarized in Table 1. The 3,3-dimethyl-1-phenyltriazene was transformed to benzylmethylamine (**1**) at a 57% yield, by treating first with *n*-BuLi and then with H_2O. The addition of $(Boc)_2O$ instead of H_2O as an electrophile greatly increased the isolated yield of **3** (81%) (entries 1 and 3). When *o*- and *p*-methylphenyltriazenes were treated as above, *m*-methylbenzylamines (**6** and **9**) were formed in both cases, but the yield of the product was superior in the latter case (entries 6, 9, and 10).

Other *o*- and *p*-substituted triazenes also gave *m*-substituted benzylamine derivatives (**11**, **12**, **14**, and **16**) (entries 12, 13, 15, 16, 18, 19, and 21). In entry 13, the desired product was obtained in trace amount and the most of substrate was remained. In the case of the *m*-methyl- and *m*-chlorophenyltriazenes, the mixtures of *o*- and *p*-substituted benzylamine derivatives (**7**, **8**, and **15**) were obtained (entries 7, 8, and 17). In contrast, *m*-methoxy- and *m*-fluorophenyltriazenes created the *o*-substituted benzylamines (**10** and **13**) exclusively in moderate to good yields, and *m*-trimethylsilylphenyltriazene gave *p*-trimethylsilylbenzylamine (**17**) as the sole product (entries 11, 14, and 20). When *o*-, *m*-, and *p*-bromophenyltriazenes were treated with *n*-BuLi (1 equiv) as above, non-substituted triazene was obtained at yields of 60, 32, and 41%, respectively. Similarly, trimethylsilylphenyltriazenes were obtained from bromophenyltriazenes, when chlorotrimethylsilane was used as an electrophile. It seemed that these reactions proceeded via transmetallation and then protonation, or trimethylsilylation.

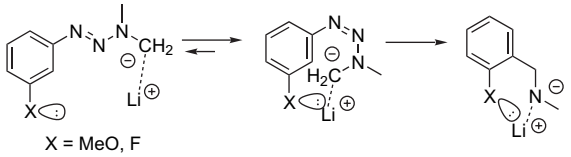
In order to explain the interesting results above, we tried to confirm the place of the first triazene deprotonations. When 3,3-dimethyl-1-phenyltriazene was treated with LDA (1 equiv) at 0 °C in the presence of chlorotrimethylsilane (1 equiv), 3-monosilylated- and 3,3-disilylated triazenes (**18** and **19**) were obtained at yields of 16 and 10%, respectively (Scheme 1). We did not observe any silylated products of the phenyl group, which are generated by the neighboring effects of nitrogen atoms. In the absence of chlorotrimethylsilane, the same procedure created benzylmethylamine at a yield of 48%. These results indicate that deprotonation of the triazene with LDA occurred at the alkyl group on the 3-nitrogen atom[14] and that the successive nucleophilic attack of the carbanion to complete the reaction proceeded on the aromatic ring.

We should also note that both Katritzky's group[15] and Johnson's group[16] reported that 1-alkylated benzotriazoles introduced some electrophiles on the 1-alkyl group via deprotonation with the base (Scheme 2). These reports support our results.



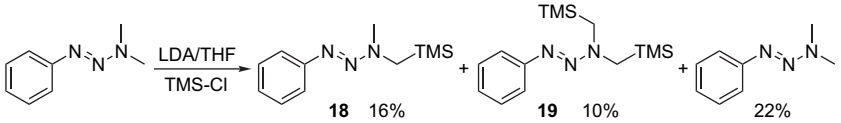
Scheme 2. Deprotonation position of 1-alkylated benzotriazoles.

As mentioned above, *m*-methoxy- and *m*-fluorophenyltriazenes exclusively created the *o*-substituted benzylamines (**10** and **13**). These results can be accounted for by the chelation control as shown in Scheme 3.

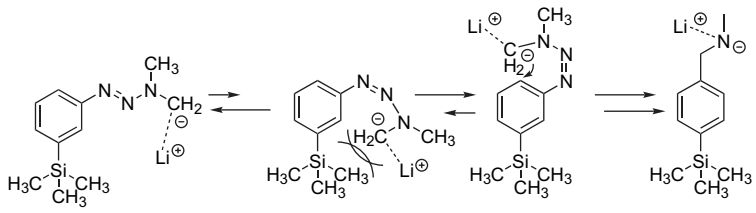


Scheme 3. Chelation control by substituents on the aromatic ring.

Conversely, the observation that *m*-trimethylsilylphenyltriazene provided *p*-trimethylsilylbenzylamine (**17**) was attributed to the steric bulk of trimethylsilyl group (Scheme 4). The regioselectivities of the carbon–carbon bond formation were attributed to the electronic and steric features of the substituents on the aromatic rings.



Scheme 1. Deprotonation position.



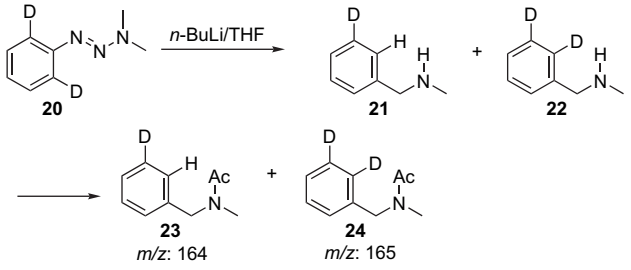
Scheme 4. Steric control by substituents on the aromatic ring.

When the substituents on the benzene ring were the strongly electron-withdrawing group like nitro group and trifluoromethyl groups, the reactions did not produce good results. In the case of the nitro and trifluoromethyl groups, the triazene reactions created complex mixtures containing unidentified polymerized products. On the other hand, the fluoro- and chlorophenyltriazenes were transformed to the corresponding benzylamines in good to moderate yields.

Also, silyl substituted benzenes are very important intermediates in organic synthesis, because the benzene nuclei are easily transformed to various electron-withdrawing groups by aromatic electrophilic *ipso*-desilylation.[17] Consequently, in order to get benzylamines, which are substituted with electron-withdrawing groups, the silyl substituted products (**16** and **17**) may become valuable compounds.

2.2. Proton source

To reveal the proton source of the products, 1-(2,6-dideuteriophenyl)-3,3-dimethyltriazene (**20**) was treated with base (Scheme 5). The ^{1}H NMR spectra of the products (**21**+**22**) indicated that the integrated value of peaks of the phenyl protons is 3.6, while that of the methyl group protons is 3 (Fig. 1). This result indicated that the products involved one or two deuterium atoms and one deuterium atom was abstracted. The MS spectra of the benzylmethylamine and the reaction products gave an (M^+-1) peak as the standard peak and the parent peaks did not appear clearly. Therefore, the amines were treated with acetic anhydride to provide the amides. The MS spectra of the acetylated compounds (**23**+**24**) and *N*-benzyl-*N*-methylacetamide are shown in Figure 2. In the former case, the parent peaks appeared at *m*/*z*=164 and 165, indicating that one or two deuterium atoms remained in the products. This suggests that the proton, located on the *ipso* position of the triazenyl group in the product, shifted from the *o*-position of the triazenyl group and/or was abstracted by the THF of the solvent.



Scheme 5. Reaction of dideuterated triazene.

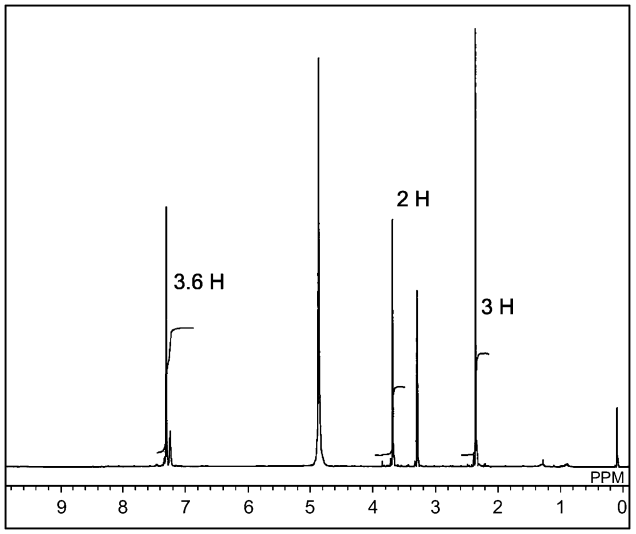


Figure 1. ^{1}H NMR spectrum of the products obtained from **20**.

2.3. Computational studies

The 1,2-proton shift on aromatic ring was proposed and verified by a computational study, because it had not yet been reported. Semiempirical PM3 optimization, as demonstrated in the WinMOPAC 2.0 program (Fujitsu Limited), was used during the preliminary studies.[18] We confirmed the reasonable reaction coordinates by searching through the transition states and conducting vibrational frequency analysis (Fig. 3). The first anion of the molecule was located on the methyl group of near side to N1–N2 double bond. In the case where the anion stayed on another methyl group, the transition state 1 (**TS1**) had no negative frequencies. When the new carbon–carbon bond formed (**I**), the configuration of methyl group and the proton of new ring junction was *syn*. When the two methyl groups were *anti* configured, we could not achieve the required optimized structure for the **TS1**. The second transition state (**TS2**) contained reasonable coordinates among the intermediate (**I**) to the product (**P**) that possessed only one negative vibrational frequency: $-2303.5\ \mathrm{cm}^{-1}$.

We performed precise ab initio calculations for the PM3 results using HyperChem software.[19] We obtained the energies (including electron correlation effects) from single point calculations using second order perturbation theory according to Møller and Plesset 2 (MP2/6-31G*).[20] The nature of each stationary point was verified by vibrational frequency analysis. As this reaction was conducted in the hexane solution, we did not consider the solvent effect. In Figure 4, the vertical line represents relative energy, and the abscissa axis is the transition of the reaction. Unexpectedly, the

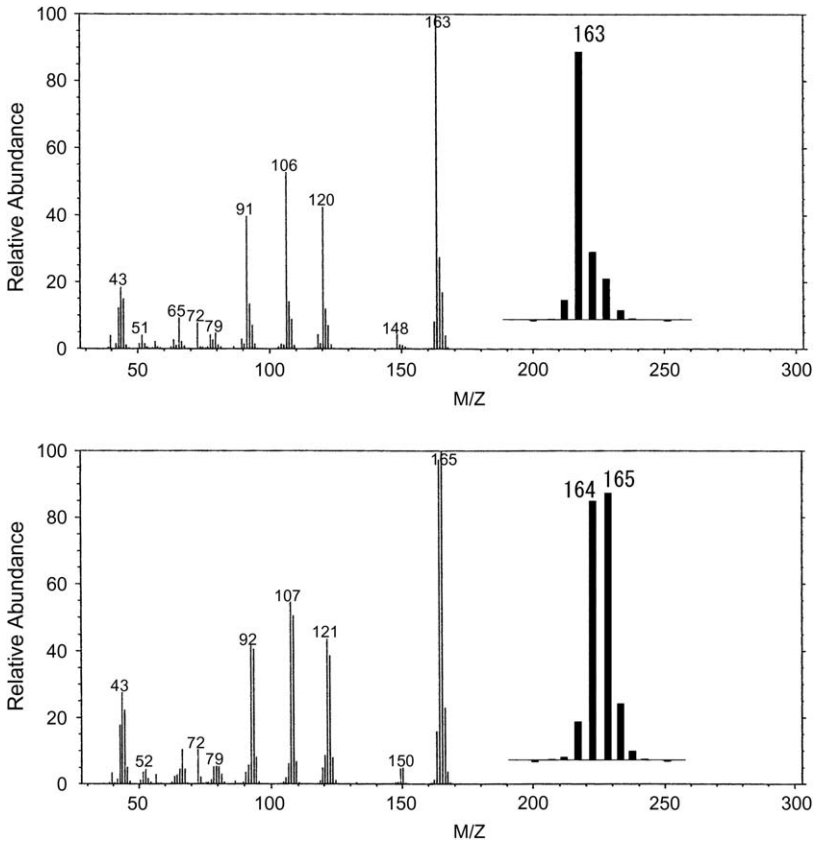


Figure 2. MS spectra of *N*-benzyl-*N*-methylacetamide (upper) and the mixture of **23** and **24** (lower).

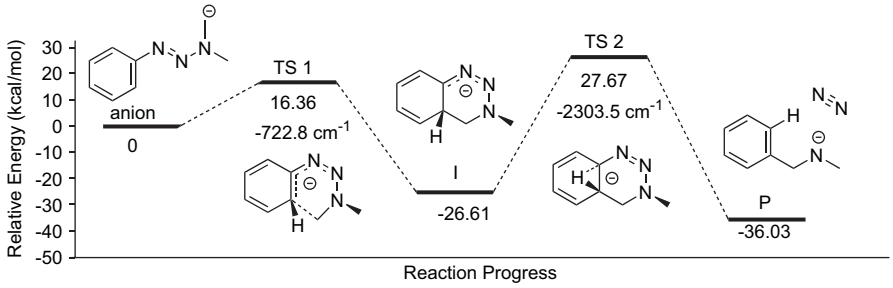


Figure 3. Potential energy correlation diagram and vibrational frequency analysis of reaction. Energy values are given in kcal/mol relative to isolated reactants.

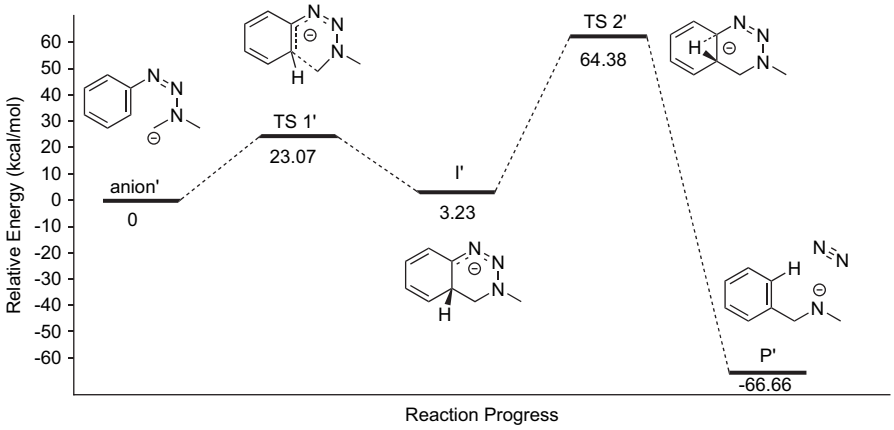
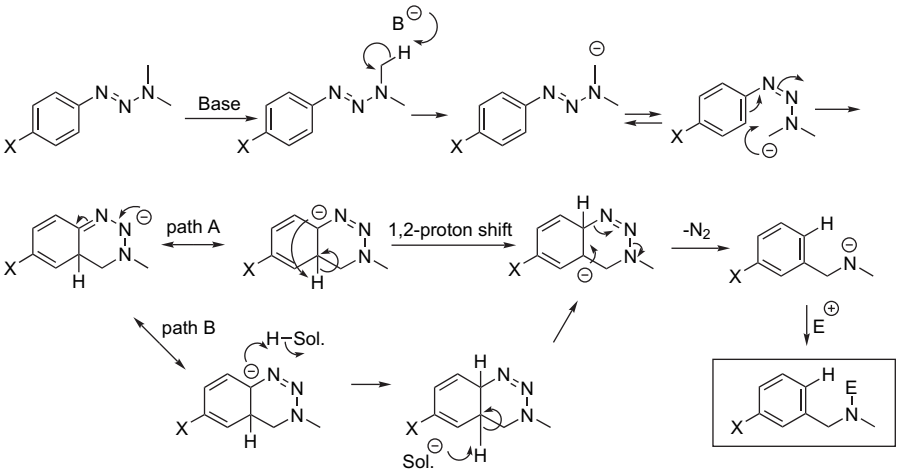


Figure 4. Potential energy surface calculated for the reaction of triazene and base via a mechanism involving 1,2-proton shift. Energy values are given in kcal/mol relative to isolated reactants.



Scheme 6. The proposed reaction mechanism.

intermediate (**I′**) was 3.23 kcal/mol higher than the starting anion, which suggests that the dearomatized compound was comparatively stable. Although the second transition state (**TS2′**) needs extremely high energy (61.15 kcal/mol) to activate **I′**, it had one negative vibration and the 1,2-shift of the proton reflected this experimental result. The release of nitrogen was the driving force of this reaction.

2.4. Reaction mechanism

Considering the above results, we proposed the reaction mechanism shown in Scheme 6. Deprotonation occurred on the α-carbon of the 3-nitrogen. This anion took place in a nucleophilic attack at the *o*-position to the triazenyl group on the aryl group, similar to the Sommelet–Hauser rearrangement[21] and formed a dearomatized intermediate like the Meisenheimer complex. For further reactions, two possible routes were considered. In path A, a 1,2-proton shift occurred. In path B, the solvent was involved in the reaction. The proton that originated from the solvent was incorporated into the anion. This was followed by the abstraction of the proton with the anion derived from the solvent. The carbanion formed a double bond to reconstruct the benzene nuclei, which released nitrogen gas continuously.

3. Conclusion

We discovered a benzylamine forming reaction from 1-aryl-3,3-dialkyltriazene. It contained an intramolecular carbon–carbon bond forming reaction. This reaction was proceeded by an unusual 1,2-proton shift, which was revealed using the deuterated triazene. In the case where the aryl group possessed a strong electron-withdrawing group as nitro or trifluoromethyl, this reaction was not permitted. These reactions provide an alternative method for the preparation of benzylamine derivatives and the new cleavage of triazenyl linkers in the solid-phase synthesis.

4. Experimental

4.1. General methods

NMR spectra were recorded on JEOL GSX-270 (^{1}H 270 MHz, ^{13}C 67.5 MHz) or JEOL GX-500 (^{1}H 500 MHz, ^{13}C 125 MHz) spectrometers in $CDCl_3$ or CD_3OD with TMS as an internal standard. Mass spectra (EI) were recorded on a JMS-HX100 spectrometer. Infrared spectra were recorded on a Shimadzu IR-435 spectrophotometer. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was freshly distilled under nitrogen from sodium benzophenone ketyl prior to use.

4.2. Preparation of triazenes

Most 1-aryl-3,3-dialkyltriazene was synthesized according to the method described in the literature.[13] And the trimethylsilylphenyltriazenes were synthesized by way of the halogen–metal exchange reaction from the corresponding bromophenyltriazene according to Welch's procedure.[3a]

4.2.1. Registry numbers of triazenes.

3,3-Dimethyl-1-phenyltriazene	7227-91-0
3,3-Diethyl-1-phenyltriazene	13056-98-9
1-(Phenylazo)pyrrolidine	36719-71-8
3,3-Dimethyl-1-(2-methylphenyl)triazene	20240-98-6
3,3-Dimethyl-1-(3-methylphenyl)triazene	20241-03-6
3,3-Dimethyl-1-(4-methylphenyl)triazene	7203-89-6
3,3-Dimethyl-1-(3-methoxyphenyl)triazene	20241-03-6
3,3-Dimethyl-1-(4-methoxyphenyl)triazene	7203-92-1
1-(2-Fluorophenyl)-3,3-dimethyltriazene	52010-51-2
1-(3-Fluorophenyl)-3,3-dimethyltriazene	52010-52-3
1-(4-Fluorophenyl)-3,3-dimethyltriazene	23456-94-2
1-(2-Chlorophenyl)-3,3-dimethyltriazene	20241-00-3
1-(3-Chlorophenyl)-3,3-dimethyltriazene	20241-05-8
1-(4-Chlorophenyl)-3,3-dimethyltriazene	7203-90-9
1-(2-Bromophenyl)-3,3-dimethyltriazene	52010-61-4
1-(4-Bromophenyl)-3,3-dimethyltriazene	7239-21-6
1-(3-Bromophenyl)-3,3-dimethyltriazene	29878-94-2

4.2.2. 3,3-Dimethyl-1-(2-trimethylsilylphenyl)triazene. To a solution of 1-(2-bromophenyl)-3,3-dimethyltriazene (3.68 g, 16.1 mmol) in dry THF (16 mL) was added dropwise a solution of *n*-BuLi (10.0 mL, 1.72 mmol, 1.72 M in hexane) at −84 °C. After stirring for 5 min, chlorotrimethylsilane (2.08 mL, 16.4 mmol) was added dropwise, and the mixture was stirred for 30 min at room temperature. The reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane)

afforded the title compound as an oil (1.78 g, 8.03 mmol, 50%); ^{1}H NMR ($CDCl_3$) δ: 7.48 (dd, J=7.0, 1.5 Hz, 1H), 7.43 (dd, J=8.0, 1.0 Hz, 1H), 7.32 (ddd, J=8.0, 7.0, 1.5 Hz, 1H), 7.12 (dd, J=7.0, 1.0 Hz, 1H), 3.35 (br s, 6H), 0.31 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.59, 134.38, 129.90, 124.82, 115.46, 41.26, 39.39, −0.22; IR (neat) cm^{-1}: 2900 (s), 1580 (m), 1460 (s), 1400 (m), 1320 (s), 1250 (s), 1060 (s), 830 (s), 750 (s); Low-MS (EI) (*m*/*z*, %): 221 (M^+, 27), 206 (21), 178 (5), 163 (16), 149 (100), 135 (9), 121 (78), 105 (5.0), 91 (5.1), 73 (7.0), 59 (3.0), 43 (8.1); HRMS: 221.1354 (Calcd for $C_{11}H_{19}N_3Si$, 221.1349).

4.2.3. 3,3-Dimethyl-1-(3-trimethylsilylphenyl)triazene. To a solution of 1-(3-bromophenyl)-3,3-dimethyltriazene (3.75 g, 16.4 mmol) in dry THF (16 mL) was added dropwise a solution of *n*-BuLi (10.0 mL, 1.72 mmol, 1.72 M in hexane) at −84 °C. After stirring for 5 min, chlorotrimethylsilane (2.10 mL, 16.6 mmol) was added dropwise, and the mixture was stirred for 30 min at room temperature. The reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane/EtOAc=19/1 as eluents) afforded the title compound as an oil (3.04 g, 13.7 mmol, 84%); ^{1}H NMR ($CDCl_3$) δ: 7.55 (m, 1H), 7.40 (m, 1H), 7.34 (dd, J=7.0, 0.8 Hz, 1H), 7.30 (dt, J=3.0, 1.0 Hz, 1H), 3.34 (s, 6H), 0.28 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 150.13, 141.09, 130.42, 128.27, 126.00, 120.33, 39.09, −1.08; IR (neat) cm^{-1}: 2900 (m), 1570 (w), 1440 (s), 1380 (s), 1300 (s), 1240 (s), 1070 (s), 820 (s), 740 (s); Low-MS (EI) (*m*/*z*, %): 221 (M^+, 26), 206 (10), 177 (5), 149 (100), 135 (5), 121 (28), 105 (3.0), 88 (5.0), 73 (45), 67 (3.0), 45 (4.0); HRMS: 221.1367 (Calcd for $C_{11}H_{19}N_3Si$, 221.1349).

4.2.4. 3,3-Dimethyl-1-(4-trimethylsilylphenyl)triazene. To a solution of 1-(4-bromophenyl)-3,3-dimethyltriazene (3.66 g, 16.0 mmol) in dry THF (16 mL) was added dropwise a solution of *n*-BuLi (10.0 mL, 1.72 mmol, 1.72 M in hexane) at −84 °C. After stirring for 5 min, chlorotrimethylsilane (2.10 mL, 16.6 mmol) was added dropwise, and the mixture was stirred for 30 min at room temperature. The reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane/$CHCl_3$=4/1 as eluents) afforded the title compound as an oil (2.68 g, 12.1 mmol, 76%); ^{1}H NMR ($CDCl_3$) δ: 7.48 (dd, J=8.0, 3.0 Hz, 2H), 7.39 (dd, J=8.0, 3.0 Hz, 2H), 3.34 (br s, 6H), 0.26 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 151.39, 136.93, 133.95, 119.86, 41.29, 37.36, −1.00; IR (neat) cm^{-1}: 2950 (s), 1590 (s), 1450 (s), 1410 (s), 1380 (s), 1320 (s), 1250 (s), 1070 (s), 820 (s), 750 (s); Low-MS (EI) (*m*/*z*, %): 221 (M^+, 28), 206 (10), 178 (5.1), 149 (100), 135 (3.0), 121 (28), 73 (15); HRMS: 221.1357 (Calcd for $C_{11}H_{19}N_3Si$, 221.1349).

4.3. General procedure for transformation of triazenes into benzylamines

To a solution (0.5–1 M) of triazene in dry THF was added dropwise a solution of *n*-BuLi/hexane (1.0 equiv) at 0 °C. After stirring for 1 h, the reaction mixture was quenched with water or each electrophile (1.5 equiv). Extractive work-up and the subsequent purification afforded benzylamine derivatives.

4.3.1. Benzylmethylamine (1). Oil, 57%; ^{1}H NMR ($CDCl_3$) δ: 7.26 (m, 5H), 4.42 (s, 2H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 139.90, 127.97, 127.76, 126.52, 55.72, 35.65; IR (neat) cm^{-1}: 3500 (m), 3000 (m), 1680 (s), 1440 (m), 1380 (m), 1250 (m), 1140 (m), 870 (m), 680 (m); Low-MS (EI) (*m*/*z*, %): 121 (M^+, 86), 120 (100), 104 (5.0), 91 (69), 77 (11), 65 (18), 51 (11), 41 (61); HRMS: 121.0897 (Calcd for $C_8H_{11}N$, 121.0892).

4.3.2. *N*-Benzyl-*N*-*n*-butylmethylamine (2). Oil, 78%; ^{1}H NMR ($CDCl_3$) δ: 7.33–7.19 (m, 5H), 3.48 (s, 2H), 2.36 (t, J=7.5 Hz, 2H), 2.18 (s, 3H), 1.50 (m, 2H), 1.33 (m, 3H), 0.90 (t, J=7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ: 139.20, 129.05, 128.18, 62.69, 57.27, 42.20, 29.54, 20.60, 14.05; IR (neat) cm^{-1}: 2950 (s), 2920 (s), 2850 (s), 1640 (br m), 1475 (br m), 1380 (m), 1260 (m); Low-MS (EI) (*m*/*z*, %): 177 (M^+, 8.8), 134 (84), 91 (100), 65 (6.3), 42 (5.0); HRMS: 177.1508 (Calcd for $C_{12}H_{19}N$, 177.1517).

4.3.3. *N*-Benzyl-*N*-(*t*-butoxycarbonyl)methylamine (3). Oil, 81%; ^{1}H NMR ($CDCl_3$) δ: 7.38–7.18 (m, 5H), 4.42 (s, 2H), 2.82 (s, 3H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.87, 138.08, 128.47, 127.14, 79.62, 52.26, 33.86, 28.42; IR (neat) cm^{-1}: 3000 (m), 1680 (s), 1440 (m), 1380 (m), 1250 (m), 1140 (m), 870 (m), 680 (m); Low-MS (EI) (*m*/*z*, %): 221 (M^+, 0.38), 165 (100), 140 (6.3), 120 (48), 91 (57), 77 (2.8), 65 (5.6), 57 (57), 51 (1.9), 41 (11); HRMS: 221.1443 (Calcd for $C_{13}H_{19}NO_2$, 221.1416).

4.3.4. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(α-methylbenzyl)amine (4). Oil, 47%; ^{1}H NMR ($CDCl_3$) δ: 7.48–7.28 (m, 5H), 5.42 (br s, 1H), 3.10 (br m, 1H), 2.93 (br m, 1H), 1.52 (d, J=2.0 Hz, 3H), 1.46 (s, 9H), 0.98 (t, J=2.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ: 155.80, 142.18, 128.21, 127.04, 126.93, 53.10, 38.13, 28.51, 17.49, 15.24; IR (neat) cm^{-1}: 2950 (m), 1680 (s), 1450 (s), 1400 (s), 1280 (s), 1130 (m), 760 (m), 700 (m); Low-MS (EI) (*m*/*z*, %): 249 (M^+, 0.63), 234 (0.44), 193 (100), 178 (75), 164 (10), 148 (14), 134 (72), 120 (7.5), 105 (87), 91 (5), 77 (15), 72 (6), 57 (78), 51 (3.5), 41 (16); HRMS: 249.1715 (Calcd for $C_{15}H_{23}NO_2$, 249.1729).

4.3.5. 1-(*t*-Butoxycarbonyl)-2-phenylpyrrolidine (5). Oil, 69%; ^{1}H NMR ($CDCl_3$) δ: 7.12–7.34 (m, 5H), 4.95 and 4.75 (br s, 1H), 3.61 (d, 3H), 2.30 (br s, 1H), 1.87 (m, 3H), 1.45 (s, 3H), 1.18 (s, 6H); ^{13}C NMR ($CDCl_3$) δ: 160.95, 152.44, 137.77, 131.16, 125.96, 121.09, 81.66, 63.96, 40.11, 33.69, 28.24, 26.93, 16.54; IR (neat) cm^{-1}: 2950 (m), 1680 (s), 1600 (w), 1480 (m), 1450 (m), 1380 (s), 1250 (m), 1150 (m), 900 (m), 730 (m); Low-MS (EI) (*m*/*z*, %): 247 (M^+, 0.23), 191 (100), 174 (27.5), 163 (4.4), 146 (85), 131 (24), 119 (37), 104 (12), 91 (23), 77 (88), 70 (21), 65 (2.5), 57 (72), 51 (3.1), 41 (20); HRMS: 247.1589 (Calcd for $C_{15}H_{21}NO_2$, 247.1572).

4.3.6. Methyl(*m*-methylbenzyl)amine (6). Oil, 74% from *o*-methylphenyltriazene, 95% from *m*-methylphenyltriazene; ^{1}H NMR ($CDCl_3$) δ: 7.05–7.24 (m, 4H), 3.71 (s, 2H), 2.45 (s, 3H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 158.21, 156.21, 147.15, 146.46, 145.89, 143.40, 74.24, 54.22, 39.55; IR (neat) cm^{-1}: 3315 (m), 2923 (s), 2790 (s), 1608 (s), 1444 (s), 1379 (w), 1351 (w); Low-MS (EI) (*m*/*z*, %): 135 (M^+, 31), 134 (58), 120 (25), 106 (19), 91 (36), 77

(31), 44 (100); HRMS: 135.1039 (Calcd for $C_9H_{13}N$, 135.1048).

4.3.7. A mixture of methyl(*o*-methylbenzyl)amine and methyl(*p*-methylbenzyl)amine (7). Oil, *o*/*p*=2/1, 71%; mixture: ^{13}C NMR ($CDCl_3$) δ: 137.95, 136.78, 136.60, 136.21, 130.23, 129.06, 128.36, 128.17, 126.97, 125.88, 55.65, 53.55, 36.30, 35.80, 21.06, 18.89; IR (neat) cm^{-1}: 3318 (m), 3019 (m), 2934 (s), 2789 (s), 1513 (w), 1460 (s), 1379 (w), 1354 (w), 1131 (w), 1096 (m), 804 (m), 743 (s).

Major product (o-isomer): ^1H NMR ($CDCl_3$) δ: 7.27–7.30 (m, 1H), 7.15–7.18 (m, 3H), 3.74 (s, 2H), 2.50 (s, 3H), 2.35 (s, 3H), 1.87 (br s, 1H); IR (neat) cm^{-1}: 3318 (m), 3019 (m), 2934 (s), 2789 (s), 1513 (w), 1460 (s), 1379 (w), 1354 (w), 1131 (w), 1096 (m), 804 (m), 743 (s); Low-MS (EI) (*m*/*z*, %): 135 (M^+, 36), 134 (14), 120 (11), 104 (100), 91 (19), 77 (28), 51 (17), 44 (67); HRMS: 135.1053 (Calcd for $C_9H_{13}N$, 135.1048).

Minor product (p-isomer): ^1H NMR ($CDCl_3$) δ: 7.20 (d, *J*=8.0 Hz, 2H), 7.13 (d, *J*=8.0 Hz, 2H), 3.71 (s, 2H), 2.44 (s, 3H), 2.33 (s, 3H), 1.87 (br s, 1H); Low-MS (EI) (*m*/*z*, %): 135 (M^+, 35), 134 (56), 120 (27), 105 (56), 91 (36), 77 (33), 51 (25), 44 (86), 42 (100); HRMS: 135.1032 (Calcd for $C_9H_{13}N$, 135.1048).

4.3.8. A mixture of *N*-(*t*-butoxycarbonyl)-*N*-methyl (*o*-methylbenzyl)amine and *N*-(*t*-butoxycarbonyl)-*N*-methyl(*p*-methylbenzyl)amine (8). Oil, *o*/*p*=2/1, 90%; ^1H NMR ($CDCl_3$) δ (*major product*): 7.14 (m, 4H), 4.44 (2H), 2.79 (br s, 3H), 2.28 (2H), 1.53 (s, 9H); (*minor product*): 7.14 (m, 4H), 3.38 (2H), 2.79 (br s, 3H), 2.34 (2H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.91, 136.77, 135.00, 129.16, 127.10, 125.96, 79.59, 49.84, 28.45, 28.41, 27.40, 18.98; IR (neat) cm^{-1}: 2950 (m), 1780 (br s), 1690 (s), 1460 (s), 1370 (s), 1280 (s), 1250 (s), 840 (m), 760 (m).

4.3.9. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*m*-methylbenzyl)amine (9). Oil, 95%; ^1H NMR ($CDCl_3$) δ: 7.22 (t, *J*=8.0 Hz, 1H), 7.10–6.97 (m, 3H), 4.39 (br s, 2H), 2.81 (br s, 3H), 2.34 (s, 3H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.98, 138.08, 137.98, 128.36, 127.86, 124.34, 79.55, 52.47, 33.83, 28.42, 21.38; IR (neat) cm^{-1}: 2900 (m), 1680 (s), 1650 (m), 1380 (br s), 1250 (s), 1120 (br s), 870 (m), 760 (m), 730 (m); Low-MS (EI) (*m*/*z*, %): 235 (M^+, 1.3), 179 (100), 164 (7.5), 146 (3.0), 134 (46), 120 (15), 105 (75), 91 (11), 77 (13), 65 (5.0), 57 (74), 51 (2.5), 41 (20); HRMS: 235.1581 (Calcd for $C_{14}H_{21}NO_2$, 235.1572).

4.3.10. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*o*-methoxybenzyl)amine (10). Oil, 70%; ^1H NMR ($CDCl_3$) δ: 7.23 (br t, *J*=7.9 Hz, 1H), 7.14 (br d, *J*=7.0 Hz, 1H), 6.93 (dt, *J*=1.0, 8.0 Hz, 1H), 6.88 (d, *J*=8.0 Hz, 1H), 4.44 (s, 2H), 3.82 (s, 3H), 2.85 (br s, 3H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 157.30, 156.17, 128.05, 126.16, 120.43, 113.87, 110.14, 79.34, 55.19, 47.34, 34.30, 28.43; IR (neat) cm^{-1}: 2950 (m), 1690 (s), 1600 (m), 1460 (s), 139 (s), 1250 (s), 1140 (s), 1020 (m), 880 (m), 750 (m); Low-MS (EI) (*m*/*z*, %): 251 (M^+, 2.5), 195 (61), 180 (1.9), 150 (100), 136 (39), 121 (58), 108 (3.1), 91 (40), 78 (8.1), 65 (8.8), 57 (56), 51 (3.8), 41 (11); HRMS: 251.1506 (Calcd for $C_{14}H_{21}NO_3$, 251.1521).

4.3.11. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*m*-methoxybenzyl)amine (11). Oil, 96%; ^1H NMR ($CDCl_3$) δ: 7.24 (t, *J*=7.8 Hz, 1H), 7.24 (m, 3H), 4.40 (s, 2H), 3.80 (s, 3H), 2.82 (br s, 3H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 159.82, 139.75, 129.47, 119.59, 112.94, 112.56, 79.62, 55.13, 52.48, 33.88, 28.43; IR (neat) cm^{-1}: 2900 (m), 1680 (s), 1600 (s), 1420 (br s), 1280 (br s), 1040 (m), 870 (m), 760 (m); Low-MS (EI) (*m*/*z*, %): 251 (M^+, 5.0), 195 (100), 170 (6.3), 150 (35), 136 (5.0), 122 (58), 107 (2.5), 91 (10), 78 (63), 57 (44), 41 (10); HRMS: 251.1503 (Calcd for $C_{14}H_{21}NO_3$, 251.1521).

4.3.12. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*m*-fluorobenzyl)amine (12). Oil, trace from *o*-fluorophenyltriazene, from 89% *p*-fluorophenyltriazene; ^1H NMR ($CDCl_3$) δ: 7.29 (dt, *J*=6.0, 7.8 Hz, 1H), 6.89–7.03 (m, 3H), 4.41 (br s, 2H), 2.83 (br s, 3H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 163.26 (d, J_{CF}=246 Hz), 155.70, 146.80, 140.82 (d, J_{CF}=7.2 Hz), 129.96 (d, J_{CF}=8.3 Hz), 122.89 (d, J_{CF}=20.8 Hz), 114.02 (d, J_{CF}=20.8 Hz), 79.84, 52.24, 52.17, 34.02, 28.36; IR (neat) cm^{-1}: 3020 (m), 2930 (m), 1690 (s), 1620 (w), 1590 (m), 1490 (m), 1450 (m), 1390 (s), 1370 (m), 1295 (w), 1250 (m), 1170 (m), 1145 (m), 1070 (m), 980 (w), 870 (w); Low-MS (EI) (*m*/*z*, %): 239 (M^+, 0.57), 183 (98), 166 (8.6), 138 (43), 109 (80), 83 (14), 57 (100), 41 (41); HRMS: 239.1331 (Calcd for $C_{13}H_{18}FNO_2$, 239.1322).

4.3.13. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*o*-fluorobenzyl)amine (13). Oil, 41%; ^1H NMR ($CDCl_3$) δ: 7.24 (m, 1H), 7.12 (dd, *J*=7.5, 1.5 Hz, 1H), 6.99–7.09 (m, 2H), 4.48 (br s, 2H), 2.87 (br s, 3H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 160.67 (d, J_{CF}=246 Hz), 155.80, 129.48 (d, J_{CF}=32.2 Hz), 128.77 (d, J_{CF}=8.3 Hz), 124.94 (d, J_{CF}=15.6 Hz), 124.11, 115.21 (d, J_{CF}=21.8 Hz), 79.71, 46.18, 45.33, 34.13, 28.35; IR (neat) cm^{-1}: 2990 (m), 2930 (m), 1710 (s), 1690 (s), 1620 (w), 1590 (m), 1490 (m), 1445 (m), 1395 (m), 1370 (m), 1250 (m), 1225 (m), 1175 (m), 1145 (m), 1050 (w), 1025 (w), 875 (w), 830 (w), 750 (m); Low-MS (EI) (*m*/*z*, %): 239 (M^+, 0.28), 183 (98), 166 (6.0), 138 (57), 109 (86), 83 (13), 57 (100), 41 (37); HRMS: 239.1346 (Calcd for $C_{13}H_{18}FNO_2$, 239.1322).

4.3.14. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*m*-chlorophenylmethyl)amine (14). Oil, 29% from *o*-chlorophenyltriazene; 54% from *p*-chlorophenyltriazene; ^1H NMR ($CDCl_3$) δ: 7.22 (m, 4H), 4.40 (s, 2H), 2.86 (s, 3H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.62, 140.21, 134.36, 129.74, 128.43, 127.31, 125.28, 79.87, 52.09, 34.02, 28.35; IR (neat) cm^{-1}: 2900 (m), 1680 (s), 1600 (m), 1580 (m), 1420 (br m), 1250 (s), 1140 (s), 880 (m), 780 (m), 680 (m); Low-MS (EI) (*m*/*z*, %): 255 (M^+, 0.23), 199 (82), 182 (8.8), 164 (5.0), 154 (33), 125 (70), 111 (3.1), 99 (5.0), 89 (15), 77 (3.5), 57 (100), 51 (2.5), 41 (26); HRMS: 255.1031 (Calcd for $C_{13}H_{18}ClNO_2$, 255.1026).

4.3.15. A mixture of *N*-(*t*-butoxycarbonyl)-*N*-methyl (*o*-chlorobenzyl)amine and *N*-(*t*-butoxycarbonyl)-*N*-methyl(*p*-chlorobenzyl)amine (15). Oil, *o*/*p*=1/1, 45%; ^1H NMR ($CDCl_3$) δ: 7.27 (m, 4H), 4.55 (d, 2H), 2.87 (d, 3H), 1.46 (m, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.95, 135.44, 129.50, 128.47, 128.23, 127.75, 126.91, 79.82, 50.18, 34.38, 28.36; IR (neat) cm^{-1}: 2950 (m), 1680 (s), 1480 (br s), 1390 (s), 1250 (s), 1140 (br s), 1040 (m), 880 (m), 740 (m).

4.3.16. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*m*-trimethylsilylbenzyl)amine (16). Oil, 67% from *o*-trimethylsilylphenyltriazene, 70% from *p*-trimethylsilylphenyltriazene; ^{1}H NMR ($CDCl_3$) δ: 7.31 (m, 4H), 4.42 (br s, 2H), 2.81 (br s, 3H), 1.48 (s, 9H), 0.26 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.82, 140.78, 137.21, 132.17, 127.86, 79.63, 52.79, 33.91, 28.45, −1.15; IR (neat) cm^{-1}: 2950 (m), 1690 (s), 1460 (s), 1390 (s), 1250 (s), 1150 (s), 820 (s), 750 (s); Low-MS (EI) (*m*/*z*, %): 293 (M^+, 0.13), 237 (100), 222 (48), 206 (3.5), 192 (28), 178 (23), 163 (39), 149 (13), 135 (5.0), 120 (37), 103 (13), 91 (3.8), 86 (9.4), 73 (21), 57 (68), 41 (10); HRMS: 293.1817 (Calcd for $C_{16}H_{27}NO_2Si$, 293.1811).

4.3.17. *N*-(*t*-Butoxycarbonyl)-*N*-methyl(*p*-trimethylsilylbenzyl)amine (17). Oil, 81%; ^{1}H NMR ($CDCl_3$) δ: 7.49 (d, J=7.5 Hz, 2H), 7.21 (d, J=7.5 Hz, 2H), 4.42 (s, 2H), 2.82 (br s, 3H), 1.48 (s, 9H), 0.26 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 155.91, 139.20, 138.63, 133.54, 79.63, 52.54 (d), 33.91, 28.45, 28.07, −1.13; IR (neat) cm^{-1}: 2950 (m), 1690 (s), 1470 (s), 1350 (s), 1130 (m), 830 (s), 750 (m); Low-MS (EI) (*m*/*z*, %): 293 (M^+, 0.44), 237 (97), 222 (100), 206 (2.5), 192 (25), 178 (28), 163 (31), 147 (12), 135 (15), 120 (27), 103 (7.5), 91 (3.5), 73 (14), 57 (51), 41 (8.8); HRMS: 293.1806 (Calcd for $C_{16}H_{27}NO_2Si$, 293.1811).

4.3.18. 3-Methyl-1-phenyl-3-(trimethylsilylmethyl)triazene (18) and 1-phenyl-3,3-bis-(trimethylsilylmethyl)-triazene (19). To a solution of lithium diisopropylamide [prepared from diisopropylamine (0.14 mL, 1.00 mmol) and *n*-BuLi (0.64 mL, 1.01 mmol, 1.58 M in hexane)] in dry THF (1 mL) was added chlorotrimethylsilane (0.13 mL, 1.03 mmol). 3,3-Dimethyl-1-phenyltriazene (0.147 mg, 0.99 mmol) was added dropwise to the above solution at 0 °C. The mixture was stirred for 30 min and warmed up to room temperature. The reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane/EtOAc=99/1 as eluents) afforded **18** (0.034 g, 16%) and **19** (0.029 g, 10%).

18: ^{1}H NMR ($CDCl_3$) δ: 7.32–7.15 (m, 4H), 7.00 (tt, J=8.2, 1.8 Hz, 1H), 3.00–3.50 (envelope, 5H), 0.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 151.09, 128.77, 124.70, 120.28, 39.97, 11.7, −1.05; IR (neat) cm^{-1}: 2952 (s), 1594 (s), 1457 (s), 1436 (s), 1387 (s), 1346 (s), 852 (s); Low-MS (EI) (*m*/*z*, %): 221 (M^+, 6.1), 153 (6.9), 135 (8.3), 105 (40), 77 (100), 73 (93), 59 (15), 51 (23), 43 (31); HRMS: 221.1330 (Calcd for $C_{11}H_{19}N_3Si$, 221.1348).

19: ^{1}H NMR ($CDCl_3$) δ: 7.31–6.95 (m, 5H), 3.24 (s, 2H), 3.10 (s, 2H), 0.11 (s, 9H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 151.22, 128.75, 124.06, 120.09, 49.13, 44.96, −0.52, −1.29; IR (neat) cm^{-1}: 3065 (s), 2897 (m), 1595 (m), 1482 (m), 1455 (s), 1415 (s), 1388 (s), 1358 (s), 1248 (s), 1183 (s), 853 (s); Low-MS (EI) (*m*/*z*, %): 293 (M^+, 0.4), 278 (0.9), 188 (18), 105 (27), 73 (100), 69 (25), 59 (28), 45 (29); HRMS: 293.1730 (Calcd for $C_{14}H_{27}N_3Si_2$, 293.1776).

4.3.19. 1-(2,6-Dideuteriophenyl)-3,3-dimethyltriazene (20). To a solution of 1-(2,6-dibromophenyl)-3,3-dimethyltriazene (1.08 g, 3.50 mmol) in dry THF (2.9 mL) was added dropwise a solution of *n*-BuLi (3.50 mL, 5.10 mmol, 1.45 M in hexane) at −80 °C. After stirring for an additional 1 h, MeOD (0.29 mL, 7.00 mmol) was added and the mixture was stirred for 30 min at room temperature. The reaction was quenched with water and extracted with ether. The extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. To a solution of the residue in dry THF (2.9 mL) was added dropwise a solution of *n*-BuLi (3.50 mL, 5.10 mmol, 1.45 M in hexane) at −80 °C. After stirring for an additional 1 h, MeOD (0.29 mL, 7.00 mmol) was added and the mixture was stirred for 30 min at room temperature. The reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane/EtOAc=19/1 as eluents) afforded **20** (0.312 g, 59%); ^{1}H NMR ($CDCl_3$) δ: 7.33 (d, J=7.2 Hz, 2H), 7.105–7.165 (t, J=7.2 Hz, 1H), 3.33 (br s, 6H); ^{13}C NMR ($CDCl_3$) δ: 150.81, 128.77, 128.67, 125.34, 120.5, 120.20 (t, J_{CD}=23.9 Hz), 120.0, 41.13; IR (neat) cm^{-1}: 760 (s), 640 (w), 610 (m), 560 (w); Low-MS (EI) (*m*/*z*, %): 151 (M^+, 28), 107 (50), 79 (100), 52 (10), 42 (4); HRMS: 151.1050 (Calcd for $C_8H_9N_3D_2$, 151.1076).

4.3.20. *N*-Benzyl-*N*-methylacetamide. To a solution of *N*-methylbenzylamine (0.121 g, 1.00 mmol) in Et_3N (1 mL) was added dropwise Ac_2O (0.15 mL, 2.00 mmol) at 0 °C. After stirring for 1 h, the reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane/EtOAc=3/2 as eluents) afforded the title compound (0.123 g, 76%); ^{1}H NMR (500 MHz; CD_3OD) δ: 7.19–7.37 (m, 5H), 4.61 and 4.57 (2H), 2.97 and 2.97 (3H), 2.16 and 2.15 (3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ: 170.92, 170.61, 137.15, 136.36, 128.78, 128.41, 127.83, 127.46, 127.17, 126.13, 54.06, 50.41, 35.35, 33.56, 21.63, 21.25; Low-MS (EI) (*m*/*z*, %): 163 (M^+, 100), 148 (5), 120 (43), 106 (53), 91 (40), 79 (5), 72 (8), 65 (10), 51 (5), 43 (20).

4.3.21. A mixture of *N*-methyl-(3-deuteriobenzyl)amine (21) and *N*-methyl-(2,3-dideuteriobenzyl)amine (22). To a solution of 1-(2,6-dideuteriophenyl)-3,3-dimethyltriazene (**20**) (0.324 g, 2.14 mmol) in dry THF (2.1 mL) was added dropwise a solution of *n*-BuLi (1.47 mL, 2.14 mmol, 1.45 M in hexane) at 0 °C. After stirring for 1 h, the reaction was quenched with water. Extractive work-up and subsequent short path distillation (0.77 mmHg) afforded the mixture of title compounds (0.099 g); ^{1}H NMR (500 MHz; CD_3OD) δ: 7.34–7.21 (m, 4H), 3.68 (s, 2H), 2.36 (s, 3H); Low-MS (EI) (*m*/*z*, %): 124 (12.5), 123 (M^+, 37), 122 (100), 105 (5), 93 (34), 92 (55), 78 (10), 66 (10), 51 (8), 44 (58); HRMS: 123.1025 (Calcd for $C_8H_9ND_2$, 123.1025).

4.3.22. A mixture of *N*-(3-deuteriobenzyl)-*N*-methylacetamide (23) and *N*-(2,3-dideuteriobenzyl)-*N*-methylacetamide (24). To a solution of a mixture of *N*-methyl (3-deuteriobenzyl)amine and *N*-methyl(2,3-dideuteriobenzyl)amine (0.220 g) in Et_3N (1.78 mL) was added dropwise Ac_2O (0.19 mL, 2.00 mmol) at 0 °C. After stirring for 1 h, the reaction was quenched with water. Extractive work-up and subsequent flash silica gel chromatography (hexane/EtOAc=3/2 as eluents) afforded the title compounds (0.065 g); ^{1}H NMR (500 MHz; CD_3OD) δ: 7.20–7.40 (m, 4H), 4.59 and 4.53 (s, 2H), 2.95 and 2.92 (s, 3H), 2.163 and 2.158 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ: 170.92,

170.62, 137.23, 137.16, 136.44, 136.35, 128.82, 128.53, 128.45, 128.34, 128.17, 127.88, 127.76, 127.41, 127.21, 127.11, 126.19, 126.08, 54.14, 54.09, 50.46, 50.42, 35.40, 33.60, 24.03, 21.70, 21.31, 13.56; Low-MS (%) 167 (3.5), 166 (22), 165 (M^+, 100), 164 (M^+, 98), 163 (16), 150 (5), 122 (38), 121 (43), 108 (50), 107 (54), 93 (39), 92 (41), 79 (5), 72 (10), 52 (5), 43 (27).

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Synthesis, properties, and hepatic metabolism of strongly fluorescent fluorodipyrrinones

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Abstract—From non-fluorescent 8-*H* fluorophenyldipyrrinones, highly fluorescent (ϕ_F 0.4–0.6) analogs have been synthesized by reaction with 1,1′-carbonyldiimidazole to bridge the dipyrrinone nitrogens and form an *N*,*N*′-carbonyldipyrrinone (3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]-pyrimidine-3,5-dione). Amphiphilic, water-soluble 8-sulfonic acid derivatives are then obtained by reaction with concd H_2SO_4. The resulting fluorinated and sulfonated *N*,*N*′-carbonyl-bridged dipyrrinones, isolated as their sodium salts, are potential cholephilic fluorescence and ^{19}F MRI imaging agents for use in probing liver and biliary metabolism. After intravenous injection in the rat they were excreted rapidly and largely unchanged in bile. ^{19}F NMR spectroscopy of a pentafluorophenyl-tosylpyrrolinone synthetic precursor exhibited rarely seen diastereotopicity.

1. Introduction

The natural pigment bilirubin (Fig. 1A) is produced continuously in animals during normal catabolism of heme.[1] It is a bichromophoric structure comprised of two *Z*-dipyrrinones bearing intramolecularly hydrogen-bonded propionic acid side-chains.[2,3] It is lipophilic and has low solubility in water at physiologic pH. In humans accumulation of bilirubin is thwarted by its efficient clearance from the blood by the liver and elimination in bile as ester glucuronide conjugates of the propionic acid side-chains.[1,4] Formation of the glucuronides is catalyzed by a specific glucuronosyl transferase enzyme (UGT1A1)[5] and their excretion from the liver is mediated by a membrane transporter known as MRP2 (multidrug resistance associated protein 2).[6] These two proteins are important in the metabolism and hepatic elimination of a large number of xenobiotics in addition to bilirubin. Genetic deficiencies of either UGT1A1 or MRP2 and a variety of liver disorders can cause accumulation of bilirubin or its glucuronides and clinical jaundice.[5,6] In the absence of UGT1A1, elimination of bilirubin is almost totally impaired. If the concentration of unconjugated bilirubin in the circulation exceeds the binding capacity of serum albumin, movement of the pigment across the blood–brain barrier and deposition within the brain can cause toxicity.[1] In contrast to bilirubin, xanthobilirubic acid (Fig. 1B),[7] a polar but water-insoluble synthetic dipyrrinone analog for one-half of bilirubin, is readily excreted intact in bile without the need for glucuronidation.[8] Thus, bilirubin and its analogs are useful probes for hepatobiliary disfunction and mechanisms of glucuronidation and biliary excretion.

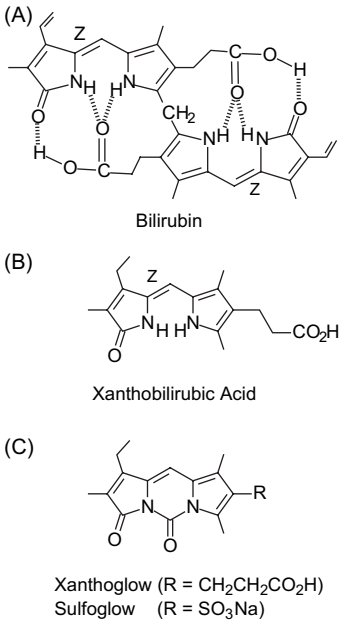

Figure 1. (A) Bilirubin. (B) Xanthobilirubic acid, a dipyrrinone model for bilirubin. (C) Xanthoglow, a highly fluorescent (ϕ_F 0.80, cyclohexane) *N*,*N*′-carbonyl-bridged analog of xanthobilirubic acid and sulfoglow, a water-soluble analog.

Keywords: Dipyrroles; Perfluorophenyl; ^{19}F NMR; Fluorescence.
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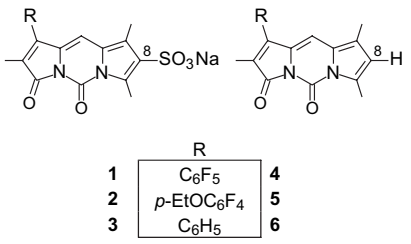
Though brightly colored, bilirubin and xanthobilirubic acid are essentially non-fluorescent at room temperature, e.g., with fluorescence quantum yields of $\phi_F<10^{-3}$ because of rapid $Z\rightarrow E$ isomerization in the excited states.[2,3,9] Restricting $Z\rightarrow E$ isomerization by bridging the two nitrogens of a dipyrrinone causes a dramatic increase in fluorescence (to $\phi_F\sim1$). Recently we found that a carbonyl group is a convenient bridge, easily inserted by reaction of the dipyrrinone with 1,1′-carbonyldiimidazole (CDI) in the presence of strong base (such as DBU) in CH_2Cl_2, and is very effective in enhancing fluorescence.[10] Thus, xanthobilirubic acid methyl ester was readily converted to xanthoglow (Fig. 1C) methyl ester, with $\phi_F=0.80$ in cyclohexane at 25 °C for λ_{exc} 410 nm and λ_{em} 473 nm.[10]

Seeking a water-soluble xanthoglow analog, we prepared the first 'sulfoglow' (Fig. 1C),[10a] a xanthoglow analog with the C(8) propionic acid replaced by sodium sulfonate. When injected intravenously into rats it was rapidly excreted intact into bile and urine, which became highly fluorescent.[11] These preliminary studies suggested that it might be possible to develop highly fluorescent cholephilic ('bile-loving') analogs useful for fluorescence imaging of renal and hepatic metabolism or for early detection of cholestatic liver disease. Were they to contain fluorine, such agents might also be useful for ^{19}F-magnetic resonance imaging. To this end we set out to synthesize cholephilic highly fluorinated sulfoglows ('fluoroglows'). From our experience and perspective, a logical target was pentafluoroglow **1**. In the course of this study, we learned, however, that its synthesis was compromised in the presence of nucleophilic base (ethoxide) in favor of *p*-ethoxytetrafluoroglow (**2**) and regioisomers. We also present the synthesis of pure **2** here because it provides a key intermediate for synthesizing *p*-alkoxy analogs of **2** of differing lipophilicity and hence regulating hepatic versus renal excreability.[11] In the following, we describe the syntheses and spectroscopic properties of new fluorinated, fluorescent dipyrrinones **4–6** and the sulfoglow sodium salts **1** and **2**, with pentafluoro- and tetrafluorophenyl groups on the lactam ring. We also present preliminary studies on the metabolism and hepatobiliary excretion of **1** and **2**. This work is part of a more comprehensive investigation of cholephilic fluorescent pharmacophores for understanding transhepatic uptake and transport and cholestatic disorders, particularly in the newborn.[11]

Target Compounds



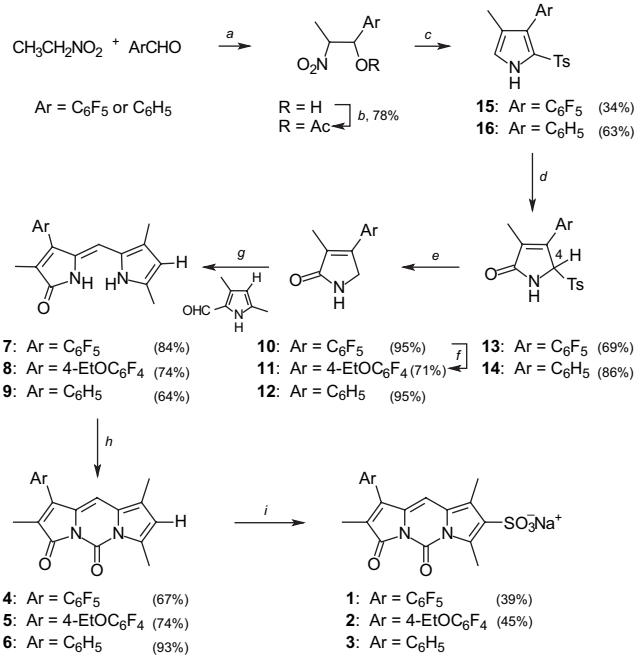
	R	
1	C_6F_5	4
2	*p*-$EtOC_6F_4$	5
3	C_6H_5	6

2. Results and discussion

2.1. Syntheses

The syntheses of **4** and **5** employed a Barton–Zard pyrrole-forming reaction sequence explored earlier with benzaldehyde.[12] Commercial pentafluorobenzaldehyde was condensed with nitroethane in the presence of DBU (Scheme 1), a Henry reaction that afforded an alcohol that was acetylated and used without purification in a Barton–Zard reaction with *p*-toluenemethylisocyanide (TosMIC) to give **15**. Replacing nitroacetate in Scheme 1 with a nitroalkene from a Knoevenagel reaction between pentafluorobenzaldehyde and nitroethane using ammonium acetate[13] might also have provided a suitable precursor to pyrrole **15**; however, this nitroalkene synthesis was unsuccessful. α-Bromination followed by treatment with TFA/H_2O is a typical way[12,14] of transforming an α-free pyrrole into a pyrrolinone (e.g., **13**), and this route was successfully applied in transforming **16** into **14**. The same approach was found earlier by our group to give unsatisfactory results with **15**. Therefore, direct oxidation of **15** to **13** using hydrogen peroxide was examined. This reaction was optimized over many runs, particularly by changing the contact time from 12 to 30 h. The yield seemed dependent also on the quality of the peroxide; an aged (lower percent peroxide?) reagent gave the best results. Chromatographic purification was necessary to obtain a 69% yield of **13**. Quantitative removal of the tosyl group to yield **10** was achieved using sodium borohydride in ethanol.[14]

Base-catalyzed condensation of **10** with 3,5-dimethyl-2-formyl-1*H*-pyrrole[15] should have led directly to **7**, but replacement of aromatic ring fluorine also occurred. Although substitution of the *para*-fluorine might have been expected, we observed a lack of regioselective replacement during test condensation reactions of pyrrolinone **10**. With a 2-fold excess of aldehyde versus pyrrolinone **10**, freshly prepared sodium methoxide or ethoxide, and a reaction time of 20 h at reflux, the yield of isolated yellow pigment from methanol was lower than from higher boiling ethanol, from which ~58% of a yellow pigment was obtained after



Scheme 1. Reagents and conditions: (a) DBU; (b) Ac_2O, DMAP; (c) TosMIC, TMG; (d) H_2O_2 or i: $PhNMe_3^+Br_3^-$, ii: TFA, H_2O; (e) $NaBH_4$; (f) NaOEt; (g) *N*-methylmorpholine; (h) CDI, DBU; (i) concd H_2SO_4, then Na_2CO_3.

chromatography and crystallization. Although the pigment from each solvent was crystalline and homogeneous by TLC, ^{1}H NMR spectroscopy revealed only ~80–85% *p*-alkoxy regioisomeric purity. Recrystallizations, even with ~50% loss, improved the purity of the ethoxy pigment **8** to only ~90%. ^{1}H NMR spectroscopy, in particular the C(5)-methine signal, was indicative of the presence of three different dipyrrinones, most likely due to *ortho*- as well as *para*-substitution and *ortho*, *para*-disubstitution of fluorine by ethoxy. The ^{13}C NMR spectral lines of the minor isomeric impurities, heavily split by the fluorines, were very weakly intense and were thus of little use in analyzing different substitution patterns. But ^{19}F NMR spectroscopy, with its wide dispersion range, revealed unequivocally the presence of the mono *ortho*-substituted analog of **8**, in addition to *para*.

The pentafluorophenyl ring is known to be susceptible to aromatic nucleophilic substitution, and so we considered pentafluorobenzaldehyde[16] reactivity with nucleophiles to be a model for that of a pentafluorophenyl β-substituent of a dipyrrinone, where in both cases the carbon attached to the fluorinated benzene ring is sp^2 hybridized. Typical fluorine-replacing nucleophilic aromatic substitution reactions including the reaction of pentafluorobenzaldehyde with (i) sodium hydrosulfide in DMF and (ii) sodium thiophenolate in CH_3OH yielded predominantly *para*-thiophenol and *para*-phenylthioether, respectively.[17] A different *ortho*–*para* selectivity was found in the reaction of dimethylamine with pentafluorobenzaldehyde in diethyl ether at room temperature for 1 h: the *ortho*-amino isomer dominated over the *para* by a 3:2 ratio.[17] No clear-cut procedure has been reported for the reaction of pentafluorobenzaldehyde with sodium methoxide in methanol; however, the *para*-methoxy derivative has been isolated in low yield after careful distillation.[17] Similarly, methoxide ion replaced predominantly the *para*-fluorine in bromopentafluorobenzene after reflux in methanol for 8 h and isolation in 70% yield by preparative gas-chromatography.[16] Recent papers discussed the regioselectivity of nucleophilic replacement (including by ethoxide) in di-, tri-, tetra-, and pentafluoropyridines.[18] On the basis of the available literature and the preliminary work described above, we deemed it advantageous to find conditions for regioselective replacement of *para*-fluorine only.

Instead of synthesizing the dipyrrinone while simultaneously and non-selectively replacing fluorine with an ethoxy group, regioselective *para*-fluorine substitution was achieved in an earlier step, under milder conditions. A significant improvement in regioselectivity was achieved when the pyrrolinone **10** was treated at sub-ambient temperature with a freshly prepared solution of sodium ethoxide in ethanol. Optimized parameters included the exact amount of ethoxide and the duration of contact, with best results being obtained from 5 equiv of sodium ethoxide and a 1-h reaction time at ~10 °C to give a 71% yield of **11** from **10**. In light of recent literature data, the reaction might be successful even at a lower temperature. After optimization, similar conditions were also found to give good regioselectivity for *para*-substitution relative to *ortho* in recently published work.[18]

With *p*-ethoxypyrrolinone **11** now isolated in pure form, we examined ways to carry it forward to dipyrrinone **8** while avoiding strongly nucleophilic ethoxide/ethanol (or sodium hydroxide/ethanol) because we suspected that a high reaction temperature and a nucleophilic base[16–18] would facilitate further substitution of (*ortho*) fluorines. Thus, condensation using a non-nucleophilic base in a non-nucleophilic solvent was sought. With a strong non-nucleophilic base (DBU) in DMF or CH_3CN solvent at 85–90 °C, condensation of **11** with 2-formyl-3,5-dimethylpyrrole (Scheme 1) led mostly to decomposition products and no formation of dipyrrinone. Hünig's base (*N*,*N*-diisopropylethylamine) in CH_3CN led to no reaction, and the strong dibasic amine, 1,4-diazabicyclo[2.2.2]octane (DABCO) in CH_3CN at 90 °C resulted mostly in decomposition products. However, with *N*-methylmorpholine in dimethylformamide at 90 °C, a promising 31% yield of dipyrrinone was obtained. With a change of solvent to CH_3CN and increased reaction time to 84 h, **11** was converted smoothly into dipyrrinone **8** in 74% yield.

With the proper conditions for dipyrrinone formation from a fluorine-containing pyrrolinone having been found, they were successfully employed on the pentafluorophenyl substituted starting material (**10**). Using pure pyrrolinone **10** and 3 equiv of 3,5-dimethylpyrrole-2-aldehyde in presence of *N*-methylmorpholine in CH_3CN at 95 °C for 84 h, the desired dipyrrinone **7** bearing a C(3)-pentafluorophenyl group was isolated in 84% yield (which was even higher than that obtained with *p*-ethoxy analog **8**). The solubility of **7** in chloroform (or dichloromethane) is very low, however, in comparison with **8**.

Although the yield at the condensation step was higher for **7** than **8**, its conversion to the *N*,*N*′-carbonyl bridged fluorophore **4** was not as clean and high-yielding as is usually observed.[10,11] Only 67% of purified tricycle **4** was isolated from a reaction, which usually gives >90% of product. The starting material **7** was almost completely consumed, and a significant amount of rather polar side product was isolated. Its structure was deduced from ^{1}H and ^{13}C NMR spectra to be the analog of **5** with a 1-imidazolyl ring replacing ethoxy at the *para*-position. Thus, nucleophilic substitution of the *para*-fluorine cannot be completely suppressed even under the mild conditions used. The adventitious imidazole nucleophile apparently is released from CDI after the carbonyl transfer reaction that forms the bridged dipyrrinone.

For comparison of spectroscopic data, the non-fluorinated parent of **7** was prepared from the known pyrrolinone **12**.[12] Here, too, condensation with 3,5-dimethylpyrrole-2-aldehyde was conducted using *N*-methylmorpholine in CH_3CN at 95 °C for 48 h to give the then unknown C(3)-phenyl-substituted dipyrrinone (**9**) in 64% yield. The typical reaction conditions (KOH/EtOH) gave unacceptable results: a mixture of two products in low yield. Cyclization of **9**, unlike **7** or **8**, smoothly afforded tricyclic **6** in 93% yield.

Insertion of the carbonyl bridge by reacting **8** with CDI and DBU was sluggish and afforded the *N*,*N*′-carbonyl-bridged dipyrrinone **5**, which was isolated in moderate 74% yield. A similar slow carbonyl insertion and comparable product yield (67%) were found in the conversion of **7** to **4**. Sulfonation of **5** using concd H_2SO_4 at 0 °C, followed by an alkaline quench, gave sodium sulfonate **2**, which seemed to be less soluble in water than its C(3)-*n*-decyl analog

synthesized earlier.[11] After quenching the sulfonation reaction, the organic material separated as a solid from both aqueous and organic (chloroform) phases. More complete extraction into chloroform was achieved by addition of methanol. The crude product was purified by radial chromatography to afford a 45% yield of the yellow sulfonate salt. Its purity was determined by ^{1}H NMR to be >90%. Sulfonation of **4** (to **1**) was found to be more sluggish, and under the reaction conditions of **5**→**2**, no product (**1**) was found. At 25 °C for 4 h, however, **4** was transformed to **1** in 39% yield. In contrast to these two reactions, attempted conversion of **6** to **3** resulted in products that were not extractable, presumably due to multiple sulfonation, of the phenyl ring and other sites.

2.2. Constitutional structures

The constitutional structures of **1**, **2**, **4**–**16** follow from the method of synthesis and their ^{1}H and ^{13}C NMR spectra. Pyrrolinone **12** was reported previously.[12] The ^{19}F NMR spectra of **1**, **2**, **4**, **5**, **7**, **8**, **10**, **11**, **13**, and **15** were also obtained and correlated with the assigned structures. The ^{19}F NMR spectrum of **13** differed in significant ways from the other nine. In particular, whereas **10** showed three ^{19}F NMR signals with second order ^{19}F{^{19}F} couplings (−139.51 ppm, *ortho*; −152.89 ppm, *para*; −161.51 ppm, *meta*), **13** exhibited two sharp and two very broad signals at 298 K (Fig. 2). The unexpected appearance of four chemical shifts at ambient temperature was clarified by lowering the temperature to 223 K where five chemical shifts were found, with the two most downfield signals showing fine structure. From this apparently more complex spectrum, it became clear that a dynamic process occurs in **13**. Although the same process might occur in the five other pentafluorophenyl substituted compounds (**1**, **4**, **7**, **10**, and **15**), it was not detected by NMR. What makes **13** unique is the presence of a stereogenic center at C(4) bearing the tosyl group. Stereogenic centers are absent in **1**, **4**, **7**, **10**, and **15**—as well as in **2**, **5**, **8**, and **11** (the *p*-ethoxy analog of **13** was not prepared). The stereogenic center in **13**, combined with the axis of rotation defined by C(3)–C(*ipso*) bond (an axis of chirality), renders the *ortho*- and *meta*-fluorines diastereotopic. They lie in different electronic environments in the preferred conformation of **13**, and ^{19}F NMR is sufficiently sensitive to detect different chemical shifts even at room temperature. Rotation around the C(3)–C(*ipso*) bond is rather uninhibited at 298 K, and only the *ortho*-fluorines, being closest to the stereogenic center, are anisochronous. At lower temperature, the hindered rotation extends the diastereotopicity to meta-fluorines as well (at −160.57 ppm and −160.75 ppm). In principle, the phenyl *ortho* and *meta* hydrogens, as well as those from the tosyl group of **14** are diastereotopic, but the ^{1}H NMR spectrum was too complicated to resolve due to overlapping signals.

The coalescence temperature (T_c) of *ortho*- and *ortho*′-^{19}F NMR signals of **13** was 313 K in $CDCl_3$. The first order rate constant (k_c) of the exchange process (i.e., the rotation rate) at this temperature is given by: $k_c = \pi \times \Delta\nu/(2)^{1/2}$ where $\Delta\nu$ is the signal separation in hertz at stop-exchange regime.[19] From the low temperature experiments $\Delta\nu$=841 Hz, which gives a rate k_c=1870 s^{-1}. Dynamic ^{19}F NMR also allows for calculation of the activation barrier to rotation[19] at coalescence according to: $\Delta G^{\ddagger}=RT_c[22.96+\ln(T_c/\Delta\nu)]$. Substituting T_c and $\Delta\nu$ gives a barrier $\Delta G^{\ddagger}$=13.7 kcal mol^{-1}, which indicates only a moderately hindered atropisomerization process.

Typically, when an AB-spin system is envisioned, methylene protons in the neighborhood of an element of chirality come first to mind. However, ^{19}F NMR, with intrinsically high receptivity of fluorine nuclei, has been used for stereochemical purposes and for testing basic NMR concepts chronologically in parallel to ^{1}H NMR. Yet, despite the enormous quantity of ^{19}F NMR spectral data accumulated,[20,21] including material clearly related to stereochemistry and conformational analysis,[19,22] there are few clear-cut examples of AB fluorine spectra.

The closest literature examples of diastereotopic fluorines in a CF_2 group are in perfluorinated compounds. Variable temperature ^{19}F NMR has been reported for 1-perfluorohexyl-1-phenylethanol,[23] which has one stereogenic center and five pairs of prochiral fluorines at increasing distance. At 353 K only the two nearest CF_2 show diastereotopicity and at 193 K only the central in the chain CF_2 continues to show a singlet with small satellites as a sign of nonequivalence. The structure of perfluoro 4-ethyl-3,4-dimethylhexan-3-yl carbanion has also been studied by dynamic ^{19}F NMR.[24] At 298 K only the two CF_2 groups furthest from the carbanionic center exhibit diastereotopicity (ABX_3 spin system). The CF_2 closest to the negative charge shows anisochronous fluorines below 193 K, and this was interpreted as an indication of frozen rotation around the relevant bonds at the carbanionic center. The only AB fluorine spin system from an intentionally labeled CF_2 was found in a difluoromethionine incorporated in three different sites in a recombinant protein.[25] The degree of chemical shift difference is small when the amino acid is at relatively free surface positions, but the anisochronicity is enhanced for a methionine incorporated in tightly packed protein core where there is less conformational freedom. Variable temperature ^{19}F NMR and ^{19}F{^{19}F} COSY experiments of free difluoromethionine have been also reported.[25]

Although difficult to perform at sub-ambient temperatures, a ^{19}F{^{19}F} COSY spectrum was acquired on a sample of pyrrolinone **13** in $CDCl_3$ at 223 K (Fig. 2). The experiment unequivocally confirmed the assignments made earlier on the ground of substituent chemical shift increments. At low temperature the two downfield signals with chemical shifts at −136.92 ppm and −138.71 ppm correlated with the most shielded fluorine nuclei at −160.57 ppm and −160.75 ppm. With scale expansion it becomes evident that the signal at −136.92 ppm correlates with that at −160.57 ppm, and the downfield signal at −138.71 ppm correlates with that at −160.75 ppm. Both upfield signals (−160.75 ppm and −160.57 ppm) correlate with the signal at −150.28 ppm but the latter did not show any other correlations. This means that the latter signal belongs to the fluorine nucleus attached to the *para*-position, which does not show long range (^4J) coupling to the *ortho*-fluorines. Such coupling (^4J=2.9 Hz), however, was detected at 323 K in the 1D high resolution ^{19}F NMR spectrum (Fig. 2A, upper middle trace). From the assignment of the *para*-fluorine signal, it follows that the signals at −160.75 ppm

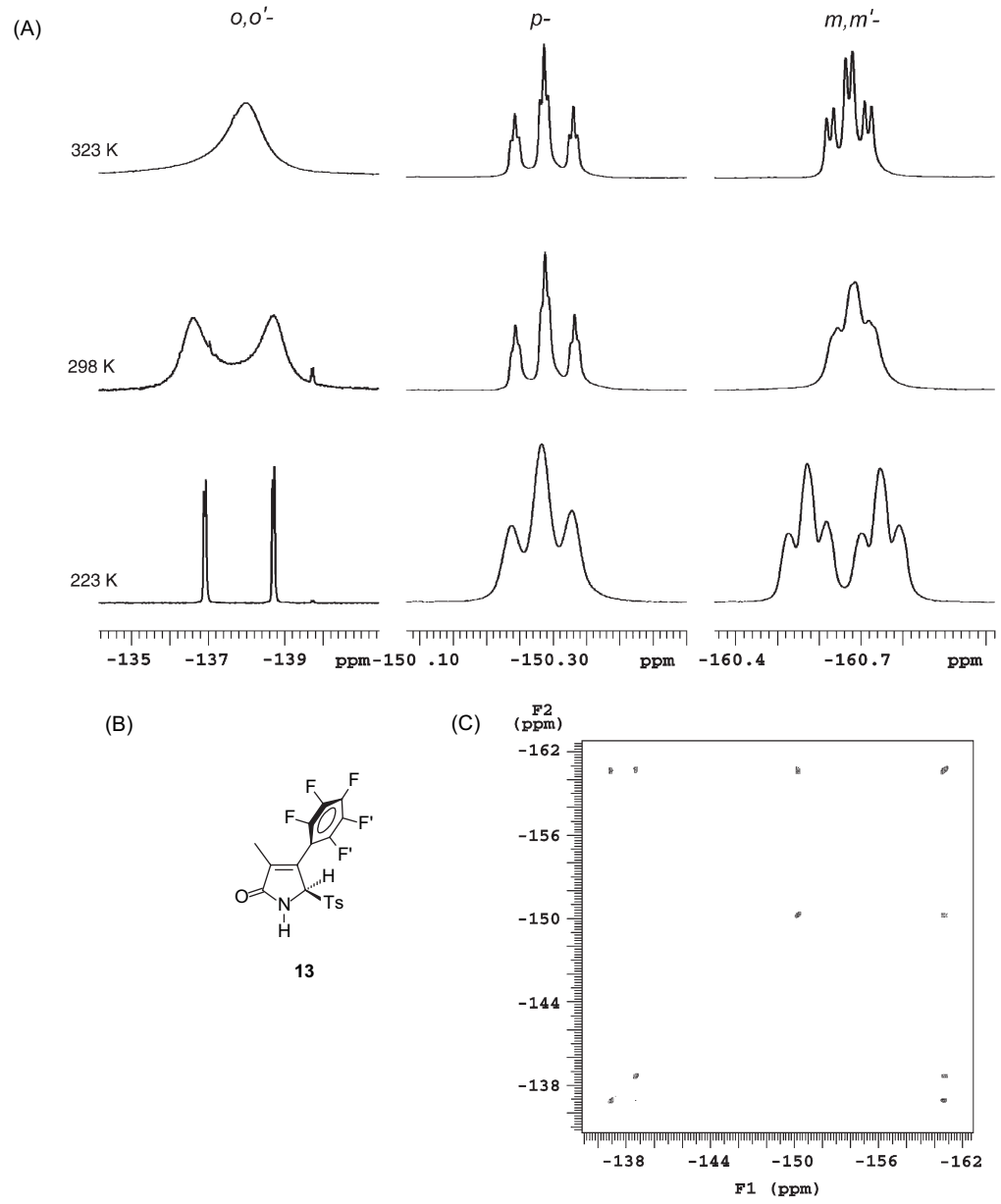


Figure 2. (A) Expanded partial ^{19}F NMR spectra of tosylpyrrolinone **13** in $CDCl_3$ at 323 K (upper traces), at 298 K (middle traces), and at 223 K (lower traces), referenced to external C_6F_6 in $CDCl_3$ at −162.90 ppm. (B) Stereochemical drawing of **13**. (C) Full $^{19}F\{^{19}F\}$ COSY spectrum of **13** in $CDCl_3$ at 223 K.

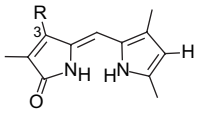
and −160.57 ppm are from *m*,*m*′-fluorines and those at −138.71 ppm and −136.92 ppm from *o*,*o*′-fluorines. In other words, only the most shielded signals (those assigned to *m*,*m*′-fluorines) exhibit a full complement of off-diagonal peaks, all of which are the result of spin–spin coupling via three bonds.

The structures of **4**–**6** also followed from the method of synthesis and from ^{13}C NMR spectra that were characteristic of dipyrrinones (**7**–**9**)[7,11] and *N*,*N*′-carbonyl-bridged dipyrrinones (**4**–**6**),[10,11] with the latter showing a urea-type carbonyl at ∼143 ppm. In the ^1H NMR, **7**–**9** exhibited more shielded C(5)-hydrogens compared to their C(3)-alkyl counterparts. Thus, C(3)-phenyl **9** showed this proton at 6.06 ppm, the pentafluorophenyl **7** at 5.78 ppm, and its *p*-ethoxy analog **8** at 5.82 ppm versus the normally encountered chemical shift, e.g., 6.13 ppm for the C(3)-*n*-decyl substituted dipyrrinone analog.[11] The shieldings in **7**–**9** are due to the influence of the aromatic π-system attached at C(3) of the dipyrrinone, because the aromatic ring faces the C(5)-hydrogen, as supported by molecular mechanics conformational analysis using PC Model.[26] PC Model revealed two isoenergetic minima when the aromatic ring of **7** was allowed to rotate with respect to the lactam, with dihedral angles C(2)–C(3)–C(*ipso*)–C(*ortho*-upper)=+65° and −65°.

2.3. ^1H NMR chemical shifts and hydrogen-bonded dimers

The dipyrrinone NH ^1H NMR chemical shifts in $CDCl_3$ reveal much about the extensively studied monomer⇆dimer equilibrium.[27] Comparison of dipyrrinone pyrrole and lactam NH ^1H NMR chemical shifts in **7**–**9** and in the

Table 1. Comparison of dipyrrinone ^{1}H NMR NH chemical shifts[a] for **7–9** and their C(3)-*n*-decyl analog in $CDCl_3$ at 25 °C



Chemical shift	M	**7** (R=C_6F_5)	**8** (R=4-EtOC_6F_4)	**9** (R=C_6H_5)	R=$(CH_2)_9CH_3$
Lactam NH	1×10^{-2}	11.97	11.91	11.78	11.30
	1×10^{-3}	11.84	11.76	11.53	11.08
	1×10^{-4}	11.61	11.51	11.02	10.58
Pyrrole NH	1×10^{-2}	10.51	10.51	10.56	10.44
	1×10^{-3}	10.42	10.40	10.43	10.30
	1×10^{-4}	10.22	10.17	10.11	9.97

[a] δ, ppm downfield from Me_4Si.

C(3)-decyldipyrrinone synthesized earlier[11] was made at the same sample concentration (Table 1). In all four examples, evidence of intermolecular hydrogen bonding is evident from the deshielded NH resonances at the typical chemical shifts.[27] A 10-fold dilution, from 10^{-3} to 10^{-4} M causes the expected slight upfield shift, again a shift that correlates with a monomer⇆dimer equilibrium. The C(3)-*n*-decyl substituted dipyrrinone exhibited somewhat less deshielded NH chemical shifts than **7–9**, suggesting slightly stronger intramolecular hydrogen bonding in the latter.

If the aromatic substituents at C(3) increase the donor ability of the lactam NH (or the acceptor ability of the carbonyl oxygen), then the dimerization constant is expected to increase. In fact, the lactam NH appears to be more sensitive to C(3) substituent and concentration than the pyrrole NH. The largest lactam NH deshielding difference ($\Delta\delta\sim0.5$ ppm) occurs when changing from C(3)-*n*-decyl to C(3)-phenyl and not between, e.g., phenyl and pentafluorophenyl, suggesting that the relative NH deshielding has an origin other than simply stronger association. A deshielding edge effect from the aromatic ring might be felt by the NH, a view supported by the gradual increase of chemical shift in changing the unsubstituted phenyl to *p*-ethoxytetrafluorophenyl and to pentafluorophenyl. Based on data at 10^{-3} M concentration, the electronegative fluorinated rings of **7** and **8** are more effective in deshielding the lactam NH to 11.84 ppm and 11.76 ppm than the unsubstituted phenyl ring (11.53 ppm). The calculated distances, lactam NH to *ortho*-F, are 4.8–5.0 Å (PC Model).[26] It seems probable that both the shielding of C(5)-H and the deshielding of lactam NH are expressions of the same magnetic anisotropy of the aryl ring on C(3).

2.4. UV–vis absorption and fluorescence emission

All the dipyrrinones and *N*,*N*′-carbonyl-bridged dipyrrinones are yellow compounds forming yellow solutions. The latter are highly fluorescent. Comparison of their UV–vis data (Table 2), particularly those in chloroform (Fig. 3), shows only small perturbations of λ_{max} and ε_{max} due to the C(3)

Table 2. Comparison of UV–vis spectral data of *N*,*N*′-carbonyl-bridged dipyrrinones **1–2**, **4–6**, and **7–9**[a]

Pigment	ε^{max} (λ^{max}, nm)					
	Cyclohexane	C_6H_6	$CHCl_3$	CH_3OH	$(CH_3)_2SO$	H_2O
1		17,600 (447)	17,700 (437)	18,600 (433)	18,400 (447)	17,700 (433)
		10,700 (282)	12,300 (278)	12,900 (275)	12,100 (281)	22,900 (271)
2		13,500 (440)	13,700 (436)	14,400 (432)	13,800 (444)	13,400 (431)
		10,800 (283)	11,500 (280)	11,600 (276)	11,300 (281)	12,000 (273)
4	20,400 (451)	19,200 (449)	19,200 (447)	19,400 (443)	19,300 (445)	
	20,000 (432)					
	10,900 (277)	sh 19,000 (439)	12,300 (279)	13,000 (277)	12,400 (276)	
5	20,100 (448)	19,500 (444)	19,000 (443)	19,200 (441)	19,100 (443)	
	20,200 (428)					
	13,600 (278)	13,600 (281)	14,000 (281)	14,200 (279)	13,500 (279)	
6	19,200 (437)	18,200 (433)	18,000 (433)	17,900 (431)	18,200 (433)	
	19,500 (419)					
	sh 12,900 (281)		13,800 (279)	13,600 (278)	13,600 (278)	
7	sh 24,900 (453)	sh 22,000 (456)	39,200 (425)	38,200 (431)	35,800 (429)	
	48,000 (427)	42,300 (429)				
	6800 (299)	6200 (299)	6200 (290)	6000 (290)	sh 6100 (287)	
8	sh 24,700 (447)	sh 23,000 (452)	37,200 (423)	37,700 (429)	35,300 (429)	
	46,100 (423)	42,000 (427)				
	7600 (297)	7000 (298)	7400 (285)	6900 (286)	7400 (282)	
9	sh 22,600 (439)	sh 22,400 (441)	35,700 (411)	36,900 (417)	34,400 (417)	
	43,600 (412)	40,700 (415)				
	7500 (290)	7100 (292)	7200 (282)	6800 (282)	7200 (286)	

[a] Concentration range $2.3–3.8\times10^{-5}$ M at 20 °C.

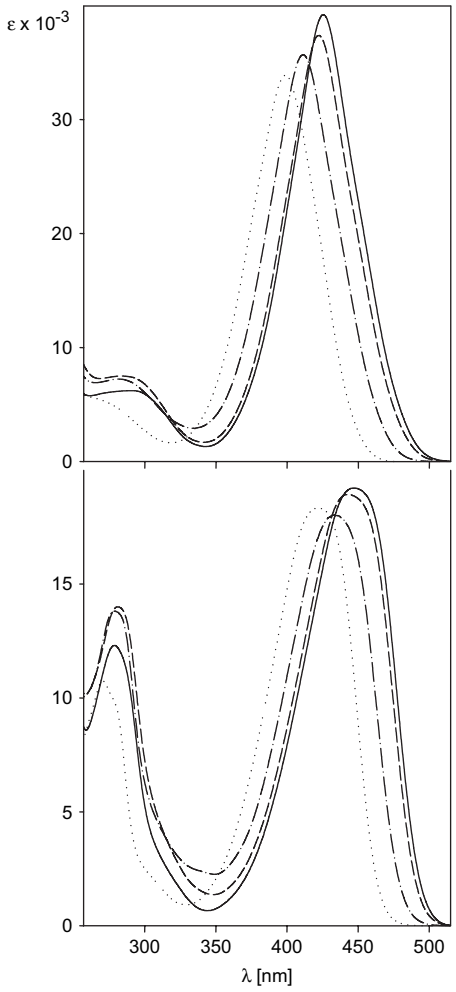


Figure 3. Comparison of UV–vis spectra in chloroform of dipyrrinones (upper panel) and their *N*,*N*′-carbonyl-bridged derivatives (lower panel) substituted at C(3) with: *n*-decyl (dotted line), phenyl (dash–dotted line), *p*-ethoxytetrafluorophenyl (dashed line), and pentafluorophenyl (solid line).

substituents. The parent dipyrrinone **9** in $CHCl_3$ has ε=35,700 (411 nm), the tetrafluorinated **8** has ε=37,200 (423 nm), and the pentafluorinated analog **7** has ε=39,200 (425 nm); and in DMSO **9** has ε=34,400 (417 nm), tetrafluorinated **8** has ε=35,300 (429 nm), and the pentafluorinated **7** has ε=35,800 (429 nm)—evidence that the electron-withdrawing phenyl substituents exert a significant bathochromic shift (of 12–14 nm) and that non-polar solvents cause a gradual but considerable hyperchromic shift, compared to the C(3)-*n*-decyl substituted dipyrrinone in chloroform: ε=33,800 (399 nm) (Fig. 3). A significant (11–23 nm) bathochromic shift is seen in DMSO, as compared to C(3)-*n*-decyl dipyrrinone, ε=34,600 (406 nm).

The influence of the nature of the *para*-substituent (F vs EtO vs H) on the absorption wavelength suggests (at least partial) conjugation between the dipyrrinone π-system and the C(3)-aryl group π-system. Similar effects are observed in the *N*,*N*′-carbonyl-bridged dipyrrinones (Table 2 and Fig. 3) and correlate directly with a decreased reactivity of **4** versus **5** in the sulfonation step.

Fluorescence of *N*,*N*′-carbonyl-bridged dipyrrinones **4**–**6** was measured in five solvents, and the intensity of fluorescence as fluorescence quantum yields (ϕ_F) was quantified using 9,10-diphenylanthracene standard as described previously.[10c] Their excitation, emission wavelengths, and calculated quantum yields in cyclohexane, benzene, chloroform, methanol, and dimethylsulfoxide are given in Table 3. These data might be compared with similar dipyrrinones described earlier,[10,28] but the best reference compound is the dipyrrinone analog with C(8)-free and C(3) substituted with C(3)-*n*-decyl, which showed ϕ_F=0.66 and emission λ_{max}^{em} = 490 nm in chloroform whereas the **6** analog showed ϕ_F=0.57 and λ_{max}^{em} = 502 nm. The corresponding *p*-ethoxytetrafluorophenyl tricycle **5** exhibited ϕ_F=0.59 with λ_{max}^{em} = 508 nm, and the pentafluorophenyl analog **4** had ϕ_F=0.57 and maximum emission at λ_{max}^{em} = 509 nm (Fig. 4). These data show that the quantum yields are of similar magnitude whether the C(3) substituent is aryl, fluorinated aryl or alkyl, and the emission wavelength $\left(\lambda_{max}^{em}\right)$ follows the same trend observed in the UV spectra: a significant bathochromic shift accompanies the replacement of C(3)-*n*-decyl with phenyl group, and an additional but smaller bathochromic shift occurs with fluorine substitution on the aryl group.

Sodium sulfonates **1** and **2** exhibited similar high fluorescence quantum yields in organic solvents: ϕ_F~0.4–0.6. Even in non-polar benzene, the values are in agreement with those of **4**–**6**, thus indicating that aggregation or solubility issues are not influencing the emission. In dimethylsulfoxide solvent somewhat enhanced fluorescence of **1** and **2** was detected in comparison to **4**–**6**. In aqueous solutions the fluorescence efficiency is diminished to about 0.3 but is still sufficient for detection in biological samples—and this solvent offers the largest Stokes shift. The fluorescence emission wavelength from **1** to **2** is not affected significantly by

Table 3. Fluorescence data for *N*,*N*′-carbonyl-bridged dipyrrinones[a] of this work and their C(3)-*n*-decyl analog

Pigment	Cyclohexane		C_6H_6		$CHCl_3$		CH_3OH		$(CH_3)_2SO$	
	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}
1[b]	—	—	0.51	516	0.43	513	0.40	536	0.67	537
2[c]	—	—	0.52	513	0.57	510	0.41	534	0.62	532
4	0.58	480	0.58	508	0.57	509	0.39	541	0.56	533
5	0.67	475	0.61	504	0.59	508	0.39	539	0.59	530
6	0.60	466	0.60	494	0.57	502	0.39	533	0.56	521
C(3)-n-decyl	0.78	463	0.74	472	0.66	490	0.36	523	0.67	501

[a] Concentration range 7.7–9.0×10^{-7} M; excitation λ_{ex}=413–450.
[b] In H_2O, ϕ_F=0.30, λ_{em}=546 nm.
[c] In H_2O, ϕ_F=0.29, λ_{em}=542 nm.

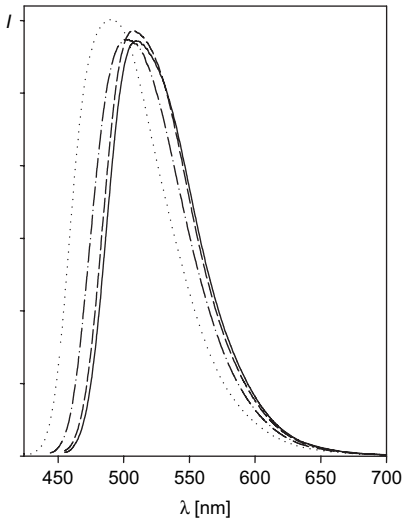


Figure 4. Comparison of fluorescence emission band in chloroform of *N*,*N*′-carbonyl-bridged dipyrrinones substituted at C(3) with: *n*-decyl (dotted line), phenyl (dash–dotted line), *p*-ethoxytetrafluorophenyl (dashed line), and pentafluorophenyl (solid line). The vertical axis plots relative intensity (*I*) of fluorescence emission.

the presence of the C(8) sulfonate group as compared to the C(8)-free parents **4**–**6**.

2.5. Hepatic excretion

When **1**, dissolved in serum, was injected intravenously as a bolus in the rat, the endogenous fluorescence of bile rapidly increased and a prominent peak, followed by a smaller broader peak, was detected in bile by HPLC within 3 min of the injection (Fig. 5a). The prominent peak was identified as unchanged **1**, based on its absorption spectrum and retention time. The highest concentration of **1** in bile was observed in the first sample collected, 3 min after the intravenous injection. Since bile flows only slowly through the short biliary cannula, onset of the efflux of **1** from the liver must occur within less than 3 min. The smaller less polar peak in the chromatograms is yet to be identified. Its normalized absorption spectrum in the HPLC mobile phase is almost superimposable on that of **1**, suggesting no major structural modification. Although it could be a metabolite of **1**, Phase I and Phase II metabolic reactions in the liver generally generate products *more* polar than the precursor. The shape of the peak suggests that it might also be a chromatographic artifact caused by colorless co-migrating constituents of bile. Figure 5b shows a biliary excretion curve for **1**, obtained by plotting HPLC peak areas of **1** in bile normalized to the sample with the maximum peak area. Most pigment was excreted within the first 30 min after injection and the fraction of the administered dose excreted unchanged in bile in two animals in 4 h was 0.5. Unchanged **1** and its possible metabolite was also detectable by HPLC and by fluorescence in urine samples collected intermittently during the experiment. Thus, **1** is cleared rapidly and preferentially from the circulation by the liver and eliminated predominantly by biliary excretion, with a smaller fraction appearing in urine.

The tetrafluorophenyl analog **2** behaved qualitatively similarly to **1**, being excreted rapidly in bile in unchanged form after intravenous injection, with an apparently smaller fraction appearing in urine. However, significant amounts of metabolites of **2**, especially less polar metabolites, were not detected. Determination of the fraction of the dose of **2** excreted in bile was hampered by poor recoveries in the analyses of **2** in the serum injectate. The reason for this is presently unclear, but may be related to the high affinity of **2** for serum albumin. However, it was clear from the bile chromatograms that **2** is excreted preferentially via the liver into bile. Thus, both **1** and **2**, like the sulfoglows reported previously, are cholephilic. They appear to be taken up rapidly by hepatocytes in vivo in the rat, and then excreted rapidly across the biliary canalicular membrane into bile.

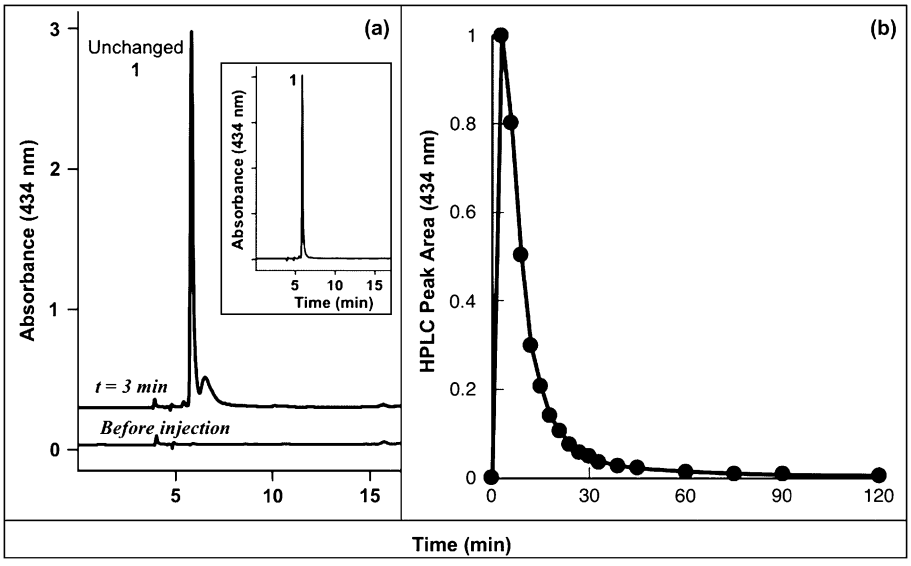


Figure 5. Hepatobiliary excretion of **1** in the homozygous Gunn rat. (a) HPLC chromatograms of bile collected just before (lower trace) and 3 min after (upper trace) intravenous injection of ~0.25 mg **1** dissolved in 1 mL rat serum. The inset shows a chromatogram of a sample of the serum solution of **1** that was injected. (b) Biliary excretion curve for **1** generated by plotting HPLC peak areas of unchanged **1** in bile before and after its injection. Values were normalized to the maximum peak area (at 3 min).

3. Concluding comments

Two new, highly fluorinated (perfluorophenyl and *p*-ethoxytetrafluorophenyl substituents) *N*,*N*′-carbonyl-bridged dipyrrinones were converted to amphiphilic derivatives by sulfonation. The highly fluorescent sulfoglows (**1** and **2**) so obtained are excreted rapidly and principally in unchanged form in bile in the rat after intravenous infusion. Some excretion in urine also was observed. These compounds may form the basis for development of ^{19}F MRI and fluorescence agents for probing liver metabolism and disease.

4. Experimental

4.1. General procedures

Nuclear magnetic resonance spectra were acquired on a Varian Unity Plus spectrometer at 11.75 T magnetic field strength operating at an ^{1}H frequency of 500 MHz, ^{13}C frequency of 125 MHz, and ^{19}F frequency of 470 MHz in solutions of $CDCl_3$ (referenced at 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C) or $(CD_3)_2SO$ (referenced at 2.49 ppm for ^{1}H and 39.50 ppm for ^{13}C). All ^{13}C NMR spectra were broadband ^{1}H decoupled, and *J* constants indicated for some signals are from ^{13}C–^{19}F coupling. The ^{19}F NMR spectra were referenced to external C_6F_6 in $CDCl_3$ at −162.90 ppm. UV–vis spectra were recorded on a Perkin Elmer Lambda 12 spectrophotometer. All fluorescence spectra were measured on a Jobin Yvon FluoroMax 3 by using constant spectral parameters: step resolution (increment) of 1 nm, both excitation and emission slits of 2 nm, and integration time of 0.5 s. Radial chromatography was carried out on Merck preparative layer grade silica gel PF_{254} with $CaSO_4$ binder, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2 or 4 mm thick rotors. Analytical thin-layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125 μm layer). Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. High resolution mass-spectra were obtained from Nebraska Center for Mass Spectrometry, Lincoln, NE.

Spectral data were obtained in spectral grade solvents (Aldrich or Fischer), which were distilled under Ar just prior to use. Before distillation, $CHCl_3$ was passed through a basic alumina column (Woelm, Eschwege, Act. 0). Distillation of $(CH_3)_2SO$ solvent was carried out under vacuum (0.5 mmHg) collecting the solvent at 0 °C and thawing it under Ar. Pyrrole **16**,[12] tosylpyrrolinone **14** (derived from **16**),[12] and pyrrolinone **12**,[12] as well as 3,5-dimethyl-2-formyl-1*H*-pyrrole,[15] were synthesized according to previously published methods.

The concentration study (Table 1) of dipyrrinones **7**–**9** NH ^{1}H NMR chemical shifts was conducted as follows. All solutions were prepared in $CDCl_3$ that had been freshly treated (freshly distilled in two cases) by passing through Woelm Activity 0 basic alumina. Stock solutions concentrated to approximately the upper limit of solubility at 25 °C were prepared (in the case of **9** this limit was 20 mM and for pentafluorophenyl **7** it was only 10 mM) then consecutively diluted in 10-fold increments to 10 μM. The ^{1}H NMR spectra were acquired using a high sensitivity probe for indirect detection. The pulse width was set at about the optimum 65° flip angle and the total repetition time was kept at 8–10 times the *T*1 values of NH signals to allow for complete relaxation. Artificial high line broadening was used in the processing to smoothen the low intensity signals, in particular at low concentrations. Overall, meticulously identical experimental conditions with samples at the same concentrations were used and all spectra were referenced to the residual $CHCl_3$ signal at 7.260 ppm. This precision was necessary to measure the chemical shifts with 10^{-3} ppm confidence.

4.1.1. 4-Methyl-3-pentafluorophenyl-2-(4-toluenesulfonyl)-1*H*-pyrrole (15).

4.1.1.1. 2-Nitro-1-pentafluorophenylpropanol. To a solution of 23.53 g (120 mmol) of perfluorobenzaldehyde and 11.26 g (150 mmol) of nitroethane in 12 mL of anhyd CH_3CN was slowly added 1.8 mL (12 mmol) of DBU, and the mixture was stirred for 30 h at room temperature. The mixture was diluted with 200 mL of CH_2Cl_2, washed with 3% aq HCl (100 mL), H_2O (3×100 mL), dried over anhyd $MgSO_4$, filtered, and the solvent was evaporated under vacuum (1 h at 40 °C). The crude nitropropanol is a 1:1 mixture of diastereomers: ^{1}H NMR *δ*: 1.40, 1.76 (2×1.5H, 2×d, *J*=6.9 Hz), 5.03–5.08 (1H, m), 5.43–5.52 (1H, m) ppm; ^{13}C NMR *δ*: 16.1, 19.5, 61.2, 67.3, 85.0, 86.0, 112.4 (m), 136.1 (dm, ^{1}J=250.7 Hz), 143.4 (dm, ^{1}J=249.8 Hz), 146.7 (dm, ^{1}J=250.2 Hz) ppm and was used directly in the next step.

4.1.1.2. 1-Acetoxy-2-nitro-1-pentafluorophenylpropane. To a solution of the alcohol (26.1 g) from above in 70 mL of anhyd CH_2Cl_2 and 50 mg of DMAP was added acetic anhydride (18.1 mL, 193 mmol) during 10 min, and the mixture was stirred for 16 h. Methanol (25 mL) was added during 15 min, and after 1 h stirring the mixture was diluted with 200 mL of CH_2Cl_2 and then carefully poured into 150 mL of satd aq $NaHCO_3$. The organic layer was washed with H_2O (3×100 mL), dried over anhyd $MgSO_4$, filtered, and the solvent was evaporated under vacuum to give 29.3 g (~78%) of a 1:1 diastereomeric mixture of nitroacetates. ^{1}H NMR *δ*: 1.46, 1.48 (2×1.5H, 2×d, *J*=6.8 Hz), 2.04, 2.21 (2×1.5H, 2×s), 4.91–4.99 (1H, m), 5.19–5.29 (1H, m) ppm; ^{13}C NMR *δ*: 14.6, 14.7, 20.0, 21.8, 67.4, 67.7, 83.8, 84.0, 108.5 (m), 136.0 (dm, ^{1}J=250.9 Hz), 143.6 (dm, ^{1}J=248.7 Hz), 145.9 (dm, ^{1}J=251.1 Hz), 167.0, 168.1 ppm. This mixture was used directly in the next step without further purification.

4.1.1.3. Pyrrole ring formation. To a solution of 24.40 g (125 mmol) of 4-toluenesulfonylmethyl isocyanide (TosMIC)[29] and 25 mL (200 mmol) of tetramethylguanidine in 100 mL of a mixture anhyd THF/isopropyl alcohol=1:1 (v/v) kept at 0 °C was added over 1 h a solution of the nitroacetate from above dissolved in 25 mL of the same solvents. The mixture was warmed slowly to room temperature and stirred for 28 h. Then it was diluted with 400 mL of CH_2Cl_2, poured into 300 mL of ice-5% aq HCl, and the organic layer was washed with 5% aq $NaHCO_3$ (100 mL) and H_2O (3×100 mL). The extract was dried (anhyd $MgSO_4$), filtered through a silica pad, evaporated, and the residue was recrystallized from ethyl acetate/hexane to afford

16.38 g (34% based on perfluorobenzaldehyde) of pyrrole **15**. It had mp 253–255 °C (dec); ^{1}H NMR δ: 1.87 (3H, s), 2.40 (3H, s), 6.89 (1H, d, J=1.8 Hz), 7.23 (2H, m, ^3J=8.3 Hz), 7.50 (2H, m, ^3J=8.3 Hz), 9.24 (1H, br s) ppm; ^{1}H NMR ($(CD_3)_2SO$) δ: 1.79 (3H, s), 2.34 (3H, s), 7.09 (1H, dq, ^3J=2.2 Hz, ^4J=0.8 Hz), 7.35 (2H, m, ^3J=8.4 Hz), 7.55 (2H, m, ^3J=8.4 Hz), 12.61 (1H, br s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.5, 20.9, 108.3 (td, ^2J=19.6 Hz, ^4J= 3.5 Hz), 111.6, 120.6, 123.1, 125.7, 126.4, 129.8, 136.9 (dm, ^1J=249.4 Hz), 138.9, 140.2 (dm, ^1J=251.4 Hz), 143.9, 144.2 (dm, ^1J=248.6 Hz) ppm. ^{19}F NMR δ: −163.4 (m), −154.8 (m, ^3J=21.0 Hz), −138.7 (m, ^3J=22.9 Hz) ppm.

Anal. Calcd for $C_{18}H_{12}F_5NO_2S$ (401.3): C, 53.86; H, 3.01; N, 3.49.

Found: C, 53.71; H, 2.96; N, 3.41.

4.1.2. 3-Methyl-4-pentafluorophenyl-5-(4-toluenesulfonyl)-3-pyrrolin-2-one (13). A mixture of 2.41 g (6 mmol) of pyrrole **15**, 240 mL of acetic acid, 120 mL of CH_2Cl_2, and 120 mL of 30% H_2O_2 was heated at reflux for 24 h. The mixture was cooled, diluted with 200 mL of H_2O, and solid NaCl (~50 g) was added. The product was extracted with CH_2Cl_2 (5×70 mL), and the combined extracts were washed with 5% aq $NaHCO_3$ (100 mL) and H_2O (2×100 mL). The solution was dried (anhyd $MgSO_4$), filtered, and the residue, after evaporation of solvent, was purified in three portions by radial chromatography eluting with a gradient of 1–4% CH_3OH in CH_2Cl_2 (v/v). The pure fractions, after evaporation, were crystallized from ethyl acetate/hexane to afford 1.73 g (69%) of **13**. It had mp 205–206 °C (dec); ^{1}H NMR δ: 1.77 (3H, q, J=1.4 Hz), 2.44 (3H, s), 5.72 (1H, br t, J=1.5 Hz), 7.31 (2H, m, ^3J=8.4 Hz), 7.58 (2H, m, ^3J=8.4 Hz), 7.66 (1H, br s) ppm; ^{13}C NMR δ: 10.9 (t, J=2.9 Hz), 21.7, 76.9 (t, J=3.2 Hz), 106.7, 129.4, 129.9, 130.5, 130.9, 137.9 (dm, ^1J=251.4 Hz), 141.8, 142.1 (dm, ^1J=250.7 Hz), 144.0 (dm, ^1J=247.6 Hz), 146.5, 171.3; ^{19}F NMR δ: −160.9 (m, ^3J=20.5 Hz), −150.9 (m, ^3J=21.1 Hz), −138.5 (v br s), −136.4 (v br s) ppm.

Anal. Calcd for $C_{18}H_{12}F_5NO_3S$ (417.3): C, 51.80; H, 2.90; N, 3.36.

Found: C, 52.25; H, 2.93, N, 3.23.

4.1.3. 3-Methyl-4-pentafluorophenyl-3-pyrrolin-2-one (10). To a solution of 417 mg (1.0 mmol) of toluenesulfonylpyrrolinone **13** in 60 mL of abs ethanol under N_2 was added sodium borohydride (114 mg, 3.0 mmol) during 10 min. The mixture was stirred for 10 more minutes, the solvent was evaporated under vacuum (<35 °C), and the residue was partitioned between 100 mL of 1% aq HCl and 100 mL of $CHCl_3$. The organic layer was washed with 3×50 mL of H_2O, dried over anhyd Na_2SO_4, filtered, and solvent was evaporated under vacuum. The crude material was purified by radial chromatography eluting with 1–2% CH_3OH in CH_2Cl_2 (v/v), and the polar band, after evaporation, was crystallized from ethyl acetate/hexane to afford 249 mg (95%) of pyrrolinone **10**. It had mp 133–134 °C; ^{1}H NMR δ: 1.88 (3H, m), 4.22 (2H, m), 7.78 (1H, br s) ppm; ^{13}C NMR δ: 10.2 (t, J=2.0 Hz), 48.4 (t, J=2.6 Hz), 108.4 (td, ^2J=18.2 Hz, ^4J=3.9 Hz), 135.7, 137.5, 137.9 (dm, ^1J=253.8 Hz), 141.4 (dm, ^1J=256.7 Hz), 143.9 (dm, ^1J=250.3 Hz), 174.4 ppm; ^{19}F NMR δ: −161.5 (m), −152.9 (m, ^3J=20.8 Hz), −139.5 (m, ^3J=21.7 Hz) ppm.

Anal. Calcd for $C_{11}H_6F_5NO$ (263.2): C, 50.20; H, 2.29; N, 5.31.

Found: C, 50.01; H, 2.59; N, 5.40.

4.1.4. 3-Methyl-4-(4-ethoxytetrafluorophenyl)-3-pyrrolin-2-one (11). To a solution of 789 mg (3.0 mmol) of pentafluorophenylpyrrolinone **10** in 10 mL of abs ethanol (cooled to 10 °C) was added during 1 h a freshly prepared solution of 15.0 mmol sodium ethoxide (from 345 mg of sodium) in 15 mL of abs ethanol. The mixture was stirred for an additional 1 h, before being diluted with 100 mL of $CHCl_3$ and poured into 100 mL of 1% aq HCl. The organic layer was washed with H_2O (3×50 mL), dried over anhyd Na_2SO_4, and filtered. The solvent was evaporated under vacuum and the residue was purified by radial chromatography eluting with 0.5–1.5% CH_3OH in CH_2Cl_2 (v/v). After solvent evaporation, the combined pure fractions were crystallized from ethyl acetate/hexane to give 617 mg (71%) of pyrrolinone **11**. It had mp 128–129 °C; ^{1}H NMR δ: 1.45 (3H, t, J=7.0 Hz), 1.87 (3H, s), 4.20 (2H, br s), 4.36 (2H, q, J=7.0 Hz), 7.58 (1H, br s) ppm; ^{13}C NMR δ: 10.3 (t, J=2.4 Hz), 15.4, 48.4 (t, J=2.9 Hz), 71.0 (t, J=3.6 Hz), 106.4 (t, ^2J=18.3 Hz), 136.5, 136.8 (t, ^3J=1.0 Hz), 137.9 (tt, ^2J=12.1 Hz, ^3J=3.6 Hz), 141.3 (dm, ^1J=248.2 Hz), 144.0 (dm, ^1J=248.2 Hz), 174.6 ppm; ^{19}F NMR δ: −157.4 (m, ^3J=20.4 Hz), −141.6 (m, ^3J= 20.4 Hz) ppm.

Anal. Calcd for $C_{13}H_{11}F_4NO_2$ (289.2): C, 53.98; H, 3.83; N, 4.84.

Found: C, 53.75; H, 4.07; N, 4.86.

4.2. General procedure for syntheses of dipyrrinones

A mixture of 1 mmol of the corresponding pyrrolinone, 369 mg (3 mmol) of 3,5-dimethyl-2-formyl-1*H*-pyrrole,[15] 2.5 mL of anhyd CH_3CN, and 1.1 mL (10 mmol) of *N*-methylmorpholine was heated under Ar at 90–95 °C in a sealed thick wall tube for 84 h (48 h for **9**). After being cooled and opened to atmospheric pressure, the mixture was chilled at −20 °C for 3 h, and the separated product was collected by filtration. It was washed on the filter with CH_3CN/H_2O/AcOH (5 mL/2 mL/2 mL), then dried under vacuum, and purified in several portions by radial chromatography eluting with gradient 1–3% CH_3OH in CH_2Cl_2 (v/v). The fractions containing pure pigment were combined, evaporated under vacuum, and the residue was crystallized from CH_3OH/CH_2Cl_2 to give pure bright yellow dipyrrinone.

4.2.1. 3-Pentafluorophenyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (7). This compound was isolated in 84% yield. It decomposed without melting at >321–324 °C; ^{1}H NMR δ: 1.94 (3H, s), 2.05 (3H, s), 2.46 (3H, s), 5.78 (1H, s), 5.86 (1H, d, ^4J=1.8 Hz), 10.51 (1H, br s), 11.97 (1H, br s) ppm; ^{13}C NMR δ: 9.8 (br), 11.5, 13.6, 105.0, 107.3 (td,

^2J=19.2 Hz, ^4J=3.3 Hz), 111.1, 123.2, 124.9, 128.2, 129.4, 130.8 (m), 136.4, 137.8 (dm, ^1J=252.5 Hz), 141.6 (dm, ^1J=250.1 Hz), 144.2 (dm, ^1J=248.5 Hz), 172.3 ppm; ^{1}H NMR ($(CD_3)_2SO$) δ: 1.76 (3H, s), 1.92 (3H, s), 2.22 (3H, s), 5.60 (1H, s), 5.74 (1H, d, ^4J=0.9 Hz), 10.48 (1H, s), 10.55 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.2 (br), 11.1, 12.9, 101.0, 106.8 (td, ^2J=19.4 Hz, ^4J=3.3 Hz), 110.3, 122.4, 125.4, 125.7, 129.3, 129.9 (t, ^3J=1.9 Hz), 133.8, 137.5 (dm, ^1J=250.3 Hz), 140.9 (dm, ^1J=251.8 Hz), 143.6 (dm, ^1J=245.1 Hz), 170.1 ppm; ^{19}F NMR δ: −161.6 (m), −153.0 (m, ^3J=21.0 Hz), −138.4 (m, ^3J=22.8 Hz) ppm.

Anal. Calcd for $C_{18}H_{13}F_5N_2O$ (368.3): C, 58.70; H, 3.56; N, 7.61.

Found: C, 58.46; H, 3.94; N, 7.54.

4.2.2. 3-(4-Ethoxytetrafluorophenyl)-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (8). This pigment was obtained in 74% yield. It had mp 287–289 °C (dec); ^{1}H NMR δ: 1.49 (3H, t, J=7.1 Hz), 1.94 (3H, s), 2.05 (3H, s), 2.46 (3H, s), 4.42 (2H, q, J=7.1 Hz), 5.82 (1H, s), 5.84 (1H, d, ^4J=2.3 Hz), 10.54 (1H, br s), 11.96 (1H, br, s) ppm; ^{13}C NMR δ: 9.8, 11.4, 13.5, 15.5, 71.0, (t, J=3.5 Hz), 104.9, 105.2 (t, ^2J=19.1 Hz), 110.9, 123.3, 125.3, 127.9, 128.9, 132.0 (t, ^3J=2.0 Hz), 136.1, 137.9 (tt, ^2J=12.1 Hz, ^3J=3.4 Hz), 141.3 (dm, ^1J=248.1 Hz), 144.3 (dm, ^1J=247.8 Hz), 172.6 ppm; ^{19}F NMR δ: −157.5 (m, ^3J=21.3 Hz), −140.5 (m, ^3J=21.3 Hz) ppm.

Anal. Calcd for $C_{20}H_{18}F_4N_2O_2$ (394.4): C, 60.91; H, 4.60; N, 7.10.

Found: C, 60.99; H, 4.88; N, 7.11.

4.2.3. 3-Phenyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (9). This compound was isolated in 64% yield. It had mp 293–295 °C (dec); ^{1}H NMR δ: 2.01 (3H, s), 2.02 (3H, s), 2.47 (3H, s), 5.82 (1H, d, ^4J=1.8 Hz), 6.06 (1H, s), 7.37 (2H, m), 7.44 (1H, m), 7.49 (2H, m), 10.61 (1H, br s), 11.81 (1H, br s) ppm; ^{13}C NMR δ: 9.4, 11.4, 13.5, 105.5, 110.4, 123.4, 123.6, 127.3, 127.8, 128.2, 128.3, 129.8, 132.7, 135.1, 146.7, 173.3 ppm.

Anal. Calcd for $C_{18}H_{18}N_2O$ (278.3): C, 77.67; H, 6.52; N, 10.06.

Found: C, 77.76; H, 6.28; N, 10.20.

4.3. General procedure for insertion of *N*,*N*′-carbonyl bridge

A mixture of 1 mmol of dipyrrinones **7**–**9**, 1,1′-carbonyldiimidazole (0.81 g, 5 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.75 mL, 5 mmol), and 80 mL of anhydrous CH_2Cl_2 was heated under N_2 at reflux for 16 h. After cooling, the mixture was washed with 100 mL of 1% aq HCl, then with H_2O (3×100 mL), and the solution was dried over anhyd $MgSO_4$. After filtration and evaporation of the solvent under vacuum, the residue was purified by radial chromatography on silica gel eluting with gradient 0.2–0.8% CH_3OH in CH_2Cl_2 (v/v). The fractions containing non-polar fluorescent band were combined, the solvents were evaporated under vacuum, and the residue was crystallized from ethyl acetate/hexane or methanol/water to afford pure bright yellow-orange tricyclic compounds.

4.3.1. 1-Pentafluorophenyl-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (4). This tricycle was isolated in 67% yield. It had mp 255–256 °C; ^{1}H NMR δ: 1.98 (3H, s), 2.10 (3H, s), 2.70 (3H, s), 6.06 (1H, br s), 6.16 (1H, s) ppm; ^{13}C NMR δ: 10.0 (br), 10.8, 15.7, 99.7, 105.3 (td, ^2J=18.7 Hz, ^4J=4.0 Hz), 117.9, 123.2, 126.9, 128.1, 129.0 (br), 132.5, 136.4, 138.1 (dm, ^1J=250.3 Hz), 142.1 (dm, ^1J=252.8 Hz), 143.1, 144.1 (dm, ^1J=250.5 Hz), 165.9 ppm; ^{19}F NMR δ: −160.4 (m), −151.1 (m, ^3J=21.0 Hz), −137.8 (m, ^3J=21.0 Hz) ppm.

Anal. Calcd for $C_{19}H_{11}F_5N_2O_2$ (394.3): C, 57.87; H, 2.81; N, 7.10.

Found: C, 57.84; H, 3.19; N, 7.13.

4.3.2. 1-(4-Ethoxytetrafluorophenyl)-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (5). This compound was obtained in 74% yield. It had mp 192–193 °C; ^{1}H NMR δ: 1.49 (3H, t, J=7.1 Hz), 1.97 (3H, s), 2.10 (3H, s), 2.69 (3H, s), 4.42 (2H, q, J=7.1 Hz), 6.04 (1H, br s), 6.18 (1H, s) ppm; ^{13}C NMR δ: 10.0 (t, J=1.6 Hz), 10.8, 15.5, 15.6, 71.1 (t, J=3.6 Hz), 99.7, 102.9 (t, ^2J=18.6 Hz), 117.7, 122.7, 126.9, 128.4, 130.2 (t, ^3J=2.2 Hz), 132.0, 136.1, 138.9 (tt, ^2J=11.9 Hz, ^3J=3.5 Hz), 141.4 (dm, ^1J=249.1 Hz), 143.2, 144.1 (dm, ^1J=249.1 Hz), 166.2 ppm; ^{19}F NMR δ: −156.5 (m, ^3J=20.1 Hz), −139.9 (m, ^3J=20.1 Hz) ppm.

Anal. Calcd for $C_{21}H_{16}F_4N_2O_3$ (420.4): C, 60.00; H, 3.84; N, 6.66.

Found: C, 59.61; H, 4.16; N, 6.68.

4.3.3. 1-Phenyl-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (6). This tricycle was isolated in 93% yield. It had mp 196–197 °C; ^{1}H NMR δ: 2.04 (3H, s), 2.08 (3H, s), 2.70 (3H, s), 6.03 (1H, br s), 6.34 (1H, s), 7.42 (2H, m), 7.53 (3H, m) ppm; ^{13}C NMR δ: 9.4, 10.7, 15.6, 99.9, 117.4, 121.7, 126.9, 127.1, 128.8, 128.9, 129.4, 130.3, 130.5, 135.3, 143.5, 143.8, 167.3 ppm.

Anal. Calcd for $C_{19}H_{16}N_2O_2$ (304.3): C, 74.98; H, 5.30; N, 9.21.

Found: C, 75.00; H, 5.42; N, 9.29.

4.4. Sodium 2,7,9-trimethyl-1-pentafluorophenyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione-8-sulfonate (1)

Finely powdered bridged dipyrrinone **4** (100 mg, 0.25 mmol) was added to 6 mL of concd H_2SO_4, and the mixture was stirred at 25 °C for 4 h. Then the temperature was lowered from −10 °C to −15 °C, and the solution was neutralized to pH 7–8 with satd aq Na_2CO_3 while introducing a stream of air in order to reduce foaming. When foaming became excessive, methanol was added in 1 mL portions on four occasions. After dilution with H_2O (50 mL), the mixture was

extracted with $CHCl_3/CH_2Cl_2$ 1:1 (7×50 mL), adding 10 mL portions of CH_3OH after each extraction. The combined extracts were evaporated under vacuum, and the residue was purified by radial chromatography by eluting with gradient 5–20% CH_3OH/CH_2Cl_2 (v/v). The polar band was collected, evaporated to dryness, and triturated with ethyl acetate. The solid product was collected by filtration to afford 49 mg (39%) of sulfonate **1**. It decomposed at >277–285 °C; ^{1}H NMR ($(CD_3)_2SO$) δ: 1.87 (3H, s), 2.19 (3H, s), 2.83 (3H, s), 6.88 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.5, 10.2, 13.5, 100.7, 104.9 (m, ^2J=18.7 Hz), 121.8, 125.9, 128.2, 128.8, 131.5, 132.7, 134.6 (br), 137.5 (dm, ^1J=251.4 Hz), 141.3 (dm, ^1J=250.5 Hz), 142.6, 143.8 (dm, ^1J=248.9 Hz), 165.3 ppm; ^{19}F NMR ($(CD_3)_2SO$) δ: −161.6 (m), −153.8 (m, ^3J=22.1 Hz), −138.2 (m, ^3J=22.1 Hz) ppm. FAB-HRMS (3-NBA+Na) calcd for $C_{19}H_{10}F_5N_2Na_2O_5S$ $(M+Na)^+$, *m/z*: 519.0026, found: 519.0021, Δ=0.5 mDa, error 1.0 ppm.

4.5. Sodium 1-(4-ethoxytetrafluorophenyl)-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione-8-sulfonate (2)

Finely ground bridged dipyrrinone **5** (105 mg, 0.25 mmol) was added to 5 mL of concd H_2SO_4 precooled at 0 °C, and the magenta colored mixture was stirred for 2 h. Then the temperature was lowered from −10 °C to −15 °C, and the solution was neutralized to pH 7–8 with concd aq Na_2CO_3 while introducing a stream of air in order to reduce foaming. Methanol was added in 1 mL portions on four occasions to wash the foam down. After dilution with H_2O (50 mL), the mixture was extracted with $CHCl_3/CH_2Cl_2$ 1:1 (7×50 mL) adding 10 mL portions of CH_3OH after each extraction. The combined extracts were evaporated under vacuum and the residue was purified by radial chromatography eluting with gradient 5–20% CH_3OH in CH_2Cl_2 (v/v). The polar band was collected, evaporated to dryness, and triturated with CH_2Cl_2/hexane. The solid product was collected by filtration to give (after drying under vacuum at 80 °C) 59 mg (45%) of sulfonate **2**. It had mp 258–261 °C (dec); ^{1}H NMR ($(CD_3)_2SO$) δ: 1.39 (3H, t, J=7.0 Hz), 1.86 (3H, s), 2.20 (3H, s), 2.83 (3H, s), 4.40 (2H, q, J=7.0 Hz), 6.87 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.5, 10.2, 13.5, 15.3, 71.1, 100.7, 102.7 (t, ^2J=18.9 Hz), 121.7, 125.9, 128.4, 129.8 (br t), 131.0, 132.6, 134.7 (br), 137.9 (m), 141.1 (dm, ^1J=246.6 Hz), 142.7, 143.9 (dm, ^1J=243.3 Hz), 165.4 ppm; ^{19}F NMR ($(CD_3)_2SO$) δ: −156.7 (m, ^3J=21.9 Hz), −140.1 (m, ^3J=21.9 Hz) ppm. FAB-HRMS (3-NBA+Na) calcd for $C_{21}H_{15}F_4N_2Na_2SO_6$ $(M+Na)^+$, *m/z*: 545.0383; found: 545.0393, Δ=−1.0 mDa, error −1.9 ppm.

4.6. Metabolism studies

The procedure for metabolism studies in the rat has been described in detail elsewhere.[11,12] Solutions of **1** and **2** in 1 mL rat serum, prepared by dissolving ~0.25 mg of each pigment in 0.1 mL $(CH_3)_2SO$ and diluting this solution slowly into 1 mL rat serum, were injected intravenously as a bolus into the femoral vein and bile was collected from a short indwelling cannula in frequent 20-μL aliquots under safelights. Bile samples were flash frozen at once in dry-ice and stored at <−50 °C until analyzed by HPLC as previously described[11,12] using absorbance at 434 nm for detection. Studies were conducted in adult male homozygous Gunn rats. Gunn rats were used to simplify the appearance and integration of chromatograms since these rats lack UGT1 isozymes and do not excrete bilirubin glucuronides in bile.[1,5] No metabolism of **1** or **2** by UGT1 isozymes would be expected and control experiments indicated that hepatic metabolism and excretion of **2** was qualitatively similar in homozygous, heterozygous, and wild-type rats.

Acknowledgements

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Synthetic study of hetisine-type aconite alkaloids. Part 1: Preparation of tetracyclic intermediate containing the C14–C20 bond

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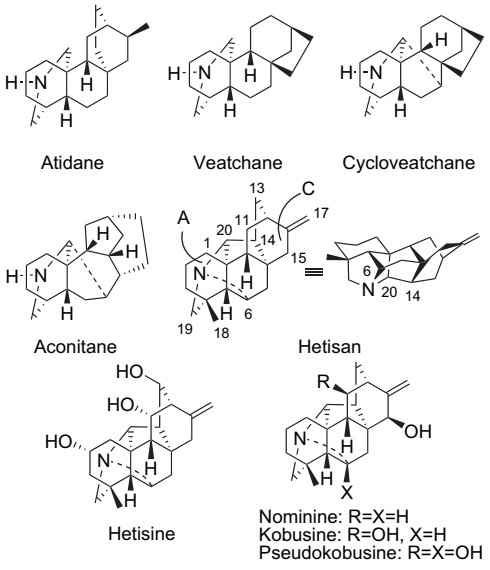
Abstract—Full details for the total synthesis of (±)-nominine, a hetisine-type aconite alkaloid, are presented in three parts. Here (part 1), we describe the preparation of the key tetracyclic intermediate **6**. Our palladium-catalyzed intramolecular α-arylation was adopted for preparation of the intermediate **4** with an angular formyl group. An acetal–ene reaction was then employed for C14–C20 bond formation to secure **6** from **5**. The reaction mechanism of the acetal–ene reaction is discussed, and a method for removal of the 2-hydroxyethyl group from **6** is developed.

1. Introduction

1.1. The aconite alkaloids

Aconitum, which has a beautiful blue-purple flower, is well-known as a poisonous herb, which occasionally results in fatalities following accidental ingestion. The aconite alkaloids, mainly contained in the tuberous root, have long been of interest to researchers, because of both their pharmacological activity and their structural complexity.[1,2] Alkaloids with similar structures have also been isolated from plants of the species *Delphinium*, *Consolida*, *Thalictrum*, and *Spiraea*, and these are included in the so-called aconite alkaloids. Over 400 aconite alkaloids have so far been isolated and structurally characterized.[1,2]

The fundamental structural frameworks of these alkaloids are generally classified into five skeletons, i.e., atidane, veatchane, cycloveatchane, aconitane, and hetisan (Scheme 1). Extensive synthetic studies of these pharmacologically important alkaloids for about 40 years have led to the total synthesis of several alkaloids belonging to the first four of the above five groups: atisine[3] (atidane), veatchine[4] (veatchane), garryine[5] (veatchane), napelline[6] (cycloveatchane), delphinine[7] (aconitane), talatisamine[8] (aconitane), and chasmanine[9] (aconitane). However, attempts to construct even a simple hetisan skeleton (the name of which is derived from hetisine), not to mention total synthesis of a hetisan alkaloid, have remained unsuccessful since the structure of hetisine was first clarified by X-ray crystal-structure analysis in 1962.[10] The heptacyclic hetisan skeleton is the most structurally complex among the above five frameworks, and incorporates two additional bonds, N–C6 and C14–C20, relative to the atidane skeleton, as exemplified by nominine, kobusine, and pseudokobusine (Scheme 1). The synthetic difficulty of the skeleton stems from the presence of these two bonds.



Scheme 1. Five representative aconite skeletons and examples of hetisine-type alkaloids.

Keywords: Aconite; Alkaloid; α-Arylation; Palladium catalyst; Acetal–ene reaction.
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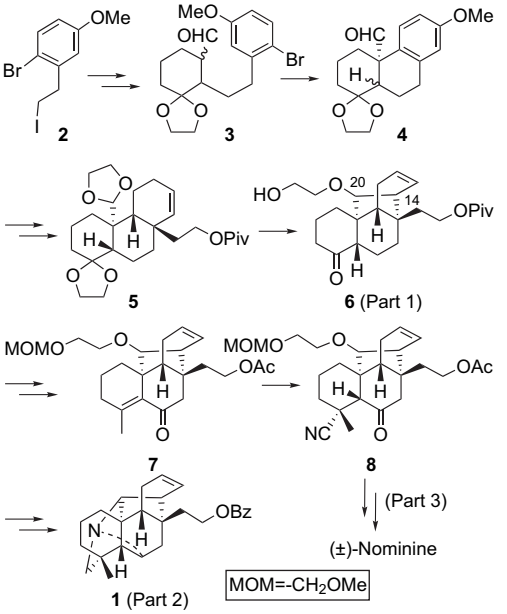
1.2. Synthetic background

Several years ago, we developed a novel palladium-catalyzed intramolecular α-arylation of aliphatic ketone, formyl, and nitro groups[11] during the course of our synthetic studies of duocarmycin SA analogs.[12] As an application of this reaction, we embarked on synthetic studies of the hetisine-type aconite alkaloids. Our fundamental synthetic strategy was to form the N–C6 and C14–C20 bonds at an early stage of the synthetic route, because it would become more difficult to connect them at a later stage, as these two bonds greatly restrict the molecular conformation. Thus, stereocontrol was expected to be easier with early introduction of these two bonds.

We first reported the preparation of the hexacyclic compound **1** lacking the C-ring of the hetisan skeleton, starting from compound **2** by way of the intermediates **3**–**8** (Scheme 2).[13] Our further synthetic efforts culminated in a total synthesis of (±)-nominine (Scheme 1), the simplest hetisine-type aconite alkaloid.[14] In this and the next papers (parts 1 and 2), we present full details for the preparation of **1**. In part 3, we describe the total synthesis of (±)-nominine, diverging from the intermediate **8**. These three papers thus describe the first synthesis of a hetisine-type aconite alkaloid. Five synthetic investigations have been reported so far, leading toward the total synthesis of this class of aconite alkaloids.[15–19]

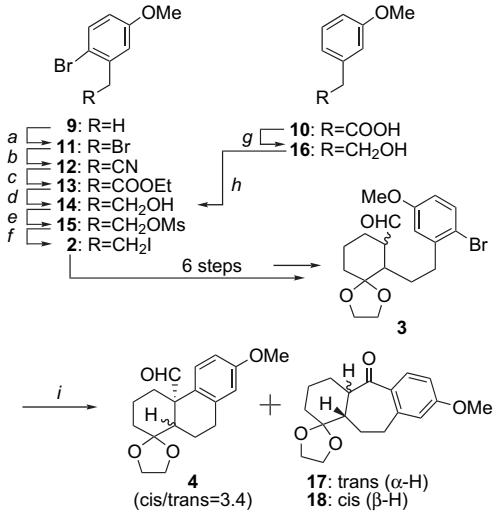


Scheme 2. Outline of the preparation of **1** from **2**.

2. Results and discussion

2.1. Preparation of tetracyclic intermediate 4

We have already reported in detail the preparation of compound **4**, starting from **2** via the precursor **3** by means of a palladium-catalyzed intramolecular cyclization reaction (Scheme 2).[11b,c] While the starting material **2** is a known compound,[21] the literature methods seemed inappropriate for large-scale preparation.[20,21] Therefore, we prepared it from 1-bromo-4-methoxy-2-methylbenzene (**9**) or 3-methoxyphenylacetic acid (**10**) by modification of the method of Ghatak[20] or Meyers[21] (Scheme 3), respectively. The cyclization step of **3** to **4** was also improved for the large-scale preparation.



Scheme 3. Large-scale preparation of **2** and **4**: (a) NBS, BPO, CCl_4, Ref. 20; (b) KCN, EtOH–H_2O, **12** 92%; (c) H_2SO_4, EtOH, **13** 90%; (d) DIBAL-H, CH_2Cl_2, **14** 96%; (e) MsCl, Et_3N, CH_2Cl_2, **15** 99%; (f) NaI, acetone, **2** 96%; (g) ClCOOMe, Et_3N, THF, then $NaBH_4$, THF–H_2O, **16** 95%; (h) Br_2, $CHCl_3$, Ref. 21; (i) $PdCl_2(Ph_3P)_2$, Cs_2CO_3, Ph_3P, THF, **4** 71%, **17** 4%, **18** 2%.

2.1.1. Preparation of 1-bromo-2-(2-iodoethyl)-4-methoxybenzene (2). 1-Bromo-2-bromomethyl-4-methoxybenzene (**11**), prepared from **9** according to the literature,[20] was treated with potassium cyanide (KCN) in ethanol–water (EtOH–H_2O) to afford **12** in 92% yield (Scheme 3). Alcoholysis of **12** with sulfuric acid (H_2SO_4) in EtOH afforded the ethyl ester **13** in 90% yield. Transformation of **13** to **2** was carried out readily by (i) reduction with diisobutylaluminum hydride (DIBAL-H), (ii) methanesulfonylation of the resulting primary alcohol to form **15**, and (iii) iodination with sodium iodide (NaI) in acetone to give **2** in yields of 96, 99, and 96%, respectively. Alternatively, compound **14** was prepared from **10** as follows: reduction of the carboxylic acid **10** to an alcohol **16** was carried out with (i) methyl chloroformate (ClCOOMe), triethylamine (Et_3N), and (ii) sodium borohydride ($NaBH_4$) in 95% yield, and subsequent bromination according to the literature[21] readily afforded the intermediate **14**.

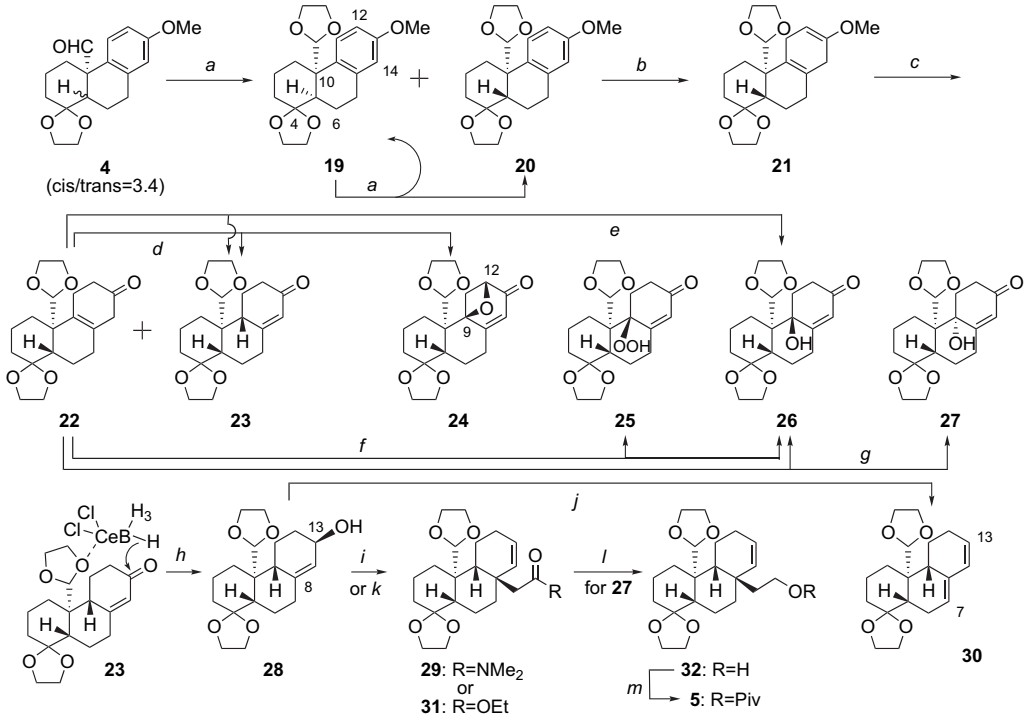
2.1.2. Palladium-catalyzed cyclization of 3. Transformation of the above-obtained **2** to the precursor **3** was executed according to the reported route in six steps.[11c] In our previous reports, **4** (cis/trans = 4.2, the structure of the major cis isomer had been established by the X-ray analysis[11c]) was obtained by the treatment of **3** with dichlorobis(triphenylphosphine)palladium(II) [$PdCl_2(Ph_3P)_2$], cesium carbonate (Cs_2CO_3) in tetrahydrofuran (THF) in 65% yield. On a larger scale, however, precipitation of black palladium metal was observed and the reaction ceased in mid-course. The reaction proceeded reproducibly to completion in the presence of triphenylphosphine [Ph_3P, 0.2 equiv, $PdCl_2(Ph_3P)_2$

(9 mol %), Cs_2CO_3 (1.6 equiv) in refluxing THF for 60 h on a 32 mmol scale], though a longer reaction time was required than in the case without the addition of Ph_3P. Under these conditions, the desired **4** (inseparable mixture of cis and trans isomers in a ratio of cis/trans = 3.4) was obtained in 71% yield, accompanied with by-products **17** (4%) and **18** (2%).

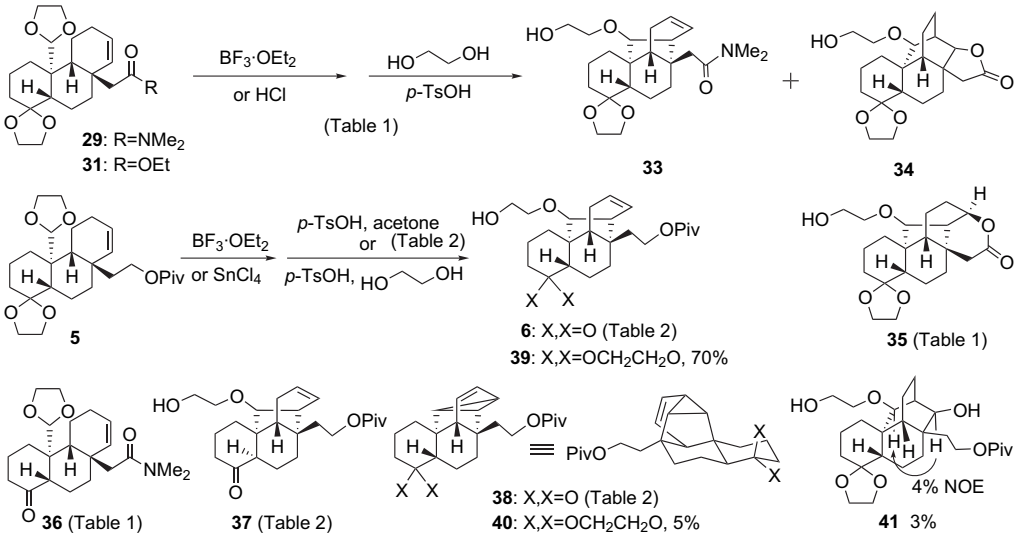
2.2. Transformation of 4 to 5

2.2.1. Transformation of 4 to the enone–diacetal 23. Acetalization of **4** (cis/trans = 3.4) with *p*-toluenesulfonic acid (*p*-TsOH) and ethylene glycol in refluxing benzene afforded readily separable *cis* (**19**) and *trans* (**20**) diacetals in 61% and 34% yields, respectively (Scheme 4). An acid-catalyzed equilibration was observed at the C5 position adjacent to the original acetal group at C4, and the ratio of the desired trans isomer **20** improved (**19**/**20**=1.8). This equilibration was conveniently leveraged for the conversion of **19** to **20** by repeated acetalization of the isolated cis isomer **19** to give recovered **19** (61%) and **20** (35%). For the elaboration of the anisole ring, the diacetal **20** was submitted to Birch reduction with lithium (Li) metal in liq. ammonia (NH_3) and THF–EtOH to give the dihydro compound **21** in 92% yield. Brief exposure of **21** to 0.5% hydrochloric acid (HCl) in THF–H_2O (4:1) afforded the β,γ-enone **22** (88%) along with the α,β-enone **23** (7%), the two acetal groups being kept intact. The former was treated with sodium methoxide (NaOMe) in methanol (MeOH) to yield **23** (55%) and an oxetane **24** (10%), accompanied with recovery of **22** (10%). It is likely that partial air oxidation took place at C9 of **23** during the alkaline treatment, and the resulting hydroperoxide **25** was attacked nucleophilically by the enolate anion at C12 (hetisan numbering) to yield **24**. This was confirmed by the fact that on further addition of dimethyl sulfide (Me_2S) to the alkaline treatment, the γ-hydroxy-α,β-enone **26** was obtained in 14% yield in place of **24**, in addition to **23** (58%) and recovered **22** (13%). Treatment of **22** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in benzene gave **25** (18%), **26** (19%), and recovered **22** (40%).[22] To prove the structure, compound **26** was synthesized through an alterative approach, as follows. Epoxidation of **22** was carried out with *m*-chloroperbenzoic acid (*m*-CPBA) as usual, and then the resulting mixture was treated with DBU to provide **26** (61%) along with its stereoisomer **27** (34%).

2.2.2. Preparation of 5 from 23. Reduction of the above-obtained enone **23** with $NaBH_4$ in the presence of cerium chloride ($CeCl_3$) was found to afford the desired β-allyl alcohol **28** exclusively in 94% yield. The half height *J* value (19 Hz) of H13 (hetisan numbering) in the ^1H NMR spectrum of **28** implies an axial orientation, and this means that the 13-hydroxy group must take equatorial β-configuration. The reason why we need the β-allyl alcohol **28** is that the stereochemistry of C8 accurately reflects that of the angular C13 in the next two carbons (corresponding to C15 and C16 of the hetisan skeleton) elongation at C13 by Claisen rearrangement. Coordination of the oxygen atom of the 1,3-dioxolanyl group at C10 to the cerium borohydride species resulted in the desired one-sided reduction of the carbonyl group from the α side to give **28**, as depicted in Scheme 4.[23]



Scheme 4. Transformation of **4** to **5**: (a) $(CH_2OH)_2$, *p*-TsOH, benzene, **19** 61% and **20** 34% from **4**; **19** 61% and **20** 35% from **19**; (b) Li, EtOH, liq. NH_3–THF, **21** 92%; (c) 0.5% HCl, THF–H_2O (4:1), **22** 88%, **23** 7%; (d) NaOMe, MeOH, **23** 55%, **24** 10%, recovery of **22** 10%; (e) NaOMe, Me_2S, MeOH, **23** 58%, **26** 14%, recovery of **22** 13%; (f) DBU, benzene, **25** 18%, **26** 19%, recovery of **22** 40%; (g) *m*-CPBA, CH_2Cl_2, then DBU, benzene, **26** 61%, **27** 34%; (h) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, **28** 94%; (i) *N*,*N*-Dimethylacetamide dimethyl acetal, toluene, 160 °C (sealed tube), **29** 69%; (j) Ac_2O, pyridine, CH_2Cl_2, **30** 86%; (k) $MeC(OEt)_3$, *t*-BuCOOH, 160 °C (sealed tube), **31** 21%; (l) $BH_3 \cdot NH_3$, BuLi, THF, **32** 94%. (m) Piv_2O, Et_3N, 4-DMAP, CH_2Cl_2, **5** 98%.



Scheme 5. Acetal–ene reaction of **29**, **31**, and **5**.

Then **28** was exposed to *N*,*N*-dimethylacetamide dimethyl acetal in toluene at 160 °C (sealed tube) to obtain the acetamide **29** in 69% yield.[24] An inseparable mixture of dehydrated dienes was formed as by-products. Of the two dienes, the $\Delta_{7,8}$ and $\Delta_{13,14}$ isomer **30** was formed exclusively on acetylation of **28** with acetic anhydride (Ac_2O) in pyridine by spontaneous elimination of acetic acid from the intermediary allyl acetate. Usual Claisen reaction with ethyl orthoacetate and pivalic acid afforded only 21% yield of **31**. As the next acetal–ene reaction of **29** was subject to a serious side reaction due to the side chain at C8 (vide infra), the amide **29** was reduced with the reagent prepared in situ from *n*-butyllithium (*n*-BuLi) and borane–ammonia complex ($BH_3\cdot NH_3$) to provide **32** in 94% yield.[25] The alcohol **32** was protected as the pivaloate with pivalic anhydride (Piv_2O) and Et_3N in the presence of 4-dimethylaminopyridine (4-DMAP) to afford **5** in 98% yield. The bulky pivaloyl protecting group was selected to prevent the side reaction in the next acetal–ene reaction. Pivaloylation with pivaloyl chloride (PivCl) and Et_3N is much faster than that with Piv_2O, but it occasionally afforded an intractable mixture, especially on large-scale reaction, probably due to ring opening of the dioxolane ring at C4 to form (2-pivaloyloxy)ethyl enol ether.

2.3. Formation of the C14–C20 bond

2.3.1. Acetal–ene reaction of 29, 31, and 5. First, the substrate **29** was subjected to the acetal–ene reaction[26] (Scheme 5). A dichloromethane (CH_2Cl_2) solution of **29** was treated with boron trifluoride diethyl etherate ($BF_3\cdot OEt_2$) at −18 °C to provide the expected compound **33** in only 21% yield, along with the pentacyclic lactone **34** in 72% yield as a by-product after reacetalization of the partially deprotected 4-carbonyl group (Table 1, run d). Since the employment of CH_2Cl_2 as the reaction solvent resulted in the desired **33** being a minor product, we looked for other reaction solvents in order to improve the yield of **33** (Table 1). Among the solvents tested, toluene was found to be the best, giving **33** (41%) and **34** (48%) (run b). The reaction temperature also affected the yield, and reaction at −18 °C (run b) gave the best yield of **33** among runs b, f, and g. A less polar solvent, cyclohexene–$PhCH_3$ (6:1) resulted in a lower yield of **33** with a new by-product **35** in 10% yield. The acetal–ene reaction of the ester **31** under the best conditions for **29** gave no desired compound corresponding to **33**, but only **34** in 66% yield (run i). Treatment of **29** with dilute HCl also afforded the lactone **34** in 64% yield, together with a ketone **36** in 26% (run h).

Table 1. Acetal–ene reaction of **29**, **31** to form **33**, **34**, **35**, **36** (Scheme 5)

Run	Substrate	Acid	Solvent	Temp (°C)	Time (h)	Yield (%)			
						33	**34**	**35**	**36**
a	**29**	$BF_3\cdot OEt_2$		−18	2	18	63	10	—
b	**29**	$BF_3\cdot OEt_2$	$PhCH_3$	−18	2	41	48	—	—
c	**29**	$BF_3\cdot OEt_2$	$ClCH_2CH_2Cl$	−18	3	14	69	—	—
d	**29**	$BF_3\cdot OEt_2$	CH_2Cl_2	−18	2	21	72	—	—
e	**29**	$BF_3\cdot OEt_2$	CH_3NO_2	−18	2	—	77	—	—
f	**29**	$BF_3\cdot OEt_2$	$PhCH_3$	−80 to −40	4	16	62	—	—
g	**29**	$BF_3\cdot OEt_2$	PhH	19	1	28	42	—	—
h[a]	**29**	0.5% HCl	THF–H_2O (4:1)	0	1	—	64	—	26
i	**31**	$BF_3\cdot OEt_2$	$PhCH_3$	−18	2	—	66	—	—

[a] Products were separated without reacetalization with ethylene glycol and *p*-TsOH.

Judging from the above result that even an ester group cyclized to form the lactone **34**, it seemed possible that not only the alcohol **32** but also its acetate would give the corresponding cyclized products in their acetal–ene reactions. Therefore, as mentioned in Section 2.2, we selected a bulky pivaloyl group to protect the alcohol **32**, affording **5**, which was subjected to the acetal–ene reaction under a variety of reaction conditions (Table 2). The products were, this time, purified after deprotection of the C4 acetal group by treatment with *p*-TsOH in acetone. When the reaction was carried out in toluene at below −18 °C, we obtained compound **6** in a good yield of 66% (runs d, e). Careful separation of the reaction products of run d provided two more compounds, **37** (3%) and **38** (3%), as by-products. The ene reaction under the conditions of run d followed by reacetalization as above provided **39** (70%), together with **40** (5%) and **41** (3%).

Table 2. Acetal–ene reaction of **5** to form **6**

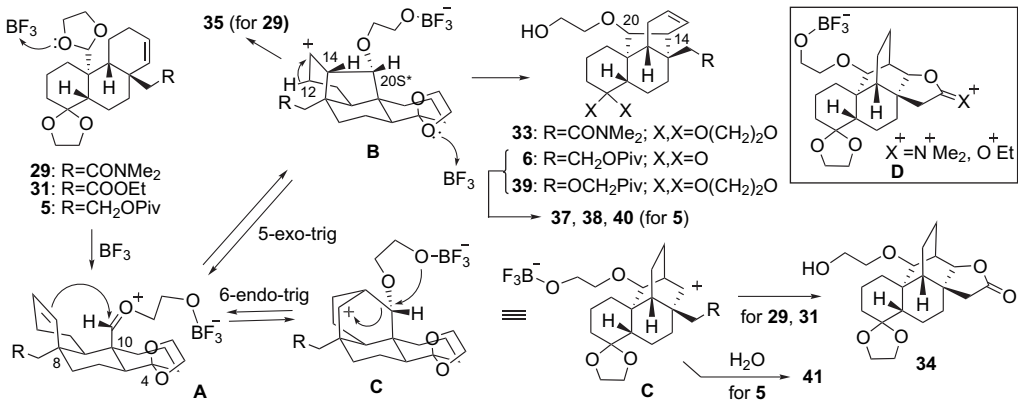
Run	Lewis acid	Solvent	Temp (°C)	Time (h)	**6** (%)
a	$BF_3\cdot OEt_2$	CH_2Cl_2	−18	2	55
b	$BF_3\cdot OEt_2$	$ClCH_2CH_2Cl$	−18	2	50
c	$BF_3\cdot OEt_2$	CS_2	−18	2	55
d	$BF_3\cdot OEt_2$	$PhCH_3$	−18	1	66[a]
e	$BF_3\cdot OEt_2$	$PhCH_3$	−70 to −50	2	66
f	$BF_3\cdot OEt_2$	$PhCH_3$	−18	0.17	59
g	$SnCl_4$	$PhCH_3$	−18	2	45

[a] By-products **37** (3%) and **38** (3%) were also isolated.

2.3.2. Reaction mechanism of the acetal–ene reaction. The reaction mechanism for the acetal–ene reaction is considered to be as follows. Coordination of the dioxolane oxygen at C10 (hetisan numbering) of **29**, **31**, and **5** to BF_3 gives rise to an intermediate **A**, in which the oxonium cation is arrayed so as to circumvent the steric congestion between the two 1,3-diaxial substituents at C4 and C8, as depicted in **A** (Scheme 6). Nucleophilic attack from the olefin to the oxonium cation takes place in two different modes, 5-*exo-trig* and 6-*endo-trig*, giving intermediates **B** and **C**, respectively. These reaction paths can explain the *S** stereochemistry of C20 in **B** and the corresponding carbon in **C**. Deprotonation from C12 of **B** affords **33** from **29** and **6** from **5**. In the case of **C**, on the other hand, there are no eliminable protons to form an olefin, because the two carbons lying adjacent to the cation are a tertiary bridgehead carbon and a quaternary carbon. Therefore, when the intermediate **C** carrying a nucleophilic C8 side chain is derived from **29** and **31**, intramolecular cyclization occurs to form the lactone **34** by way of the intermediary cation **D**. On the contrary, this cyclization is not able to occur from **C**, derived from **5**, because the bulkiness of the pivaloyl group prevents the cyclization and results in retro-ene reaction to afford **6** eventually, by way of **A** and **B**. The stereochemistry of **33** was born out by the fact that 4% NOE enhancement was observed at H20 (δ 4.44, d, J=6.5 Hz) on irradiation at H14 (δ 2.91, ddd, J=7, 6.5, 1.5 Hz) in the ^{1}H NMR spectrum. Analogously, observation of the NOE enhancement between two protons of **34** at δ 3.85 (d, J=3 Hz) and at δ 4.26 (d, J=4 Hz) supports the structure; they were assigned to the two protons on the carbons bearing the (2-hydroxyethyl)oxy group and the lactone O atom, respectively. The IR spectrum of **34** (ν_{max}=1767 cm^{-1}) is also consistent with the 5-membered lactone structure.

Partial cyclization from the intermediate **B** from **29** gives rise to the by-product **35**, whose IR spectrum (ν_{max}=1719 cm^{-1}) is consistent with the 6-membered lactone structure. Formation of the cis isomer **37** is attributable to enolization brought about by coordination of the C4 acetal or carbonyl group to BF_3. A sequence of reactions on **6**, coordination of the oxygen at C20 to BF_3 followed by cyclization from $\Delta_{12,13}$ to C20 in 3-*exo-trig* mode, and deprotonation from C11 provides an explanation for the formation of the by-products **38** and **40**. Compound **41**, obtained in a small amount, is a by-product generated by trapping of the intermediary cation of the 6-*endo-trig* route with water at quenching of the reaction. The orientation of the secondary hydroxy group of **41** was determined by the fact that there is a 4% NOE enhancement between the two protons on the carbons bearing oxygen in the bicyclo[2.2.2]octane framework, as depicted in Scheme 5.

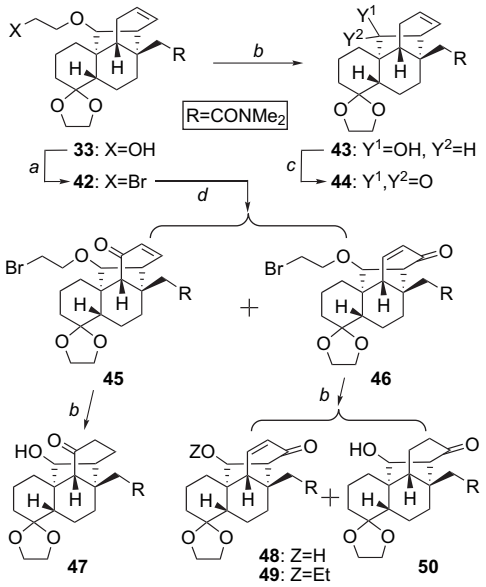
2.3.3. Suitability of 2-hydroxyethyl group to protect the C20 hydroxy group and model study for its removal. The above acetal–ene reaction furnished the desired tetracyclic products **33**, **6**, and **39** with a 2-hydroxyethyl protecting group at the C20 hydroxy group. The ene reaction proceeds, needless to say, starting from an aldehyde as well as an acetal. However, this protecting group plays a critical role in the total synthesis of (±)-nominine in the following respects. (1) The compound without a protecting group on the C20 hydroxy group is in danger of undergoing C14–C20 bond fission through the retro-ene reaction. In addition, it would



Scheme 6. Reaction mechanism of the acetal–ene reaction to form the C14–C20 bond.

be difficult to protect the hydroxy group on C20 because of steric hindrance. In the case of the compounds **33**, **6**, and **39**, the 2-hydroxyethyl group (originated from ethylene glycol) conveniently protected the hydroxy group. (2) As the 2-hydroxyethyl group is an extremely stable protecting group, provided the terminal primary hydroxy group is protected appropriately, it can readily tolerate a variety of reaction conditions encountered in the subsequent synthetic route. (3) The 2-hydroxyethyl group can be removed with ease by bromination followed by mild reduction with zinc in alcohol–H_2O.

We confirmed these features by means of the following model reactions with compound **33** (Scheme 7). Compound **33** was readily brominated with carbon tetrabromide (CBr_4) and Ph_3P to afford **42** in 87% yield. Then smooth removal of the 2-bromoethyl group was carried out by exposure of **42** to zinc (Zn) in refluxing 2-propanol–H_2O (14:1) with ammonium chloride (NH_4Cl) to afford **43** in the high yield of 94%. Taking into consideration the prospective construction of the azabicyclo ring system of the hetisan framework, we tried to protect this hydroxy group with an acyl group, such as acetyl, methanesulfonyl, or trifluoromethanesulfonyl. However, these trials resulted in a recovery of **43** due to the steric congestion around the hydroxy group. Oxidation of **43** with Dess–Martin periodinane[27] provided **44** in a good yield.



Scheme 7. Model deprotection of the 2-hydroxyethyl group and allylic oxidation of **33**: (a) Br_4C, Ph_3P, CH_2Cl_2, **42** 87%; (b) Zn, NH_4Cl, 2-PrOH-H_2O (14:1), **43** 94% from **42**; **47** 89% from **45**; **48** 49%, **49** 9%, **50** 39% from **46**; (c) Dess–Martin periodinane, CH_2Cl_2, **44** 92%; (d) CrO_3, 3,5-dimethylpyrazole, **45** 28%, **46** 28%, recovery of **42** 29%.

2.4. Further transformation from the acetal–ene reaction products

With the desired compounds **33**, **6**, and **39** in hand, we next examined further transformation toward the hetisan skeleton, including nominine. The basicity of the nitrogen on the azabicyclo ring system is so strong that it would be troublesome to carry on the synthesis employing the intermediates bearing the ring system. So we envisioned construction of the azabicyclo ring preferably at a later stage of the total synthesis. Thus, we directed our attention to functionalization of C11, C6, or the side chain at C8. Here, we describe an attempt to functionalize C11 of **33** by allylic oxidation.

A number of aconite alkaloids, such as kobusine and pseudokobusine, carry an oxygen function (carbonyl or hydroxy group) at C11 (Scheme 1). To begin with, allylic oxidation of **42** was examined (Scheme 7). Chromium trioxide (CrO_3, 12 equiv) oxidation in the presence of 3,5-dimethylpyrazole[28] gave favorable results, giving **45** and **46** in 28% yield each, along with a recovery of **42** in 29% yield. Further excess of the oxidizing reagent caused a significant drop in the yields. Although the oxidation did not afford the desired product **45** in satisfactory yield and the regioisomeric enone **46** was obtained in the same amount, some alkaloids such as hetisine and spirasine IV have an oxygen function at this position, C13.[1,2]

The above reductive deprotection of the hydroxy group at C20 was carried out for **45** and **46**. The sole product from **45** was a saturated keto–alcohol **47**: the enone group was incompatible with the reductive conditions. The enone–alcohol **48** was produced from **46**, but compound **50**, corresponding to **47** and the debrominated product **49**, were also isolated. Thus, it proved complicated to functionalize C11 of **33**. However, some clues were obtained for functionalization of C11 and for C-ring formation by the connection of C12 and the side chain at C8.

In part 2, we will describe some attempts at C-ring formation and the functionalization of C6 for the construction of the pyrrolidine ring, leading to the synthesis of a hexacyclic compound **1** lacking the C-ring of the hetisan skeleton.

3. Conclusion

In summary, we have synthesized the tetracyclic synthetic intermediates **33**, **6**, and **39** for hetisine-type aconite alkaloids, utilizing the following key reactions: (i) palladium-catalyzed intramolecular α-arylation at the formyl group (**3**→**4**), and (ii) acetal–ene reaction to form the C14–C20 bond (**29**→**33**, **5**→**6**, **5**→**39**). This was a substantial step toward the first total synthesis of hetisine-type aconite alkaloids. In the subsequent papers, we report further efforts toward this goal.

4. Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus (hot plate), and are not corrected. MS and high-resolution MS (HRMS) were recorded on a Hitachi M-80B spectrometer in a gas chromatography (GC) or direct inlet (DI) mode at an ionizing voltage of 70 eV, and figures in parentheses indicate the relative intensities. IR spectra were measured on a Hitachi 215 or Shimadzu IR-460 spectrophotometer. ^1H NMR spectra were obtained on a Varian Mercury 300 (300 MHz) in $CDCl_3$ unless otherwise specified and coupling constants (J values) are rounded to the nearest 0.5 Hz. ^{13}C NMR spectra were measured on a Varian Mercury 300 (75 MHz) in

$CDCl_3$ and ^{13}C multiplicities are shown in parentheses as CH_3 (primary), CH_2 (secondary), CH (tertiary), and C (quaternary). The NMR signals were assigned using proton decoupling techniques, as well as gCOSY, DEPT, gHSQC, gHMBC, and/or NOESY spectra. Some characteristic ^1H and ^{13}C NMR signals were selected and assigned as HX and CX, respectively, where X represents hetisan carbon numbering. Column chromatography was conducted on silica gel (SiO_2, Fuji Davison BW 200), and the weight of SiO_2 and the eluting solvent is indicated in parentheses. Preparative TLC (PTLC) was carried out on glass plates (20×20 cm) coated with Merck Silica gel $60PF_{254}$ (0.8 mm thick) unless otherwise specified, and the developing solvent is indicated in parentheses. Usual work-up refers to washing of the organic layers with water or brine, drying over anhydrous Na_2SO_4, and evaporating off the solvents under reduced pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use.

4.1. Large-scale preparation of 2 and 4 (Scheme 3)

4.1.1. (2-Bromo-5-methoxy)phenylacetonitrile (12). KCN (7.48 g, 0.115 mol) was added to a solution of **11**[21] (7.48 g, 36.7 mmol) in EtOH (30 ml) and H_2O (15 ml) and the mixture was refluxed with stirring for 5 h. After having been cooled, the mixture was extracted with EtOAc. Usual work-up followed by recrystallization provided **12** (5.58 g, 92%) as colorless prisms, mp 54–55 °C (Et_2O–hexane). Anal. Calcd for C_9H_8BrNO: C, 47.81; H, 3.57; Br, 35.35; N, 6.20. Found: C, 47.66; H, 3.56; Br, 35.34; N, 6.21. GC–HRMS Calcd for C_9H_8BrNO: 226.9770, 224.9789. Found: 226.9780, 224.9814. GC–MS *m/z*: 227, 225 (M^+, 100, 95), 212, 210 (12, 13), 184, 182 (28, 30), 146 (34), 116 (23), 103 (41), 76 (26), 63 (25). IR (KBr) cm^{-1}: 2240. ^1H NMR δ: 3.80 (2H, s), 3.82 (3H, s), 6.77 (1H, dd, *J*=9, 3 Hz), 7.07 (1H, d, *J*=3 Hz), 7.47 (1H, d, *J*=9 Hz). ^{13}C NMR δ: 24.9 (CH_2), 55.5 (CH_3), 113.5 (C, C2), 115.2 (CH, C6), 115.4 (CH, C4), 116.7 (C, CN), 130.5 (C, C1), 133.4 (CH, C3), 159.1 (C, C5).

4.1.2. Ethyl (2-bromo-5-methoxy)phenylacetate (13). A solution of **12** (13.54 g, 59.9 mmol) in EtOH (40 ml) and concd H_2SO_4 (16.0 ml, 0.300 mol) was stirred under reflux for 6 h. The mixture was cooled in an ice bath and was diluted with H_2O. Extraction with EtOAc, washing with saturated $NaHCO_3$–H_2O, usual work-up, and distillation afforded **13** (14.71 g, 90%) as a colorless oil, bp 174–176 °C/13 mmHg. GC–HRMS Calcd for $C_{11}H_{13}BrO_3$: 274.0028, 272.0048. Found: 274.0032, 272.0050. GC–MS *m/z*: 274, 272 (M^+, 13, 12), 201, 199 (46, 48), 193 (50), 165 (100), 77 (31), 51 (27). IR (neat) cm^{-1}: 1737. ^1H NMR δ: 1.26 (3H, t, *J*=7 Hz), 3.73 (2H, s), 3.77 (3H, s), 4.18 (2H, q, *J*=7 Hz), 6.70 (1H, dd, *J*=9, 3 Hz), 6.84 (1H, d, *J*=3 Hz), 7.43 (1H, d, *J*=9 Hz). ^{13}C NMR δ: 14.2 (CH_3), 41.8 (CH_2), 55.3 (CH_3), 60.9 (CH_2), 114.3 (CH), 115.2 (C), 116.9 (CH), 133.0 (CH), 134.9 (C, C1), 158.6 (C), 170.1 (C).

4.1.3. 2-(2-Bromo-5-methoxyphenyl)ethanol (14). DIBAL-H (0.94 M in hexane, 93.0 ml, 87.4 mmol) was added dropwise to a cooled (−78 °C) solution of **13** (9.50 g, 34.8 mmol) in CH_2Cl_2 (120 ml) under an Ar atmosphere, and the mixture was stirred at −78 to −8 °C for 3 h. The mixture was slowly poured into saturated NH_4Cl–H_2O containing NH_4Cl solid with efficient stirring to decompose an excess reagent. The mixture was dredged with Celite (200 g) and the whole was filtered through a Celite bed in vacuo. The filtered Celite was washed thoroughly with CH_2Cl_2. Usual work-up and separation by SiO_2 column chromatography [120 g, benzene–hexane (19:1)] gave **14** (7.74 g, 96%) as a colorless oil. GC–HRMS Calcd for $C_9H_{11}BrO_2$: 231.9923, 229.9942. Found: 231.9923, 229.9926. GC–MS *m/z*: 232, 230 (M^+, 51, 53), 201, 199 (58, 56), 150 (72), 121 (100), 91 (51), 77 (65), 51 (55), 31 (74). ^1H NMR δ: 2.95 (2H, t, *J*=6.5 Hz), 3.76 (3H, s), 3.82 (2H, t, *J*=6.5 Hz), 7.76 (1H, br s, OH), 6.64 (1H, dd, *J*=9, 3 Hz), 6.82 (1H, d, *J*=3 Hz), 7.40 (1H, d, *J*=9 Hz). ^{13}C NMR δ: 39.4 (CH_2), 55.3 (CH_3), 61.8 (CH_2), 113.5 (CH, C4), 114.8 (C, C2), 116.7 (CH, C6), 133.1 (CH, C3), 138.6 (C, C1), 158.6 (C, C5).

4.1.4. 2-Bromo-5-methoxyphenethyl methanesulfonate (15). A solution of MsCl (2.85 ml, 36.8 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a cooled (−20 °C) solution of **14** (7.74 g, 33.5 mmol) and Et_3N (6.06 ml, 43.6 mmol) in CH_2Cl_2 (45 ml) and the mixture was stirred for 1 h under an Ar atmosphere. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. The organic layer was washed with saturated $CuSO_4$–H_2O and then with saturated $NaHCO_3$–H_2O. Usual work-up and purification by SiO_2 column chromatography [100 g, hexane–EtOAc (5:2)] gave **15** (10.27 g, 99%) as a colorless syrup. DI-HRMS Calcd for $C_{10}H_{13}BrO_4S$: 309.9698, 307.9717. Found: 309.9684, 307.9692. DI-MS *m/z*: 310, 308 (M^+, 21, 23), 214, 212 (90, 100), 201, 199 (18, 19), 79 (33), 51 (22). ^1H NMR δ: 2.91 (3H, s), 3.16 (2H, t, *J*=7 Hz), 3.78 (3H, s), 4.44 (2H, t, *J*=7 Hz), 6.70 (1H, dd, *J*=9, 3 Hz), 6.84 (1H, d, *J*=3 Hz), 7.43 (1H, d, *J*=9 Hz). ^{13}C NMR δ: 36.0 (CH_2), 37.2 (CH_3), 55.4 (CH_3), 68.3 (CH_2), 114.4 (CH), 114.5 (C), 116.9 (CH), 133.3 (CH), 136.2 (C), 158.8 (C).

4.1.5. 1-Bromo-2-(2-iodoethyl)-4-methoxybenzene (2). NaI (7.48 g, 49.9 mmol) was added to a solution of **15** (10.27 g, 33.2 mmol) in acetone (120 ml) and the mixture was refluxed with stirring for 8 h. After the mixture had been cooled in an ice bath, H_2O was added and the whole was extracted with EtOAc. Usual work-up and separation by SiO_2 column chromatography [100 g, hexane–EtOAc (99.9:0.1)] yielded **2** (10.91 g, 96%) as a colorless syrup. The product **2** is a known compound.[20] GC–HRMS Calcd for $C_9H_{10}BrIO$: 341.8942, 339.8962. Found: 341.8930, 339.8954. GC–MS *m/z*: 342, 340 (M^+, 42, 44), 215, 213 (77, 79), 134 (100), 91 (42), 63 (48). ^1H NMR δ: 3.21–3.29 (2H, m), 3.31–3.39 (2H, m), 3.79 (3H, s), 6.70 (1H, dd, *J*=9, 3 Hz), 6.79 (1H, d, *J*=3 Hz), 7.42 (1H, d, *J*=9 Hz). ^{13}C NMR δ: 3.0 (CH_2, *C*H_2I), 40.7 (CH_2), 55.4 (CH_3), 114.0 (CH), 114.2 (C), 116.2 (CH), 133.3 (CH), 140.5 (C, C1), 158.7 (C).

4.1.6. 2-(3-Methoxyphenyl)ethanol (16). A solution of methyl chloroformate (24.5 ml, 0.317 mol) in THF (50 ml) was added slowly to a cooled (−20 °C) solution of 3-methoxyphenylacetic acid (50.0 g, 0.301 mol) and Et_3N (44.5 ml, 0.320 mol) in THF (400 ml) and the mixture was stirred at −20 °C for 1 h. The precipitate was filtered under reduced pressure and the filtered salt was washed with THF (50 ml). The combined THF solution was added to a slurry

of $NaBH_4$ (57.2 g, 1.51 mol) in H_2O (300 ml) and the mixture was stirred at 0–24 °C for 40 h. Another portion of $NaBH_4$ (11.5 g, 0.303 mol) was added and the resulting mixture was further stirred for 24 h. The mixture was gradually poured into saturated NH_4Cl–H_2O and the whole was extracted with CH_2Cl_2. Distillation afforded **16** (43.6 g, 96%) as a colorless oil (bp: 135–140 °C/10 mmHg). The compound **16** was prepared in the literature[20] by treatment of 3-methoxyphenylacetic acid with $NaBH_4$ and I_2 in 89% yield, and is also available from Aldrich Chemical Co. (bp: 141–143 °C/12 mmHg).

4.1.7. Improved Pd cyclization of 3 to form 4 and by-products 17, 18. A slurry of **3** (12.24 g, 32.0 mmol), $PdCl_2(Ph_3P)_2$ (2.00 g, 2.85 mmol), Cs_2CO_3 (16.70 g, 51.2 mmol), and Ph_3P (1.67 g, 6.37 mmol) in THF (200 ml) was refluxed with vigorous stirring under an Ar atmosphere for 60 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the mixture was extracted with EtOAc. Usual work-up followed by purification by SiO_2 column chromatography (250 g, benzene) and PTLC provided **4** (6.83 g, 71%, cis/trans = 3.4), **17** (61 mg, 4%), and **18** (29 mg, 2%) in order of increasing polarity. Spectral data of the three products and single crystal X-ray analysis data of *cis*-**4** have already been reported.[11c]

4.2. Transformation of 4 to 5 (Scheme 4)

4.2.1. Acetalization of 4 to form 19 and 20. Ethylene glycol (2.50 ml, 44.9 mmol) and *p*-TsOH·H_2O (160 mg, 0.842 mmol) were added to a solution of **4** (2.68 g, 8.87 mmol, cis/trans = 3.4) in benzene (40 ml), and the mixture was refluxed with stirring for 6 h with a Dean–Stark apparatus. After the mixture had been cooled in an ice bath, saturated $NaHCO_3$–H_2O was added and the whole was extracted with EtOAc. Usual work-up and subsequent SiO_2 column chromatography [80 g, hexane–EtOAc (14:1∼9:1)] afforded **20** (1.05 g, 34%) and **19** (1.87 g, 61%) in order of increasing polarity. **19**: Colorless prisms, mp 99–100 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.18; H, 7.53. GC–HRMS Calcd for $C_{20}H_{26}O_5$: 346.1779. Found: 346.1781. GC–MS *m*/*z*: 346 (M^+, 1), 273 (17), 99 (100), 73 (28), 55 (9), 45 (10). ^{1}H NMR δ: 1.41–1.56 (2H, m), 1.60–1.75 (2H, m), 1.77–1.94 (2H, m), 2.04–2.19 (2H, m), 2.27 (1H, dd, *J*=5.5, 5.5 Hz, H5), 2.75 (1H, ddd, *J*=17, 6.5, 6.5 Hz, H7), 2.88 (1H, ddd, *J*=17, 7.5, 7.5 Hz, H7), 3.52–3.62 (1H, m), 3.68–3.97 (7H, m), 3.76 (3H, s), 5.24 (1H, s), 6.58 (1H, d, *J*=3 Hz), 6.68 (1H, dd, *J*=9, 3 Hz), 7.41 (1H, d, *J*=9 Hz). ^{13}C NMR δ: 19.0 (CH_2, C6), 19.2 (CH_2, C3), 28.6 (CH_2, C7), 28.8 (CH_2, C1), 33.7 (CH_2, C2), 43.2 (CH, C5), 45.4 (C, C10), 54.8 (CH_3), 64.0 (CH_2), 64.5 (CH_2), 65.0 (CH_2), 65.1 (CH_2), 107.7 (CH), 111.2 (×2, CH and C, C4 and C12), 112.8 (CH, C14), 128.3 (CH, C11), 129.8 (C), 140.2 (C, C8), 157.1 (C). **20**: Colorless needles, mp 108–109 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.25; H, 7.52. GC–HRMS Calcd for $C_{20}H_{26}O_5$: 346.1779. Found: 346.1783. GC–MS *m*/*z*: 346 (M^+, 1), 273 (18), 99 (100), 73 (29), 55 (10), 45 (9). ^{1}H NMR δ: 1.31 (1H, ddd, *J*=13.5, 13.5, 4 Hz, H1), 1.42–1.55 (1H, m), 1.66–1.97 (5H, m), 2.10–2.27 (1H, m), 2.67–2.78 (1H, m, dioxolane proton, anisotropy), 2.76–2.99 (3H, m), 3.50–3.64 (3H, m), 3.77 (3H, s), 3.82–3.92 (1H, m), 3.94–4.03 (2H, m), 4.06–4.16 (1H, m), 5.80 (1H, s), 6.60 (1H, d, *J*=2.5 Hz), 6.63 (1H, dd, *J*=8.5, 2.5 Hz), 7.29 (1H, d, *J*=8.5 Hz). ^{13}C NMR δ: 16.7 (CH_2, C6), 19.7 (CH_2, C2), 29.4 (CH_2, C7), 33.9 (CH_2, C1), 35.6 (CH_2, C3), 44.8 (C, C10), 49.0 (CH, C5), 54.9 (CH_3), 64.1 (CH_2), 64.4 (CH_2), 64.9 (CH_2), 65.6 (CH_2), 105.0 (CH), 110.3 (CH, C12), 112.6 (CH), 128.1 (C), 128.3 (CH), 131.6 (C, C9), 139.4 (C, C8), 157.4 (C). Under the same conditions, **19** (920 mg, 2.66 mmol) was acetalized to give **20** (323 mg, 35%) and recovered **19** (561 mg, 61%).

4.2.2. Birch reduction of 20 to form 21. Li (7.89 g, 1.13 mol) was added in small portions during 2.5 h to a cooled (−78 °C) solution of **20** (6.50 g, 18.8 mmol) in liq. NH_3 (ca. 150 ml), THF (80 ml), and EtOH (80 ml) with efficient stirring by a mechanical stirrer. After the addition completed, stirring was continued for 3 h. Solid NH_4Cl (10.0 g) was slowly added and the cooling bath was removed. Stirring was further continued at room temperature with trapping evaporating NH_3 with concd HCl and ice. Saturated NH_4Cl–H_2O was added and the mixture was filtered through a Celite bed, and the Celite was washed with $CHCl_3$. Extraction with $CHCl_3$ followed by usual work-up afforded crystalline material, which was purified by recrystallization and SiO_2 column chromatography [hexane–EtOAc (8:1)] to yield **21** (5.99 g, 92%) as colorless prisms, mp 167–168.5 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.73; H, 8.07. GC–HRMS Calcd for $C_{20}H_{28}O_5$: 348.1935. Found: 348.1923. GC–MS *m*/*z*: 348 (M^+, 4), 275 (35), 213 (10), 99 (100), 73 (38), 55 (12), 45 (18). IR (KBr) cm^{-1}: 1697, 1663. ^{1}H NMR δ: 1.17 (1H, ddd, *J*=13, 13, 4.5 Hz, H1), 1.43 (1H, ddd, *J*=13, 13, 5 Hz, H3), 1.59–2.04 (8H, m), 2.45 (1H, dddd, *J*=13, 3, 3, 1.5 Hz, H1), 2.60 (2H, dd, *J*=7.5, 7 Hz, H14), 2.74–3.04 (2H, m, H11), 3.54 (3H, s), 3.61–4.11 (8H, m), 4.60 (1H, dd, *J*=4, 3.5 Hz, H12), 5.66 (1H, s). ^{13}C NMR δ: 16.4 (CH_2), 19.6 (CH_2), 28.0 (CH_2, C11), 30.2 (CH_2), 32.3 (CH_2), 34.3 (CH_2, C14), 35.6 (CH_2), 44.8 (C, C10), 49.5 (CH), 53.6 (CH_3), 63.6 (CH_2), 64.3 (CH_2), 65.1 (CH_2), 65.5 (CH_2), 90.9 (CH, C12), 105.5 (CH), 110.3 (C, C4), 127.4 (C, C9), 127.9 (C), 151.8 (C, C13).

4.2.3. Hydrolysis of vinyl ether 21 to form 22 and 23. HCl–H_2O (2.5%, 5.0 ml) was added to a cooled (0 °C) solution of **21** (590 mg, 1.70 mmol) in THF (20 ml) and the mixture was stirred at the temperature for 1.5 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and separation by SiO_2 column chromatography [25 g, hexane–EtOAc (4:1∼1:1)] afforded **22** (497 mg, 88%) and **23** (41 mg, 7%) in order of increasing polarity. **22**: Colorless prisms, mp 142–144 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84. Found: C, 68.18; H, 7.82. GC–HRMS Calcd for $C_{19}H_{26}O_5$: 334.1779. Found: 334.1772. GC–MS *m*/*z*: 334 (M^+, 1), 261 (2), 199 (1), 99 (22), 73 (100), 55 (7), 45 (11). IR ($CHCl_3$) cm^{-1}: 1713. ^{1}H NMR δ: 1.15 (1H, ddd, *J*=13, 13, 4.5 Hz, H1), 1.44 (1H, ddd, *J*=13, 13, 5.5 Hz, H3), 1.60–1.80 (4H, m), 1.82–2.05 (4H, m), ca. 2.26–2.48 (3H, m), 2.50 (1H, dddd, *J*=13, 3, 3, 1 Hz, H1), 2.64–2.74 (1H, m), 2.72 (1H, d, *J*=21 Hz, H14), 2.82 (1H, d, *J*=21 Hz, H14), 3.65–4.10 (8H, m), 5.56 (1H, s). ^{13}C NMR δ: 16.4 (CH_2), 19.5 (CH_2), 26.1 (CH_2, C12), 30.5 (CH_2), 32.1 (CH_2), 35.6 (CH_2), 39.4 (CH_2), 45.0 (C), 45.1 (CH_2, C14), 49.7 (CH), 63.4 (CH_2), 64.3 (CH_2), 65.3 (CH_2), 65.5 (CH_2), 105.2 (CH), 110.0 (C),

128.9 (C), 131.7 (C), 211.9 (C). **23**: Colorless prisms, mp 172–173 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84. Found: C, 67.78; H, 7.71. GC–HRMS Calcd for $C_{19}H_{26}O_5$: 334.1779. Found: 334.1782. GC–MS *m/z*: 334 (M^+, 10), 289 (4), 261 (4), 99 (21), 73 (100), 55 (8), 45 (15). IR ($CHCl_3$) cm^{-1}: 1652, 1611. ^1H NMR δ: 1.03–1.14 (1H, m), 1.40–1.52 (1H, m), 1.53–1.69 (2H, m), 1.72 (1H, dd, *J*=13, 3 Hz, H5), 1.75–1.96 (3H, m), 1.99–2.21 (4H, m), 2.29–2.47 (2H, m), 2.54 (1H, br dddd, *J*=13, 3, 3, 1.5 Hz, H1), 2.64 (1H, br ddd, *J*=17, 5, 1.5 Hz, H7), 3.66–4.05 (8H, m), 5.46 (1H, s), 5.73 (1H, br s, H14). ^{13}C NMR δ: 19.0 (CH_2), 19.6 (CH_2), 23.4 (CH_2), 34.15 (CH_2), 34.18 (CH_2), 35.6 (CH_2), 38.2 (CH_2), 44.9 (C), 49.7 (CH, C9), 51.4 (CH), 62.8 (CH_2), 64.2 (CH_2), 64.6 (CH_2), 65.5 (CH_2), 104.9 (CH), 109.6 (C), 122.5 (CH, C14), 168.1 (C, C8), 199.8 (C).

4.2.4. NaOMe treatment of 22. NaOMe (150 mg, 2.78 mmol) was added to a cooled (0 °C) slurry of **22** (305 mg, 0.913 mmol) in MeOH (15 ml) and the mixture was stirred at 0 °C for 0.5 h and at 18 °C for 16 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [benzene–EtOAc (5:1)] gave recovered **22** (30 mg, 10%), **23** (169 mg, 55%), and an oxetane **24** (31 mg, 10%) in order of increasing polarity. **24**: Colorless glass. DI-HRMS Calcd for $C_{19}H_{24}O_6$: 348.1571. Found: 348.1557. DI-MS *m/z*: 348 (M^+, 2), 257 (5), 99 (100), 73 (75), 55 (11), 45 (20). IR ($CHCl_3$) cm^{-1}: 1651. ^1H NMR δ: 1.52–1.71 (2H, m), 1.75–1.92 (3H, m), 2.04–2.17 (2H, m), 2.21–2.42 (4H, m), 2.71 (1H, ddd, *J*=18.5, 13, 7 Hz, H7), 2.86 (1H, dd, *J*=12, 6 Hz, H5), 3.40–3.54 (1H, m), 3.63–3.87 (4H, m), 3.91–4.01 (2H, m), 4.03–4.14 (1H, m), 5.20 (1H, d, *J*=7 Hz, H12), 5.51 (1H, s), 5.52 (1H, s, H14). ^{13}C NMR δ: 19.9 (CH_2), 24.8 (CH_2), 28.9 (CH_2), 29.0 (CH_2), 32.9 (CH_2), 36.0 (CH_2), 45.9 (C), 50.2 (CH), 62.7 (CH_2), 63.5 (CH_2), 64.4 (CH_2), 65.9 (CH_2), 88.0 (C, C9), 95.0 (CH, C12), 103.9 (CH), 106.9 (CH, C14), 110.0 (C, C4), 164.5 (C, C8), 199.1 (C).

4.2.5. NaOMe treatment of 22 in the presence of Me_2S. NaOMe (40 mg, 0.741 mmol) was added to a cooled (0 °C) slurry of **22** (210 mg, 0.629 mmol) and Me_2S (0.23 ml, 3.14 mmol) in MeOH (8 ml) and the mixture was stirred at 0 °C for 0.5 h and at 18 °C for 14 h. The same work-up as above afforded recovered **22** (28 mg, 13%), **23** (122 mg, 58%), and **26** (31 mg, 14%) in order of increasing polarity. **26**: Colorless needles, mp 234–235 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.12; H, 7.48. Found: C, 64.75; H, 7.35. DI-HRMS Calcd for $C_{19}H_{26}O_6$: 350.1728. Found: 350.1737. DI-MS *m/z*: 350 (M^+, 8), 288 (11), 260 (11), 165 (11), 99 (100), 73 (83), 55 (19), 45 (35). IR ($CHCl_3$) cm^{-1}: 1660. ^1H NMR δ: 1.39–1.51 (1H, m), 1.57–1.91 (6H, m), 1.97–2.05 (1H, m), 2.12–2.18 (1H, m), 2.16 (1H, br s, OH), 2.26–2.69 (6H, m), 3.67–4.04 (8H, m), 5.51 (1H, s), 5.66 (1H, br d, *J*=2 Hz, H14). ^{13}C NMR δ: 19.1 (CH_2), 19.3 (CH_2), 26.3 (CH_2), 30.0 (CH_2), 31.2 (CH_2), 33.5 (CH_2, C12), 35.3 (CH_2), 43.8 (CH), 48.9 (C, C10), 62.7 (CH_2), 64.0 (CH_2), 64.3 (CH_2), 65.5 (CH_2), 71.5 (C, C9), 104.0 (CH), 110.6 (C), 122.9 (CH, C14), 165.2 (C, C8), 199.3 (C, C13).

4.2.6. DBU treatment of 22 to form hydroperoxide 25 and 26. A solution of **22** (10 mg, 29.9 μmol) and DBU (9 μl, 60.3 mmol) in benzene (2.5 ml) was refluxed with stirring for 4 h. Addition of H_2O, extraction with EtOAc, successive washing with $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O, and PTLC [benzene–EtOAc (4:1)] gave recovered **22** (4 mg, 40%), **25** (2 mg, 18%), and **26** (2 mg, 19%) in order of increasing polarity. **25**: Colorless glass. DI-HRMS Calcd for $C_{19}H_{26}O_7$: 366.1677. Found: 366.1695. DI-MS *m/z*: 366 (M^+, 2), 350 (3), 293 (3), 277 (3), 251 (4), 99 (62), 73 (100), 55 (17), 45 (25). IR ($CHCl_3$) cm^{-1}: 1653. ^1H NMR δ: 1.43 (1H, ddd, *J*=12.5, 12.5, 5.5 Hz, H1), 1.49–1.92 (6H, m), 2.17 (1H, br d, *J*=12.5 Hz, H1), 2.24–2.89 (7H, m), 3.64–4.03 (8H, m), 5.52 (1H, s), 5.95 (1H, d, *J*=2 Hz, H14), 7.59 (1H, br s, OOH).

4.2.7. Alternative preparation of 26 and 27 by *m*-CPBA oxidation of 22. *m*-CPBA (26 mg, 0.151 mmol) was added to a solution of **22** (18 mg, 53.9 μmol) in CH_2Cl_2 (3 ml) and the mixture was stirred at 0 °C for 1 h, and at 20 °C for 2.5 h. Saturated $Na_2S_2O_3$–H_2O and saturated $NaHCO_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up and rough separation by PTLC [hexane–EtOAc (1:1)] afforded a mixture of products (20 mg), which were treated with DBU (40 μl, 0.292 mmol) in refluxing benzene (3 ml) for 30 min. The same work-up as in Section 4.2.5 and purification by PTLC [1.5% MeOH–CH_2Cl_2] furnished **26** (11.5 mg, 61%) and **27** (6.5 mg, 34%) in order of decreasing polarity. **27**: Colorless prisms, mp 194–196 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.12; H, 7.48. Found: C, 64.85; H, 7.38. DI-HRMS Calcd for $C_{19}H_{26}O_6$: 350.1728. Found: 350.1724. DI-MS *m/z*: 350 (M^+, 3), 260 (2), 133 (7), 99 (100), 73 (23), 55 (11), 45 (14). IR ($CHCl_3$) cm^{-1}: 1665, 1620. ^1H NMR δ: 1.34 (1H, ddd, *J*=12.5, 12.5, 5 Hz, H1), 1.45–1.71 (4H, m), 1.77–2.02 (3H, m), 2.03 (1H, dddd, *J*=13, 13, 4.5, 1.5 Hz, H12), 2.17 (1H, ddd, *J*=13, 4.5, 2.5 Hz, H12), 2.19–2.39 (3H, m), 2.90 (1H, ddd, *J*=17, 13, 4.5 Hz, H11), 3.32 (1H, dddd, *J*=15.5, 12, 7, 2.5 Hz, H7), 3.76–3.87 (2H, m), 3.89–4.07 (5H, m), 4.08–4.17 (1H, m), 4.55 (1H, d, *J*=1.5 Hz, OH), 5.53 (1H, s). ^{13}C NMR δ: 18.1 (CH_2, C6), 19.7 (CH_2, C2), 28.3 (CH_2), 31.5 (CH_2), 33.2 (CH_2, C12), 34.0 (CH_2, C11), 35.7 (CH_2), 44.4 (CH, C5), 46.7 (C), 62.6 (CH_2), 64.2 (CH_2), 65.0 (CH_2), 65.7 (CH_2), 74.9 (C, C9), 106.1 (CH), 110.0 (C), 126.6 (CH), 166.4 (C, C8), 199.4 (C).

4.2.8. Reduction of 23 to form allylalcohol 28. $CeCl_7 \cdot H_2O$ (408 mg, 1.10 mmol) and $NaBH_4$ (43 mg, 1.13 mmol) were added to a cooled (0 °C) solution of **23** (330 mg, 0.988 mmol) in MeOH (25 ml) and the mixture was stirred at that temperature for 30 min. Quenching by successive addition of saturated NH_4Cl–H_2O and saturated $NaHCO_3$–H_2O followed by extraction with CH_2Cl_2, usual work-up, and PTLC [benzene–EtOAc (2:1)] provided **28** as colorless prisms, mp 191–192 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{19}H_{28}O_5 \cdot 1/4H_2O$: C, 66.93; H, 8.43. Found: C, 67.08; H, 8.37. DI-HRMS Calcd for $C_{19}H_{28}O_5$: 336.1935. Found: 336.1936. DI-MS *m/z*: 336 (M^+, 1), 318 (8), 184 (22), 99 (100), 73 (98), 45 (28). ^1H NMR δ: 0.93–1.05 (1H, m), 1.11–1.28 (1H, m), 1.38–1.50 (1H, m), 1.55–1.94 (10H, m, including OH), ca. 2.01–2.14 (1H, m), ca. 2.12–2.25 (1H, m), 2.41 (1H, ddd, *J*=15, 3, 3 Hz, H7), 2.46 (1H, br dddd, *J*=13, 3, 3, 1.5 Hz, H1), 3.65–3.73 (1H, m), 3.77–3.96 (6H, m), 3.97–4.03 (1H, m), 4.14–4.23 (1H, m, $W_{1/2}$=19 Hz, H13), 5.21–5.25 (1H, m, $W_{1/2}$=5.5 Hz, H14), 5.45 (1H, s).

^{13}C NMR δ: 19.7 (CH_2), 19.8 (CH_2), 22.1 (CH_2, C11), 33.9 (CH_2), 34.3 (CH_2), 34.5 (CH_2), 35.8 (CH_2), 44.4 (C), 48.9 (CH, C9), 52.8 (CH), 62.6 (CH_2), 64.1 (CH_2), 65.1 (CH_2), 65.4 (CH_2), 67.9 (CH, C13), 105.7 (CH), 110.0 (C), 122.3 (CH, C14), 141.3 (C, C8).

4.2.9. Claisen rearrangement of 28 under neutral conditions to form 29. A solution of **28** (500 mg, 1.49 mmol) and *N*,*N*-dimethylacetamide dimethyl acetal (1.53 ml, 10.48 mmol) in toluene (20 ml) was heated with stirring at 160 °C (bath temperature) in a sealed tube for 4 h. The volatile materials were removed in vacuo and resulting residue was purified by SiO_2 column chromatography [25 g, benzene–EtOAc (4:1)] to yield **29** (418 mg, 69%) as colorless needles, mp 140.5–141.5 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{23}H_{35}NO_5$: C, 68.12; H, 8.70; N, 3.45. Found: C, 68.04; H, 8.62; N, 3.48. DI-HRMS Calcd for $C_{23}H_{35}NO_5$: 405.2513. Found: 405.2521. DI-MS *m*/*z*: 405 (M^+, 4), 360 (6), 332 (20), 319 (15), 245 (56), 99 (82), 73 (100), 45 (34). IR ($CHCl_3$) cm^{-1}: 1622. ^{1}H NMR δ: 1.09 (1H, ddd, *J*=13, 13, 4.5 Hz, H1), 1.48 (1H, ddd, *J*=13, 13, 5 Hz, H3), 1.52–2.02 (12H, m), 2.08–2.19 (1H, m), 2.32 (1H, d, *J*=14 Hz, CH_2CON), 2.51 (1H, br dddd, *J*=13, 3, 3, 1 Hz, H1), 2.56 (1H, d, *J*=14 Hz, CH_2CON), 2.91 (3H, s), 2.98 (3H, s), 3.65–4.07 (8H, m), 5.34 (1H, s), 5.45 (1H, br dd, *J*=10, 1.5 Hz, H14), 5.67 (1H, ddd, *J*=10, 5, 2.5 Hz, H13). ^{13}C NMR δ: 16.9 (CH_2), 20.0 (CH_2), 20.7 (CH_2), 23.6 (CH_2), 34.3 (CH_2), 34.5 (CH_3), 35.9 (CH_2), 38.1 (C), 38.2 (CH_2), 38.5 (CH_3), 43.8 (C, C8), 46.2 (CH_2, *C*H_2CON), 49.7 (CH), 50.6 (CH), 62.3 (CH_2), 63.9 (CH_2), 64.0 (CH_2), 65.6 (CH_2), 104.9 (CH), 110.5 (C), 126.3 (CH, C13), 136.6 (CH, C14), 171.5 (C).

4.2.10. Acetylation of 28 to form diene 30. Ac_2O (0.10 ml, 1.06 mmol) was added to a solution of **28** (5.5 mg, 16.4 μmol) and pyridine (0.30 ml, 3.71 mmol) in CH_2Cl_2 (1 ml) and the mixture was stirred at 20 °C for 2 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–CH_2Cl_2 (1:2)] gave **30** (4.5 mg, 86%) as a labile colorless syrup. GC–HRMS Calcd for $C_{19}H_{26}O_4$: 318.1830. Found: 318.1818. GC–MS *m*/*z*: 318 (M^+, 8), 245 (11), 211 (10), 184 (24), 99 (84), 73 (100), 45 (35). ^{1}H NMR δ: 0.96–1.08 (1H, m), 1.41–1.71 (4H, m), 1.85–2.26 (8H, m), 2.59 (1H, dddd, *J*=13, 3, 3, 1.5 Hz, H1), 3.67–3.86 (4H, m), 3.90–4.06 (4H, m), 5.43 (1H, s), 5.50–5.55 (1H, m, H7), 5.61–5.69 (1H, m, H13), 5.99 (1H, dd, *J*=9.5, 1.5 Hz, H14). ^{13}C NMR δ: 19.6, 22.0, 24.1, 27.3, 35.4, 36.0, 41.9, 48.6, 48.7, 62.1, 64.1, 65.46, 65.52, 105.3, 109.9, 122.6, 126.4, 129.7, 136.0.

4.2.11. Claisen rearrangement of 28 under acidic conditions to form 31. A solution of **28** (20 mg, 59.5 μmol) and pivalic acid (2 mg, 19.6 μmol) in triethyl orthoacetate (1.50 ml, 8.19 mmol) was heated with stirring at 160 °C (bath temperature) in a sealed tube for 15 h. After the mixture had been cooled, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–CH_2Cl_2 (1:1)] gave **31** (5 mg, 21%) as a colorless syrup. DI-HRMS Calcd for $C_{23}H_{34}O_6$: 406.2353. Found: 406.2342. DI-MS *m*/*z*: 406 (M^+, 7), 361 (12), 344 (6), 333 (16), 319 (15), 245 (21), 99 (79), 73 (100), 45 (37). IR ($CHCl_3$) cm^{-1}: 1720. ^{1}H NMR δ: 1.03 (1H, ddd, *J*=13, 13, 4.5 Hz, H1), 1.24 (3H, dd, *J*=7, 7 Hz, OCH_2CH_3), 1.42–1.94 (13H, m), 2.12 (1H, ddd, *J*=13.5, 6.5, 6.5 Hz, H7), 2.29 (1H, d, *J*=13.5 Hz, CH_2COO), 2.51 (1H, d, *J*=13.5 Hz, CH_2COO), 2.52 (1H, br d, *J*=13 Hz, H1), 3.63–4.04 (8H, m), 4.02–4.14 (2H, m, OCH_2CH_3), 5.34 (1H, s), 5.40 (1H, br d, *J*=10 Hz, H14), 5.69 (1H, ddd, *J*=10, 5, 2.5 Hz, H13). ^{13}C NMR δ: 14.3 (CH_3), 16.8 (CH_2), 20.0 (CH_2), 20.6 (CH_2), 23.6 (CH_2), 34.1 (CH_2), 35.9 (CH_2), 37.6 (C), 38.4 (CH_2), 43.8 (C), 49.5 (CH), 49.7 (CH_2, *C*H_2COO), 50.4 (CH), 59.8 (CH_2, O*C*H_2CH$_3$), 62.3 (CH_2), 63.9 (CH_2), 64.1 (CH_2), 65.7 (CH_2), 104.9 (CH), 110.4 (C), 126.4 (CH, C13), 136.1 (CH, C14), 171.8 (C).

4.2.12. Reduction of acetamide 29 to form 32. *n*-BuLi (1.5 M in hexane, 5.57 ml, 8.36 mmol) was added to a cooled (0 °C) slurry of $BH_3 \cdot NH_3$ (90%, 307 mg, 8.97 mmol) in THF (12 ml) in a round bottom flask (300 ml). The mixture was stirred under an Ar atmosphere at that temperature for 5 min and at 20 °C for 10 min, and then was allowed to cool again to 0 °C. A THF (8 ml) solution of **29** (480 mg, 1.19 mmol) was added dropwise to this and the mixture was stirred for 10 min. The cooling bath was removed and the mixture was further stirred at 22 °C for 19 h. After the mixture had been cooled in an ice bath again, saturated NH_4Cl–H_2O (25 ml) and NH_4Cl powder (ca. 2 g) were gradually added to this with efficient stirring. SiO_2 (15 g) and CH_2Cl_2 (30 ml) were further added and the whole was vigorously stirred at 20 °C for 3 h. The whole was filtered under reduced pressure and filtered SiO_2 was washed thoroughly with CH_2Cl_2. Usual work-up followed by SiO_2 column chromatography [30 g, 1% MeOH–CH_2Cl_2] yielded **32** (404 mg, 94%) as a colorless viscous syrup. DI-HRMS Calcd for $C_{21}H_{32}O_5$: 364.2248. Found: 364.2247. DI-MS *m*/*z*: 364 (M^+, 6), 319 (27), 291 (17), 185 (6), 112 (21), 99 (99), 73 (100), 55 (21), 45 (46). ^{1}H NMR δ: 1.00 (1H, ddd, *J*=13, 13, 4.5 Hz, H1), 1.31 (1H, ddd, *J*=13.5, 13, 6.5 Hz, H7), 1.46 (1H, ddd, *J*=13, 13, 5 Hz, H3), 1.48–1.81 (9H, m, including OH), 1.81–2.01 (5H, m), 2.09 (1H, ddd, *J*=13.5, 7, 7 Hz, H7), 2.53 (1H, dddd, *J*=13, 3, 3, 1.5 Hz, H1), 3.66–3.83 (4H, m), 3.87–3.97 (3H, m), 3.99–4.07 (1H, m), 5.33 (1H, s), 5.37 (1H, dd, *J*=10, 1.5 Hz, H14), 5.70 (1H, ddd, *J*=10, 5, 2.5 Hz, H13). ^{13}C NMR δ: 16.7 (CH_2), 20.0 (CH_2), 21.0 (CH_2), 26.4 (CH_2), 34.2 (CH_2), 36.0 (CH_2), 36.9 (C, C8), 38.7 (CH_2), 43.7 (C), 49.0 (CH_2, *C*H_2CH$_2$OH), 49.3 (CH), 51.8 (CH), 59.9 (CH_2, CH_2*C*H_2OH), 62.3 (CH_2), 63.9 (CH_2), 64.2 (CH_2), 65.7 (CH_2), 104.9 (CH), 110.4 (C), 126.4 (CH), 137.2 (CH).

4.2.13. Pivaloylation of 32 to form 5. Piv_2O (1.22 ml, 6.02 mmol) was slowly added to a cooled (0 °C) solution of Et_3N (3.30 ml, 23.7 mmol) in CH_2Cl_2 (3 ml) under an Ar atmosphere and the mixture was stirred at that temperature for 15 min. The resulting solution was slowly added dropwise to a cooled (−18 °C) solution of **32** (560 mg, 1.54 mmol) and 4-DMAP (28 mg, 0.230 mmol) in CH_2Cl_2 (2 ml). The whole was stirred at −18 to 23 °C for five days. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and SiO_2 column chromatography [40 g, hexane–EtOAc (8:1)] afforded **5** (674 mg, 98%) as a colorless needles, mp 93–94 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 69.40; H, 9.02. DI-HRMS Calcd for $C_{26}H_{40}O_6$: 448.2823. Found: 448.2832. DI-MS *m*/*z*: 448 (M^+, 3), 403 (3), 375

(8), 319 (5), 99 (61), 73 (100), 57 (35), 45 (24), 41 (17). IR ($CHCl_3$) cm^{-1}: 1719. ^1H NMR δ: 1.01 (1H, ddd, J=13.5, 13, 4.5 Hz, H1), 1.18 (9H, s), 1.34 (1H, ddd, J=13.5, 13.5, 6.5 Hz, H7), 1.47 (1H, ddd, J=13, 13, 5 Hz, H3), 1.48–1.80 (8H, m), 1.81–2.00 (1H, m), 2.10 (1H, ddd, J=13.5, 7, 7 Hz, H7), 2.53 (1H, br d, J=13 Hz, H1), 3.66–3.84 (4H, m), 3.87–3.97 (3H, m), 4.00–4.12 (3H, m), 5.31 (1H, br dd, J=9.5, 1.5 Hz, H14), 5.34 (1H, s), 5.70 (1H, ddd, J=9.5, 5, 2.5 Hz, H13). ^{13}C NMR δ: 16.7 (CH_2), 20.0 (CH_2), 21.0 (CH_2), 23.9 (CH_2), 27.2 ($CH_3\times3$), 34.1 (CH_2), 36.0 (CH_2), 36.8 (C, C8), 38.6 (C, CO*C*Me$_3$), 38.7 (CH_2), 43.8 (C), 44.1 (CH_2, *C*H_2CH$_2$OPiv), 49.4 (CH), 51.5 (CH), 62.0 (CH_2, *C*H_2OPiv), 62.3 (CH_2), 63.9 (CH_2), 64.2 (CH_2), 65.7 (CH_2), 104.9 (CH), 110.4 (C), 126.4 (CH, C13), 136.4 (CH, C14), 178.4 (C).

4.3. Acetal–ene reactions of 29, 31, and 5 (Scheme 5)

4.3.1. BF_3 treatment of 29 and 31 to form 33 and 34 (35) (Table 1). The procedure for Table 1, run b was described as a representative example. $BF_3\cdot OEt_2$ (105 μl, 0.828 mmol) was added to a cooled (−18 °C) solution of **29** (56 mg, 0.138 mmol) in toluene (5 ml) under an Ar atmosphere with stirring. After stirring for 2 h at −18 °C, saturated $NaHCO_3$–H_2O (8 ml) and EtOAc (10 ml) were added and resulting mixture was vigorously stirred for 2 h at an ambient temperature. Extraction with EtOAc followed by usual work-up gave a residue (66 mg). The residue was dissolved in benzene (6 ml) and to this were added ethylene glycol (0.40 ml, 7.18 mmol) and *p*-TsOH·H_2O (4 mg, 21.1 μmol). The mixture was stirred under reflux using a Dean–Stark apparatus for 2 h. After the mixture had been cooled, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC (1.8% MeOH–CH_2Cl_2) afforded **33** (23 mg, 41%) and **34** (25 mg, 48%) in order of decreasing polarity. **33**: Colorless glass. DI-HRMS Calcd for $C_{23}H_{35}NO_5$: 405.2513. Found: 405.2522. DI-MS *m/z*: 405 (M^+, 19), 360 (20), 343 (12), 318 (9), 273 (9), 257 (14), 99 (100), 87 (58), 72 (33), 55 (19), 45 (34). IR ($CHCl_3$) cm^{-1}: 1627. ^1H NMR δ: 1.06 (1H, ddd, J=13, 12, 5 Hz, H1), 1.19–1.31 (1H, m), 1.46 (1H, br d, J=5 Hz, H9), 1.50–1.80 (7H, m), 1.85–1.98 (1H, m), 2.07 (1H, br d, J=13 Hz, H1), 2.18 (1H, d, J=15.5 Hz, C*H*$_2$CON), 2.20 (1H, dddd, J=19, 5, 2.5, 2.5 Hz, H11), 2.38 (1H, br d, J=19 Hz, H11), 2.48 (1H, d, J=15.5 Hz, C*H*$_2$CON), 2.89 (1H, br s, OH), 2.91 (1H, ddd, J=7, 6.5, 1.5 Hz, H14), 2.93 (3H, s), 3.00 (3H, s), 3.38 (1H, ddd, J=11, 6, 3.5 Hz), 3.57 (1H, ddd, J=11, 6, 3 Hz), 3.62–3.76 [2H, m, changed with D_2O to 3.66 (1H, ddd, J=12, 6, 3 Hz) and 3.72 (1H, ddd, J=12, 6, 3.5 Hz)], 3.74–3.86 (1H, m), 3.86–4.00 (3H, m), 4.44 (1H, d, J=6.5 Hz, H20), 5.59 (1H, ddd, J=9.5, 3, 2.5 Hz, H12), 5.70 (1H, dddd, J=9.5, 7, 2, 1.5 Hz, H13). ^{13}C NMR δ: 18.3 (CH_2, C6), 21.3 (CH_2, C2), 27.1 (CH_2, C11), 28.8 (CH_2, C1), 33.8 (CH_2, C7), 34.8 (CH_2, C3), 35.3 (CH_3, N*C*H$_3$), 37.4 (CH_2, *C*H_2CON), 37.8 (CH_3, N*C*H$_3$), 43.6 (C, C8), 48.1 (CH, C14), 48.2 (C, C10), 50.5 (CH, C5), 54.5 (CH, C9), 62.2 (CH_2, *C*H_2OH), 63.9 (CH_2), 65.4 (CH_2), 69.6 (CH_2, *C*H_2CH$_2$OH), 80.3 (CH, C20), 110.5 (C, C4), 125.7 (CH, C12), 128.7 (CH, C13), 172.5 (C, *C*ON). **34**: Colorless prisms, mp 206–207 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{21}H_{30}O_6$: C, 66.64; H, 7.99. Found: C, 66.36; H, 8.02. DI-HRMS Calcd for $C_{21}H_{30}O_6$: 378.2041. Found: 378.2043. DI-MS *m/z*: 378 (M^+, 3), 333 (25), 318 (6), 113 (16), 112 (14), 99 (100), 86 (10), 55 (10), 45 (17). IR ($CHCl_3$) cm^{-1}: 1767. ^1H NMR δ: 0.94–0.97 (1H, m), 1.25–1.92 (13H, m), 2.04 (1H, ddddd, J=14, 14, 14, 4, 4 Hz, H2), 2.28 (1H, d, J=19 Hz, C*H*$_2$COO), 2.29–2.37 (2H, m), 2.69 (1H, d, J=19 Hz, C*H*$_2$COO), 3.33–3.41 (1H, m), 3.35 (1H, br s, OH), 3.61–3.79 (2H, m, sharpened with D_2O), 3.71–3.77 (1H, m), 3.81–3.89 (1H, m), 3.85 (1H, d, J=3 Hz, H corresponding to H20), 3.90–4.03 (3H, m), 4.26 (1H, d, J=4 Hz, C*H*OCO). ^{13}C NMR δ: 9.8 (CH_2, C corresponding to C12), 15.3 (CH_2, C corresponding to C11), 17.1 (CH_2, C6), 22.1 (CH_2, C2), 31.9 (CH_2, C1), 33.1 (CH, C corresponding to C13), 34.6 (CH_2, C3), 37.9 (CH_2, C7), 38.9 (C, C8), 40.6 (CH_2, *C*H_2COO), 40.9 (C, C10), 47.4 (CH, C9), 50.5 (CH, C5), 61.9 (CH_2, *C*H_2OH), 64.2 (CH_2), 65.4 (CH_2), 70.6 (CH_2, *C*H_2CH$_2$OH), 75.5 (CH, C corresponding to C20), 84.1 (CH, C corresponding to C14), 110.1 (C, C4), 176.3 (C, *C*OO). Another by-product **35** with polarity between **33** and **34** was obtained as a colorless glass employing cyclohexene–toluene (6:1) as the reaction solvent (run a). DI-HRMS Calcd for $C_{21}H_{30}O_6$: 378.2041. Found: 378.2027. DI-MS *m/z*: 378 (M^+, 5), 333 (42), 317 (4), 289 (2), 99 (100), 55 (13), 45 (20). IR ($CHCl_3$) cm^{-1}: 1719. ^1H NMR δ: 0.82–0.91 (1H, m), 1.07–2.07 (12H, m), 2.23 (1H, d, J=19.5 Hz, C*H*$_2$COO), 2.24–2.38 (2H, m), 2.47–2.58 (3H, m), 2.70 (1H, d, J=19.5 Hz, C*H*$_2$COO), 3.58–3.65 (1H, m), 3.68–3.85 (4H, m), 3.89–4.01 (3H, m), 4.58 (1H, br dd, J=4.5, 4 Hz, H13), 4.68 (1H, d, J=6.5 Hz, H20).

4.3.2. HCl treatment of 29 to form 34 and 36 (Table 1, run h). HCl–H_2O (2.5% 0.5 ml) was added to a cooled (0 °C) solution of **29** (30 mg, 74.1 μmol) in THF (2 ml) and the mixture was stirred for 1 h. Saturated $NaHCO_3$–H_2O (5 ml) and EtOAc (5 ml) were added and resulting mixture was vigorously stirred for 2 h at an ambient temperature. Extraction with EtOAc followed by usual work-up and PTLC (0.8% MeOH–CH_2Cl_2) afforded **34** (18 mg, 64%) and **36** (7 mg, 26%) in order of increasing polarity. **36**: Colorless glass. DI-HRMS Calcd for $C_{21}H_{31}NO_4$: 361.2251. Found: 361.2264. DI-MS *m/z*: 361 (M^+, 1), 333 (2), 288 (7), 274 (11), 261 (7), 246 (19), 218 (8), 201 (18), 91 (21), 87 (85), 73 (100), 45 (38). IR ($CHCl_3$) cm^{-1}: 1704, 1631. ^1H NMR δ: 1.19–1.39 (2H, m), 1.72 (1H, ddd, J=13, 13, 4 Hz), 1.80–2.11 (8H, m), 2.14–2.24 (2H, m), 2.24 (1H, d, J=15 Hz), 2.29–2.45 (2H, m), 2.53 (1H, d, J=15 Hz), 2.65 (1H, br ddd, J=13, 3.5, 3.5 Hz, H1), 2.92 (3H, s), 2.99 (3H, s), 3.59–3.77 (3H, m), 3.96–4.06 (1H, m), 5.29 (1H, s), 5.71 (1H, ddd, J=10, 3.5, 2.5 Hz), 5.59 (1H, br d, J=10 Hz). ^{13}C NMR δ: 17.4 (CH_2), 17.5 (CH_2), 21.3 (CH_2), 22.4 (CH_2), 33.3 (CH_2), 35.4 (CH_3), 37.3 (C), 37.9 (CH_2), 38.1 (CH_3), 39.2 (CH_2, C3), 45.3 (CH_2), 45.9 (CH), 47.9 (C), 57.8 (CH, C5), 62.7 (CH_2), 65.2 (CH_2), 105.0 (CH), 129.5 (CH), 136.1 (CH), 170.8 (C), 209.8 (C, C4).

4.3.3. Acetal–ene reaction of 5 to form 6 (37, 38) (Table 2). The procedure for Table 2, run d was described as a representative example. $BF_3\cdot OEt_2$ (392 μl, 3.09 mmol) was added to a cooled (−18 °C) solution of **5** (231 mg, 0.516 mmol) in toluene (10 ml) under an Ar atmosphere with stirring. After stirring for 1 h at −18 °C, saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. After usual work-up, obtained residue (260 mg) was dissolved in acetone

(10 ml) and to this was added *p*-TsOH·H_2O (21 mg, 0.111 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min and at 19 °C for 3.5 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (2:1)] yielded crude **38** (12 mg), **6** (138 mg, 66%), and crude **37** (18 mg) in order of increasing polarity. The crude **37** was further purified by PTLC (0.3% MeOH–CH_2Cl_2) to give **37** (6 mg, 3%). The crude-**38** was also separated by PTLC [hexane-1,2-dimethoxyethane (DME) (19:1)] to provide **38** (6 mg, 3%). **6**: Colorless glass. DI-HRMS Calcd for $C_{24}H_{36}O_5$: 404.2561. Found: 404.2569. DI-MS *m*/*z*: 404 (M^+, 3), 369 (5), 302 (5), 275 (8), 240 (31), 91 (39), 73 (36), 57 (100), 45 (41), 41 (47). IR ($CHCl_3$) cm^{-1}: 1717, 1705. ^1H NMR δ: 1.18 (9H, s), 1.45–1.98 (8H, m, including OH), 1.68 (1H, ddd, J=13.5, 8.5, 6 Hz, CH_2CH$_2$OPiv), 1.98–2.09 (1H, m), 2.04 (1H, ddd, J=13, 5, 8.5, 6.5 Hz, CH_2CH$_2$OPiv), 2.21–2.36 (5H, m), 2.42 (1H, br d, J=19 Hz, H11), 2.56 (1H, ddd, J=6.5, 6.5, 1.5 Hz, H14), 3.25 (1H, ddd, J=9, 5, 4.5, 3 Hz, CH_2CH$_2$OH), 3.35 (1H, ddd, J=9.5, 7.5, 3 Hz, CH_2CH$_2$OH), 3.54–3.72 [2H, m, changed with D_2O to 3.58 (1H, ddd, J=11.5, 4.5, 3 Hz) and 3.66 (1H, ddd, J=11.5, 7.5, 3 Hz)], 3.62 (1H, d, J=6.5 Hz, H20), 4.00 (1H, ddd, J=11, 8.5, 6.5 Hz, CH_2OPiv), 4.14 (1H, ddd, J=11, 8.5, 6 Hz, CH_2OPiv), 5.60 (1H, ddd, J=9.5, 3, 3 Hz, H12), 5.69 (1H, dddd, J=9.5, 6.5, 2, 1.5 Hz, H13). ^{13}C NMR δ: 19.1 (CH_2, C6), 25.6 (CH_2, C2), 27.17 ($CH_3\times3$), 27.23 (CH_2, C11), 28.6 (CH_2, C1), 33.6 (CH_2, *C*H$_2$CH$_2$OPiv), 33.8 (CH_2, C7), 38.6 (C, *C*Me$_3$), 41.5 (CH_2, C3), 42.6 (C, C8), 47.7 (CH, C14), 53.8 (CH, C9), 54.0 (C, C10), 56.5 (CH, C6), 62.0 (CH_2, *C*H$_2$OH), 62.5 (CH_2, *C*H$_2$OPiv), 69.8 (CH_2, *C*H$_2$CH$_2$OH), 81.2 (CH, C20), 126.2 (CH, C12), 126.9 (CH, C13), 178.4 (C), 212.1 (C). **37**: Colorless glass. DI-HRMS Calcd for $C_{24}H_{36}O_5$: 404.2561. Found: 404.2580. DI-MS *m*/*z*: 404 (M^+, 3), 342 (16), 302 (8), 275 (14), 240 (28), 213 (39), 91 (37), 73 (32), 57 (100), 45 (49), 41 (48). IR ($CHCl_3$) cm^{-1}: 1708. ^1H NMR δ: 1.18 (9H, s), 1.40–1.73 (6H, m), 1.41 (1H, br d, J=4.5 Hz, H9), 1.62 (1H, br s, OH), 1.98–2.42 (8H, m), 2.53 (1H, br d, J=6 Hz, H5), 2.55 (1H, ddd, J=7, 6, 1.5 Hz, H14), 3.41 (1H, ddd, J=9.5, 5, 3.5 Hz), 3.46 (1H, ddd, J=9.5, 6.5, 3.5 Hz), 3.62–3.79 [2H, m, changed with D_2O to 3.66 (1H, ddd, J=11.5, 5, 3.5 Hz) and 3.73 (1H, ddd, J=11.5, 6.5, 3.5 Hz)], 3.96 (1H, d, J=6 Hz), 4.00 (1H, ddd, J=10.5, 8, 6.5 Hz), 4.09 (1H, ddd, J=10.5, 8.5, 6 Hz), 5.60 (1H, ddd, J=9.5, 3, 3 Hz), 5.69 (1H, dddd, J=9.5, 7, 2, 1.5 Hz). ^{13}C NMR δ: 17.8 (CH_2), 23.7 (CH_2), 25.9 (CH_2), 27.2 ($CH_3\times3$), 28.4 (CH_2), 32.3 (CH_2), 33.5 (CH_2), 38.6 (C), 41.3 (CH_2), 43.3 ($\times2$, CH and C, C8 and C9), 46.4 (CH), 53.1 (C), 57.3 (CH, C5), 61.9 (CH_2), 62.4 (CH_2), 70.3 (CH_2), 87.6 (CH), 126.2 (CH), 126.7 (CH), 178.4 (C), 210.7 (C). **38**: Colorless glass. DI-HRMS Calcd for $C_{22}H_{30}O_3$: 342.2193. Found: 342.2201. DI-MS *m*/*z*: 342 (M^+, 2), 240 (56), 212 (20), 129 (25), 91 (31), 57 (100), 41 (64). IR ($CHCl_3$) cm^{-1}: 1709. ^1H NMR δ: 1.06–1.15 (2H, m, H14 and H20), 1.18 (9H, s), 1.32–1.42 (1H, m), 1.37–1.52 (2H, m), 1.52–1.73 (5H, m), 1.80–2.06 (3H, m), 1.99 (1H, dd, J=7, 1.5 Hz), 2.20–2.39 (3H, m), 3.94–4.09 (2H, m), 5.67 (1H, ddd, J=8, 7, 2 Hz, H11), 6.11 (1H, ddd, J=8, 5.5, 1.5 Hz, H12). ^{13}C NMR δ: 16.4 (CH, C13), 18.8 (CH, C20), 19.8 (CH_2, C6), 23.7 (CH_2, C2), 24.0 (CH, C14), 27.2 ($CH_3\times3$), 29.3 (CH_2), 31.9 (CH_2), 32.8 (CH_2), 38.6 (C), 39.2 (C), 41.8 (CH_2), 48.7 (C), 53.2 (CH), 54.0 (CH), 62.2 (CH_2), 124.3 (CH, C11), 125.7 (CH, C12), 178.3 (C), 212.4 (C).

4.3.4. Acetal–ene reaction, reacetalization of 5 to form 39, 40, and 41. The crude ene reaction product (592 mg) prepared as above from **5** (582 mg, 1.30 mmol) and $BF_3\cdot OEt_2$ (0.80 ml, 6.50 mmol) was dissolved in benzene (40 ml). Ethylene glycol (3.60 ml, 64.6 mmol) and *p*-TsOH·H_2O (30 mg, 0.16 mmol) were added to the solution and the mixture was heated under reflux for 1.5 h with Dean–Stark apparatus. The same work-up as before followed by separation by PTLC [benzene–EtOAc (8:1)] furnished crude-**40** (42 mg), **39** (408 mg, 70%), and crude **41** (35 mg) in order of increasing polarity. The crude-**40** was purified by PTLC [hexane–CH_2Cl_2 (1:1)] to yield **40** (25 mg, 3%). Crude **41** was also separated by PTLC [benzene–EtOAc (3:1)] to afford **41** (18 mg, 3%). **39**: Colorless glass. DI-HRMS Calcd for $C_{26}H_{40}O_6$: 448.2823. Found: 448.2831. DI-MS *m*/*z*: 448 (M^+, 5), 403 (11), 386 (3), 363 (2), 319 (3), 285 (3), 284 (3), 257 (4), 112 (16), 99 (100), 57 (50), 45 (23), 41 (20). IR ($CHCl_3$) cm^{-1}: 1711. ^1H NMR δ: 1.00–1.11 (1H, m), 1.17–1.28 (2H, m), 1.18 (9H, s), 1.51–1.89 (9H, m), 2.06 (1H, ddd, J=13.5, 8.5, 6.5 Hz), 2.07 (1H, br d, J=13.5 Hz, H1), 2.18 (1H, dddd, J=19, 5, 2.5, 2.5 Hz, H11), 2.36 (1H, br d, J=19 Hz, H11), 2.50 (1H, ddd, J=6.5, 6, 1 Hz, H14), 2.85 (1H, t, J=6.5 Hz, OH), 3.33–3.41 (1H, m), 3.51–3.59 (1H, m), 3.66–3.73 (2H, m), 3.77–3.87 (1H, m), 3.87–4.00 (3H, m), 4.00 (1H, ddd, J=10.5, 8.5, 6.5 Hz), 4.13 (1H, ddd, J=10.5, 8.5, 6 Hz), 4.42 (1H, d, J=6.5 Hz), 5.59 (1H, br ddd, J=9.5, 2.5, 2.5 Hz, H12), 5.66 (1H, br dd, J=9.5, 6.5 Hz, H13). ^{13}C NMR δ: 18.3 (CH_2), 21.2 (CH_2), 26.9 (CH_2), 27.2 ($CH_3\times3$), 28.8 (CH_2), 33.5 (CH), 34.1 (CH_2), 34.7 (CH_2, C3), 38.5 (C), 42.3 (C), 47.6 (CH), 48.4 (C), 50.6 (CH, C5), 54.8 (CH), 62.3 (CH_2), 62.6 (CH_2), 63.9 (CH_2), 65.4 (CH_2), 69.7 (CH_2), 80.6 (CH), 110.4 (C, C5), 125.9 (CH), 127.4 (CH), 178.4 (C). **40**: Colorless glass. DI-HRMS Calcd for $C_{24}H_{34}O_4$: 386.2455. Found: 386.2460. DI-MS *m*/*z*: 386 (M^+, 4), 284 (27), 257 (5), 195 (6), 99 (100), 57 (38), 41 (17). IR ($CHCl_3$) cm^{-1}: 1712. ^1H NMR δ: 0.82–0.96 (1H, m), 1.07 (1H, ddd, J=7, 5, 1 Hz, H14), 1.17 (9H, s), 1.19–1.82 (15H, m), 3.80–4.05 (6H, m), 5.57 (1H, ddd, J=8, 6.5, 2 Hz, H11), 6.08 (1H, ddd, J=8, 5.5, 1.5 Hz, H12). ^{13}C NMR δ: 16.1 (CH, C13), 18.8 (CH_2), 19.1 (CH, C20), 20.4 (CH_2), 24.2 (CH, C14), 27.2 ($CH_3\times3$), 29.8 (CH_2), 32.6 (CH_2), 32.9 (CH), 35.5 (CH_2), 38.5 (C), 39.2 (C), 43.8 (C), 48.7 (CH), 54.0 (CH), 62.4 (CH_2), 64.4 (CH_2), 65.4 (CH_2), 110.5 (C, C4), 123.8 (CH), 125.8 (CH), 178.4 (C). **41**: Colorless glass. DI-HRMS Calcd for $C_{26}H_{42}O_7$: 466.2928. Found: 466.2924. DI-MS *m*/*z*: 466 (M^+, 2), 421 (18), 405 (2), 364 (11), 319 (7), 302 (6), 112 (30), 99 (100), 73 (16), 57 (84), 45 (15), 41 (21). IR ($CHCl_3$) cm^{-1}: 1713. ^1H NMR δ: 0.76 (1H, dd, J=2.5, 2.5 Hz, H9), 0.80 (1H, ddd, J=13, 13, 4.5 Hz, H1), 1.21–1.41 (4H, m), 1.23 (9H, s), 1.41–1.89 (11H, m, including secondary OH), 1.90–2.03 (2H, m), 2.33 (1H, br ddd, J=13, 3, 3 Hz, H1), 3.24 (1H, br dd, J=5.5, 5.5 Hz, primary OH), 3.35–3.44 (1H, m), 3.56–3.65 (2H, m), ca. 3.63–3.74 (3H, m), 3.79–3.99 (5H, m), 4.70 (1H, d, J=2.5 Hz, C*H*OH). ^{13}C NMR δ: 10.5 (CH_2, C corresponding to C12), 14.8 (CH_2, C corresponding to C11), 16.6 (CH_2), 22.2 (CH_2), 27.2 ($CH_3\times3$), 32.2 (CH_2), 33.8 (CH, C corresponding to C13), 34.9 (CH_2), 35.0 (CH_2), 36.9 (CH_2), 37.1 (C), 39.1 (C), 40.6 (C), 46.7 (CH), 50.8 (CH), 59.9 (CH_2), 61.9

(CH_2), 64.3 (CH_2), 65.4 (CH_2), 70.2 (CH_2), 74.6 (CH, *C*HOH), 75.8 (CH), 110.4 (C), 177.8 (C).

4.4. Model deprotection of 2-hydroxyethyl group and allylic oxidation of 33 (Scheme 7)

4.4.1. Bromination of 33 to form 42. Ph_3P (33 mg, 0.143 mmol) and CBr_4 (33 mg, 99.4 μmol) were added to a cooled (0 °C) solution of **33** (20 mg, 49.4 μmol) in CH_2Cl_2 (4 ml) and the mixture was stirred at 20 °C for 1 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [CH_2Cl_2–EtOAc (19:1)] gave **42** (20 mg, 87%) as a colorless glass. DI-HRMS Calcd for $C_{23}H_{34}BrNO_4$: 469.1650, 467.1670. Found: 469.1654, 467.1671. DI-MS *m*/*z*: 469, 467 (M^+, 10, 10), 424, 422 (3, 2), 388 (16), 360 (23), 344 (8), 273 (11), 257 (11), 99 (100), 87 (67), 72 (27), 45 (28). IR ($CHCl_3$) cm^{-1}: 1632. ^1H NMR δ: 1.02 (1H, ddd, J=13, 13, 4 Hz, H1), 1.22 (1H, ddd, J=13.5, 12.5, 4 Hz, H3), 1.42 (1H, br d, J=5.5 Hz, H9), 1.50–1.79 (7H, m), 1.86–1.96 (1H, m), 2.04 (1H, br dddd, J=13, 3, 3, 1.5 Hz, H1), 2.17 (1H, dddd, J=18, 5.5, 2.5, 2 Hz, H11), 2.36 (1H, br d, J=18.5 Hz, H11), 2.45 (1H, d, J=15.5 Hz), 2.69 (1H, d, J=15.5 Hz), 2.90 (1H, ddd, J=6.5, 6.5, 1.5 Hz), 2.92 (3H, s), 3.00 (3H, s), 3.38–3.54 (2H, m, C*H*$_2$Br), 3.55–3.64 (1H, m), 3.67–3.81 (2H, m), 3.85–3.98 (3H, m), 4.33 (1H, d, J=6.5 Hz), 5.55 (1H, ddd, J=9.5, 3, 2.5 Hz), 5.68 (1H, dddd, J=9.5, 6.5, 2, 2 Hz). ^{13}C NMR δ: 18.2 (CH_2), 21.0 (CH_2), 27.0 (CH_2), 28.7 (CH_2), 30.4 (CH_2, *C*H_2Br), 33.9 (CH_2), 34.9 (CH_2), 35.3 (CH_3), 37.5 (CH_2), 37.8 (CH_3), 43.7 (C), 48.3 (CH), 48.4 (C), 50.6 (CH), 54.6 (CH), 63.9 (CH_2), 65.4 (CH_2), 69.6 (CH_2, *C*H_2CH$_2$Br), 81.3 (CH), 110.3 (C), 125.3 (CH), 129.0 (CH), 172.5 (C).

4.4.2. Reductive deprotection of 42 to form 43. Zn dust (252 mg, 3.85 mg atom) and NH_4Cl (10 mg, 0.187 mmol) were added to a solution of **42** (18 mg, 38.5 μmol) in 2-propanol–H_2O (14:1, 5 ml) and the mixture was refluxed with stirring for 3 h. Saturated NH_4Cl–H_2O was added and the whole was filtered under reduced pressure. Extraction with CH_2Cl_2 followed by PTLC [hexane-DME (2:1)] afforded **43** (13 mg, 94%) as a colorless glass. DI-HRMS Calcd for $C_{21}H_{34}NO_4$: 361.2251. Found: 361.2240. DI-MS *m*/*z*: 361 (M^+, 56), 332 (24), 316 (41), 274 (15), 245 (22), 183 (23), 99 (87), 87 (100), 72 (46), 55 (29), 45 (50). IR ($CHCl_3$) cm^{-1}: 1626. ^1H NMR δ: 1.09 (1H, ddd, J=13, 13, 4 Hz), 1.23 (1H, ddd, J=13, 12.5, 4 Hz), 1.51 (1H, br d, J=5 Hz), 1.54–1.94 (9H, m), 2.12 (1H, br d, J=11.5 Hz, OH), 2.28 (1H, dd, J=19.5, 5 Hz), 2.38 (1H, br d, J=19.5 Hz), 2.49 (1H, d, J=15.5 Hz), 2.59 (1H, d, J=15.5 Hz), 2.85–2.92 (1H, m), 2.93 (3H, s), 3.01 (3H, s), 3.73–3.81 (1H, m), 3.84–4.00 (3H, m), 4.70 (1H, br dd, J=11.5, 7 Hz, changed to d, J=7 Hz with D_2O, H20), 5.72–5.83 (2H, m). ^{13}C NMR δ: 18.0 (CH_2), 21.1 (CH_2), 27.5 (CH_2), 29.1 (CH_2), 33.9 (CH_2), 34.9 (CH_2), 35.3 (CH_3), 37.6 (CH_2), 37.9 (CH_3), 43.7 (C), 48.4 (C, C10), 50.0 (CH), 50.5 (CH, C14), 53.8 (CH), 63.9 (CH_2), 65.5 (CH_2), 72.6 (CH, C20), 110.1 (C), 128.6 (CH), 129.6 (CH), 172.4 (C).

4.4.3. Dess–Martin oxidation of 43 to form 44. A solution of **43** (12 mg, 33.2 μmol) and Dess–Martin periodinane (70 mg, 0.165 mmol) in CH_2Cl_2 (4 ml) was refluxed with stirring for 8 h. Saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–DME (3:2)] provided **44** (11 mg, 92%) as colorless prisms, mp 195–196 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{21}H_{29}NO_4$: C, 70.19; H, 8.13; N, 3.90. Found: C, 70.03; H, 8.23; N, 3.94. DI-HRMS Calcd for $C_{21}H_{29}NO_4$: 359.2095. Found: 359.2084. DI-MS *m*/*z*: 359 (M^+, 3), 331 (27), 244 (21), 129 (27), 99 (100), 91 (24), 87 (67), 72 (34), 55 (25), 45 (37). IR ($CHCl_3$) cm^{-1}: 1727, 1635. ^1H NMR δ: 1.25 (1H, ddd, J=13, 13, 4 Hz), 1.34 (1H, ddd, J=13.5, 13.5, 4.5 Hz), 1.51–1.66 (2H, m), 1.69–1.92 (6H, m), 2.09 (1H, br dd, J=5.5, 3 Hz), 2.22–2.40 (3H, m), 2.47 (1H, d, J=16 Hz), 2.52 (1H, d, J=16 Hz), 2.94 (1H, dd, J=7.5, 2.5 Hz, H14), 2.94 (3H, s), 3.02 (3H, s), 3.73–3.81 (1H, m), 3.83–4.02 (3H, m), 5.70 (1H, ddd, J=9.5, 3, 2.5 Hz), 5.80 (1H, dddd, J=9.5, 7.5, 2, 1.5 Hz). ^{13}C NMR δ: 19.5 (CH_2), 20.0 (CH_2), 26.1 (CH_2), 29.8 (CH_2), 34.0 (CH_2), 34.8 (CH_2), 35.4 (×2, CH_3 and CH_2), 37.8 (CH_3), 41.1 (C), 51.1 (CH), 52.7 (CH), 54.9 (C, C10), 56.7 (CH, C14), 64.0 (CH_2), 66.0 (CH_2), 109.2 (C), 126.8 (CH), 128.1 (CH), 171.5 (C), 211.7 (C, C20).

4.4.4. Oxidation of 42 with CrO_3 and 3,5-dimethylpyrazole. To a cooled (−18 °C) slurry of CrO_3 (49 mg, 0.490 mmol) in CH_2Cl_2 was added 3,5-dimethylpyrazole (55 mg, 0.573 mmol) and the mixture was stirred for 15 min. A solution of **42** (19 mg, 40.6 μmol) in CH_2Cl_2 (2 ml) was added to this and the resulting mixture was stirred at −18 to 27 °C for 38 h. Saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC (0.5% MeOH–CH_2Cl_2) provided recovery of **42** (5.5 mg, 29%) and a mixture of **45** and **46** (15 mg) in order of increasing polarity. The latter was further purified by PTLC [hexane–2-propanol (6:1)] and yielded **45** (5.5 mg, 28%) and **46** (5.5 mg, 28%) in order of decreasing polarity. **45**: Colorless glass. DI-HRMS Calcd for $C_{23}H_{32}BrNO_5$: 483.1443, 481.1463. Found: 483.1434, 481.1461. DI-MS *m*/*z*: 483, 481 (M^+, 6, 6), 402 (16), 397, 395 (6, 6), 271 (84), 109, 107 (17, 17), 99 (100), 87 (84), 72 (41), 55 (25), 45 (32). IR ($CHCl_3$) cm^{-1}: 1661, 1639. ^1H NMR δ: 1.20–1.38 (3H, m), 1.47–1.82 (7H, m), 2.08 (1H, br dd, J=1.5, 1.5 Hz, H9), 2.19 (1H, ddd, J=13.5, 6.5, 2 Hz), 2.25 (1H, d, J=16 Hz), 2.57 (1H, d, J=16 Hz), 2.89 (3H, s), 2.91 (3H, s), 3.39 (1H, dt, J=10, 6.5 Hz), 3.45 (1H, dt, J=10, 6.5 Hz), 3.72 (2H, dd, J=6.5, 6.5 Hz), 3.76–3.85 (2H, m), 3.88–4.00 (3H, m), 4.66 (1H, d, J=6.5 Hz), 6.11 (1H, dd, J=9.5, 1.5 Hz, H12), 7.16 (1H, dd, J=9.5, 7.5 Hz, H13). ^{13}C NMR δ: 18.0 (CH_2), 20.7 (CH_2), 29.5 (CH_2), 30.2 (CH_2), 34.5 (CH_2), 34.9 (CH_2), 35.3 (CH_3), 37.6 (CH_3), 38.0 (CH_2), 47.7 (C), 49.6 (CH), 50.0 (CH), 51.8 (C), 64.0 (CH_2), 65.5 (CH_2), 70.1 (CH_2), 74.6 (CH, C9), 79.6 (CH, C20), 109.6 (C), 129.4 (CH, C12), 153.7 (CH, C13), 170.8 (C), 202.0 (C, C11). **46**: Colorless glass. DI-HRMS Calcd for $C_{23}H_{32}BrNO_5$: 483.1443, 481.1463. Found: 483.1418, 481.1461. DI-MS *m*/*z*: 483, 481 (M^+, 13, 10), 402 (11), 374 (16), 358 (11), 271 (12), 109, 107 (14, 14), 99 (100), 87 (33), 72 (32), 55 (11), 45 (17). IR ($CHCl_3$) cm^{-1}: 1677, 1638. ^1H NMR δ: 1.19–1.89 (10H, m), 2.01 (1H, br d, J=13 Hz), 2.32 (1H, d, J=15.5 Hz), 2.39 (1H, d, J=15.5 Hz), 2.66 (1H, dd, J=7, 1.5 Hz, H9), 2.89 (3H, s), 2.92 (3H, s), 3.18 (1H, ddd, J=7, 1.5, 1.5 Hz, H14), 3.32 (2H, dd, J=6.5, 6.5 Hz), 3.56 (1H, dt, J=10.5, 6.5 Hz),

3.75 (1H, dt, J=10.5, 6.5 Hz), 3.76–3.85 (1H, m), 3.88–4.01 (3H, m), 4.61 (1H, d, J=7 Hz), 6.10 (1H, dd, J=9.5, 1.5 Hz, H12), 7.08 (1H, dd, J=9.5, 7 Hz, H11). ^{13}C NMR δ: 17.9 (CH_2), 20.7 (CH_2), 29.7 (CH_2), 29.9 (CH_2), 33.9 (CH_2), 35.0 (CH_2), 35.3 (CH_3), 36.3 (CH_2), 37.7 (CH_3), 49.4 (CH), 51.5 (C), 53.1 (C), 56.7 (CH, C9), 64.0 (CH_2), 65.4 (CH_2), 66.1 (CH, C14), 70.8 (CH_2), 77.5 (CH), 109.7 (C), 131.1 (CH, C12), 151.1 (CH, C11), 170.8 (C), 200.4 (C, C13).

4.4.5. Zn reduction of 45 to form 47. In a similar manner as for the preparation of **43** from **42** (Section 4.4.2), **45** (6.5 mg, 13.5 μmol) was treated with Zn (220 mg, 3.36 mg atom) and NH_4Cl (11 mg, 0.206 mmol) for 3 h. The same work-up and PTLC (2% MeOH–CH_2Cl_2) gave **47** (4.5 mg, 89%) as a colorless glass. DI-HRMS Calcd for $C_{21}H_{31}NO_5$: 377.2200. Found: 377.2209. DI-MS m/z: 377 (M^+, 22), 332 (6), 276 (7), 180 (10), 99 (100), 87 (61), 72 (41), 55 (17), 46 (23), 45 (21). IR ($CHCl_3$) cm^{-1}: 1689, 1640. ^{1}H NMR δ: 1.06–1.21 (2H, m), 1.32 (1H, ddd, J=12.5, 12.5, 5 Hz, H3), 1.46–1.74 (6H, m), 1.66 (1H, br s, OH), 1.75–1.84 (3H, m), 2.15 (1H, d, J=16.5 Hz), 2.15–2.32 (3H, m), 2.28 (1H, d, J=16.5 Hz), 2.64 (1H, ddd, J=19, 10, 10 Hz, H12), 2.92 (3H, s), 2.94 (3H, s), 2.98–3.05 (1H, m), 3.77–3.85 (1H, m), 3.86–4.01 (3H, m), 4.95 (1H, br d, J=7 Hz, H20). ^{13}C NMR δ: 17.9 (CH_2), 20.8 (CH_2), 20.9 (CH_2, C13), 27.0 (CH_2), 34.4 (CH_2), 35.2 (CH_2), 35.3 (CH_3), 36.9 (CH_2, C12), 37.6 (CH_3), 38.5 (CH_2), 45.5 (CH, C14), 46.3 (C), 48.7 (C), 49.7 (CH), 64.0 (CH_2), 65.5 (CH_2), 70.2 (CH), 74.2 (CH, C9), 109.7 (C), 170.2 (C), 215.0 (C).

4.4.6. Zn reduction of 46 to form 48, 49, 50. In the same manner as for the procedure of Section 4.4.5, **46** (6.5 mg, 13.5 μmol) was treated with Zn (220 mg, 3.36 mg atom) and NH_4Cl (11 mg, 0.206 mmol) for 4 h. The same work-up and PTLC (2% MeOH–CH_2Cl_2) gave **48** (2.5 mg, 49%), **50** (2 mg, 39%), and **49** (0.5 mg, 9%) in order of decreasing polarity. **48**: Colorless glass. DI-HRMS Calcd for $C_{21}H_{29}NO_5$: 375.2044. Found: 375.2057. DI-MS m/z: 375 (M^+, 25), 347 (8), 330 (4), 289 (9), 271 (22), 227 (11), 99 (100), 87 (76), 72 (28), 45 (22). IR ($CHCl_3$) cm^{-1}: 1675, 1634. ^{1}H NMR δ: 1.25–1.92 (11H, m, including OH), 2.12 (1H, br d, J=13 Hz, H1), 2.34 (1H, d, J=15.5 Hz), 2.41 (1H, d, J=15.5 Hz), 2.71 (1H, br d, J=7 Hz), 2.89 (3H, s), 2.92 (3H, s), 3.11 (1H, ddd, J=7, 1.5, 1.5 Hz, H14), 3.77–3.85 (1H, m), 3.87–3.98 (3H, m), 5.05 (1H, br d, J=7 Hz, H20), 6.12 (1H, dd, J=9.5, 1.5 Hz), 7.14 (1H, dd, J=9.5, 7 Hz). ^{13}C NMR δ: 17.8 (CH_2), 20.9 (CH_2), 29.6 (CH_2), 33.9 (CH_2), 35.1 (CH_2), 35.3 (CH_3), 36.3 (CH_2), 37.7 (CH_3), 49.5 (CH), 51.6 (C), 52.1 (C), 56.9 (CH), 64.1 (CH_2), 65.4 (CH_2), 68.2 (CH, C14), 69.3 (CH, C20), 109.7 (C), 170.9 (C), 130.9 (CH), 151.8 (CH), 201.5 (C). **49**: Colorless glass. DI-HRMS Calcd for $C_{23}H_{33}NO_5$: 403.2357. Found: 403.2367. DI-MS m/z: 403 (M^+, 25), 374 (15), 358 (7), 317 (21), 99 (100), 87 (22), 72 (33). IR ($CHCl_3$) cm^{-1}: 1672, 1633. ^{1}H NMR δ: 1.04 (3H, dd, J=7, 7 Hz, OCH_2CH_3), 1.20–1.88 (10H, m), 2.00 (1H, br d, J=13 Hz), 2.32 (1H, d, J=15.5 Hz), 2.39 (1H, d, J=15.5 Hz), 2.64 (1H, br d, J=7 Hz), 2.89 (3H, s), 2.92 (3H, s), 3.16 (1H, ddd, J=7, 1.5, 1.5 Hz), 3.30 (1H, dq, J=9, 7 Hz, OCH_2CH_3), 3.49 (1H, dq, J=9, 7 Hz, OCH_2CH_3), 3.74–3.85 (1H, m), 3.87–3.99 (3H, m), 4.54 (1H, d, J=7 Hz), 6.09 (1H, dd, J=9.5, 1.5 Hz), 7.06 (1H, dd, J=9.5, 7 Hz). **50**: Colorless glass. DI-HRMS Calcd for $C_{21}H_{31}NO_5$: 377.2200. Found: 377.2197. DI-MS m/z: 377 (M^+, 19), 332 (10), 192 (7), 168 (12), 99 (100), 87 (40), 72 (32), 55 (15), 46 (22). IR ($CHCl_3$) cm^{-1}: 1701, 1640. ^{1}H NMR δ: 1.23 (1H, ddd, J=13.5, 13.5, 4.5 Hz), 1.32 (1H, ddd, J=13, 13, 4 Hz), 1.42–2.27 (14H, m, including OH), 2.08 (1H, d, J=15.5 Hz), 2.30 (1H, d, J=15.5 Hz), 2.67 (1H, ddd, J=18, 10, 10 Hz, H12), 2.83 (1H, d, J=7.5 Hz, H14), 2.92 (3H, s), 2.96 (3H, s), 3.76–3.85 (1H, m), 3.86–4.00 (3H, m), 4.94 (1H, br d, J=7.5 Hz, H20). ^{13}C NMR δ: 17.9 (CH_2), 19.5 (CH_2, C11), 20.9 (CH_2), 27.7 (CH_2), 33.3 (CH_2), 34.8 (CH_2), 35.4 (CH_3), 36.2 (CH_2, C12), 36.6 (CH_2), 37.8 (CH_3), 47.2 (C), 47.7 (C), 50.0 (CH), 52.5 (CH), 63.9 (CH_2), 65.4 (CH_2), 67.9 (CH, C14), 68.8 (CH, C20), 110.1 (C), 170.4 (C), 212.4 (C).

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Synthetic study of hetisine-type aconite alkaloids. Part 2: Preparation of hexacyclic compound lacking the C-ring of the hetisan skeleton

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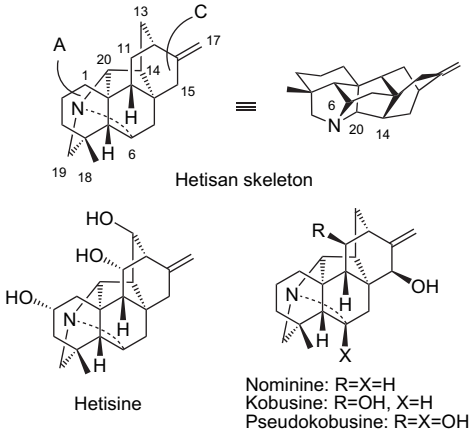
Abstract—A hexacyclic compound **1**, having almost the full hetisine-type aconite alkaloid framework lacking only the C-ring with an *exo*-methylene group, was synthesized from the intermediate **3** reported in the preceding paper. The synthesis involved the following key reactions the crucial conversion of **3** to **4**, a stereoselective hydrocyanation reaction to obtain **5** from **4**, and construction of the azabicyclic ring system (**5**→**1**).

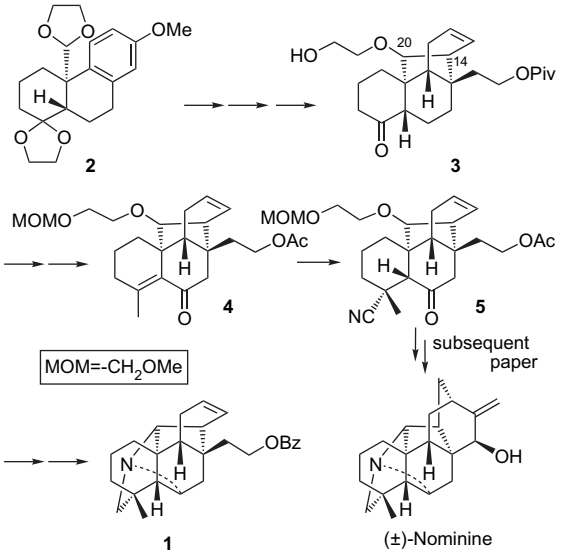
1. Introduction

Over 400 aconite alkaloids have been isolated so far from *Aconitum*, *Delphinium*, *Consolida*, *Thalictrum*, and *Spiraea*. They are generally classified into five skeletons, i.e., atidane, veatchane, cycloveatchane, aconitane, and hetisan (Scheme 1).[1,2] Extensive synthetic investigations over the last 40 years have led to total syntheses of the first four.[3] However, attempts to synthesize hetisine-type aconite alkaloids had met with no success until we recently reported the total synthesis of (±)-nominine.[4] Five synthetic investigations leading toward the total synthesis of this class of aconite alkaloids have been reported so far.[5–9]



Scheme 1. Representative hetisine-type alkaloids.

It was several years ago that we set out to synthesize hetisine-type aconite alkaloids by applying our palladium-catalyzed intramolecular α-arylation of aliphatic ketone, formyl, and nitro groups.[10] Our synthetic strategy was based on early formation of the N–C6 and C14–C20 bonds, which are characteristic of the hetisan skeleton.[3] We first reported the preparation of the hexacyclic compound **1** lacking the C-ring of the hetisan skeleton by way of the intermediates **2**–**5** (Scheme 2).[11] Further synthetic efforts culminated in a total synthesis of (±)-nominine.[4] We described in the preceding paper the preparation of the intermediates, such as **3**, with the C14–C20 bond. In this paper (Part 2), we



Scheme 2. Outline of the preparation of **1**.

Keywords: Aconite; Alkaloid; Hetisan; Hydrocyanation; Azabicyclic ring.
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present full details of the synthesis of **1** from the intermediate **3**.[3] In the final paper (Part 3), we will deal with the total synthesis of (±)-nominine diverging from the intermediate **5**. These three papers describe in detail the first total synthesis of a hetisine-type aconite alkaloid.

2. Results and discussion

2.1. Seeking a route from the acetal–ene reaction product

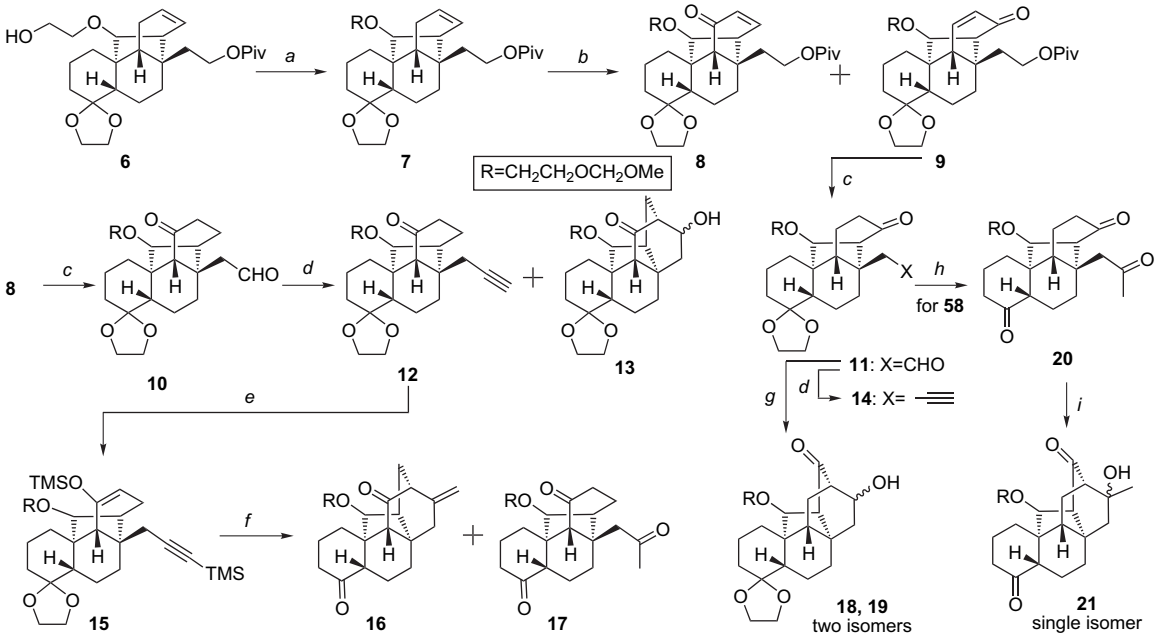
2.1.1. Seeking a method for the C-ring formation. As described in the preceding paper,[3] we planned to form the azabicyclic ring in the final stage of the synthesis, due to its strong basicity. Consequently, for further transformation from the acetal–ene reaction product (e.g., **3**) toward the hetisan skeleton, we considered the following three routes: (1) functionalization of C11, aiming at formation of the C-ring, as well as the synthesis of the alkaloids having a C11 hydroxy group, such as kobusine; (2) elaboration of the C8 side-chain for the C-ring formation; and (3) dehydration to form the $\Delta_{5,6}$ enone for construction of the pyrrolidine ring. After several trials on route (1), we found that chromium trioxide (CrO_3)-3,5-dimethylpyrazole functioned successfully in the oxidation of C11 and C13.[3] Therefore, we examined C-ring formation based on route (1) followed by route (2), starting from compound **6** prepared in the preceding paper (Scheme 3).

After protection of hydroxy group of **6** as methoxymethyl (MOM) ether as usual, the product **7** was oxidized as before[3] with CrO_3 and 3,5-dimethylpyrazole to obtain **8** (43%) and **9** (37%). In order to apply route (2), compound **8** was transformed to the keto-aldehyde **10** via three sequential operations: (i) hydrogenation on palladium (Pd), (ii) methanolysis with potassium carbonate (K_2CO_3), and (iii) oxidation with pyridinium chlorochromate (PCC) supported on alumina (20 wt % PCC–Al_2O_3).[12] The same treatment of **9** provided **11** without difficulty. The additional carbon corresponding to C17 of the hetisan skeleton was introduced at this stage as an alkyne carbon. Thus, dimethyl (1-diazo-2-oxopropyl)phosphonate[13] was allowed to react in the presence of K_2CO_3 with **10** and **11** to afford **12** and **14**, respectively, in high yields. A small quantity of the aldol product **13** was also isolated from **10**.

The carbomercuration and aldol reactions were examined for the C-ring formation from **11** and **12**. Although treatment of the silyl enol ether **15** derived from **12** with mercuric chloride ($HgCl_2$) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) according to the literature[14] gave no cyclized product, stirring of **15** with mercuric triflate–*N*,*N*,*N*′,*N*′-tetramethylurea complex [$Hg(OTf)_2(TMU)_2$][15] and then with HCl–H_2O secured the desired **16** in 50% yield along with the triketone **17** in 12% yield.

The aldol reaction was also applicable for the C-ring formation. On treatment of **11** with K_2CO_3 in boiling MeOH, two isomeric aldol products **18** and **19** were obtained in 56% and 31% yields, respectively. A similar aldol reaction of **20** with LDA, prepared from **14** with the above $Hg(OTf)_2(TMU)_2$, provided a single isomer **21** in 46% yield together with the recovery of **20** (21%). The stereochemistry of **18**, **19**, and **21** remains unclear.

2.1.2. Dehydrogenative oxidation of 22, 32, and 3 to form the $\Delta_{5,6}$ enone and attempted introduction of a nitrogen function at C6. The hetisan skeleton possesses a nitrogen function at C6 and a pyrrolidine ring, built onto the



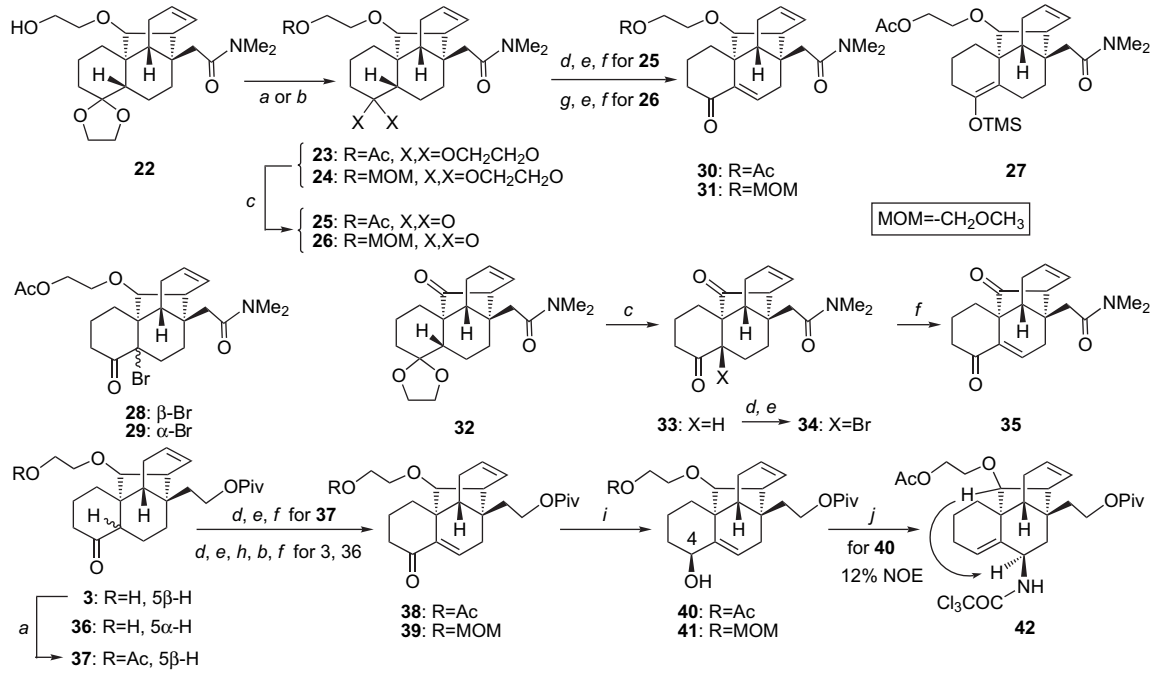
Scheme 3. Seeking a method for the C-ring formation. Reagents and conditions: (a) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, **7** 98%; (b) CrO_3, 3,5-dimethylpyrazole, **8** 43%, **9** 37%; (c) (i) H_2 (1 atm), Pd–C, MeOH; (ii) K_2CO_3, MeOH; (iii) 20% PCC–Al_2O_3, CH_2Cl_2, **10** (i) 95%, (ii) 98%, (iii) 85%; **11** (i) 97%, (ii) 95%, (iii) 90%; (d) dimethyl(1-diazo-2-oxopropyl)phosphonate, K_2CO_3, MeOH, **12** 83%, **13** 7%, **14** 98%; (e) LDA, TMSCl, THF; (f) $Hg(OTf)_2(TMU)_2$, CH_3CN–CH_2Cl_2; then 5% HCl–H_2O, **16** 50% from **12**, **17** 12% from **12**; (g) K_2CO_3, MeOH, **18** 56%, **19** 31%; (h) $Hg(OTf)_2(TMU)_2$, CH_3CN–CH_2Cl_2–H_2O, **20** 80%; (i) LDA, THF, **21** 46%, recovery of **20** 21%.

azabicyclic ring system, is present involving C4, C5, and C6. The N–C6 bond is characteristic of the hetisan skeleton and its early formation accords with our fundamental synthetic strategy. We therefore tried to introduce the $\Delta_{5,6}$ olefin from **22**, **32**, and **3**, which were prepared in the preceding paper[3] (Scheme 4).

The primary alcohol of **22** was protected as an acetate or a MOM ether to obtain **23** and **24**, respectively. These compounds were deprotected with *p*-toluenesulfonic acid (*p*-TsOH) in acetone to yield ketones **25** and **26**, respectively. Acetate **25** was then treated with iodotrimethylsilane (TMSI) and HMDS to afford the thermodynamic enolate **27** selectively.[16] The enolate was allowed to react with *N*-bromosuccinimide (NBS) to acquire the 5-bromo derivatives **28** and **29**. These were separately led to the desired enone **30** by treatment with 1,8-diazabicyclo[5,4,0]undecene-7 (DBU) in benzene. The overall yield of **30** from **25** was improved to 62% without separation at the stage of bromides **28** and **29**. On the other hand, as the MOM group of **26** cannot tolerate the above conditions with TMSI, enolization of **26** was carried out with bromomagnesium diisopropylamide[17] to afford the silyl enol ether corresponding to **27**. This product could be transformed to **31** as above, but in only 27% overall yield from **26**. The four sequential reactions employed for the transformation of **23** to **30** was applied to compound **32** to furnish the keto-enone derivative **35**, though the overall yield was not so good, and only the β-isomer **34** was isolated as the intermediary bromide.

As mentioned above, the best overall yield of enone from the corresponding ketone was obtained in the acetyl series (**25**→**30**), as compared with the MOM (**26**→**31**) or ketone series (**33**→**35**). So, compound **3** was led to **38** by way of **37** in the same manner as above in good yield. But we considered that a MOM group would be preferable as the protecting group for the primary alcohol, rather than acyl groups such as acetyl, because (i) the previously reported ene reaction proceeded in better yield for **3** than for **22**,[3] (ii) discretionary removal of the protecting groups of the two primary alcohol of **3** would be easier in the MOM series than in the acetyl series, as compound **3** bears another acyl function, the pivaloyl group, and (iii) furthermore, the acetyl protecting group would be cleaved during prospective introduction of the C18-methyl group as a carbanion. We therefore devised a suitable reaction sequence to get **39** containing the MOM ether. Thus, after treatment with TMSI, HMDS, and then NBS, the resulting products (mixture of α- and β-bromo derivatives) were stirred briefly with dilute HCl–H_2O to cleave the trimethylsilyl (TMS) group from the 2-(trimethylsilyloxy)ethyl group. The MOM group was introduced at this point, and the resulting mixture was led to the desired enone **39** in a good overall yield of 57% after DBU treatment. In the same manner, **39** was also obtained from the cis isomer **36** in 45% overall yield. This compound **39** became the intermediate in the finally settled synthetic route to (±)-nominine.[4]

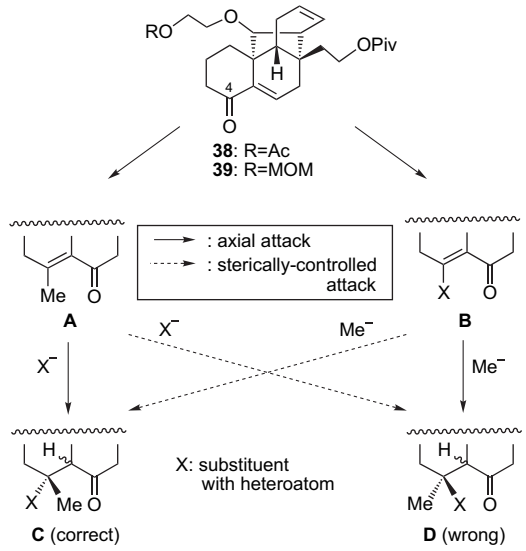
Using compound **38**, we attempted to introduce a nitrogen function at C6 (Scheme 4). Reduction of the enones **38** and **39** with sodium borohydride ($NaBH_4$) and cerium chloride ($CeCl_3$) was found to afford stereoselectively the 4β-OH derivatives **40** and **41**, respectively. Although the stereochemistry of **40** was unclear at this point, it was confirmed after derivation of **40** to **42** as follows. Thus, **40** was reacted with trichloroacetonitrile (CCl_3CN) and DBU[18] in order to



Scheme 4. Dehydrogenative oxidation of **22**, **32**, and **3** to form the $\Delta_{5,6}$-enone and attempted introduction of nitrogen function at C6. Reagents and conditions: (a) Ac_2O, pyridine, CH_2Cl_2, **23** 96% from **22**, **37** 95% from **3**; (b) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, **24** 94% from **22**; (c) *p*-TsOH, acetone, **25** 96% from **23**, **26** 95% from **24**, **33** 94% from **32**; (d) TMSCl, NaI, HMDS, CH_3CN; (e) NBS, THF, **28** 33% overall from **25**, **29** 21% overall from **25**, **34** 70% overall from **33**; (f) DBU, benzene, **30** 87% from **28**, **30** 80% from **29**, **30** 62% overall from **25**, **31** 27% overall from **26**, **35** 69% from **34**, **38** 64% overall from **37**, **39** 57% overall from **3**, **39** 45% overall from **36**; (g) *i*-Pr_2NMgBr, TMSCl, Et_3N, Et_2O-HMPA; (h) 0.2% HCl, THF–H_2O (12:1); (i) $NaBH_4$, $CeCl_3\cdot 7H_2O$, MeOH, **40** 92% from **38**, **41** 93% from **39**; (j) CCl_3CN, DBU, CH_2Cl_2, **42** 57% from **40**.

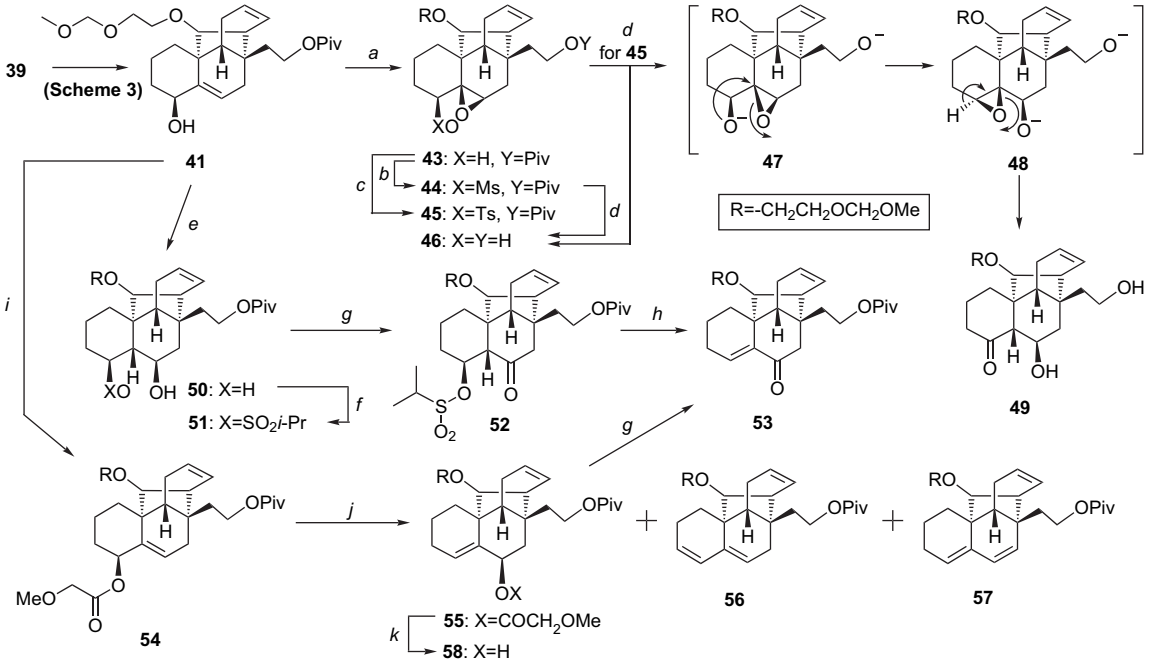
introduce a N-function at C6, affording **42**, whose stereochemistry was proved to be the undesired unnatural β-configuration by means of NOE experiments: 12% NOE enhancement was observed at H6 (δ 4.74–4.87, m) in the ^{1}H NMR NOE difference spectrum on irradiation at H20 (δ 3.91, d, J=6 Hz) of **42**. As this rearrangement reaction proceeds with stereoretention, this means that the stereochemistry of the hydroxy group at C4 of **40** is β. The stereoselectivity is consistent with the 'axial attack of small nucleophiles to cyclohexanone carbonyl group' reported by Cieplak.[19] The knowledge that small nucleophiles attack the C4 carbonyl not from the less-hindered β-side, but from the apparently congested α-side is very important for the subsequent synthetic route toward (±)-nominine, as described below.

2.1.3. Efforts aiming at carbonyl 1,3-transposition from 39. Our next task was to construct the pyrrolidine ring with the correct C4 stereochemistry from the above enone **38** or **39**. The fact that simple reduction of the C4 carbonyl group of **38** and **39** gave stereoselectively the 4β-hydroxy compounds **40** and **41** provided an important clue (Scheme 4). That is, if the 'axial attack of the small nucleophile' was also applicable to the enone **A** or **B** derived from **38** or **39**, the desired **C** with the correct C4 stereochemistry could be secured by the Michael addition of X to the enone **A** carrying a methyl group at C4, where X is a substituent with a heteroatom (Scheme 5). On the other hand, if the sterically controlled attack took precedence over the axial attack, **D** with the wrong stereochemistry would be formed from **A**, and so we would have to allow the enone **B** to react with methyl carbanion in a 1,4-addition manner to obtain compound **C**.



Scheme 5. Construction of C4 quaternary carbon with correct stereochemistry.

With this in mind, we first sought to obtain the 6-oxo-4-ene compound from **39** by means of 1,3-carbonyl transposition reaction (Scheme 6). The allyl alcohol **41** derived from **39** was oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) to afford **43** in 90% yield together with the enone **39** in 4% yield. The stereochemistry of **43** was confirmed by observation of a weak NOE enhancement (1.4%) at H6 (δ 3.25, d, J=3.5 Hz) on irradiation at H20 (δ 3.83, d, J=6 Hz) in the ^{1}H NMR spectrum. Then, **43** was readily led to the mesylate **44** with methanesulfonyl chloride (MsCl) and triethylamine



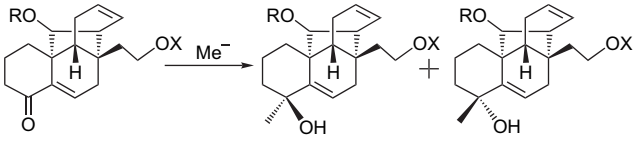
Scheme 6. Efforts aiming at carbonyl 1,3-transposition from **41** to form **53**. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, **43** 90%, **39** 4%; (b) MsCl, Et_3N, CH_2Cl_2, **44** 86%; (c) *p*-TsCl, Et_3N, CH_2Cl_2, **45** 94%; (d) Na, liq. NH_3, THF, **46** 78% from **44**, **46** 54% from **45**, **49** 36% from **45**; (e) $BH_3\cdot SMe_2$, THF; then H_2O_2, NaOH, **50** 50%, recovery of **41** 20%; (f) *i*-$PrSO_2Cl$, Et_3N, CH_2Cl_2, **51** 86%; (g) Dess–Martin periodinane, CH_2Cl_2, **52** quant. from **51**, **53** quant. from **58**; (h) DBU, benzene, **53** 91% from **52**; (i) $MeOCH_2COCl$, pyridine, CH_2Cl_2, **54** 86%; (j) $Eu(fod)_3$, $CHCl_3$, **55** 33%, **56** 20%, **57** 20%; (k) K_2CO_3, MeOH, **58** 77%.

(Et_3N). Although we tried to transform **44** to the 6-hydroxy-4-ene derivative under the Birch reduction conditions according to the literature,[20] the sole product was epoxy-diol **46**. This compound was also obtained from tosylate **45** under the same conditions, but in this case, the 6-hydroxy-4-one derivative **49** was also isolated as another product. It is likely that not the C4-oxygen bond, but the sulfone–oxygen bond was cleaved to form **47** due to steric congestion around C4, then intramolecular epoxy-alcohol rearrangement (**48**) and subsequent epoxy-ketone rearrangement took place during post-treatment to yield **49**.

The hydroboration–oxidation protocol afforded the 4,6-di-β-hydroxy compound **50** when an appropriate amount of borane–methyl sulfide complex ($BH_3 \cdot SMe_2$) was used. Selective protection of the 4-hydroxy group was attained by using isopropylsulfonyl chloride (*i*-$PrSO_2Cl$) and Et_3N to furnish **51** in 86% yield. Reaction of **50** with MsCl, Et_3N or TsCl, Et_3N resulted in a dimesylate or recovery of **50**, respectively. Compound **52**, obtained from **51** quantitatively by means of the Dess–Martin oxidation,[21] was led to the desired enone **53** by treatment with DBU. Alternatively, compound **53** was also prepared from **41** as follows. The methoxyacetyl derivative **54** was prepared from **41**, and was subjected to 1,3-allylic rearrangement[22] catalyzed with tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III) [$Eu(fod)_3$] to yield the desired **55**, though in only 33% yield, along with dienes **56** and **57** in 20% yield each. The methoxyacetyl group of **55** was removed and the resulting allyl alcohol **58** was oxidized with Dess–Martin periodinane to yield **53**.

2.1.4. Preparation of the β-methylenone 4 from 39. Since the preparation of **53** from **39** was difficult, as described in Scheme 6, we sought to obtain **4** in a more straightforward manner. Reaction of **39** with methylmagnesium iodide (MeMgI) in THF stereoselectively afforded the 4α-methyl derivative **59** in 38% yield together with recovery of **39** in 30% yield (Table 1, run b). The direction of the methyl carbanion attack is also in accordance with the above-mentioned 'axial attack of small nucleophile'. The recovery of **39** is attributable to enolization of **39** brought about by proton abstraction from C3 or C7 with MeMgI. With the more nonpolar solvent toluene, the enolization is liable to increase (run a). Employment of methyl lithium (MeLi) in place of MeMgI in diethyl ether (Et_2O) provided **59** (48%), its stereoisomer **60** (16%), and recovery of **39** (25%) (run c). Reaction of MeLi in THF, meanwhile, involved cleavage of the pivaloyl group to give **61** (59%), **62** (11%), and **63** (6%), corresponding to recovered enone (run d). The obtained **63** could be reused to furnish **61** (60%) and **62** (12%) (run e). The stereochemistry of **59** and **61** was confirmed by the observation of a NOESY cross peak between C4-α-methyl (axial) and H20 in the ^1H NMR spectra. On the other hand, in the ^1H NMR spectra of **60** and **62**, a NOESY cross peak was observed between C4-β-methyl (equatorial) and H6 olefin proton. We adopted the reaction conditions of runs d and e in Table 1 for this step toward the hetisine-type aconite alkaloids.

Table 1. Introduction of C18-methyl group into **39** (yield: %)



Run	Substrate	Me^-	Solvent	**59**	**60**	**61**	**62**	**39**	**63**
a	**39**	MeMgI	Toluene	19	—	—	—	50	—
b	**39**	MeMgI	THF	38	—	—	—	30	—
c	**39**	MeLi	Et_2O	48	16	—	—	25	—
d	**39**	MeLi	THF	—	—	59	11	—	6
e	**63**	MeLi	THF	—	—	60	12	—	—

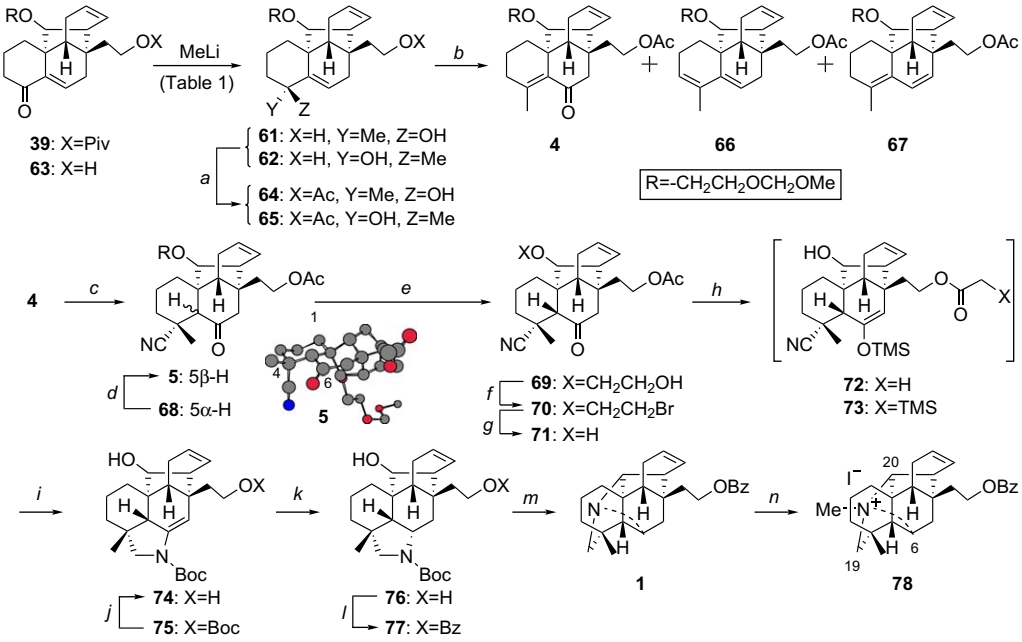
The intermediates **61** and **62** were acetylated as usual to afford **64** and **65** in yields of 93% and 90%, respectively (Scheme 7). Although the resulting tertiary allyl alcohols **64** and **65** were subjected to conventional PCC oxidation in dichloromethane (CH_2Cl_2), the desired enone **4** was obtained only in a trace amount and the major products were the dehydrated dienes **66** and **67**. An extensive search for the optimum oxidizing agent and reaction solvent led to oxidation with 20 wt % PCC–Al_2O_3 in benzene as the conditions of choice. With these conditions, the desired compound **4** was obtained in 63% and 65% yields from **64** and **65** with concomitant formation of **66** and **67** in yields of over 10% each, respectively.

2.2. Transformation of 4 to 1 by way of 5

Our remaining tasks from the intermediate **4** are formation of a pyrrolidine ring bridging between C4 and C6, construction of the azabicyclic ring, and creation and elaboration of the C-ring. Among these, we first focused on the pyrrolidine ring formation by use of the hydrocyanation reaction.

2.2.1. Stereoselective hydrocyanation reaction of 4. Hydrocyanation reaction[23] with diethylaluminum cyanide (Et_2AlCN) in toluene converted **4** to afford the trans isomer **5** with the desired C4 stereochemistry in 94% yield, together with its C5-stereoisomer **68** in 2% yield (Scheme 7). The structure of **5** was proved by the observation of a NOESY cross peak between H5 and C4-methyl as well as between H5 and H9. In the ^1H NMR spectrum of **68**, a NOESY cross peak was observed between H5 and H20. No C4-stereoisomer was formed, in complete concordance with the above-described 'axial attack of small nucleophile'. Use of tetrahydrofuran (THF) in place of toluene as the solvent resulted in an intractable reaction mixture. The minor isomer **68** was readily isomerized to thermodynamically more stable **5** on treatment with DBU in boiling benzene.

The 2-(methoxymethoxy)ethyl group at the C20-hydroxy group of **5** was removed at this stage according to the established procedure described in the preceding paper.[3] Thus, the MOM group was first cleaved to **69** with TMSI prepared in situ from chlorotrimethylsilane (TMSCl) and sodium iodide (NaI) in acetonitrile (CH_3CN). HCl treatment of **5** resulted in concomitant cleavage of the acetyl group. The obtained primary alcohol was brominated with carbon tetrabromide (Br_4C) and triphenylphosphine (Ph_3P) to afford **70**, and the

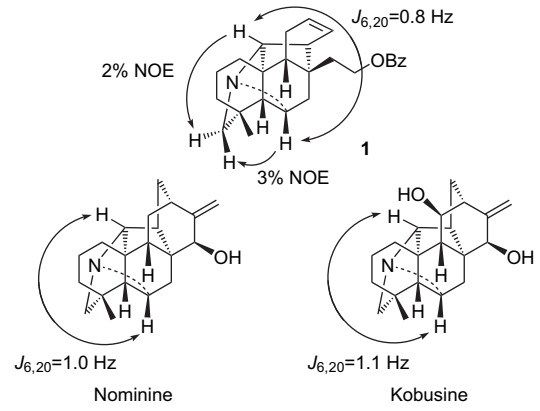


Scheme 7. Preparation of **1** from **61** and **62** by way of stereoselective hydrocyanation of **4** to form **5**. Reagents and conditions: (a) Ac_2O, pyridine, CH_2Cl_2, **64** 93% from **61**, **65** 90% from **62**; (b) 20 wt % PCC–Al_2O_3, benzene, **4** 63%, **66** 16%, **67** 13% from **64**; **4** 65%, **66** 15%, **67** 15% from **65**; (c) Et_2AlCN, toluene, **5** 94%, **68** 2%; (d) DBU, benzene, **5** 92% from **68**; (e) TMSCl, NaI, CH_3CN, **69** 92%; (f) Br_4C, Ph_3P, CH_2Cl_2, **70** 93%; (g) Zn, NH_4Cl, *i*-PrOH–H_2O (14:1), **71** 95%; (h) TMSCl, LDA, THF; (i) $LiAlH_4$, THF; Boc_2O, Et_3N, CH_2Cl_2, **74** 31% overall from **71**, **75** 25% overall from **71**; (j) K_2CO_3, MeOH, **74** 89% from **75**; (k) $NaBH_3CN$, 1% HCl, MeOH–H_2O (8:1), **76** 85%; (l) BzCl, pyridine, CH_2Cl_2, **77** 95%; (m) CF_3COOH, CH_2Cl_2; $SOCl_2$, pyridine, CH_2Cl_2, **1** 78% overall; (n) MeI, MeOH, **78** 73%.

resulting bromide was exposed to zinc (Zn) in the presence of ammonium chloride (NH_4Cl) in 2-propanol–H_2O (14:1) to afford **71** in the high yield of 95%.

2.2.2. Pyrrolidine ring formation from 71. It is necessary to protect the C6 carbonyl group of **71** prior to reduction of the cyano group. However, usual acetalization conditions such as ethylene glycol, *p*-TsOH in boiling benzene or methyl orthoformate, *p*-TsOH in MeOH ended in recovery of **71**, probably due to steric hindrance around the C6 carbonyl group. So the carbonyl group of **71** was protected as the silyl enol ether **72** by reaction with TMSCl and lithium diisopropylamide (LDA) (Scheme 7). At this juncture, formation of a small amount of **73** was observed, but the mixture of **72** and **73** was subjected without further purification to the next step, as the ester function would be cleaved under the reduction conditions of the cyano group. The mixture of **72** and **73** was reduced with lithium aluminum hydride (LAH) in boiling THF. Quenching of the reaction with water-saturated Et_2O involved concomitant cleavage of the silyl enol ether and the resulting amino-carbonyl compound cyclized spontaneously to an intermediary imine compound. The products after stirring the imine with di-*tert*-butyl dicarbonate (Boc_2O) and Et_3N were enamino-carbamates **74** (31% from **71**) and **75** (25% from **71**). The latter carbonate **75** was easily led to the former alcohol **74** on treatment with K_2CO_3 in MeOH. Then, the alkene conjugated to the nitrogen was reduced with sodium cyanoborohydride ($NaBH_3CN$) in a weak acid medium to provide **76** with the pyrrolidine ring.

2.2.3. Synthesis of 1 and its quaternization. Now the synthesis of **1** is in its final stage. After protection of the primary hydroxy group of **76** as the benzoate, the Boc group of the resulting **77** was cleaved with trifluoroacetic acid (CF_3COOH) to give an amino-alcohol (Scheme 7). This was exposed to thionyl chloride ($SOCl_2$) and pyridine[24] in CH_2Cl_2 at an ambient temperature to secure **1** in a good yield. In the ^1H NMR spectrum of **1**, the observations of long-range coupling (0.8 Hz) between H6 and H20 as well as the NOE enhancements at 19β-H and 19α-H on irradiation at H6 and H20, respectively, substantiated the structure (Scheme 8). Long-range coupling between H6 and H20 is also observed in the ^1H NMR spectra of the natural alkaloids nominine and kobusine. All other spectral data of **1** are also consistent with the structure depicted, but nevertheless, definitive structure proof is not an easy matter. The nitrogen involved in the azabicyclo ring system is known to be highly basic. So, the obtained **1** was led to the quaternary salt with methyl iodide (MeI) to get **78** in order to prove the complex



Scheme 8. Long-range coupling and NOE enhancement of **1**.

structure of **1**. In the ^{1}H and ^{13}C NMR spectra of **78**, the proton and carbon signals assigned to positions adjacent to the quaternary nitrogen, H6, H19 (×2), H20, and C6, C19, C20 were all shifted downfield, as described in the experimental section, giving explicit support to the azabicyclic ring structure of **1**.

3. Conclusion

In summary, we have synthesized compound **1** bearing the essential structural features of the hetisine-type aconite alkaloids, by utilizing the following key reactions: (i) dehydrogenative carbonyl 1,3-transposition reaction (**3**→**4**), (ii) stereoselective hydrocyanation reaction to form the natural-type C4 quaternary carbon (**4**→**5**), and (iii) construction of the azabicyclic ring system from **5**. The first total synthesis of hetisine-type aconite alkaloid is still in mid-course. In the subsequent paper, we show how our project was brought to a conclusion, culminated in a total synthesis of (±)-nominine from the intermediate **5**.

4. Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus (hot plate), and are not corrected. MS and high-resolution MS (HRMS) were recorded on a Hitachi M-80B spectrometer in direct inlet mode at an ionizing voltage of 70 eV, and figures in parentheses indicate the relative intensities. IR spectra were measured on a Hitachi 215 or Shimadzu IR-460 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian Mercury 300 (300 MHz) in $CDCl_3$ unless otherwise specified and coupling constants (*J* values) are rounded to the nearest 0.5 Hz. ^{13}C NMR spectra were measured on a Varian Mercury 300 (75 MHz) in $CDCl_3$ and ^{13}C multiplicities are shown in parentheses as CH_3 (primary), CH_2 (secondary), CH (tertiary), and C (quaternary). The NMR signals were assigned using proton decoupling techniques as well as gCOSY, DEPT, gHSQC, gHMBC, and/or NOESY spectra. Some characteristic signals for ^{1}H and ^{13}C NMR were selected and assigned as HX and CX, respectively, where X stands for hetisan carbon numbering. Column chromatography was conducted on silica gel (SiO_2, Fuji Davison BW 200), and the weight of SiO_2 and the eluting solvent are indicated in parentheses. Preparative TLC (PTLC) was carried out on glass plates (20×20 cm) coated with Merck silica gel 60PF_{254} (0.8 mm thick) unless otherwise specified, and the developing solvent is indicated in parentheses. Usual work-up refers to washing of the organic layers with water or brine, drying over anhydrous Na_2SO_4, and evaporating off the solvents under reduced pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use.

4.1. Seeking a method for the C-ring formation (Scheme 3)

4.1.1. Protection of 6 with MOMCl to form 7. MOMCl (64 μl, 0.843 mmol) was added during 1 min to a cooled (−18 °C) solution of **6** (25 mg, 55.8 μmol) and *i*-Pr_2NEt (0.24 ml, 1.38 mmol) in CH_2Cl_2 (4 ml) under an Ar atmosphere. The solution was stirred for 15 h at −18 to 15 °C, then saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. The organic layer was washed successively with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O, and was treated as usual. The resulting residue was separated by PTLC [hexane–EtOAc (3:1)] to give **7** (27 mg, 98%) as a colorless glass. HRMS Calcd for $C_{28}H_{44}O_7$: 492.3085. Found: 492.3073. MS *m*/*z*: 492 (M^+, 3), 477 (2), 447 (1), 431 (2), 423 (1), 407 (2), 403 (10), 386 (3), 285 (5), 257 (4), 112 (19), 99 (100), 89 (21), 57 (47), 45 (73). IR ($CHCl_3$) cm^{-1}: 1709. ^{1}H NMR *δ*: 0.99 (1H, ddd, *J*=13, 13, 4 Hz), 1.10 (9H, s), 1.16–1.27 (2H, m), 1.46–1.90 (9H, m), 2.01–2.13 (2H, m), 2.15 (1H, dddd, *J*=19, 5, 3, 2.5 Hz), 2.33 (1H, br d, *J*=19 Hz), 2.47 (1H, ddd, *J*=7, 6, 1.5 Hz), 3.37 (3H, s, $CH_2OC\mathit{H}_3$), 3.45 (1H, ddd, *J*=11, 5.5, 5.5 Hz), 3.54 (1H, ddd, *J*=11, 6, 4.5 Hz), 3.58–3.71 (2H, m), 3.73–3.83 (1H, m), 3.84–3.98 (3H, m), 3.99 (1H, ddd, *J*=11, 8.5, 6.5 Hz), 4.13 (1H, ddd, *J*=11, 9, 6 Hz), 4.26 (1H, d, *J*=6 Hz), 4.64 (1H, d, *J*=6.5 Hz, $OC\mathit{H}_2OCH_3$), 4.67 (1H, d, *J*=6.5 Hz, $OC\mathit{H}_2OCH_3$), 5.53 (1H, ddd, *J*=9.5, 3, 3 Hz), 5.64 (1H, br dd, *J*=9.5, 7 Hz). ^{13}C NMR *δ*: 18.3 (CH_2), 20.9 (CH_2), 26.9 (CH_2), 27.2 (CH_3×3), 28.9 (CH_2), 33.7 (CH_2), 34.4 (CH_2), 34.9 (CH_2), 38.6 (C), 42.4 (C), 47.8 (CH), 48.6 (C), 50.8 (CH), 54.8 (CH), 55.0 (CH_3, CH_2OCH_3), 62.7 (CH_2), 63.8 (CH_2), 65.4 (CH_2), 66.7 (CH_2, $C\mathit{H}_2$OMOM), 68.8 (CH_2), 81.5 (CH), 96.4 (CH_2, $OC\mathit{H}_2OCH_3$), 110.3 (C), 125.3 (CH), 127.8 (CH), 178.4 (C).

4.1.2. Oxidation of 7 to form 8 and 9. In the same manner as reported in the preceding paper,[3] **7** (48 mg, 97.6 μmol) was oxidized with CrO_3 (146 mg, 1.46 mmol) and 3,5-dimethylpyrazole (169 mg, 1.76 mmol) in CH_2Cl_2 (5 ml) at −18 to 22 °C for 63 h. The same work-up as before and purification by PTLC [hexane–DME (5:1)] provided **8** (21 mg, 43%) and **9** (18.5 mg, 37%) in order of decreasing polarity. **8**: Colorless glass. HRMS Calcd for $C_{28}H_{42}O_8$: 506.2877. Found: 506.2875. MS *m*/*z*: 506 (M^+, 18), 417 (4), 299 (3), 271 (6), 243 (3), 99 (100), 89 (14), 57 (38), 45 (59), 41 (14). IR ($CHCl_3$) cm^{-1}: 1716, 1662. ^{1}H NMR *δ*: 1.17 (9H, s), 1.22–1.33 (2H, m), 1.45–1.83 (9H, m), 1.86–1.98 (2H, m), 2.09 (1H, br s, H9), 3.10 (1H, ddd, *J*=7, 6, 2 Hz, H14), 3.35 (3H, s), 3.54–3.60 (2H, m), 3.61–3.66 (2H, m), 3.76–3.86 (1H, m), 3.88–4.04 (5H, m), 4.62 (2H, s), 4.62 (1H, br d, *J*=6 Hz), 6.12 (1H, dd, *J*=9.5, 1.5 Hz, H12), 7.09 (1H, dd, *J*=9.5, 7 Hz, H13). ^{13}C NMR *δ*: 18.0 (CH_2), 20.5 (CH_2), 27.1 (CH_3×3), 29.6 (CH_2), 34.2 (CH_2), 34.4 (CH_2), 34.8 (CH_2), 38.5 (C), 48.1 (C), 50.0 (CH, C9), 50.1 (CH, C14), 50.7 (C), 55.0 (CH_3), 61.6 (CH_2), 64.0 (CH_2), 65.4 (CH_2), 66.6 (CH_2), 69.6 (CH_2), 74.0 (CH), 79.8 (CH), 96.4 (CH_2), 109.6 (C), 129.7 (CH, C12), 151.8 (CH, C13), 178.2 (C), 201.6 (C, C11). **9**: Colorless glass. HRMS Calcd for $C_{28}H_{42}O_8$: 506.2877. Found: 506.2881. MS *m*/*z*: 506 (M^+, 6), 417 (28), 405 (8), 299 (7), 271 (5), 112 (10), 99 (100), 89 (22), 57 (53), 45 (87), 41 (17). IR ($CHCl_3$) cm^{-1}: 1718, 1679. ^{1}H NMR *δ*: 1.16 (9H, s), 1.21 (1H, ddd, *J*=13, 13, 5 Hz), 1.32 (1H, ddd, *J*=13.5, 13.5, 5 Hz), 1.46–1.57 (3H, m), 1.57–1.83 (7H, m), 1.98–2.06 (1H, m), 2.01 (1H, dd, *J*=6.5, 1.5 Hz, H9), 3.07 (1H, ddd, *J*=6.5, 1.5, 1.5 Hz, H14), 3.33 (3H, s), 3.39–3.47 (1H, m), 3.49–3.63 (3H, m), 3.77–3.86 (1H, m), 3.89–4.02 (5H, m), 4.58 (1H, d, *J*=6.5 Hz), 4.59 (2H, s), 6.12 (1H, dd, *J*=9.5, 1.5 Hz, H12), 6.96 (1H, dd, *J*=9.5, 6.5 Hz, H11).

^{13}C NMR δ: 17.9 (CH_2), 20.3 (CH_2), 27.1 ($CH_3\times3$), 29.8 (CH_2), 32.9 (CH_2), 34.3 (CH, C9), 35.1 (CH_2), 38.5 (C), 49.7 (CH), 50.3 (C), 53.3 (C), 55.0 (CH_3), 57.3 (CH), 61.6 (CH_2), 64.1 (CH_2), 65.4 (CH_2), 65.6 (CH, C14), 66.2 (CH_2), 70.4 (CH_2), 77.8 (CH), 96.3 (CH_2), 109.6 (C), 131.8 (CH, C12), 148.7 (CH, C11), 178.2 (C), 200.0 (C, C13).

4.1.3. Transformation of 8 to 10 by (i) hydrogenation, (ii) alcoholysis, and (iii) oxidation. (i) *Hydrogenation*: A solution of **8** (21 mg, 41.5 μmol) in MeOH (5 ml) was hydrogenated over 10% Pd–C (5 mg, 4.7 μg atom) under a hydrogen atmosphere (1 atm) at 20 °C for 1.5 h. The mixture was filtered through a Celite pad and the pad was rinsed with CH_2Cl_2. Evaporation of the combined organic layers followed by separation by PTLC [hexane–EtOAc (2:1)] furnished the *dihydro derivative* (20 mg, 95%) as a colorless glass. HRMS Calcd for $C_{28}H_{44}O_8$: 508.3034. Found: 508.3020. MS *m*/*z*: 508 (M^+, 5), 447 (5), 419 (19), 407 (3), 301 (4), 99 (100), 57 (40), 45 (56), 41 (13). IR ($CHCl_3$) cm^{-1}: 1718, 1687. ^{1}H NMR δ: 1.04 (1H, ddd, *J*=13, 13, 4.5 Hz), 1.17 (9H, s), 1.23–1.42 (2H, m), 1.45–1.88 (11H, m), 1.78 (1H, s, H9), 2.01 (1H, ddd, *J*=13, 10, 3 Hz, H13), 2.27 (1H, dd, *J*=18.5, 7.5 Hz, H12), 2.40–2.47 (1H, m), 2.63 (1H, ddd, *J*=18.5, 10, 9.5 Hz, H12), 3.36 (3H, s), 3.60–3.85 (5H, m), 3.87–4.01 (3H, m), 4.05 (1H, ddd, *J*=11, 8.5, 6 Hz), 4.47 (1H, d, *J*=6.5 Hz, H20), 4.65 (2H, s). ^{13}C NMR δ: 17.8 (CH_2), 20.2 (CH_2), 20.5 (CH_2, C13), 27.1 ($CH_3\times3$), 27.3 (CH_2), 33.8 (CH_2), 34.6 (CH_2), 35.1 (CH_2), 36.5 (CH_2, C12), 38.6 (C, *C*Me_3), 44.6 (CH, C14), 45.2 (C), 49.0 (C), 49.8 (CH), 55.0 (CH_3), 60.7 (CH_2), 63.9 (CH_2), 65.4 (CH_2), 66.8 (CH_2), 71.0 (CH_2), 73.4 (CH), 78.3 (CH), 96.4 (CH_2), 109.7 (C), 178.4 (C), 214.1 (C). (ii) *Alcoholysis*: A solution of the above dihydro derivative (44 mg, 86.6 μmol) in 2% w/v K_2CO_3–MeOH (5 ml) was stirred under reflux for 2 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and PTLC [benzene–EtOAc (2:3)] yielded the *primary alcohol* (36 mg, 98%) as a colorless glass. HRMS Calcd for $C_{23}H_{36}O_7$: 424.2459. Found: 424.2456. MS *m*/*z*: 424 (M^+, 4), 363 (5), 335 (18), 319 (3), 99 (100), 55 (14), 45 (67). IR ($CHCl_3$) cm^{-1}: 1683. ^{1}H NMR δ: 1.03 (1H, ddd, *J*=13, 13, 4 Hz), 1.28 (1H, ddd, *J*=13, 13, 4 Hz), 1.33–1.44 (1H, m), 1.46–1.80 (12H, m, including OH), 1.80 (1H, s, H9), 1.99 (1H, ddd, *J*=13.5, 10, 3 Hz), 2.26 (1H, dd, *J*=18.5, 7.5 Hz), 2.37–2.44 (1H, m), 2.62 (1H, ddd, *J*=18.5, 10, 9.5 Hz), 3.36 (3H, s), 3.59–3.85 (7H, m), 3.87–3.99 (3H, m), 4.45 (1H, d, *J*=6.5 Hz), 4.65 (2H, s). ^{13}C NMR δ: 17.9 (CH_2), 20.2 (CH_2), 20.5 (CH_2), 27.4 (CH_2), 33.8 (CH_2), 35.1 (CH_2), 36.5 (CH_2), 38.9 (CH_2, *C*H_2CH_2OH), 45.2 (CH), 45.3 (C), 49.1 (C), 49.7 (CH), 55.0 (CH_3), 58.9 (CH_2, CH_2OH), 63.9 (CH_2), 65.4 (CH_2), 66.8 (CH_2), 71.0 (CH_2), 73.1 (CH), 78.2 (CH), 96.4 (CH_2), 109.7 (C), 214.5 (C). (iii) *Oxidation*: PCC–Al_2O_3 (20 wt %, 252 mg, 0.234 mmol) was added to a cooled (0 °C) solution of the above alcohol (33 mg, 77.8 μmol) in CH_2Cl_2 (5 ml) and the mixture was stirred at 0 °C for 30 min and at 18 °C for 1 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (4:3)] provided **10** (28 mg, 85%) as a colorless glass. HRMS Calcd for $C_{23}H_{34}O_7$: 422.2303. Found: 422.2304. MS *m*/*z*: 422 (M^+, 2), 361 (5), 333 (13), 99 (100), 89 (8), 55 (14), 45 (77). IR ($CHCl_3$) cm^{-1}: 1717, 1686. ^{1}H NMR δ: 1.06 (1H, ddd, *J*=13, 13, 4.5 Hz), 1.23–1.41 (2H, m), 1.47–1.81 (8H, m), 1.87 (1H, br s, H9), 1.95–2.04 (1H, m), 2.06 (1H, ddd, *J*=13.5, 9.5, 3.5 Hz), 2.26 (1H, dd, *J*=19, 7.5 Hz), 2.37 (1H, d, *J*=2 Hz, C*H*$_2$CHO), 2.68 (1H, ddd, *J*=19, 9.5, 9.5 Hz), 2.72–2.78 (1H, m), 3.36 (3H, s), 3.62–3.83 (5H, m), 3.87–4.02 (3H, m), 4.50 (1H, d, *J*=7 Hz), 4.64 (2H, s), 9.76 (1H, t, *J*=2 Hz, C*H*O). ^{13}C NMR δ: 17.9 (CH_2), 20.1 (CH_2), 20.9 (CH_2), 27.3 (CH_2), 34.9 (CH_2), 35.0 (CH_2), 36.4 (CH_2), 44.4 (CH), 45.4 (C), 49.0 (C), 49.5 (CH), 50.0 (CH_2, *C*H_2CHO), 55.1 (CH_3), 63.9 (CH_2), 65.5 (CH_2), 66.8 (CH_2), 71.1 (CH_2), 72.7 (CH), 78.0 (CH), 96.4 (CH_2), 109.6 (C), 200.4 (CH, CHO), 213.5 (C).

4.1.4. Transformation of 9 to 11. In the same manner as described for the preparation of **10** from **8** (Section 4.1.3), **9** (43 mg, 85.0 μmol) was hydrogenated to the dihydro derivative (42 mg, 97%). This compound (24 mg, 47.2 μmol) was subjected to alcoholysis to get the primary alcohol (19 mg, 95%), which was then oxidized to **11** (17 mg, 90%). The *dihydro derivative*: colorless glass. HRMS Calcd for $C_{28}H_{44}O_8$: 508.3034. Found: 508.3050. MS *m*/*z*: 508 (M^+, 3), 493 (2), 463 (2), 419 (23), 317 (6), 301 (8), 99 (100), 57 (47), 45 (73), 41 (17). IR ($CHCl_3$) cm^{-1}: 1714, 1699. ^{1}H NMR δ: 1.08–1.20 (1H, m), 1.17 (9H, s), 1.23–1.35 (1H, m), 1.42–1.46 (1H, m, H9), 1.46–1.91 (11H, m), 1.96–2.11 (1H, m), 2.11 (1H, br d, *J*=13 Hz), 2.21 (1H, dd, *J*=18, 9 Hz, H12), 2.66 (1H, ddd, *J*=18, 10, 10 Hz, H12), 2.83 (1H, d, *J*=7 Hz, H14), 3.35 (3H, s), 3.43–3.63 (4H, m), 3.75–3.85 (1H, m), 3.88–4.00 (3H, m), 4.01 (1H, ddd, *J*=11, 8, 6.5 Hz), 4.13 (1H, ddd, *J*=11, 8, 7 Hz), 4.47 (1H, d, *J*=7 Hz, H20), 4.60 (1H, d, *J*=6.5 Hz), 4.62 (1H, d, *J*=6.5 Hz). ^{13}C NMR δ: 18.0 (CH_2), 19.2 (CH_2, C11), 20.2 (CH_2), 27.1 ($CH_3\times3$), 28.1 (CH_2), 33.3 (CH_2), 33.5 (CH_2), 34.8 (CH_2), 35.5 (CH_2, C12), 38.5 (C), 45.8 (C), 48.0 (C), 50.2 (CH), 53.5 (CH, C9), 54.9 (CH_3), 60.9 (CH_2), 63.9 (CH_2), 65.1 (CH, C14), 65.4 (CH_2), 66.3 (CH_2), 69.9 (CH_2), 77.3 (CH), 96.1 (CH_2), 109.8 (C), 178.2 (C), 210.7 (C). The *primary alcohol*: colorless glass. HRMS Calcd for $C_{23}H_{36}O_7$: 424.2459. Found: 424.2453. MS *m*/*z*: 424 (M^+, 3), 409 (3), 335 (30), 319 (7), 112 (11), 99 (100), 55 (20), 45 (90). IR ($CHCl_3$) cm^{-1}: 1696. ^{1}H NMR δ: 1.08–1.19 (1H, m), 1.23–1.34 (1H, m), 1.38–1.43 (1H, m), 1.43–1.83 (9H, m, including OH), 1.53 (2H, t, *J*=7.5 Hz, C*H*$_2$CH$_2$OH), 1.85 (1H, dddd, *J*=14.5, 10, 9, 4.5 Hz, H11), 2.01 (1H, br dd, *J*=14.5, 10 Hz, H11), 2.20 (1H, dd, *J*=18, 9 Hz), 2.67 (1H, ddd, *J*=18, 10, 10 Hz), 2.89 (1H, d, *J*=7 Hz), 3.35 (3H, s), 3.43–3.63 (4H, m), 3.68 (2H, t, *J*=7.5 Hz, C*H*$_2$OH), 3.75–3.84 (1H, m), 3.87–3.98 (3H, m), 4.46 (1H, d, *J*=7 Hz, H20), 4.59 (1H, d, *J*=6.5 Hz), 4.63 (1H, d, *J*=6.5 Hz). ^{13}C NMR δ: 18.0 (CH_2), 19.3 (CH_2), 20.2 (CH_2), 28.1 (CH_2), 33.7 (CH_2, *C*H_2CH_2OH), 34.9 (CH_2), 35.7 (CH_2), 37.7 (CH_2), 46.1 (C), 47.7 (C), 50.3 (CH), 54.3 (CH), 55.0 (CH_3), 59.0 (CH_2, CH_2OH), 63.9 (CH_2), 64.7 (CH), 65.4 (CH_2), 66.3 (CH_2), 69.9 (CH_2), 77.6 (CH), 96.2 (CH_2), 109.9 (C), 211.9 (C). **11**: Colorless glass. HRMS Calcd for $C_{23}H_{34}O_7$: 422.2303. Found: 422.2302. MS *m*/*z*: 422 (M^+, 3), 407 (4), 333 (26), 112 (20), 99 (100), 89 (12), 86 (13), 55 (16), 45 (90). IR ($CHCl_3$) cm^{-1}: 1717, 1700. ^{1}H NMR δ: 1.11–1.23 (1H, m), 1.24–1.36 (1H, m), 1.43–1.83 (10H, m), 2.02–2.17 (2H, m), 2.22 (1H, dd, *J*=18, 9 Hz), 2.27 (1H, dd,

J=16, 2.5 Hz, CH_2CHO), 2.37 (1H, dd, J=16, 2 Hz, CH_2CHO), 2.73 (1H, ddd, J=18, 10.5, 10.5 Hz), 3.02 (1H, d, J=7 Hz, H14), 3.35 (3H, s), 3.46–3.64 (4H, m), 3.75–3.85 (1H, m), 3.88–3.99 (3H, m), 4.51 (1H, d, J=7 Hz, H20), 4.61 (1H, d, J=6.5 Hz), 4.63 (1H, d, J=6.5 Hz), 9.73 (1H, dd, J=2.5, 2 Hz, CHO). ^{13}C NMR δ: 18.0 (CH_2), 19.5 (CH_2), 20.2 (CH_2), 28.0 (CH_2), 34.7 (CH_2), 34.8 (CH_2), 35.5 (CH_2), 46.1 (C), 47.7 (C), 48.7 (CH_2, CH_2CHO), 50.1 (CH), 53.3 (CH), 55.0 (CH_3), 63.9 (CH_2), 64.5 (CH, C14), 65.4 (CH_2), 66.3 (CH_2), 70.1 (CH_2), 77.1 (CH, C20), 96.2 (CH_2), 109.8 (C), 200.6 (CH, CHO), 210.5 (C).

4.1.5. Preparation of acetylene 12 from 10. K_2CO_3 (18 mg, 0.130 mmol) was added to a solution of **10** (14 mg, 33.2 μmol) and dimethyl (1-diazo-2-oxopropyl)-phosphonate (38 mg, 0.198 mmol) in MeOH (3 ml) and the mixture was stirred at 19 °C under an Ar atmosphere for 3.5 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (3:2)] afforded **12** (11.5 mg, 83%) and **13** (1 mg, 7%) in order of increasing polarity. **12**: Colorless glass. HRMS Calcd for $C_{24}H_{34}O_6$: 418.2353. Found: 418.2348. MS m/z: 418 (M^+, 11), 357 (6), 329 (21), 99 (100), 55 (16), 45 (76). IR ($CHCl_3$) cm^{-1}: 2116, 1686. ^{1}H NMR δ: 1.05 (1H, ddd, J=13, 13, 4.5 Hz), 1.23–1.43 (2H, m), 1.47–1.85 (8H, m), 1.77 (1H, br s, H9), 1.97 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 1.97–2.17 (4H, m), 2.26 (1H, dd, J=18.5, 7.5 Hz, H12), 2.51–2.57 (1H, m), 2.65 (1H, ddd, J=18.5, 9.5, 9.5 Hz, H12), 3.37 (3H, s), 3.60–3.84 (5H, m), 3.88–4.00 (3H, m), 4.47 (1H, d, J=7 Hz, H20), 4.65 (2H, s). ^{13}C NMR δ: 17.9 (CH_2), 20.2 (CH_2), 20.6 (CH_2), 26.2 (CH_2, CH_2C≡CH), 27.4 (CH_2), 34.4 (CH_2), 35.1 (CH_2), 36.5 (CH_2), 44.5 (CH), 46.3 (C), 49.7 (CH), 50.1 (C), 55.1 (CH_3), 63.9 (CH_2), 65.5 (CH_2), 66.8 (CH_2), 70.1 (C, C≡CH), 71.0 (CH_2), 72.0 (CH), 77.9 (CH), 80.7 (CH, C≡CH), 96.4 (CH_2), 109.7 (C), 213.8 (C). **13**: Colorless glass. HRMS Calcd for $C_{23}H_{34}O_7$: 422.2303. Found: 422.2303. MS m/z: 422 (M^+, 2), 407 (3), 361 (9), 333 (33), 99 (100), 69 (18), 55 (16), 45 (71). IR ($CHCl_3$) cm^{-1}: 1701. ^{1}H NMR δ: 1.21–1.95 (15H, m, including OH), 1.52 (1H, dd, J=14.5, 6 Hz, H15), 1.91 (1H, dd, J=14.5, 9.5 Hz, H15), 2.27–2.31 (1H, m), 2.35 (1H, dddd, J=7, 7, 2, 2 Hz, H14), 3.35 (3H, s), 3.40–3.48 (1H, m), 3.54–3.71 (3H, m), 3.74–3.84 (1H, m), 3.86–3.98 (3H, m), 3.99–4.07 (1H, m, H16), 4.20 (1H, d, J=7 Hz, H20), 4.62 (2H, s). ^{13}C NMR δ: 12.7 (CH_2, C13), 17.8 (CH_2, C6), 20.0 (CH_2, C2), 28.1 (CH_2, C1), 34.5 (CH_2, C7), 35.2 (CH_2, C3), 37.4 (CH_2, C15), 43.0 (C, C8), 45.2 (CH, C14), 49.2 (C, C10), 50.1 (CH, C5), 52.5 (CH, C12), 55.0 (CH_3), 63.9 (CH_2), 65.1 (CH, C9), 65.4 (CH_2), 66.5 (CH_2), 67.8 (CH, C16), 70.3 (CH_2), 77.7 (CH, C20), 96.4 (CH_2), 110.0 (C), 214.1 (C).

4.1.6. Preparation of acetylene 14 from 11. In the same manner as above (Section 4.1.5), **11** (40 mg, 94.8 μmol) was led to **14** (39 mg, 98%) as a colorless glass. HRMS Calcd for $C_{24}H_{34}O_6$: 418.2353. Found: 418.2334. MS m/z: 418 (M^+, 6), 329 (36), 313 (10), 112 (12), 99 (100), 89 (17), 55 (14), 45 (85). IR ($CHCl_3$) cm^{-1}: 2122, 1698. ^{1}H NMR δ: 1.09–1.21 (1H, m), 1.23–1.35 (1H, m), 1.43–1.67 (5H, m), 1.71–2.15 (9H, m), 1.99 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.21 (1H, dd, J=18, 9 Hz, H12), 2.68 (1H, ddd, J=18, 10.5, 10.5 Hz, H12), 2.83 (1H, d, J=6.5 Hz, H14), 3.35 (3H, s), 3.43–3.63 (4H, m), 3.76–3.86 (1H, m), 3.88–4.01 (3H, m), 4.51 (1H, d, J=6.5 Hz, H20), 4.60 (1H, d, J=6.5 Hz), 4.62 (1H, d, J=6.5 Hz). ^{13}C NMR δ: 17.9 (CH_2), 19.3 (CH_2), 20.2 (CH_2), 25.2 (CH_2), 28.1 (CH_2), 33.9 (CH_2), 34.9 (CH_2), 35.7 (CH_2), 46.8 (C), 47.9 (C), 50.2 (CH), 52.5 (CH), 55.0 (CH_3), 63.9 (CH_2), 64.3 (CH, C14), 65.4 (CH_2), 66.3 (CH_2), 70.0 (CH_2), 70.3 (C, C≡CH), 77.8 (CH, C20), 80.7 (CH, C≡CH), 96.2 (CH_2), 109.9 (C), 210.6 (C).

4.1.7. Carbomercuration of 12 to form 16 and 17. BuLi (1.57 M, 0.55 ml, 0.864 mmol) was added to a cooled (−18 °C) solution of diisopropylamine (151 μl, 1.08 mmol) in THF (2 ml) under an Ar atmosphere and the mixture was stirred at the same temperature for 10 min. The resulting solution was cooled to −78 °C and to this were added TMSCl (0.27 ml, 2.13 mmol) and then a THF (2 ml) solution of **12** (9 mg, 21.5 μmol). The mixture was stirred at −78 °C for 30 min, then Et_3N (0.60 ml, 4.31 mmol) was added, and the resulting mixture was further stirred for 5 min. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $CuSO_4$–H_2O, saturated $NaHCO_3$–H_2O and H_2O, and treated as usual to give crude **15** (15 mg). Aside from this, trifluoromethanesulfonic anhydride (Tf_2O, 8 μl, 47.6 μmol) was added to a slurry of mercury(II) oxide (HgO, 10.5 mg, 48.5 μmol) in CH_3CN (1.5 ml) at 0 °C and the mixture was stirred at the same temperature under an Ar atmosphere for 3 min. *N*,*N*,*N*′,*N*′-Tetramethylurea (TMU, 12 μl, 0.100 mmol) was further added, and the whole was stirred at 0 °C for 3 min and at 20 °C for 10 min, then cooled again in an ice bath. The crude **15** (15 mg) in CH_2Cl_2 (2 ml) was added and the resulting mixture was stirred at 0 °C for 1 h and at 22 °C for 15 h. After the mixture had been cooled to 0 °C, 2.5% HCl–H_2O (0.48 ml, 0.324 mmol) was added and the mixture was stirred at 0 °C for 10 min and at 18 °C for 2 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (3:2)] afforded **16** (2 mg) and a mixture of organo-mercury compounds (12 mg). The latter was dissolved in MeOH (2 ml), and to this was added *p*-TsOH·H_2O (10 mg, 52.6 μmol). The resulting solution was stirred at 19 °C for 1.5 h. Quenching with saturated $NaHCO_3$–H_2O, extraction with CH_2Cl_2, usual work-up, and PTLC [hexane–EtOAc (5:2)] gave a further crop of **16** (2 mg, total 4 mg, 50%) and **17** (1 mg, 12%) in order of increasing polarity. **16**: Colorless glass. HRMS Calcd for $C_{22}H_{30}O_5$: 374.2092. Found: 374.2088. MS m/z: 374 (M^+, 0.5), 342 (1), 329 (4), 268 (6), 239 (14), 91 (14), 45 (100). IR ($CHCl_3$) cm^{-1}: 1704, 1693, 1646. ^{1}H NMR δ: 1.37–1.47 (1H, m, H7), 1.54 (1H, dd, J=13, 9 Hz, H13), 1.59–1.81 (5H, m), 1.84 (1H, br s, H9), 1.94–2.03 (1H, m, H2), 2.06–2.14 (1H, m, H1), 2.20 (1H, ddd, J=16.5, 1.5, 1.5 Hz, H15), 2.21–2.43 (4H, m), 2.23 (1H, dd, J=13, 5 Hz, H13), 2.52 (1H, ddd, J=16.5, 3, 3 Hz, H15), 2.89 (1H, d, J=5 Hz, H12), 3.28 (1H, ddd, J=10, 5.5, 4 Hz), 3.33 (3H, s), 3.42 (1H, dd, J=6.5, 0.5 Hz, H20), 3.49 (1H, ddd, J=10, 6, 4 Hz), 3.53–3.64 (2H, m), 4.58 (2H, s), 4.72 (1H, br s, C=CH_2), 4.91 (1H, br s, C=CH_2). ^{13}C NMR δ: 19.1 (CH_2, C6), 20.9 (CH_2, C13), 23.7 (CH_2, C2), 28.1 (CH_2, C1), 33.6 (CH_2, C7), 35.6 (CH_2, C15), 41.9 (CH_2, C3), 43.4 (C, C8), 44.1 (CH, C14), 53.1 (C, C10), 54.3 (CH, C12), 55.1 (CH_3),

55.7 (CH, C5), 64.8 (CH, C9), 66.3 (CH_2), 70.3 (CH_2), 79.0 (CH, C20), 96.3 (CH_2), 109.0 (CH_2, C17), 143.8 (C, C16), 209.5 (C, C11), 211.4 (C, C4). **17**: Colorless glass. HRMS Calcd for $C_{22}H_{32}O_6$: 392.2197. Found: 392.2207. MS *m/z*: 392 (M^+, 1), 360 (1), 347 (7), 302 (5), 289 (4), 91 (10), 89 (9), 73 (10), 59 (13), 55 (12), 45 (100), 43 (49). IR ($CHCl_3$) cm^{-1}: 1696. ^1H NMR δ: 1.20 (1H, ddd, J=13, 13, 7 Hz, H7), 1.41–1.90 (5H, m), 1.91–2.47 (8H, m), 2.01 (1H, br s, H9), 2.11 (3H, s, $COCH_3$), 2.38 (1H, d, J=18 Hz, CH_2COMe), 2.47 (1H, d, J=18 Hz, CH_2COMe), 2.67 (1H, ddd, J=19, 9.5, 9.5 Hz), 2.96–3.02 (1H, m), 3.34 (3H, s), 3.33–3.51 (1H, m), 3.59–3.70 (3H, m), 3.70 (1H, d, J=6.5 Hz, H20), 4.60 (2H, s). ^{13}C NMR δ: 18.7 (CH_2), 20.9 (CH_2), 23.9 (CH_2), 27.5 (CH_2), 31.4 (CH_3, CO*C*H_3), 33.4 (CH_2), 36.6 (CH_2), 41.8 (CH_2, C3), 43.8 (CH), 45.7 (C), 48.9 (CH_2, *C*H_2COMe), 52.9 (C), 55.1 (CH_3), 55.3 (CH, C5), 66.7 (CH_2), 71.1 (CH_2), 72.0 (CH), 79.2 (CH), 96.3 (CH_2), 206.1 (C, *C*OMe), 210.6 (C, C4), 213.2 (C, C11).

4.1.8. Aldol reaction of 11 to form 18 and 19. A solution of **11** (8 mg, 19.0 μmol) in K_2CO_3–MeOH (2% w/v, 2 ml) was stirred at 50 °C for 4 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (1:2)] afforded **18** (4.5 mg, 56%) and **19** (2.5 mg, 31%) in order of decreasing polarity. **18**: Colorless glass. HRMS Calcd for $C_{23}H_{34}O_7$: 422.2303. Found: 422.2325. MS *m/z*: 422 (M^+, 1), 333 (38), 317 (21), 99 (100), 55 (19), 45 (91). IR ($CHCl_3$) cm^{-1}: 1710. ^1H NMR δ: 0.98 (1H, ddd, J=13, 13, 4 Hz), 1.19–1.72 (11H, m), 1.74–1.84 (2H, m, including OH), 2.07 (1H, dd, J=15, 9.5 Hz), 2.14–2.24 (2H, m), 2.34 (1H, br d, J=13 Hz), 2.72 (1H, dd, J=7, 2 Hz, H14), 3.35 (3H, s), 3.38–3.46 (1H, m), 3.54–3.60 (2H, m), 3.74–3.84 (2H, m), 3.88–3.99 (3H, m), 4.00–4.09 (1H, m, H16), 4.38 (1H, dd, J=7, 1.5 Hz, H20), 4.62 (2H, s). **19**: Colorless glass. HRMS Calcd for $C_{23}H_{34}O_7$: 422.2303. Found: 422.2292. MS *m/z*: 422 (M^+, 2), 407 (3), 377 (4), 333 (44), 317 (14), 99 (100), 55 (16), 45 (77). IR ($CHCl_3$) cm^{-1}: 1714. ^1H NMR δ: 0.98 (1H, ddd, J=12.5, 12.5, 4 Hz), 1.17–1.72 (10H, m, including OH), 1.39 (1H, dd, J=15.5, 2.5 Hz, H15), 1.79 (1H, br d, J=13 Hz), 1.89 (1H, dd, J=15.5, 9.5 Hz, H15), 1.89–1.95 (1H, m), 2.11 (1H, ddd, J=14, 10, 1.5 Hz), 2.26–2.31 (1H, m, H12), 2.36 (1H, br d, J=12.5 Hz), 2.57 (1H, dd, J=7.5, 2 Hz, H14), 3.35 (3H, s), 3.37–3.46 (1H, m), 3.52–3.62 (2H, m), 3.72–3.83 (2H, m), 3.88–3.98 (3H, m), 4.01–4.10 (1H, m, H16), 4.38 (1H, dd, J=7.5, 1.5 Hz, H20), 4.62 (2H, s).

4.1.9. Hydration of 14 to form 20. Tf_2O (8 μl, 47.6 μmol) was added to a cooled (0 °C) slurry of HgO (10.5 mg, 48.5 μmol) in CH_3CN (2 ml) and the mixture was stirred for 3 min. TMU (11.5 μl, 96.3 μmol) was added to this and the resulting mixture was stirred at 0 °C for 3 min and at 20 °C for 5 min. This solution of $Hg(OTf)_2(TMU)_2$ in CH_3CN (0.20 ml) was added to a solution of **14** (20 mg, 47.8 μmol) in CH_2Cl_2 (1 ml). H_2O (9 μl, 0.50 mmol) and CH_3CN (0.8 ml) were further added and the whole was stirred at 22 °C for 40 h. Quenching with saturated $NaHCO_3$–H_2O, extraction with CH_2Cl_2, usual work-up, and PTLC [hexane–EtOAc (1:1)] yielded **20** (15 mg, 80%) as a colorless glass. HRMS Calcd for $C_{22}H_{32}O_6$: 392.2197. Found: 392.2181. MS *m/z*: 392 (M^+, 1), 347 (10), 303 (6), 245 (6), 229 (8), 91 (10), 89 (8), 73 (16), 45 (100), 43 (54). IR ($CHCl_3$) cm^{-1}: 1699. ^1H NMR δ: 1.50–1.57 (1H, m), 1.61–1.93 (6H, m), 2.00–2.14 (3H, m), 2.10 (3H, s), 2.17–2.44 (5H, m), 2.29 (1H, d, J=17.5 Hz, CH_2Ac), 2.44 (1H, d, J=17.5 Hz, CH_2Ac), 2.69 (1H, ddd, J=18.5, 10.5, 10 Hz, H12), 2.96 (1H, d, J=6.5 Hz, H14), 3.28–3.37 (1H, m), 3.32 (3H, s), 3.46–3.53 (3H, m), 3.68 (1H, d, J=6.5 Hz, H20), 4.56 (2H, s). ^{13}C NMR δ: 18.9 (CH_2), 19.7 (CH_2), 24.0 (CH_2), 28.0 (CH_2), 31.9 (CH_3), 32.7 (CH_2), 35.7 (CH_2), 41.4 (CH_2), 46.5 (C), 47.5 (CH_2, *C*H_2Ac), 51.4 (CH), 52.7 (C), 55.0 (CH_3), 55.9 (CH), 65.0 (CH), 66.3 (CH_2), 70.0 (CH_2), 78.0 (CH), 96.1 (CH_2), 206.4 (C, *C*OMe), 210.0 (C, C13), 211.5 (C, C4).

4.1.10. Aldol reaction of 20 with LDA to form 21. BuLi (1.57 M, 0.97 ml, 1.52 mmol) was added to a cooled (−18 °C) solution of i-Pr_2NH (0.28 ml, 2.00 mmol) in THF (2 ml) and the mixture was stirred under an Ar atmosphere for 10 min. After the mixture had been cooled to −78 °C, a solution of **20** (12 mg, 30.6 μmol) in THF (2 ml) was added and the whole was stirred for 1 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–EtOAc (2:3)] provided a recovery of **20** (2.5 mg, 21%) and **21** (5.5 mg, 46%) as a colorless glass in order of increasing polarity. HRMS Calcd for $C_{22}H_{32}O_6$: 392.2197. Found: 392.2177. MS *m/z*: 392 (M^+, 0.2), 329 (5), 287 (12), 241 (15), 105 (10), 91 (10), 55 (14), 45 (100), 43 (29). IR ($CHCl_3$) cm^{-1}: 1702. ^1H NMR δ: 1.21–1.78 (9H, m, including OH), 1.36 (3H, s), 1.60 (1H, d, J=15.5 Hz, H15), 1.68 (1H, d, J=15.5 Hz, H15), 1.83 (1H, ddd, J=15, 10.5, 1.5 Hz, H11), 2.14 (1H, ddd, J=15, 4, 2 Hz, H11), 2.00 (1H, dd, J=4, 1.5 Hz, H12), 2.25–2.42 (3H, m), 2.46 (1H, br ddd, J=13, 3, 3 Hz), 2.72 (1H, dd, J=7.5, 2 Hz, H14), 3.23–3.37 (1H, m), 3.33 (3H, s), 3.45–3.56 (2H, m), 3.59 (1H, dd, J=7.5, 1.5 Hz, H20), 3.80 (1H, ddd, J=10.5, 4, 4 Hz), 4.57 (2H, s). ^{13}C NMR δ: 19.1 (CH_2), 19.9 (CH_2), 23.2 (CH_2), 27.3 (CH_2), 28.7 (CH_3), 34.1 (CH_2), 41.6 (CH_2), 44.8 (C), 45.2 (CH_2), 48.8 (CH, C9), 54.2 (CH, C12), 55.0 (C), 55.1 (CH_3), 55.5 (CH, C5), 61.4 (CH, C14), 66.5 (CH_2), 71.0 (CH_2), 72.3 (C), 81.5 (CH, C20), 96.2 (CH_2), 211.4 (C), 212.0 (C).

4.2. Oxidation of 22, 32, and 3 to form the $\Delta_{5,6}$ enone and attempted introduction of nitrogen function at C6 (Scheme 4)

4.2.1. Acetylation of 22 to form 23. A solution of **22** (18 mg, 44.4 μmol), Ac_2O (0.30 ml, 0.277 mmol), and pyridine (0.50 ml, 0.510 mmol) in CH_2Cl_2 (1.5 ml) was stirred at 27 °C for 4 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–DME (2:1)] gave **23** (19 mg, 96%) as a colorless glass. HRMS Calcd for $C_{25}H_{37}NO_6$: 447.2619. Found: 447.2621. MS *m/z*: 447 (M^+, 23), 402 (4), 388 (8), 360 (30), 343 (19), 273 (9), 257 (13), 256 (12), 99 (53), 87 (100), 72 (14), 55 (9), 43 (42). IR ($CHCl_3$) cm^{-1}: 1727, 1630. ^1H NMR δ: 1.00 (1H, ddd, J=13, 13, 4 Hz, H1), 1.22 (1H, ddd, J=13, 13, 3.5 Hz, H3), 1.41 (1H, br d, J=5 Hz, H9), 1.49–1.79 (7H, m), 1.86–1.98 (1H, m), 2.05 (3H, s, COCH_3), 2.06 (1H, br d, J=13 Hz, H1), 2.17 (1H, dddd, J=19, 5, 3, 2 Hz, H11),

2.36 (1H, br d, J=19 Hz, H11), 2.46 (1H, d, J=15.5 Hz, CH_2CON), 2.69 (1H, d, J=15.5 Hz, CH_2CON), 2.89 (1H, ddd, J=7.5, 6.5, 1.5 Hz, H14), 2.92 (3H, s), 3.00 (3H, s), 3.49 (1H, ddd, J=11.5, 6, 4 Hz), 3.62 (1H, ddd, J=11.5, 6.5, 4 Hz), 3.73–3.83 (1H, m), 3.83–3.98 (3H, m), 4.15 (1H, ddd, J=11.5, 6, 4 Hz, CH_2OAc), 4.25 (1H, ddd, J=11.5, 6.5, 4 Hz, CH_2OAc), 4.30 (1H, d, J=6.5 Hz, H20), 5.54 (1H, ddd, J=9.5, 3, 3 Hz), 5.66 (1H, dddd, J=9, 5, 7.5, 2, 1.5 Hz). ^{13}C NMR δ: 18.3 (CH_2), 20.8 (CH_2), 21.0 (CH_3, COCH_3), 27.1 (CH_2), 28.8 (CH_2), 34.0 (CH_2), 35.0 (CH_2), 35.3 (CH_3), 37.5 (CH_2), 37.8 (CH_3), 43.7 (C), 48.1 (CH), 48.4 (C), 50.6 (CH), 54.5 (CH), 63.4 (CH_2, CH_2OAc), 67.0 (CH_2), 80.9 (CH), 110.3 (C), 125.2 (CH), 128.9 (CH), 170.8 (C, OCOCH$_3$), 172.6 (C, CONMe$_2$).

4.2.2. Methoxymethylation of 22 to form 24. MOMCl (42 μl, 0.553 mmol) was added to a solution of **22** (44 mg, 0.109 mmol) and *N*,*N*-diisopropylethylamine (*i*-Pr$_2$NEt) in CH_2Cl_2 (4 ml) at 0 °C under an Ar atmosphere. The mixture was stirred at that temperature for 30 min, and at 27 °C for 19 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O. Usual work-up and separation by PTLC [hexane–DME (3:1)] furnished **24** (46 mg, 94%) as a colorless glass. HRMS Calcd for $C_{25}H_{39}NO_6$: 449.2775. Found: 449.2790. MS *m*/*z*: 449 (M^+, 38), 404 (11), 388 (9), 360 (39), 343 (66), 257 (39), 99 (100), 87 (55), 72 (25), 45 (80). IR ($CHCl_3$) cm^{-1}: 1625. ^{1}H NMR δ: 1.00 (1H, ddd, J=13, 13, 4 Hz, H1), 1.22 (1H, ddd, J=13, 13, 3.5 Hz), 1.41 (1H, br d, J=5 Hz), 1.48–1.77 (7H, m), 1.85–2.00 (1H, m), 2.07 (1H, br d, J=13 Hz), 2.17 (1H, dddd, J=19, 5, 2.5, 2.5 Hz), 2.35 (1H, br d, J=19 Hz), 2.46 (1H, d, J=15.5 Hz), 2.70 (1H, d, J=15.5 Hz), 2.88 (1H, ddd, J=7, 6.5, 1.5 Hz), 2.92 (3H, s), 3.00 (3H, s), 3.36 (3H, s, OCH$_2$OCH_3), 3.42–3.50 (1H, m), 3.52–3.60 (1H, m), 3.62–3.71 (2H, m, CH_2OMOM), 3.73–3.81 (1H, m), 3.83–3.97 (3H, m), 4.28 (1H, d, J=6.5 Hz), 4.64 (1H, d, J=6.5 Hz, OCH_2OCH$_3$), 4.67 (1H, d, J=6.5 Hz, OCH_2OCH$_3$), 5.52 (1H, ddd, J=9.5, 3, 2.5 Hz), 5.68 (1H, br dd, J=9.5, 7 Hz). ^{13}C NMR δ: 18.3 (CH_2), 20.9 (CH_2), 27.1 (CH_2), 28.8 (CH_2), 34.0 (CH_2), 35.0 (CH_2), 35.3 (CH_3), 37.5 (CH_2), 37.8 (CH_3), 43.7 (C), 48.3 (CH), 48.4 (C), 50.7 (CH), 54.6 (CH), 55.0 (CH_3, OCH_3), 63.8 (CH_2), 65.4 (CH_2), 66.7 (CH_2, CH_2OMOM), 68.7 (CH_2, CH_2CH_2OMOM), 81.3 (CH), 96.4 (CH_2, OCH_2OMe), 110.3 (C), 125.0 (CH), 129.1 (CH), 172.6 (C).

4.2.3. Deacetalization of 23, 24 to form 25, 26, respectively. The procedure for the preparation of **23** is described as a representative example. *p*-TsOH·H_2O (2 mg, 10.5 μmol) was added to a cooled (0 °C) solution of **48** (18 mg, 40.3 μmol) in acetone (3 ml) and the mixture was stirred at 26 °C for 3.5 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–DME (5:2)] provided **25** (15.5 mg, 96%) as a colorless glass. HRMS Calcd for $C_{23}H_{33}NO_5$: 403.2357. Found: 403.2351. MS *m*/*z*: 403 (M^+, 16), 344 (8), 316 (30), 299 (12), 87 (100), 72 (16), 45 (21), 43 (42). IR ($CHCl_3$) cm^{-1}: 1733, 1695, 1630. ^{1}H NMR δ: 1.49 (1H, ddd, J=13, 13, 4.5 Hz, H1), 1.56–2.06 (7H, m), 2.20–2.34 (5H, m), 2.04 (3H, s), 2.42 (1H, br d, J=19.5 Hz), 2.56 (1H, d, J=15.5 Hz), 2.63 (1H, d, J=15.5 Hz), 2.84 (1H, br ddd, J=7.5, 6, 1.5 Hz), 2.93 (3H, s), 3.01 (3H, s), 3.31 (1H, ddd, J=11, 6, 4.5 Hz), 3.47 (1H, ddd, J=11, 4, 4 Hz), 3.61 (1H, d, J=6 Hz, H20), 4.03–4.20 (2H, m), 5.57 (1H, br ddd, J=9.5, 3, 2.5 Hz), 5.64 (1H, dddd, J=9.5, 7, 1.5, 1.5 Hz). ^{13}C NMR δ: 19.0 (CH_2), 20.9 (CH_3), 24.7 (CH_2), 27.4 (CH_2), 28.6 (CH_2), 33.3 (CH_2), 35.3 (CH_3), 37.4 (CH_2), 37.8 (CH_3), 41.6 (CH_2, C3), 43.8 (C), 48.8 (CH), 53.1 (CH), 53.8 (C), 56.5 (CH, C5), 63.5 (CH_2), 66.9 (CH_2), 81.9 (CH), 126.0 (CH), 127.6 (CH), 170.7 (C), 172.3 (C), 212.5 (C, C4). In the same manner, **24** (18 mg, 95%) was obtained as a colorless glass from **26** (21 mg, 46.8 mmol) after PTLC [hexane–DME (3:1)]. HRMS Calcd for $C_{23}H_{35}NO_5$: 405.2513. Found: 405.2520. MS *m*/*z*: 405 (M^+, 31), 373 (22), 344 (10), 316 (59), 299 (40), 87 (100), 72 (34), 45 (98). IR ($CHCl_3$) cm^{-1}: 1698, 1635. ^{1}H NMR δ: 1.49 (1H, ddd, J=13, 12, 4.5 Hz, H1), 1.50–2.03 (7H, m), 2.16–2.35 (5H, m), 2.47 (1H, br d, J=19.5 Hz), 2.57 (1H, d, J=15.5 Hz), 2.63 (1H, d, J=15.5 Hz), 2.84 (1H, ddd, J=7, 6.5, 1.5 Hz), 2.93 (3H, s), 3.01 (3H, s), 3.24–3.35 (1H, m), 3.34 (3H, s), 3.40–3.49 (1H, m), 3.55–3.60 (2H, m), 3.63 (1H, d, J=6.5 Hz, H20), 4.59 (1H, d, J=6.5 Hz), 4.62 (1H, d, J=6.5 Hz), 5.57 (1H, ddd, J=9.5, 3, 3 Hz), 5.67 (1H, dddd, J=9.5, 7, 1.5, 1.5 Hz). ^{13}C NMR δ: 19.1 (CH_2), 24.8 (CH_2), 27.4 (CH_2), 28.7 (CH_2), 33.3 (CH_2), 35.3 (CH_3), 37.5 (CH_2), 37.8 (CH_3), 41.5 (CH_2, C3), 43.8 (C), 48.9 (CH), 53.1 (CH), 53.7 (C), 55.0 (CH_3), 56.5 (CH, C5), 66.6 (CH_2), 68.6 (CH_2), 81.9 (CH), 96.3 (CH_2), 125.8 (CH), 128.0 (CH), 172.4 (C), 212.5 (C, C4).

4.2.4. Preparation of 28 and 29 from 25. NaI (42 mg, 0.280 mmol) and TMSCl (35 μl, 0.276 mmol) were added successively to a cooled (0 °C) solution of **25** (14 mg, 34.7 μmol) and HMDS (110 μl, 0.529 mmol) in CH_3CN (3 ml) under an Ar atmosphere. After having been stirred at that temperature for 10 min, the mixture was refluxed with stirring for 2 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Consecutive washing of the organic layer with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O, and usual work-up gave a crude enol silyl ether (**27**, 19 mg). The residue was dissolved in THF (2.5 ml) at 0 °C and NBS (10 mg, 56.2 μmol) was added to this. The mixture was stirred at 0 to 27 °C for 4 h. Saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (1:1)] provided crude **29** (5 mg) and **28** (5.5 mg, 33%) in order of increasing polarity. The crude **29** was further purified by PTLC (CH_2Cl_2) to give **29** (3.5 mg, 21%). ^{1}H NMR of crude **27** δ: 0.15 (9H, s), 1.29–1.40 (1H, m), 1.52–2.50 (12H, m), 2.04 (3H, s), 2.59 (1H, d, J=16 Hz), 2.65 (1H, d, J=16 Hz), 2.76 (1H, br dd, J=6, 5 Hz), 2.93 (3H, s), 3.00 (3H, s), 3.46 (1H, ddd, J=11, 6, 4 Hz), 3.56 (1H, ddd, J=11, 5, 4 Hz), 3.89 (1H, d, J=6 Hz, H20), 4.09–4.24 (2H, m), 5.51–5.66 (2H, m). **28**: Colorless glass. HRMS Calcd for $C_{23}H_{32}BrNO_5$: 483.1443, 481.1463. Found: 483.1456, 481.1459. MS *m*/*z*: 483, 481 (M^+, 1, 1), 402 (9), 397, 395 (4, 5), 314 (10), 297 (8), 211 (11), 87 (100), 72 (17), 45 (15), 43 (44). IR ($CHCl_3$) cm^{-1}: 1737, 1713, 1634. ^{1}H NMR δ: 1.50–1.67 (3H, m), 1.88 (1H, ddd, J=13.5, 8, 2 Hz), 1.90–2.00 (2H, m), 2.05–2.15 (1H, m), 2.08 (3H, s), 2.17–2.31 (3H, m), 2.37 (1H, br d, J=15 Hz), 2.39 (1H, d,

J=15.5 Hz), 2.70 (1H, d, J=15.5 Hz), 2.86 (1H, ddd, J=15, 7.5, 2 Hz), 2.91 (3H, s), 2.96 (3H, s), 3.12 (1H, ddd, J=7, 6.5, 1.5 Hz, H14), 3.35 (1H, ddd, J=15, 13, 7.5 Hz), 3.69 (2H, dd, J=5, 5 Hz), 4.16–4.29 (2H, m), 4.19 (1H, dd, J=6.5, 1 Hz, H20), 5.57 (1H, ddd, J=9.5, 3.5, 3 Hz), 5.72 (1H, dddd, J=9.5, 7, 2, 2 Hz). ^{13}C NMR δ: 21.0 (CH_3), 21.7 (CH_2), 24.8 (CH_2, C6), 27.0 (CH_2), 31.4 (CH_2, C1), 34.0 (CH_2), 35.3 (CH_3), 35.8 (CH_2), 37.3 (CH_2), 37.7 (CH_3), 43.7 (C), 45.5 (CH), 46.5 (CH), 56.3 (C), 63.5 (CH_2), 67.7 (CH_2), 80.3 (C, Br–C), 85.8 (CH), 124.4 (CH), 128.6 (CH), 170.7 (C), 171.6 (C), 203.4 (C). **29**: Colorless glass. HRMS Calcd for $C_{23}H_{32}BrNO_5$: 483.1443, 481.1463. Found: 483.1461, 481.1457. MS m/z: 483, 481 (M^+, 0.4, 0.4), 401 (3), 314 (4), 297 (4), 227 (5), 211 (7), 87 (100), 72 (10), 45 (11), 43 (27). IR ($CHCl_3$) cm^{-1}: 1737, 1709, 1629. ^{1}H NMR δ: 1.76 (1H, ddd, J=12.5, 12.5, 4.5 Hz), 1.78–2.14 (6H, m), 2.04 (3H, s), 2.22–2.44 (4H, m), 2.47–2.52 (1H, m), 2.48 (1H, d, J=15.5 Hz), 2.74 (1H, d, J=15.5 Hz), 2.94 (3H, s), 3.01 (3H, s), 3.16 (1H, ddd, J=7, 6, 1.5 Hz, H14), 3.28 (1H, dt, J=11, 5.5 Hz), 3.40–3.53 (1H, m), 3.46 (1H, dt, J=11, 4 Hz), 3.60 (1H, dd, J=6, 1 Hz, H20), 4.10 (2H, dd, J=5.5, 4 Hz), 5.59 (1H, ddd, J=9.5, 3, 3 Hz, H12), 5.67 (1H, dddd, J=9.5, 7, 1.5, 1.5 Hz, H13).

4.2.5. Dehydrobromination of 28 and 29 to form 30. DBU (9 μl, 60.3 μmol) was added to a solution of **28** (5.5 mg, 11.4 μmol) in benzene (2 ml) and the mixture was refluxed with stirring for 1 h. Saturated NH_4Cl–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–DME (2:1)] afforded **30** (4 mg, 87%) as a colorless glass. HRMS Calcd for $C_{23}H_{31}NO_5$: 401.2200. Found: 401.2197. MS m/z: 401 (M^+, 5), 314 (7), 297 (19), 227 (12), 211 (18), 87 (100), 72 (20), 45 (15), 43 (57). IR ($CHCl_3$) cm^{-1}: 1735, 1674, 1638, 1604. ^{1}H NMR δ: 1.44 (1H, ddd, J=13, 11.5, 3.5 Hz, H1), 1.69–1.91 (3H, m), 2.03 (3H, s), ca. 2.12–2.22 (2H, m), 2.23 (1H, ddd, J=18, 12.5, 6.5 Hz, H3), 2.36 (1H, br d, J=19 Hz), 2.48 (1H, dddd, J=18, 4.5, 3.5, 2 Hz, H3), 2.57 (1H, d, J=16 Hz), 2.61 (2H, d, J=4 Hz, H7×2), 2.82 (1H, d, J=16 Hz), ca. 2.92–3.03 (1H, m), 2.94 (3H, s), 3.01 (3H, s), 3.43 (1H, ddd, J=11, 6.5, 4 Hz), 3.54 (1H, ddd, J=11, 5, 4 Hz), 3.74 (1H, d, J=6 Hz), 4.09–4.23 (2H, m), 5.52–5.69 (2H, m), 6.84 (1H, t, J=4 Hz, H6). ^{13}C NMR δ: 19.7 (CH_2), 20.9 (CH_3), 25.8 (CH_2), 26.4 (CH_2), 35.3 (CH_3), 36.9 (CH_2), 37.6 (CH_3), 39.2 (CH_2, C3), 39.6 (CH_2, C7), 40.8 (C), 48.7 (C), 49.3 (CH), 50.4 (CH), 63.4 (CH_2), 67.4 (CH_2), 91.3 (CH), 125.2 (CH), 128.0 (CH), 137.6 (CH, C6), 148.3 (C, C5), 170.6, (C), 171.4 (C), 197.3 (C, C4). In the same manner, **30** (2 mg, 80%) was obtained from **29** (3 mg, 6.22 μmol) on treatment with DBU (5 μl, 33.5 μmol) in boiling benzene (2 ml) for 2 h.

4.2.6. Preparation of 30 from 25 without isolation of the intermediates. A solution of **25** (60 mg, 0.149 mmol) and HMDS (0.38 ml, 1.83 mmol) in CH_3CN (5 ml) was stirred as above with NaI (179 mg, 1.19 mmol) and TMSCl (151 μl, 1.19 mmol) under an Ar atmosphere at 0 °C for 5 min, and at reflux for 2 h. The same work-up gave a residue (73 mg). This was dissolved in THF (4 ml) and the solution was stirred with NBS (40 mg, 0.225 mmol) at 0–20 °C for 4 h. The same work-up afforded a mixture of bromides (87 mg), which was then treated with DBU (89 μl, 0.596 mmol) in refluxing benzene (5 ml) for 1 h to give **30** (37 mg, 62% overall) after purification by PTLC as above.

4.2.7. Preparation of 31 from 26. Methylmagnesium bromide (MeMgBr, 3 M in Et_2O, 0.35 ml, 1.05 mmol) was added to a solution of diisopropylamine (i-Pr_2NH, 0.15 ml, 1.07 mmol) in Et_2O (6 ml) under an Ar atmosphere. After the mixture had been stirred at 27 °C for 14 h, a solution of **26** (17 mg, 42.0 μmol) in Et_2O (4 ml) was added and the resulting mixture was stirred for 10 min. TMSCl (0.32 ml, 2.52 mmol), Et_3N (0.41 ml, 2.95 mmol), and hexamethylphosphoramide (HMPA, 73 μl, 0.420 mmol) were added and the whole was further stirred at 27 °C for 4 h and at reflux for 1.5 h. After the mixture had been cooled in an ice bath, saturated $NaHCO_3$–H_2O was added and the whole was extracted with EtOAc. Consecutive washing of the organic layer with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O, and usual work-up gave a crude enol silyl ether (28 mg). The residue was dissolved in THF (3 ml) at 0 °C and NBS (15 mg, 84.3 μmol) was added to this, then the mixture was stirred under an Ar atmosphere at 0–27 °C for 14 h. Saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2 to give a residue (33 mg). A solution of the residue (33 mg) was stirred with DBU (12 μl, 80.4 μmol) at reflux for 1 h. The same work-up as before and purification by PTLC [hexane–EtOAc (1:1)] provided **31** (4.5 mg, 27% overall) as a colorless glass. HRMS Calcd for $C_{23}H_{33}NO_5$: 403.2357. Found: 403.2364. MS m/z: 403 (M^+, 3), 316 (5), 297 (24), 271 (11), 227 (16), 211 (25), 210 (20), 87 (69), 72 (39), 45 (100). IR ($CHCl_3$) cm^{-1}: 1673, 1637, 1604. ^{1}H NMR δ: 1.39–1.50 (1H, m), 1.73–1.89 (3H, m), 2.11–2.26 (3H, m), 2.36 (1H, br d, J=19.5 Hz, H11), 2.47 (1H, dddd, J=18, 4, 4, 2 Hz, H3), 2.61 (2H, d, J=4 Hz, H7×2), 2.57 (1H, d, J=16 Hz), 2.82 (1H, d, J=16 Hz), 2.94 (3H, s), ca. 2.94–3.01 (1H, m), 3.01 (3H, s), 3.34 (3H, s), 3.36–3.44 (1H, m), 3.47–3.55 (1H, m), 3.58–3.68 (2H, m), 3.75 (1H, d, J=6 Hz), 4.63 (2H, s), 5.55–5.67 (2H, m), 6.83 (1H, t, J=4 Hz, H6). ^{13}C NMR δ: 19.7 (CH_2), 25.9 (CH_2), 26.4 (CH_2), 35.3 (CH_3), 37.0 (CH_2), 37.6 (CH_3), 39.1 (CH_2, C3), 39.6 (CH_2, C7), 40.8 (C), 48.7 (C), 49.4 (CH), 50.5 (CH), 55.0 (CH_3), 67.0 (CH_2), 69.1 (CH_2), 91.6 (CH), 96.4 (CH_2), 125.0 (CH), 128.3 (CH), 137.5 (CH, C6), 148.4, (C, C5), 171.5 (C), 197.4 (C, C4).

4.2.8. Deacetalization of 32 to form 33. In the same manner as described for the preparation of **25** from **23** (Section 4.2.3), **32** (28 mg, 78.0 μmol) was stirred with p-TsOH·H_2O (3 mg, 15.8 μmol) in acetone (3 ml) to afford **33** (23 mg, 94%) as a colorless glass after PTLC [hexane–DME (3:2)]. HRMS Calcd for $C_{19}H_{25}NO_3$: 315.1833. Found: 315.1830. MS m/z: 315 (M^+, 3), 287 (11), 259 (13), 201 (8), 172 (8), 129 (14), 87 (100), 72 (21), 45 (35). IR ($CHCl_3$) cm^{-1}: 1728, 1707 (sh), 1633. ^{1}H NMR δ: 1.54–1.72 (2H, m), 1.76–2.10 (6H, m), 2.27 (1H, ddd, J=13.5, 13.5, 6, 1 Hz, H3), 2.32–2.64 (5H, m), 2.45 (1H, d, J=15.5 Hz), 2.58 (1H, d, J=15.5 Hz), 2.83 (1H, dd, J=7, 2 Hz, C14), 2.95 (3H, s), 3.03 (3H, s), 5.67–5.75 (1H, m), 5.77–5.84 (1H, m). ^{13}C NMR δ: 20.1 (CH_2), 22.6 (CH_2), 26.4 (CH_2), 29.5 (CH_2), 33.2 (CH_2), 35.0 (CH_2), 35.4 (CH_3), 37.8 (CH_3), 40.6 (CH_2, C3), 41.1 (C), 50.2 (CH), 54.4 (CH, C5), 56.9 (CH), 58.1 (C), 124.8 (CH), 129.0 (CH), 171.3 (C), 209.5 (C, C4), 211.0 (C, C20).

4.2.9. Preparation of 34 from 33. In the same manner as described for the preparation of **28** and **29** from **25** (Section 4.2.4), **33** (23 mg, 73.0 μmol) was treated with NaI (88 mg, 0.587 mmol), TMSCl (74 μl, 58.4 μmol), and HMDS (0.23 ml, 1.11 mmol) in CH_3CN (3 ml) to give a crude silyl enol ether (30 mg), which was then stirred with NBS (20 mg, 0.112 mmol) in THF (3 ml) to provide **34** (20 mg, 70%) as a colorless glass after PTLC (CH_2Cl_2). HRMS Calcd for $C_{19}H_{24}BrNO_3$: 395.0919, 393.0939. Found: 395.0910, 393.0949. MS *m/z*: 395, 393 (M^+, 2, 2), 367, 365 (1, 1), 314 (18), 286 (46), 227 (19), 199 (100), 87 (53), 72 (35), 45 (50). IR ($CHCl_3$) cm^{-1}: 1729, 1715 (sh), 1636. ^1H NMR δ: 1.82–2.05 (4H, m), 2.07–2.29 (3H, m), 2.30 (1H, br d, J=19.5 Hz), 2.35–2.43 (1H, m, H3), 2.41–2.52 (1H, m), 2.53 (2H, s), 2.54–2.72 (1H, m), 2.93–2.99 (1H, m, H9), 2.96 (3H, s), 3.04 (3H, s), 3.17 (1H, dd, J=7.5, 2.5 Hz), 3.36 (1H, ddd, J=14.5, 14.5, 6.5 Hz, H3), 5.76 (1H, br ddd, J=9, 7.5, 1.5 Hz), 5.83 (1H, ddd, J=9, 3, 2.5 Hz). ^{13}C NMR δ: 21.0 (CH_2), 26.2 (CH_2), 26.6 (CH_2), 30.6 (CH_2), 30.9 (CH_2), 34.5 (CH_2), 34.8 (CH_2), 35.4 (CH_3), 37.8 (CH_3), 41.0 (C), 47.3 (CH), 56.5 (CH), 62.1 (C, C10), 73.5 (C, Br–C), 125.0 (CH), 128.8 (CH), 170.8 (C), 202.7 (C, C4), 208.0 (C, C20).

4.2.10. Dehydrobromination of 34 to form 35. In the same manner as described for the preparation of **30** from **28** and **29** (Section 4.2.5), **34** (20 mg, 50.8 μmol) was stirred with DBU (38 μl, 0.255 mmol) in refluxing benzene (3 ml) for 1 h to afford **35** (11 mg, 69%) as a colorless glass after PTLC [hexane–DME (2:1)]. HRMS Calcd for $C_{19}H_{23}NO_3$: 313.1677. Found: 313.1678. MS *m/z*: 313 (M^+, 8), 285 (3), 226 (58), 198 (81), 170 (26), 154 (47), 141 (33), 87 (100), 72 (39), 45 (77). IR ($CHCl_3$) cm^{-1}: 1725, 1678, 1636. ^1H NMR δ: 1.77–1.94 (2H, m), 2.12–2.21 (1H, m), 2.12–2.32 (1H, m), 2.32–2.59 (5H, m), 2.48 (1H, dd, J=21, 3.5 Hz, H7), 2.56 (1H, d, J=16.5 Hz), 2.63 (1H, d, J=16.5 Hz), 2.81 (1H, dd, J=21, 4.5 Hz, H7), 2.94 (3H, s), 3.02 (1H, br dd, J=7, 1.5 Hz), 3.02 (3H, s), 5.76 (1H, ddd, J=9, 7, 1.5 Hz), 5.83 (1H, ddd, J=9, 2.5, 2.5 Hz), 6.99 (1H, dd, J=4.5, 3.5 Hz, H6). ^{13}C NMR δ: 19.0 (CH_2), 25.9 (CH_2), 27.2 (CH_2), 34.7 (CH_2), 35.3 (CH_3), 37.6 (CH_3), 38.2 (CH_2, C7), 38.9 (CH_2, C3), 39.1 (C), 46.5 (CH), 54.1 (C), 58.4 (CH), 125.8 (CH), 128.4 (CH), 136.5 (C, C5), 138.5 (CH, C6), 170.6 (C), 195.8 (C, C4), 204.8 (C, C20).

4.2.11. Acetylation of 3 to form 37. In the same manner as described for the preparation of **23** from **22** (Section 4.2.1), **3** (37 mg, 91.6 μmol) was stirred with Ac_2O (0.20 ml, 2.12 mmol) and pyridine (0.30 ml, 3.88 mmol) in CH_2Cl_2 (1.5 ml) at 16 °C for 6 h. The same work-up and separation by PTLC [hexane–EtOAc (3:1)] gave **37** (39 mg, 95%) as a colorless glass. HRMS Calcd for $C_{26}H_{38}O_6$: 446.2666. Found: 446.2637. MS *m/z*: 446 (M^+, 0.1), 359 (0.2), 344 (0.4), 342 (0.4), 317 (0.3), 257 (1), 240 (8), 213 (2), 91 (6), 87 (100), 57 (22), 43 (29). IR ($CHCl_3$) cm^{-1}: 1719, 1701. ^1H NMR δ: 1.18 (9H, s), 1.42–2.11 (10H, m), 2.04 (3H, s), 2.19–2.34 (5H, m), 2.41 (1H, br d, J=19 Hz), 2.50 (1H, br dd, J=6, 6 Hz), 3.30 (1H, ddd, J=11, 5.5, 5.5 Hz, CH_2CH$_2$OAc), 3.45 (1H, ddd, J=11, 4, 4 Hz, CH_2CH$_2$OAc), 3.59 (1H, d, J=6 Hz), 4.00 (1H, ddd, J=11, 8.5, 6.5 Hz, CH_2OPiv), 4.07–4.15 (2H, m, CH_2OAc), 4.13 (1H, ddd, J=11, 9, 6 Hz, CH_2OPiv), 5.53–5.66 (2H, m). ^{13}C NMR δ: 19.1 (CH_2), 20.8 (CH_3), 24.7 (CH_2), 27.2 ($CH_3\times3$ and CH_2, Piv and C11), 28.7 (CH_2), 33.6 (CH_2), 33.9 (CH_2), 38.6 (C), 41.5 (CH_2), 42.6 (C), 47.8 (CH), 53.8 (CH), 54.0 (C), 56.5 (CH), 62.5 (CH_2, CH_2OPiv), 63.4 (CH_2, CH_2OAc), 66.9 (CH_2, CH_2CH$_2$OAc), 82.0 (CH), 126.0 (CH), 126.4 (CH), 170.7 (C, COMe), 178.4 (C, COMe$_3$), 212.2 (C, C4).

4.2.12. Preparation of 38 from 37. In the same manner as described for the preparation of **30** from **25** (Section 4.2.6), **37** (44 mg, 98.7 μmol) was led to the enone **38** (64% overall) in three steps. **38**: Colorless glass. HRMS Calcd for $C_{26}H_{36}O_6$: 444.2510. Found: 444.2509. MS *m/z*: 444 (M^+, 0.4), 340 (2), 315 (1), 238 (2), 211 (10), 87 (100), 57 (27), 43 (39). IR ($CHCl_3$) cm^{-1}: 1721, 1678, 1607. ^1H NMR δ: 1.20 (9H, s), 1.43 (1H, ddd, J=13, 12, 3 Hz), 1.55 (1H, br d, J=5 Hz, H9), 1.67–1.91 (3H, m), 2.04 (3H, s), 2.11–2.30 (4H, m), 2.33 (1H, dd, J=21, 3 Hz, H7), 2.35 (1H, br d, J=19 Hz), 2.43–2.56 (2H, m), 2.66 (1H, dd, J=21, 5 Hz, H7), 3.42 (1H, ddd, J=11, 6.5, 4.5 Hz), 3.52 (1H, ddd, J=11, 5, 4 Hz), 3.69 (1H, d, J=6 Hz), 4.03–4.23 (4H, m), 5.54–5.64 (2H, m), 6.83 (1H, dd, J=5, 3 Hz, H6). ^{13}C NMR δ: 19.6 (CH_2), 20.9 (CH_3), 25.8 (CH_2), 26.3 (CH_2), 27.2 ($CH_3\times3$), 33.2 (CH_2), 38.6 (C), 39.1 (CH_2), 39.8 (CH_2, C7), 40.3 (C), 48.9 (C), 49.5 (CH, C9), 50.0 (CH), 61.9 (CH_2), 63.3 (CH_2), 67.4 (CH_2), 91.6 (CH), 125.5 (CH), 126.6 (CH), 136.7 (CH, C6), 148.6 (C, C5), 170.6 (C), 178.3 (C), 197.2 (C).

4.2.13. Preparation of 39 from 3. In the same manner as described for the preparation of **28** and **29** from **25** (Section 4.2.4), **3** (525 mg, 1.30 μmol) was led to the crude α-bromoketone (802 mg) on treatment with NaI (585 mg, 3.90 mmol), TMSCl (0.49 ml, 3.98 mmol), and HMDS (1.10 ml, 6.23 mmol) in CH_3CN (22 ml), and then with NBS (462 mg, 2.60 mmol) in THF (25 ml). The crude bromoketone was dissolved in THF (12 ml) and 2.5% HCl–H_2O (1.00 ml) was added to this at 0 °C. After the mixture had been stirred at 0 °C for 10 min, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up gave a residue (766 mg). The residue was dissolved in CH_2Cl_2 (25 ml) and to this was added *i*-Pr_2NEt (3.38 ml, 19.4 mmol). The resulting mixture was cooled to −20 °C under an Ar atmosphere, and a solution of MOMCl (0.74 ml, 9.74 mmol) in CH_2Cl_2 (5 ml) was slowly added to this during 30 min. The mixture was stirred at −20 to 0 °C for 16 h, and the reaction was quenched by the addition of saturated $NaHCO_3$–H_2O. The whole was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O, and then was treated as usual to afford a residue, which was roughly purified by SiO_2 column chromatography [25 g, hexane–EtOAc (7:1 to 4:1)] to give a mixture of two stereoisomers (511 mg). The mixture (511 mg) was dissolved in benzene (30 ml) and DBU (289 μl, 1.02 mmol) was added to this. The resulting mixture was stirred under reflux for 1 h, and was treated as before. Purification by PTLC [hexane–EtOAc (4:1)] yielded **39** (331 mg, 57% overall from **3**) as a colorless viscous syrup. HRMS Calcd for $C_{26}H_{38}O_6$: 446.2666. Found: 446.2678. MS *m/z*: 446 (M^+, 2), 414 (2), 401 (2), 340 (6), 317 (3), 285 (5), 255 (6), 211 (35), 73 (18), 57 (62), 45 (100),

41 (22). IR ($CHCl_3$) cm^{-1}: 1719, 1675, 1606. ^1H NMR δ: 1.20 (9H, s), 1.38–1.47 (1H, m), 1.54 (1H, br d, J=5 Hz), 1.74 (1H, ddd, J=13.5, 8, 6 Hz), 1.78–1.88 (2H, m), 2.15–2.33 (5H, m), 2.33 (1H, dd, J=21, 3 Hz, H7), 2.48 (1H, dddd, J=18.5, 4, 4, 2 Hz, H3), 2.52–2.58 (1H, m), 2.65 (1H, dd, J=21, 5 Hz, H7), 3.35 (3H, s), 3.40 (1H, ddd, J=10.5, 6, 4.5 Hz), 3.50 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.58–3.69 (2H, m), 3.71 (1H, d, J=6 Hz), 4.08 (1H, ddd, J=11, 8, 6.5 Hz), 4.19 (1H, ddd, J=11, 8, 6 Hz), 4.63 (2H, s), 5.56–5.66 (2H, m), 6.82 (1H, dd, J=5, 3 Hz, H6). ^{13}C NMR δ: 19.7 (CH_2), 25.9 (CH_2), 26.3 (CH_2), 27.2 ($CH_3\times3$), 33.2 (CH_2), 38.6 (C), 39.1 (CH_2, C3), 39.8 (CH_2, C7), 40.3 (C), 48.9 (C), 49.6 (CH), 50.1 (CH), 55.1 (CH_3), 61.9 (CH_2), 66.7 (CH_2), 69.1 (CH_2), 91.9 (CH), 96.4 (CH_2), 125.3 (CH), 127.0 (CH), 136.7 (CH, C6), 148.8 (C, C5), 178.4 (C), 197.3 (C, C4). In the same manner, **36** (22 mg, 54.5 μmol) was converted to **39** (11 mg, 45% overall from **36**) in five steps.

4.2.14. Reduction of 38, 39 to form 40, 41, respectively. Preparation of **40** from **38** is described as a representative example. $CeCl_3\cdot7H_2O$ (29 mg, 77.9 μmol) and $NaBH_4$ (3 mg, 78.9 μmol) were added in this order to a cooled (0 °C) solution of **38** (26 mg, 58.6 μmol) in MeOH (3 ml). After the mixture had been stirred at 0 °C for 10 min, saturated NH_4Cl–H_2O and saturated$NaHCO_3$–H_2O were added and the mixture was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (7:4)] provided **40** (24 mg, 92%) as a slightly unstable colorless glass. HRMS Calcd for $C_{26}H_{38}O_6$: 446.2666. Found: 446.2674. MS m/z: 446 (M^+, 1), 428 (1), 340 (1), 324 (1), 222 (9), 157 (15), 87 (100), 57 (41), 43 (57). IR ($CHCl_3$) cm^{-1}: 1721. ^1H NMR δ: 1.20 (9H, s), 1.36–1.49 (2H, m), 1.49–1.66 (4H, m, including OH), 1.73 (1H, ddd, J=13.5, 8.5, 6 Hz), 1.86–1.98 (2H, m), 2.05 (3H, s), 2.11–2.28 (3H, m), 2.31 (1H, br d, J=18.5 Hz), ca. 2.47–2.53 (1H, m), 2.49 (1H, ddd, J=18.5, 4.5, 2 Hz, H7), 3.45 (1H, ddd, J=11, 5.5, 4.5 Hz), 3.52 (1H, ddd, J=11, 5, 4.5 Hz), 3.68 (1H, d, J=6 Hz), 4.07 (1H, ddd, J=11, 8.5, 6.5 Hz), ca. 4.10–4.21 (2H, m), 4.19 (1H, ddd, J=11, 8.5, 6 Hz), 4.21–4.29 (1H, m, H4), 5.49–5.53 (1H, m, H6), 5.53–5.64 (2H, m). ^{13}C NMR δ: 18.8 (CH_2), 20.9 (CH_3), 23.8 (CH_2), 26.4 (CH_2), 27.2 ($CH_3\times3$), 31.4 (CH_2), 33.7 (CH_2), 38.6 (C), 38.7 (CH_2), 40.5 (C), 48.9 (C), 49.4 (CH), 50.2 (CH), 62.2 (CH_2), 63.4 (CH_2), 67.5 (CH_2), 69.1 (CH, C4), 92.5 (CH), 117.8 (CH, C6), 125.4 (CH), 127.2 (CH), 152.7 (C, C5), 170.6 (C), 178.4 (C). In the same manner, **41** (15 mg, 93%) was obtained from **39** (16 mg, 35.9 μmol) as a colorless glass. HRMS Calcd for $C_{26}H_{40}O_6$: 448.2823. Found: 448.2804. MS m/z: 448 (M^+, 0.2), 430 (0.6), 416 (2), 403 (3), 342 (2), 258 (3), 240 (8), 222 (16), 195 (17), 157 (19), 57 (64), 45 (100), 41 (22). IR ($CHCl_3$) cm^{-1}: 1719. ^1H NMR δ: 1.20 (9H, s), 1.33–1.48 (2H, m), 1.55–1.70 (4H, m, including OH), 1.72 (1H, ddd, J=13.5, 8.5, 6 Hz), 1.88–2.00 (2H, m), 2.11–2.34 (4H, m), 2.48 (1H, ddd, J=19, 4.5, 2 Hz, H7), 2.50 (1H, dd, J=6, 6 Hz), 3.35 (3H, s), 3.42 (1H, dt, J=11, 5 Hz), 3.49 (1H, dt, J=11, 5 Hz), 3.62 (2H, dd, J=5, 5 Hz), 3.70 (1H, d, J=6 Hz), 4.08 (1H, ddd, J=11, 8.5, 6.5 Hz), 4.19 (1H, ddd, J=11, 8.5, 6 Hz), 4.21–4.31 (1H, m, H4), 4.63 (2H, s), 5.47–5.52 (1H, m, H6), 5.52–5.65 (2H, m). ^{13}C NMR δ: 18.9 (CH_2), 23.8 (CH_2), 26.4 (CH_2), 27.2 ($CH_3\times3$), 31.5 (CH_2, C3), 33.7 (CH_2), 38.6 (C), 38.7 (CH_2, C7), 40.4 (C), 49.0 (C), 49.4 (CH, C9), 50.3 (CH, C14), 55.1 (CH_3), 62.2 (CH_2), 66.8 (CH_2), 69.0 (CH, C4), 69.3 (CH_2), 92.8 (CH), 96.4 (CH_2), 117.5 (CH, C6), 125.2 (CH), 127.5 (CH), 152.9 (C, C5), 178.4 (C).

4.2.15. Rearrangement of 40 to form 42. DBU (40 μl, 0.268 mmol) and CCl_3CN (54 μl, 0.538 mmol) were added to a solution of **40** (12 mg, 26.9 μmol) in CH_2Cl_2 (3 ml) and the mixture was stirred at 22 °C for 15 h. Saturated NH_4Cl–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and PTLC [benzene–EtOAc (34:1)] gave crude **42** (10 mg), which was further purified by PTLC [hexane–EtOAc (5:1)] to yield **42** (9 mg, 57%) as a colorless glass. HRMS Calcd for $C_{28}H_{38}Cl_3NO_6$: 589.1762. Found: 589.1742. MS m/z: 595, 593, 591, 589 (M^+, 0, 0, 0.1, 0.1), 558, 556, 554 (0.2, 0.7, 1), 491, 489, 487, 485 (0, 0.5, 1, 1), 454, 452, 450 (0.5, 1, 2), 362, 360, 358, 356 (0.1, 1, 2, 2), 222 (6), 195 (7), 87 (100), 57 (35), 43 (46). IR ($CHCl_3$) cm^{-1}: 1718. ^1H NMR δ: 1.18 (9H, s), 1.33–1.43 (1H, m), 1.40 (1H, dd, J=13.5, 10 Hz, H7), 1.59–1.85 (5H, m), 1.96–2.14 (3H, m), 2.07 (3H, s), 2.20 (1H, dd, J=19, 5 Hz), 2.36 (1H, dd, J=13.5, 7.5 Hz, H7), 2.36 (1H, br d, J=19 Hz), 2.66 (1H, br dd, J=6, 6 Hz), 3.48 (1H, ddd, J=11, 6, 4 Hz), 3.54 (1H, ddd, J=11, 5, 4 Hz), 3.91 (1H, d, J=6 Hz), 4.05 (1H, ddd, J=11, 7.5, 6.5 Hz), 4.10–4.24 (3H, m), 4.74–4.87 (1H, m, H6), 5.33 (1H, ddd, J=4, 4, 2 Hz, H4), 5.56–5.68 (2H, m), 6.50 (1H, d, J=9 Hz, NH). ^{13}C NMR δ: 19.8 (CH_2), 20.9 (CH_3), 25.0 (CH_2), 25.4 (CH_2), 26.5 (CH_2), 27.2 ($CH_3\times3$), 33.1 (CH_2), 38.6 (C), 43.0 (C), 43.5 (CH_2), 47.5 (CH), 49.3 (CH, C6), 50.2 (C), 50.5 (CH), 62.2 (CH_2), 63.5 (CH_2), 67.8 (CH_2), 86.8 (CH), 92.6 (C, CCl_3), 116.5 (CH, C4), 125.7 (CH), 126.6 (CH), 142.4 (C, C5), 161.1 (C, NH*C*OCCl_3), 170.7 (C), 178.3 (C).

4.3. Efforts aiming at carbonyl 1,3-transposition from 39 to form 53 (Scheme 6)

4.3.1. Epoxidation of 41 to form 43. *m*-CPBA (16 mg, 92.8 μmol) was added to a cooled (0 °C) solution of **41** (14 mg, 31.3 μmol) in CH_2Cl_2 (3 ml) and the mixture was stirred at 0 °C for 10 min and at 27 °C for 1 h. Saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (7:4)] afforded **39** (0.5 mg, 4%) and **43** (13 mg, 90%) in order of increasing polarity. **43**: Colorless glass. HRMS Calcd for $C_{26}H_{40}O_7$: 464.2772. Found: 464.2784. MS m/z: 464 (M^+, 0.3), 446 (3), 401 (1), 358 (1), 340 (3), 303 (3), 256 (7), 229 (9), 211 (17), 89 (24), 73 (13), 57 (68), 45 (100). IR ($CHCl_3$) cm^{-1}: 1719. ^1H NMR δ: 1.14–1.31 (2H, m), 1.19 (9H, s), 1.55–1.75 (3H, m), 1.77 (1H, d, J=11.5 Hz, OH), 1.82 (1H, dd, J=16.5, 3.5 Hz, H7), 1.90–2.01 (2H, m), 2.05–2.20 (4H, m), 2.25 (1H, d, J=16.5 Hz, H7), 2.47 (1H, br dd, J=6, 5.5 Hz), 3.25 (1H, d, J=3.5 Hz, H6), 3.37 (3H, s), 3.45 (1H, ddd, J=10.5, 6, 4.5 Hz), 3.56 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.64–3.69 (2H, m), 3.83 (1H, d, J=6 Hz), 3.84 (1H, ddd, J=12, 11.5, 4.5 Hz, changed to dd, J=12, 4.5 Hz with D_2O, H4), 4.03 (1H, ddd, J=11, 8, 6.5 Hz), 4.13 (1H, ddd, J=11, 8, 6 Hz), 4.65 (2H, s), 5.52–5.63 (2H, m). ^{13}C NMR δ: 21.4 (CH_2), 26.3 (CH_2), 27.2 ($CH_3\times3$, CH_2), 32.3 (CH_2), 33.7 (CH_2), 34.9 (CH_2), 38.6 (C), 40.5 (C), 43.6 (CH), 47.3 (C), 48.3 (CH), 53.3 (CH, C6), 55.1 (CH_3), 62.1 (CH_2), 65.6 (CH, C4), 66.9 (CH_2), 67.2 (C, C5), 69.0

(CH_2), 87.8 (CH), 96.5 (CH_2), 125.9 (CH), 126.8 (CH), 178.3 (C).

4.3.2. Mesylation of 43 to form 44. MsCl (10 μl, 0.129 mmol) was added to a cooled (−20 °C) solution of **43** (3 mg, 6.47 μmol) and Et_3N (46 μl, 0.331 mmol) in CH_2Cl_2 (1.5 ml) under an Ar atmosphere. After the mixture had been stirred at that temperature for 40 min, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O. Usual work-up and separation by PTLC [hexane–EtOAc (2:1)] yielded **44** (3 mg, 86%) as a colorless glass. MS *m/z*: 497 (M^+-CH_2OMe, 0.3), 446 (3), 401 (1), 340 (2), 299 (2), 238 (6), 211 (14), 89 (29), 73 (17), 57 (67), 45 (100), 41 (19). IR ($CHCl_3$) cm^{-1}: 1713. ^1H NMR δ: 1.19 (9H, s), ca. 1.30–1.41 (2H, m), ca. 1.51–1.84 (3H, m), 1.85 (1H, dd, *J*=16, 5, 4 Hz, H7), 1.94–2.20 (6H, m), 2.22 (1H, d, *J*=16.5 Hz, H7), 2.50 (1H, dd, *J*=6, 5 Hz), 3.03 (3H, s, SO_2CH_3), 3.19 (1H, d, *J*=4 Hz, H6), 3.38 (3H, s), 3.47 (1H, ddd, *J*=11, 6.5, 4.5 Hz), 3.57 (1H, ddd, *J*=11, 4.5, 4 Hz), 3.65–3.71 (2H, m), 3.91 (1H, d, *J*=6 Hz), 4.03 (1H, ddd, *J*=11, 7.5, 7 Hz), 4.11 (1H, ddd, *J*=11, 8, 6 Hz), 4.66 (1H, d, *J*=6.5 Hz), 4.70 (1H, d, *J*=6.5 Hz), 5.07 (1H, dd, *J*=12, 4.5 Hz, H4), 5.53–5.63 (2H, m).

4.3.3. Tosylation of 43 to form 45. In a similar manner to that described above (Section 4.3.2), **43** (6 mg, 12.9 μmol) was treated with *p*-toluenesulfonyl chloride (TsCl, 25 mg, 0.131 mmol) and Et_3N (0.50 ml, 3.59 mmol) in CH_2Cl_2 (1.5 ml) at 27 °C for 23 h. The same work-up as above and purification by PTLC [hexane–EtOAc (3:1)] yielded **45** (7.5 mg, 94%) as a colorless glass. MS *m/z*: 446 (M^+-TsOH, 4), 340 (3), 255 (6), 238 (6), 211 (12), 91 (33), 89 (28), 57 (65), 45 (100), 41 (17). IR ($CHCl_3$) cm^{-1}: 1718. ^1H NMR δ: 1.14–1.24 (1H, m), 1.19 (9H, s), 1.51–1.81 (5H, m), 1.75 (1H, dd, *J*=16.5, 3.5 Hz, H7), 1.93 (1H, br d, *J*=13.5 Hz), 2.03–2.17 (4H, m), 2.18 (1H, d, *J*=16.5 Hz, H7), 2.42–2.50 (1H, m), 2.45 (3H, s), 3.09 (1H, d, *J*=3.5 Hz, H6), 3.40 (3H, s), 3.47 (1H, ddd, *J*=11, 5.5, 4.5 Hz), 3.59 (1H, ddd, *J*=11, 4.5, 4 Hz), 3.66–3.73 (2H, m), 3.84 (1H, d, *J*=6 Hz), 4.01 (1H, ddd, *J*=11, 7.5, 7 Hz), 4.08 (1H, ddd, *J*=11, 8, 6 Hz), 4.69 (1H, d, *J*=6.5 Hz), 4.73 (1H, d, *J*=6.5 Hz), 4.96 (1H, dd, *J*=12, 4.5 Hz, H4), 5.51–5.62 (2H, m), 7.30–7.36 (2H, m), 7.75–7.81 (2H, m). ^{13}C NMR δ: 21.6 (CH_3), 21.8 (CH_2), 26.2 (CH_2), 26.4 (CH_2), 27.2 ($CH_3\times3$), 28.8 (CH_2), 33.7 (CH_2), 34.6 (CH_2), 38.6 (C), 40.6 (C), 43.4 (CH), 48.3 (CH), 48.4 (C), 52.6 (CH, C6), 55.2 (CH_3), 62.0 (CH_2), 65.3 (C, C5), 67.0 (CH_2), 68.8 (CH_2), 75.5 (CH, C4), 87.6 (CH), 96.5 (CH_2), 126.0 (CH), 126.7 (CH), 127.4 (CH×2), 129.6 (CH×2), 134.0 (C), 144.5 (C), 178.3 (C).

4.3.4. Attempted Birch reduction of 44 and 45. Reduction of **45** is described as a representative example. Na (33 mg, 1.43 mg atom) was added in small portions to a cooled (−78 °C) solution of **45** (4.5 mg, 7.28 μmol) in liq. NH_3 (ca. 3 ml) and THF (1.5 ml) under an Ar atmosphere. After the mixture had been stirred for 1 h, NH_4Cl (powder, 115 mg, 2.15 mmol) was added and the cooling bath was removed. The mixture was stirred at ambient temperature for 20 min. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [CH_2Cl_2–DME (5:1)] gave **46** (1.5 mg, 54%) and **49** (1 mg, 36%) in order of increasing polarity. **46**: Colorless glass. MS *m/z*: 362 (M^+-H_2O, 1), 344 (3), 317 (2), 303 (2), 274 (3), 256 (7), 229 (6), 211 (9), 105 (10), 91 (16), 89 (11), 73 (9), 59 (11), 45 (100), 41 (11). ^1H NMR δ: 1.12–1.31 (2H, m), 1.58 (1H, ddd, *J*=13.5, 8.5, 5.5 Hz), 1.59–1.89 (4H, m, including OH×2), 1.82 (1H, dd, *J*=16.5, 4 Hz, H7), 1.90–2.11 (4H, m), ca. 2.13–2.20 (2H, m), 2.21 (1H, d, *J*=16.5 Hz, H7), 2.48 (1H, br dd, *J*=6, 5 Hz), 3.24 (1H, d, *J*=4 Hz, H6), 3.37 (3H, s), 3.44 (1H, ddd, *J*=10.5, 6, 4.5 Hz), 3.57 (1H, ddd, *J*=10.5, 4.5, 4.5 Hz), 3.62–3.77 (2H, m), 3.69–3.89 (2H, m, CH_2OH), 3.78–3.89 (1H, m, H4), 3.82 (1H, d, *J*=6 Hz), 4.65 (2H, s), 5.52–5.63 (2H, m). ^{13}C NMR δ: 21.4 (CH_2), 26.4 (CH_2), 27.2 (CH_2), 32.3 (CH_2), 35.1 (CH_2), 38.1 (CH_2), 40.5 (C), 43.7 (CH), 47.2 (C), 48.4 (CH), 53.4 (CH, C6), 55.1 (CH_3), 60.1 (CH_2, CH_2OH), 65.5 (CH, C4), 66.9 (CH_2), 67.3 (C, C5), 69.0 (CH_2), 87.9 (CH), 96.5 (CH_2), 126.0 (CH), 126.9 (CH). For structure confirmation, **46** (1.5 mg) was treated with PivCl in pyridine to yield the pivaloate (1.5 mg), whose ^1H NMR was identical with that of **43**. **49**: Colorless glass. HRMS Calcd for $C_{21}H_{32}O_6$: 380.2197. Found: 380.2183. MS *m/z*: 380 (M^+, 1), 348 (2), 335 (3), 303 (7), 275 (5), 273 (6), 256 (6), 211 (11), 91 (19), 73 (14), 45 (100), 41 (11). IR ($CHCl_3$) cm^{-1}: 1691. ^1H NMR δ: 1.42–1.64 (3H, m), 1.72 (1H, ddd, *J*=13.5, 9, 5.5 Hz), ca. 1.88–2.08 (3H, m, including), 2.08 (1H, d, *J*=9 Hz, H5), 2.15–2.43 (6H, m), 2.57 (1H, br dd, *J*=6, 6 Hz), 2.85 (1H, d, *J*=2.5 Hz, OH), 3.24 (1H, ddd, *J*=10.5, 5.5, 5 Hz), 3.34 (3H, s), 3.41 (1H, ddd, *J*=10.5, 4.5, 4.5 Hz), ca. 3.53–3.60 (2H, m), 3.54 (1H, d, *J*=6 Hz), 3.61–3.82 (2H, m, CH_2OH), 4.25 (1H, dddd, *J*=10.5, 9, 7.5, 2.5 Hz, changed to ddd, *J*=10.5, 9, 7.5 Hz with D_2O, H6), 4.58 (1H, d, *J*=6.5 Hz), 4.61 (1H, d, *J*=6.5 Hz), 5.54–5.65 (2H, m). In the same manner, **46** (2 mg, 78%) was obtained from **44** (3 mg, 5.54 μmol).

4.3.5. Hydroboration–oxidation of 41 to form 50. $BH_3\cdot SMe_2$ (11 μl, 0.186 mmol) was added to a cooled (0 °C) solution of **41** (20 mg, 44.6 μmol) in THF (2.5 ml) under an Ar atmosphere and the mixture was stirred at 0 °C for 30 min, and at 22 °C for 15 h. EtOH (20 μl, 0.533 mmol) was gradually added and the resulting mixture was stirred for 10 min. After the mixture had been cooled in an ice bath, NaOH–H_2O (1 N, 180 μl, 0.180 mmol) and H_2O_2–H_2O (30%, 40 μl, 0.353 mmol) were further added, and the whole was stirred at 0 °C for 20 min, and at 22 °C for 8 h. Saturated NH_4Cl–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–DME (3:1)] provided **50** (10.5 mg, 50%) and recovered **41** (4 mg, 20%) in order of decreasing polarity. **50**: Colorless glass. HRMS Calcd for $C_{26}H_{42}O_7$: 466.2928. Found: 466.2933. MS *m/z*: 466 (M^+, 1), 448 (2), 337 (7), 305 (7), 275 (6), 259 (7), 240 (7), 213 (10), 117 (12), 105 (13), 57 (85), 45 (100), 41 (27). IR ($CHCl_3$) cm^{-1}: 1716. ^1H NMR δ: 0.98 (1H, ddd, *J*=13, 12, 5.5 Hz), 1.19 (9H, s), 1.21 (1H, dd, *J*=10, 10 Hz, H5), 1.40–1.45 (1H, m), ca. 1.53 (1H, dd, *J*=14, 10 Hz, H7), 1.75 (1H, ddd, *J*=13.5, 8, 6 Hz), 1.82–1.93 (2H, m), 2.00 (1H, br d, *J*=13 Hz), 2.09 (1H, ddd, *J*=13.5, 8.5, 6.5 Hz), 2.21 (1H, dd, *J*=21, 4.5 Hz), 2.26 (1H, dd, *J*=14, 7 Hz, H7), 2.31 (1H, br d, *J*=21 Hz), 2.50–2.57 (2H, m, including OH, changed to δ 2.54, 1H, dd,

J=6, 5.5 Hz, H14), 3.28–3.37 (1H, m), 3.36 (3H, s), 3.42–3.47 (1H, br, OH), 3.47 (1H, dt, J=10.5, 5 Hz), ca. 3.58–3.66 (2H, m), ca. 3.65–3.75 (1H, m, H4), 3.71 (1H, d, J=6 Hz), ca. 3.98–4.08 (1H, m, H6), 4.06 (1H, ddd, J=10.5, 8, 6.5 Hz), 4.14 (1H, ddd, J=10.5, 8.5, 6 Hz), 4.63 (2H, s), 5.53–5.65 (2H, m). ^{13}C NMR δ: 21.5 (CH_2), 27.0 (CH_2), 27.2 ($CH_3\times3$), 29.0 (CH_2), 33.3 (CH_2), 35.1 (CH_2), 38.6 (C), 42.3 (C, C8), 44.0 (CH_2, C7), 47.5 (CH), 49.5 (C), 53.2 (CH), 55.1 (CH_3), 57.5 (CH, C5), 62.3 (CH_2), 66.8 (CH_2), 68.7 (CH_2), 71.3 (CH, C6), 75.4 (CH, C4), 83.7 (CH), 96.5 (CH_2), 126.1 (CH), 126.5 (CH), 178.3 (C).

4.3.6. *i*-Propylsulfonylation of 50 to form 51. *i*-$PrSO_2Cl$ (8 μl, 71.3 μmol) was added to a cooled (−18 °C) solution of **50** (9 mg, 19.3 μmol) and Et_3N (31 μl, 0.223 mmol) in CH_2Cl_2 (2 ml) under an Ar atmosphere and the mixture was stirred for 30 min. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. The organic layer was washed successively with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O and then treated as usual. Separation by PTLC [benzene–EtOAc (5:2)] afforded **51** (9.5 mg, 86%) as a colorless glass. MS *m/z*: 404 (M^+-CH_2OMe-i-$PrSO_3$, 1), 386 (1), 342 (6), 240 (15), 222 (15), 195 (14), 157 (17), 129 (19), 91 (23), 71 (25), 57 (100), 45 (92), 43 (53), 41 (53). IR ($CHCl_3$) cm^{-1}: 1717. ^{1}H NMR δ: 1.01 (1H, ddd, J=13, 12.5, 5 Hz), 1.11 (9H, s), 1.38–1.62 (4H, m), 1.44 (6H, d, J=7 Hz, SO_2CHMe_2), 1.64–1.81 (3H, m), 1.98 (1H, br ddd, J=13, 3, 3 Hz), 2.04–2.16 (2H, m), 2.18–2.34 (2H, m), 2.28 (1H, dd, J=14.5, 8 Hz), 2.58 (1H, br dd, J=6, 6 Hz), 3.13 (1H, d, J=3.5 Hz, OH), 3.27 (1H, sep, J=7 Hz, SO_2CHMe_2), 3.37 (3H, s), 3.39 (1H, ddd, J=11, 5.5, 5 Hz), 3.51 (1H, ddd, J=11, 4.5, 4 Hz), ca. 3.58–3.70 (2H, m), 3.81 (1H, d, J=6 Hz), 4.01–4.19 (3H, m), 4.64 (1H, d, J=6.5 Hz), 4.69 (1H, d, J=6.5 Hz), 4.80 (1H, ddd, J=11, 10.5, 4.5 Hz, H4), 5.53–5.66 (2H, m). ^{13}C NMR δ: 16.5 (CH_3), 16.6 (CH_3), 21.4 (CH_2), 27.0 (CH_2), 27.2 ($CH_3\times3$), 29.0 (CH_2), 32.8 (CH_2), 33.3 (CH_2), 38.6 (C), 41.9 (C), 43.8 (CH_2), 47.4 (CH), 50.4 (C), 52.9 (CH), 53.1 (CH, SO_2CHMe_2), 55.1 (CH_3), 56.9 (CH, C5), 62.2 (CH_2), 67.0 (CH_2), 68.5 (CH_2), 69.4 (CH, C6), 83.5 (CH), 84.7 (CH, C4), 96.5 (CH_2), 125.7 (CH), 126.9 (CH), 178.3 (C).

4.3.7. Dess–Martin oxidation of 51 to form 52. A solution of **51** (7 mg, 12.2 μmol) and Dess–Martin periodinane (52 mg, 0.123 mmol) in CH_2Cl_2 (3 ml) was heated under reflux with stirring for 18 h. After the mixture had been cooled, saturated $Na_2S_2O_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up followed by PTLC [hexane–EtOAc (3:2)] afforded **52** (7 mg, quant.) as a colorless glass. MS *m/z*: 446 (M^+-i-$PrSO_3H$, 2), 414 (3), 317 (3), 285 (5), 256 (5), 238 (6), 211 (15), 91 (15), 89 (17), 73 (45), 57 (76), 45 (100), 43 (43), 41 (41). IR ($CHCl_3$) cm^{-1}: 1719. ^{1}H NMR δ: ca. 1.14–1.22 (1H, m), 1.19 (9H, s), 1.33 (3H, d, J=6.5 Hz), 1.44 (3H, d, J=7 Hz), ca. 1.44–1.55 (1H, m), 1.55–1.69 (2H, m), 1.88 (1H, ddd, J=14, 7, 7 Hz), 1.93–1.97 (1H, m), ca. 2.02–2.11 (1H, m), 2.07 (1H, ddd, J=14, 7, 7 Hz), 2.28–2.48 (3H, m), 2.47 (1H, d, J=16.5 Hz, H7), 2.47 (1H, d, J=9.5 Hz, H5), 2.54 (1H, d, J=16.5 Hz, H7), 2.64 (1H, br dd, J=6, 5.5 Hz), ca. 3.32–3.39 (1H, m), 3.35 (3H, s), 3.45 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.50 (1H, d, J=6 Hz), ca. 3.54–3.65 (2H, m), 3.66 (1H, qq, J=7, 6.5 Hz), 4.04 (1H, ddd, J=11.5, 7, 7 Hz), 4.14 (1H, ddd, J=11.5, 7, 7 Hz), 4.61 (1H, d, J=6.5 Hz), 4.64 (1H, d, J=6.5 Hz), 4.74 (1H, ddd, J=11, 9.5, 5.5 Hz, H4), 5.55–5.66 (2H, m). ^{13}C NMR δ: 16.3 (CH_3), 17.0 (CH_3), 20.3 (CH_2), 27.2 ($CH_3\times3$, CH_2), 28.6 (CH_2), 32.3 (CH_2), 32.7 (CH_2), 38.6 (C), 44.3 (C), 48.4 (CH), 50.6 (C), 51.7 (CH_2, C7), 52.8 (CH), 53.7 (CH), 55.1 (CH_3), 61.6 (CH_2), 62.9 (CH, C5), 66.6 (CH_2), 68.8 (CH_2), 78.1 (CH, C4), 84.7 (CH), 96.4 (CH_2), 125.6 (CH), 126.2 (CH), 178.2 (C), 207.4 (C, C6).

4.3.8. DBU treatment of 52 to form 53. A solution of **52** (7 mg, 12.3 μmol) and DBU (9 μl, 60.3 μmol) in benzene (2.5 ml) was heated under reflux with stirring for 2 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (2:1)] afforded **53** (5 mg, 91%) as a colorless glass. HRMS Calcd for $C_{26}H_{38}O_6$: 446.2666. Found: 446.2648. MS *m/z*: 446 (M^+, 4), 414 (4), 317 (5), 285 (8), 255 (5), 211 (19), 73 (56), 57 (57), 45 (100), 41 (20). IR ($CHCl_3$) cm^{-1}: 1716, 1676, 1617. ^{1}H NMR δ: 1.20 (9H, s), 1.41 (1H, ddd, J=12.5, 9.5, 5 Hz), 1.50–1.66 (2H, m), 1.67–1.72 (1H, m), 1.76 (1H, ddd, J=13.5, 7.5, 6.5 Hz), 1.99–2.44 (4H, m), 2.28–2.35 (2H, m), 2.50 (1H, d, J=20 Hz, H7), 2.61 (1H, br dd, J=6, 6 Hz), 2.62 (1H, d, J=20 Hz, H7), 3.35 (3H, s), 3.39–3.47 (1H, m), 3.55 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.62–3.67 (2H, m), 3.75 (1H, d, J=6 Hz), 4.07 (1H, ddd, J=11, 7, 7 Hz), 4.15 (1H, ddd, J=11, 7.5, 6 Hz), 4.63 (2H, s), 5.59–5.69 (2H, m), 6.69 (1H, dd, J=5, 3.5 Hz, H4). ^{13}C NMR δ: 19.7 (CH_2), 25.2 (CH_2, C3), 25.8 (CH_2), 26.5 (CH_2), 27.2 ($CH_3\times3$), 33.0 (CH_2), 38.6 (C), 40.5 (C), 46.3 (C), 48.0 (CH), 49.0 (CH), 50.0 (CH_2, C7), 55.1 (CH_3), 61.7 (CH_2), 66.7 (CH_2), 69.5 (CH_2), 89.3 (CH), 96.4 (CH_2), 125.1 (CH), 127.0 (CH), 131.2 (CH, C4), 145.4 (C, C5), 178.3 (C), 199.2 (C, C6).

4.3.9. Methoxyacetylation of 41 to form 54. Methoxyacetyl chloride (49 μl, 0.536 mmol) was added to a cooled (0 °C) solution of **41** (8 mg, 17.9 μmol) in CH_2Cl_2 (1.5 ml) and pyridine (0.3 ml) under an Ar atmosphere. After the mixture had been stirred at 0 °C for 45 min, saturated $NaHCO_3$–H_2O was added and the whole was stirred at 19 °C for 20 min. Extraction with CH_2Cl_2, usual work-up, and separation by PTLC [hexane–EtOAc (2:1)] provided **54** (8 mg, 86%) as a colorless glass. MS *m/z*: 475 (M^+-CH_2OMe, 0.5), 430 (3), 414 (1), 398 (2), 324 (3), 222 (24), 195 (19), 157 (28), 57 (43), 45 (100), 41 (18). IR ($CHCl_3$) cm^{-1}: 1743, 1720. ^{1}H NMR δ: 1.19 (9H, s), 1.39–1.50 (1H, m), ca. 1.55–1.70 (4H, m), 1.72 (1H, ddd, J=11, 8, 6.5 Hz), 1.82–1.92 (1H, m), 1.98 (1H, ddd, J=13.5, 5, 5 Hz), 2.10–2.26 (3H, m), 2.30 (1H, br d, J=19.5 Hz), 2.46 (1H, ddd, J=18, 4, 2 Hz), 2.51 (1H, dd, J=6, 6 Hz), 3.36 (3H, s), 3.42 (1H, dt, J=10.5, 5 Hz), 3.46 (3H, s, $COCH_2OCH_3$), 3.49 (1H, dt, J=10.5, 5 Hz), 3.63 (2H, dd, J=5, 5 Hz), 3.71 (1H, d, J=6 Hz), 4.04 (2H, s, $COCH_2OMe$), 4.08 (1H, ddd, J=11, 8, 6.5 Hz), 4.17 (1H, ddd, J=11, 8.5, 6 Hz), 4.63 (1H, d, J=6.5 Hz), 4.65 (1H, d, J=6.5 Hz), 5.36–5.41 (1H, m, H6), 5.48–5.55 (1H, m, H4), 5.52–5.65 (2H, m). ^{13}C NMR δ: 18.1 (CH_2), 23.3 (CH_2), 26.3 (CH_2), 27.2 ($CH_3\times3$), 27.4 (CH_2), 33.6 (CH_2), 38.6 (C), 38.7 (CH_2), 40.5 (C), 48.7 (C), 49.5 (CH), 50.3 (CH), 55.1 (CH_3), 59.3 (CH_3, $COCH_2OCH_3$), 62.2 (CH_2), 66.9 (CH_2), 69.2 (CH_2), 69.9 (CH_2, $COCH_2OMe$), 72.0

(CH, C4), 92.7 (CH), 96.4 (CH_2), 120.7 (CH, C6), 125.2 (CH), 127.4 (CH), 147.5 (C, C5), 169.5 (C, *C*OCH_2OMe), 178.4 (C).

4.3.10. Europium-catalyzed rearrangement of 54 to form 55–57. $Eu(fod)_3$ (1.5 mg, 1.59 μmol) was added to a solution of **54** (6 mg, 11.5 μmol) in $CHCl_3$ (2 ml) under an Ar atmosphere and the mixture was stirred at 22 °C for 24 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [benzene–EtOAc (15:1)] yielded **55** (2 mg, 33%), **56** (1 mg, 20%), and **57** (1 mg, 20%) in order of decreasing polarity. **55**: Colorless glass. MS *m*/*z*: 430 (M^+−$MeOCH_2COOH$, 2), 414 (2), 386 (2), 324 (3), 222 (20), 195 (16), 157 (24), 89 (17), 73 (19), 57 (49), 45 (100), 41 (20). IR ($CHCl_3$) cm^{-1}: 1743, 1719. ^1H NMR δ: 1.18 (9H, s), 1.48–2.36 (9H, m), 1.77 (1H, dd, *J*=15, 5.5 Hz, H7), 2.09 (1H, dd, *J*=15, 7 Hz, H7), 2.22–2.26 (2H, m), 2.47–2.54 (1H, m), 3.36 (3H, s), 3.39–3.55 (2H, m), 3.46 (3H, s), 3.65 (2H, dd, *J*=5, 5 Hz), 3.79 (1H, d, *J*=6 Hz), 4.00–4.19 (2H, m), 4.04 (2H, s, $COCH_2OMe$), 4.64 (2H, s), 5.11 (1H, ddd, *J*=3.5, 3.5, 1.5 Hz, H4), 5.55–5.65 (2H, m), 5.69 (1H, ddd, *J*=7, 5.5, 1.5 Hz, H6). **56**: Colorless glass. HRMS Calcd for $C_{26}H_{38}O_5$: 430.2717. Found: 430.2701. MS *m*/*z*: 430 (M^+, 2), 398 (1), 324 (2), 222 (39), 157 (44), 91 (18), 89 (13), 73 (13), 57 (66), 45 (100), 41 (23). IR ($CHCl_3$) cm^{-1}: 1717. ^1H NMR δ: 1.20 (9H, s), ca. 1.56–1.61 (1H, m), 1.37 (1H, ddd, *J*=12.5, 12.5, 5 Hz), 1.73 (1H, ddd, *J*=13.5, 8.5, 5.5 Hz), 1.97–2.36 (6H, m), 2.36 (1H, br d, *J*=19 Hz), 2.48–2.58 (2H, m), 3.35 (3H, s), 3.38 (1H, dd, *J*=10.5, 5 Hz), 3.47 (1H, dt, *J*=10.5, 5 Hz), 3.62 (2H, dd, *J*=5, 5 Hz), 3.78 (1H, d, *J*=6.5 Hz), 4.07 (1H, ddd, *J*=10.5, 8.5, 6.5 Hz), 4.19 (1H, ddd, *J*=10.5, 9, 6 Hz), 4.63 (2H, s), 5.21 (1H, dd, *J*=4, 3.5 Hz, H6), 5.55–5.67 (2H, m), 5.77 (1H, br dd, *J*=9.5, 5 Hz, H3), 5.93 (1H, dd, *J*=9.5, 2.5 Hz, H4). **57**: Colorless glass. HRMS Calcd for $C_{26}H_{38}O_5$: 430.2717. Found: 430.2713. MS *m*/*z*: 430 (M^+, 1), 398 (1), 324 (5), 222 (40), 157 (45), 89 (32), 73 (21), 57 (41), 45 (100), 41 (20). IR ($CHCl_3$) cm^{-1}: 1720. ^1H NMR δ: 1.19 (9H, s), 1.25–1.36 (1H, m), 1.39–1.70 (2H, m), 1.68 (1H, br d, *J*=4.5 Hz), 1.84 (1H, ddd, *J*=14, 8, 6.5 Hz), 1.96–2.09 (3H, m), 2.15 (1H, br d, *J*=13 Hz), 2.19–2.30 (1H, m), 2.36 (1H, br d, *J*=19 Hz), 2.64 (1H, br dd, *J*=7, 6 Hz), 3.35 (3H, s), ca. 3.35–3.43 (1H, m), 3.50 (1H, ddd, *J*=10.5, 5, 5 Hz), 3.61–3.66 (2H, m), 3.79 (1H, d, *J*=6 Hz), 4.05–4.17 (2H, m), 4.64 (2H, s), 5.40 (1H, br dd, *J*=5, 3.5 Hz, H4), 5.64 (1H, ddd, *J*=9.5, 3, 3 Hz), 5.55 (1H, dddd, *J*=9.5, 6.5, 1.5, 1.5 Hz), 5.77 (1H, d, *J*=9.5 Hz, H6), 5.90 (1H, d, *J*=9.5 Hz, H7).

4.3.11. Alcoholysis of 55 to form 58. K_2CO_3 (10 mg, 72.5 μmol) was added to a cooled (0 °C) solution of **55** (1.5 mg, 2.88 μmol) in MeOH (2 ml) and the mixture was stirred for 1.5 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (2:1)] gave **58** (1 mg, ca. 77%) as a colorless glass. HRMS Calcd for $C_{26}H_{40}O_6$: 448.2823. Found: 448.2824. MS *m*/*z*: 448 (M^+, 0.4), 342 (4), 258 (7), 240 (13), 213 (22), 174 (18), 91 (18), 57 (78), 45 (100), 41 (27). IR ($CHCl_3$) cm^{-1}: 1720. ^1H NMR δ: 1.19 (9H, s), 1.35 (1H, d, *J*=5 Hz, OH), 1.42–1.88 (5H, m), 1.65 (1H, dd, *J*=14.5, 6.5 Hz, H7), 1.90–1.94 (1H, m), 1.95–2.08 (3H, m), 2.08 (1H, dd, *J*=14.5, 6.5 Hz, H7), ca. 2.16–2.30 (2H, m), 2.45–2.52 (1H, m), 3.36 (3H, s), 3.37–3.44 (1H, m), 3.50 (1H, ddd, *J*=10.5, 5, 5 Hz), ca. 3.60–3.69 (2H, m), 3.73 (1H, d, *J*=6 Hz), 4.10 (1H, ddd, *J*=11, 8.5, 6.5 Hz), 4.18 (1H, ddd, *J*=11, 8.5, 6.5 Hz), 4.35–4.44 (1H, m, H6), 4.64 (2H, s), 5.54 (1H, ddd, *J*=4, 3.5, 1.5 Hz, H4), 5.55–5.65 (2H, m).

4.3.12. Dess–Martin oxidation of 58 to form 53. In the same manner as described for the preparation of **52** from **51** (Section 4.3.7), **58** (1 mg, 2.23 mmol) was oxidized with Dess–Martin periodinane (10 mg, 23.7 μmol) to afford **53** (1 mg, quant.) after PTLC [hexane–EtOAc (3:1)].

4.4. Introduction of the C18-methyl group into 39 (Table 1)

4.4.1. Reaction of 39 with MeMgI (runs a and b). The reaction procedure in THF (run b) is described as a representative example. MeMgI (0.37 M in Et_2O, 0.30 ml, 0.117 mmol) was added to a cooled (0 °C) solution of **39** (5 mg, 11.2 μmol) in THF (2.5 ml) under an Ar atmosphere and the mixture was stirred at that temperature for 20 min. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [benzene–EtOAc (7:1)] yielded **59** (2 mg, 38%) and recovered **39** (1.5 mg, 30%) in order of decreasing polarity. **59**: Colorless glass. MS *m*/*z*: 444 (M^+−H_2O, 2), 356 (1), 338 (3), 236 (31), 171 (48), 57 (82), 45 (100), 41 (35). IR ($CHCl_3$) cm^{-1}: 1713. ^1H NMR δ: 1.19 (9H, s), 1.32–1.74 (7H, m, including OH), 1.34 (3H, s), 2.01–2.10 (1H, m), 2.10–2.20 (1H, m), 2.15 (1H, dd, *J*=18.5, 3 Hz, H7), 2.24 (1H, ddd, *J*=13.5, 8.5, 7 Hz), 2.33 (1H, br d, *J*=19.5 Hz, H11), 2.49 (1H, br dd, *J*=6, 6 Hz, H14), 2.49 (1H, dd, *J*=18.5, 4 Hz, H7), 3.36 (3H, s), 3.40 (1H, ddd, *J*=11, 5.5, 5.5 Hz), 3.51 (1H, ddd, *J*=11, 4.5, 4.5 Hz), ca. 3.59–3.68 (2H, m), 3.66 (1H, d, *J*=6 Hz, H20), 4.08 (1H, ddd, *J*=10.5, 8.5, 7 Hz), 4.18 (1H, ddd, *J*=10.5, 8.5, 6 Hz), 4.64 (2H, s), 5.52–5.64 (2H, m), 5.69 (1H, dd, *J*=4, 3 Hz, H6). ^{13}C NMR δ: 19.8 (CH_2), 25.9 (CH_2), 26.5 (CH_2), 27.2 ($CH_3\times3$), 31.7 (CH_3), 33.7 (CH_2), 38.6 (C), 38.8 (CH_2, C7), 39.0 (CH_2), 40.3 (C), 48.4 (C, C10), 50.3 (CH), 50.8 (CH, C9), 55.1 (CH_3), 62.2 (CH_2), 66.9 (CH_2), 69.1 (CH_2), 71.7 (C, C4), 92.6 (CH), 96.5 (CH_2), 118.7 (CH, C6), 125.4 (CH), 127.3 (CH), 156.1 (C), 178.4 (C). In a similar manner, **39** (5 mg, 11.2 μmol) was reacted with MeMgI in toluene at −18 to 0 °C to afford **59** (1 mg, 19%) and a recovery of **39** (2.5 mg, 50%).

4.4.2. Reaction of 39 with MeLi in Et_2O (run c). MeLi (1.1 M, 0.31 ml, 0.341 mmol) was added to a cooled (−78 °C) solution of **39** (6 mg, 13.5 μmol) in Et_2O (3 ml) under an Ar atmosphere and the mixture was stirred for 30 min. Quenching with saturated NH_4Cl–H_2O, extraction with CH_2Cl_2, usual work-up, and PTLC [benzene–EtOAc (6:1)] yielded **60** (1 mg, 16%), **59** (3 mg, 48%), and recovered **39** (1.5 mg, 25%) in order of decreasing polarity. **60**: Colorless glass. MS *m*/*z*: 444 (M^+−H_2O, 2), 356 (3), 338 (2), 236 (19), 171 (33), 57 (67), 45 (100), 41 (23). IR ($CHCl_3$) cm^{-1}: 1707. ^1H NMR δ: 1.20 (9H, s), ca. 1.22–1.85 (7H, m), 1.36 (3H, s), 2.09–2.36 (4H, m), 2.15 (1H, dd, *J*=19, 3.5 Hz, H7), 2.49 (1H, dd, *J*=6, 6 Hz, H14), 2.49 (1H, dd, *J*=19, 4.5 Hz, H7), 3.35 (3H, s), 3.46–3.68 (4H, m), 3.86 (1H, d, *J*=6 Hz, H20), 4.06 (1H, ddd, *J*=11,

8.5, 6.5 Hz), 4.16 (1H, ddd, *J*=11, 8.5, 6 Hz), 4.63 (1H, d, *J*=6.5 Hz), 4.65 (1H, d, *J*=6.5 Hz), 5.52–5.65 (2H, m), 5.69 (1H, dd, *J*=4.5, 3.5 Hz, H6).

4.4.3. Reaction of 39 or 63 with MeLi in THF (runs d and e). The reaction procedure of run d is described as a representative example. MeLi (1.1 M in Et_2O, 1.37 ml, 1.51 mmol) was slowly added during 1 min to a cooled (−78 °C) solution of **39** (84 mg, 0.188 mmol) in THF (8 ml) under an Ar atmosphere and the mixture was stirred at that temperature for 20 min. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [benzene–EtOAc (1:1)] furnished crude-**63** (10 mg), **61** (42 mg, 59%), and **62** (8 mg, 11%) in order of increasing polarity. The crude-**63** was further separated by PTLC (2% MeOH–CH_2Cl_2) to give **63** (4 mg, 6%). **61**: Slightly labile colorless glass. MS *m*/*z*: 360 (M^+-H_2O, 2), 315 (1), 272 (4), 254 (15), 209 (17), 189 (21), 171 (22), 45 (100). ^{1}H NMR *δ*: 1.31–1.76 (9H, m, including OH×2), 1.34 (3H, s), 2.05 (1H, ddd, *J*=12.5, 3.5, 3.5 Hz), 2.09–2.24 (2H, m), 2.13 (1H, dd, *J*=19, 3 Hz, H7), 2.33 (1H, br d, *J*=19 Hz), 2.47 (1H, dd, *J*=19, 4 Hz, H7), 2.49 (1H, dd, *J*=6, 6 Hz), 3.36 (3H, s), 3.40 (1H, dt, *J*=10.5, 5 Hz), 3.51 (1H, dt, *J*=10.5, 5 Hz), 3.64 (2H, dd, *J*=5, 5 Hz), ca. 3.64–3.82 (2H, m), 3.65 (1H, d, *J*=6 Hz), 4.64 (2H, s), 5.51–5.63 (2H, m), 5.68 (1H, dd, *J*=4, 3 Hz, H6). ^{13}C NMR *δ*: 19.8 (CH_2), 25.9 (CH_2), 26.6 (CH_2), 31.7 (CH_3), 38.3 (CH_2), 39.0 (CH_2), 39.1 (CH_2), 40.3 (C), 48.3 (C), 50.3 (CH), 50.8 (CH), 55.1 (CH_3), 60.3 (CH_2), 66.9 (CH_2), 69.1 (CH_2), 71.7 (C, C4), 92.6 (CH), 96.5 (CH_2), 118.8 (CH, C6), 125.6 (CH), 127.3 (CH), 156.1 (C, C5). **62**: Slightly labile colorless glass. MS *m*/*z*: 360 (M^+-H_2O, 2), 315 (1), 272 (3), 254 (13), 209 (14), 189 (18), 171 (17), 45 (100). ^{1}H NMR *δ*: 1.24–1.38 (1H, m), 1.36 (3H, s), 1.42–1.67 (3H, m), 1.59–1.88 (5H, m, including OH×2), 2.08–2.24 (3H, m), 2.13 (1H, dd, *J*=19, 3 Hz, H7), 2.31 (1H, br d, *J*=20 Hz), 2.45–2.51 (1H, m), 2.46 (1H, dd, *J*=19, 4.5 Hz, H7), 3.35 (3H, s), 3.45–3.83 (6H, m), 3.85 (1H, d, *J*=6 Hz), 4.63 (1H, d, *J*=6.5 Hz), 4.65 (1H, d, *J*=6.5 Hz), 5.52–5.65 (2H, m), 5.68 (1H, dd, *J*=4.5, 3 Hz, H6). ^{13}C NMR *δ*: 18.0 (CH_2), 25.3 (CH_2), 26.5 (CH_2), 28.9 (CH_3), 37.9 (CH_2), 38.2 (CH_2), 39.2 (CH_2), 40.1 (C), 47.8 (C), 50.0 (CH), 50.4 (CH), 55.1 (CH_3), 60.3 (CH_2), 67.2 (CH_2), 69.3 (CH_2), 70.8 (C, C4), 93.8 (CH), 96.4 (CH_2), 119.0 (CH, C6), 125.2 (CH), 127.9 (CH), 155.5 (C, C5). **63**: Colorless glass. HRMS Calcd for $C_{21}H_{30}O_5$: 362.2092. Found: 362.2109. MS *m*/*z*: 362 (M^+, 0.5), 330 (1), 317 (2), 285 (2), 273 (2), 256 (20), 211 (27), 185 (11), 91 (12), 73 (13), 45 (100). ^{1}H NMR *δ*: 1.43 (1H, ddd, *J*=13, 10, 5 Hz), 1.53 (1H, br d, *J*=5 Hz), 1.68 (1H, ddd, *J*=13.5, 8.5, 5.5 Hz), 1.77–1.88 (2H, m), 2.13–2.27 (5H, m, including OH), 2.28–2.39 (1H, m), 2.33 (1H, dd, *J*=21, 3 Hz, H7), 2.48 (1H, dddd, *J*=18.5, 4, 4, 2 Hz, H3), 2.56 (1H, br dd, *J*=6, 6 Hz), 2.62 (1H, dd, *J*=21, 5 Hz, H7), 3.35 (3H, s), 3.39 (1H, ddd, *J*=10.5, 6, 4.5 Hz), 3.51 (1H, ddd, *J*=10.5, 4.5, 4.5 Hz), 3.58–3.68 (2H, m), ca. 3.68–3.84 (2H, m), 3.70 (1H, d, *J*=6 Hz), 4.63 (2H, s), 5.55–5.65 (2H, m), 6.82 (1H, dd, *J*=5, 3 Hz, H6). ^{13}C NMR *δ*: 19.7 (CH_2), 25.9 (CH_2), 26.4 (CH_2), 37.5 (CH_2), 39.1 (CH_2, C3), 40.0 (CH_2, C7), 40.2 (C), 48.8 (C), 49.7 (CH), 50.2 (CH), 55.1 (CH_3), 60.1 (CH_2, CH_2OH), 66.7 (CH_2), 69.1 (CH_2), 91.9 (CH), 96.4 (CH_2), 125.4 (CH), 127.0 (CH), 136.9 (CH, C6), 148.8 (C, C5), 197.4 (C, C4). In the same manner, **63** (4 mg, 11.0 μmol) was reacted with MeLi (1.1 M in Et_2O, 0.08 ml, 88.1 μmol) to give **61** (2.5 mg, 60%) and **62** (0.5 mg, 12%) (Table 1, run e).

4.5. Preparation of 1 from 61 and 62 (Scheme 7)

4.5.1. Acetylation of 61, 62 to form 64, 65, respectively. The preparation of **64** from **61** is described as a typical procedure. Ac_2O (0.20 ml, 2.12 mmol) was added to a cooled (0 °C) solution of **61** (35 mg, 92.6 μmol) in pyridine (0.30 ml, 3.71 mmol) and CH_2Cl_2 (1.5 ml), and the mixture was stirred at 0 °C for 5 min and at 25 °C for 5 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–EtOAc (2:1)] provided **64** (36 mg, 93%) as a slightly labile colorless glass. MS *m*/*z*: 402 (M^+-H_2O, 2), 357 (2), 314 (8), 296 (5), 236 (15), 209 (23), 171 (29), 45 (100), 43 (59). IR ($CHCl_3$) cm^{-1}: 1720. ^{1}H NMR *δ*: 1.31–1.43 (1H, m), 1.34 (3H, s), 1.44–1.79 (7H, m), 2.01–2.20 (2H, m), 2.04 (3H, s), 2.14 (1H, dd, *J*=19, 3 Hz), 2.23 (1H, ddd, *J*=13.5, 9.5, 6.5 Hz), 2.33 (1H, br d, *J*=18.5 Hz), 2.47 (1H, dd, *J*=19, 4 Hz), 2.48 (1H, dd, *J*=6, 6 Hz), 3.36 (3H, s), 3.40 (1H, dt, *J*=10.5, 5 Hz), 3.51 (1H, dt, *J*=10.5, 5 Hz), 3.64 (2H, dd, *J*=5, 5 Hz), 3.66 (1H, d, *J*=6 Hz), 4.07 (1H, ddd, *J*=10.5, 9, 6.5 Hz, C*H*$_2$OAc), 4.21 (1H, ddd, *J*=10.5, 9.5, 5.5 Hz, C*H*$_2$OAc), 4.64 (2H, s), 5.51–5.63 (2H, m), 5.69 (1H, dd, *J*=4, 3 Hz). ^{13}C NMR *δ*: 19.8 (CH_2), 21.1 (CH_3, CO*C*H$_3$), 25.9 (CH_2), 26.5 (CH_2), 31.7 (CH_3), 33.8 (CH_2), 38.8 (CH_2), 39.0 (CH_2), 40.2 (C), 48.3 (C), 50.2 (CH), 50.7 (CH), 55.1 (CH_3), 62.2 (CH_2, *C*H$_2$OAc), 66.9 (CH_2), 69.1 (CH_2), 71.7 (C, C4), 92.5 (CH), 96.4 (CH_2), 118.6 (CH), 125.5 (CH), 127.2 (CH), 156.1 (C), 170.9 (C, *C*OCH$_3$). In the same manner, **62** (8 mg, 21.2 μmol) was led to **65** (8 mg, 90%), a slightly labile colorless glass. MS *m*/*z*: 402 (M^+-H_2O, 2), 357 (1), 314 (2), 296 (3), 236 (13), 209 (11), 171 (32), 45 (100), 43 (51). IR ($CHCl_3$) cm^{-1}: 1724. ^{1}H NMR *δ*: 1.25–1.38 (1H, m), 1.36 (3H, s), 1.41–1.56 (3H, m), 1.62–1.89 (4H, m, including OH), 2.04 (3H, s), ca. 2.05–2.21 (2H, m), 2.14 (1H, dd, *J*=19, 2.5 Hz), 2.23 (1H, ddd, *J*=13, 9.5, 6.5 Hz), 2.32 (1H, br d, *J*=20 Hz), 2.44–2.51 (1H, m), 2.46 (1H, dd, *J*=19, 4.5 Hz), 3.35 (3H, s), 3.46–3.60 (2H, m), 3.60–3.71 (2H, m), 3.86 (1H, d, *J*=6 Hz), 4.07 (1H, ddd, *J*=10.5, 9.5, 6.5 Hz, C*H*$_2$OAc), 4.21 (1H, ddd, *J*=10.5, 9.5, 6 Hz, C*H*$_2$OAc), 4.63 (1H, d, *J*=6.5 Hz), 4.65 (1H, d, *J*=6.5 Hz), 5.52–5.64 (2H, m), 5.68 (1H, dd, *J*=4.5, 2.5 Hz). ^{13}C NMR *δ*: 17.9 (CH_2), 21.1 (CH_3, CO*C*H$_3$), 25.3 (CH_2), 26.4 (CH_2), 28.9 (CH_3), 33.7 (CH_2), 37.9 (CH_2), 38.9 (CH_2), 40.0 (C), 47.9 (C), 49.8 (CH), 50.4 (CH), 55.1 (CH_3), 62.2 (CH_2, *C*H$_2$OAc), 67.2 (CH_2), 69.4 (CH_2), 70.7 (C, C4), 93.7 (CH), 96.4 (CH_2), 118.9 (CH), 125.1 (CH), 127.8 (CH), 155.5 (C), 170.9 (C, *C*OCH$_3$).

4.5.2. Oxidation of 64 and 65 to form 4, 66, and 67. The oxidation procedure of **64** is presented as a representative example. PCC–Al_2O_3 (20 wt %, 346 mg, 0.321 mmol) was added in one portion to a cooled (5 °C) solution of **64** (45 mg, 0.107 mmol) in benzene (8 ml), and the mixture was stirred at that temperature for 15 min and at 25 °C for 2 h. Saturated $NaHCO_3$–H_2O was added and the whole was filtered under reduced pressure. The filtered Al_2O_3 was washed with CH_2Cl_2. The filtrate was extracted with CH_2Cl_2 and the organic layer was treated as usual.

Purification by PTLC [hexane–EtOAc (9:1)] afforded **4** (28 mg, 63%), **66** (7 mg, 16%), and **67** (5.5 mg, 13%) in order of decreasing polarity. **4**: Colorless glass. HRMS Calcd for $C_{24}H_{34}O_6$: 418.2353. Found: 418.2359. MS *m/z*: 418 (M^+, 2), 386 (3), 373 (7), 299 (6), 237 (9), 225 (23), 187 (14), 73 (30), 45 (100), 43 (45). IR ($CHCl_3$) cm^{-1}: 1727, 1668. ^1H NMR δ: 1.46–1.72 (3H, m), 1.78 (1H, ddd, J=14, 8, 6.5 Hz), 1.83–1.94 (2H, m), 2.03 (3H, s), 2.03–2.17 (3H, m), 2.04 (3H, s), 2.22–2.38 (2H, m), 2.46 (1H, d, J=19.5 Hz, H7), 2.52 (1H, d, J=19.5 Hz, H7), 2.56–2.62 (1H, m), 3.35 (3H, s), 3.43 (1H, ddd, J=10.5, 5.5, 4.5 Hz), 3.53 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.61–3.66 (2H, m), 3.69 (1H, d, J=6 Hz), 4.07 (1H, ddd, J=11, 8, 6.5 Hz), 4.17 (1H, ddd, J=11, 8, 6.5 Hz), 4.62 (2H, s), 5.54–5.68 (2H, m). ^{13}C NMR δ: 19.7 (CH_2), 21.0 (CH_3), 21.9 (CH_3), 26.1 (CH_2), 26.6 (CH_2), 32.6 (CH_2), 34.2 (CH_2), 41.0 (C), 47.96 (C), 48.00 (CH), 49.6 (CH), 51.8 (CH_2, C7), 55.0 (CH_3), 61.7 (CH_2), 66.7 (CH_2), 69.5 (CH_2), 90.1 (CH), 96.4 (CH_2), 125.1 (CH), 127.1 (CH), 140.1 (C, C5), 143.4 (C, C4), 170.7 (C), 201.1 (C, C6). **66**: Slightly unstable colorless glass. HRMS Calcd for $C_{24}H_{34}O_5$: 402.2404. Found: 402.2410. MS *m/z*: 402 (M^+, 1), 342 (1), 236 (16), 209 (10), 208 (12), 171 (27), 45 (100), 43 (53). IR ($CHCl_3$) cm^{-1}: 1729. ^1H NMR δ: 1.36 (1H, ddd, J=12.5, 12.5, 5 Hz), ca. 1.52–1.61 (1H, m), 1.73 (3H, br s), 1.74 (1H, ddd, J=13, 9, 6 Hz), 1.92–2.31 (6H, m), 2.05 (3H, s), 2.49 (1H, dd, J=6, 5.5 Hz), 2.55 (1H, dd, J=19, 4.5 Hz, H7), 3.34 (3H, s), 3.38 (1H, dt, J=10.5, 5 Hz), 3.47 (1H, dt, J=10.5, 5 Hz), 3.61 (2H, dd, J=5, 5 Hz), 3.79 (1H, d, J=6 Hz), 4.09 (1H, ddd, J=10.5, 9, 6.5 Hz), 4.22 (1H, ddd, J=10.5, 9.5, 6 Hz), 4.82 (2H, s), 5.35 (1H, dd, J=4.5, 3 Hz, H6), 5.54–5.67 (3H, m). ^{13}C NMR δ: 19.3 (CH_3), 21.1 (CH_3), 23.0 (CH_2), 25.0 (CH_2), 26.5 (CH_2), 33.9 (CH_2), 39.3 (CH_2), 40.3 (C), 47.1 (C), 49.5 (CH), 50.1 (CH), 55.1 (CH_3), 62.2 (CH_2), 66.8 (CH_2), 68.9 (CH_2), 91.9 (CH), 96.4 (CH_2), 117.0 (CH, C6), 125.4 (CH), 126.3 (CH, C3), 127.5 (CH), 129.2 (C, C4), 148.5 (C, C5), 170.9 (C). **67**: Slightly unstable colorless glass. HRMS Calcd for $C_{24}H_{34}O_5$: 402.2404. Found: 402.2384. MS *m/z*: 402 (M^+, 1), 342 (1), 296 (6), 236 (16), 209 (11), 171 (48), 45 (100), 43 (40). IR ($CHCl_3$) cm^{-1}: 1722. ^1H NMR δ: 1.24 (1H, ddd, J=12.5, 12.5, 3.5 Hz), 1.46–1.70 (3H, m), 1.68 (3H, br s), 1.84 (1H, dt, J=14, 7.5 Hz), 1.88–2.11 (3H, m), 2.03 (3H, s), 2.12 (1H, br d, J=12.5 Hz), 2.20 (1H, dddd, J=19.5, 5, 2.5, 2.5 Hz), 2.36 (1H, br d, J=19.5 Hz), 2.62 (1H, dd, J=6, 6 Hz), 3.35 (3H, s), 3.38 (1H, ddd, J=10.5, 5, 4.5 Hz), 3.49 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.60–3.66 (2H, m), 3.78 (1H, d, J=6 Hz), 4.13 (2H, dd, J=7.5, 7.5 Hz), 4.64 (2H, s), 5.53 (1H, dddd, J=9.5, 6.5, 1.5, 1.5 Hz), 5.61 (1H, ddd, J=9.5, 2.5, 2.5 Hz), 5.74 (1H, d, J=9.5 Hz, H7), 6.29 (1H, d, J=9.5 Hz, H6). ^{13}C NMR δ: 18.8 (CH_3), 20.4 (CH_2), 21.1 (CH_3), 26.7 (CH_2), 27.0 (CH_2), 31.3 (CH_2), 32.0 (CH_2), 43.4 (C), 48.7 (C), 48.8 (CH), 49.4 (CH), 55.0 (CH_3), 62.4 (CH_2), 66.8 (CH_2), 69.2 (CH_2), 89.5 (CH), 96.5 (CH_2), 124.8 (CH, C6), 125.6 (CH), 125.9 (CH), 126.7 (CH, C4), 135.1 (CH, C7), 137.6 (C, C5), 170.9 (C). In the same manner, oxidation of **65** (14 mg, 33.3 μmol) provided **4** (9 mg, 65%), **66** (2 mg, 15%), and **67** (2 mg, 15%).

4.5.3. Hydrocyanation of 4 to form 5 and 68. Et_2AlCN (1 M in toluene, 1.03 ml, 1.03 mmol) was added to a cooled (−18 °C) solution of **4** (54 mg, 0.129 mmol) in toluene (8 ml) under an Ar atmosphere and the mixture was stirred at that temperature for 30 min, and at 24 °C for 1.5 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (7:4)] afforded **5** (54 mg, 94%) and **68** (1 mg, 2%) in order of increasing polarity. **5**: Colorless glass. HRMS Calcd for $C_{25}H_{35}NO_6$: 445.2462. Found: 445.2443. MS *m/z*: 445 (M^+, 1), 413 (2), 400 (5), 385 (1), 356 (2), 326 (2), 296 (2), 280 (4), 252 (4), 175 (5), 117 (6), 105 (9), 89 (20), 73 (16), 59 (17), 45 (100), 43 (38). IR ($CHCl_3$) cm^{-1}: 1733, 1706. ^1H NMR δ: 1.14–1.29 (2H, m), 1.56 (3H, s), 1.56–1.68 (2H, m), 1.76–1.81 (1H, m), 1.80–2.15 (3H, m), 2.04 (3H, s), 2.15 (1H, s, H5), 2.21 (1H, br d, J=13 Hz), 2.25–2.35 (1H, m), 2.43 (1H, br d, J=20 Hz), 2.45 (1H, d, J=17.5 Hz, H7), 2.63 (1H, d, J=17.5 Hz, H7), 2.68 (1H, br dd, J=6, 6 Hz), 3.35 (3H, s), 3.46–3.74 (4H, m), 3.88 (1H, d, J=6 Hz), 4.05 (1H, ddd, J=11.5, 7.5, 7 Hz), 4.17 (1H, ddd, J=11.5, 7.5, 6.5 Hz), 4.62 (1H, d, J=6.5 Hz), 4.66 (1H, d, J=6.5 Hz), 5.57–5.68 (2H, m). ^{13}C NMR δ: 20.1 (CH_2), 21.0 (CH_3), 27.0 (CH_2), 28.3 (CH_3, C4-methyl), 29.4 (CH_2), 32.2 (CH_2), 33.3 (C, C4), 38.5 (CH_2), 43.1 (C), 47.9 (C), 48.3 (CH), 51.6 (CH_2, C7), 54.1 (CH), 55.0 (CH_3), 61.6 (CH_2), 66.1 (CH, C5), 66.5 (CH_2), 68.8 (CH_2), 84.0 (CH), 96.2 (CH_2), 122.8 (C, CN), 125.1 (CH), 126.9 (CH), 170.6 (C), 206.0 (C, C6). **68**: Colorless glass. HRMS Calcd for $C_{25}H_{35}NO_6$: 445.2462. Found: 445.2466. MS *m/z*: 445 (M^+, 1), 413 (1), 400 (3), 385 (1), 371 (2), 340 (3), 326 (3), 296 (3), 280 (3), 252 (6), 105 (8), 89 (15), 73 (17), 59 (13), 45 (100), 43 (37). IR ($CHCl_3$) cm^{-1}: 2234, 1729, 1702. ^1H NMR δ: 1.25–1.46 (2H, m), 1.37 (3H, s), 1.66–1.85 (3H, m), 1.91 (1H, ddd, J=13, 12.5, 3.5 Hz, H3), 1.97–2.05 (2H, m), 2.04 (3H, s), 2.16 (1H, ddd, J=14, 7.5, 7 Hz), 2.21 (1H, br d, J=19 Hz), 2.34 (1H, dd, J=19, 5 Hz), 2.47 (1H, d, J=20 Hz, H7), 2.52–2.58 (1H, m), 2.63 (1H, d, J=20 Hz, H7), 2.73 (1H, s, H5), 3.37 (3H, s), 3.44–3.61 (2H, m), 3.52 (1H, d, J=6 Hz), 3.62–3.68 (2H, m), 4.06 (1H, ddd, J=11, 7.5, 7 Hz), 4.16 (1H, ddd, J=11.5, 7.5, 6.5 Hz), 4.63 (1H, d, J=6.5 Hz), 4.66 (1H, d, J=6.5 Hz), 5.58–5.68 (2H, m). ^{13}C NMR δ: 17.9 (CH_2), 21.0 (CH_3), 21.2 (CH_3, C4-methyl), 26.1 (CH_2), 27.5 (CH_2), 32.7 (CH_2), 34.8 (C, C4), 37.6 (CH_2), 40.2 (C), 43.0 (CH), 45.5 (CH), 47.2 (C), 52.0 (CH_2, C7), 55.2 (CH_3), 61.3 (CH_2), 63.2 (CH, C5), 66.7 (CH_2), 69.9 (CH_2), 93.6 (CH), 96.4 (CH_2), 124.8 (CH), 125.1 (C, CN), 127.3 (CH), 170.6 (C), 209.7 (C, C6).

4.5.4. Isomerization of 68 to 5 with DBU. A solution of **68** (6 mg, 13.5 μmol) and DBU (10 μl, 67.0 μmol) in benzene (2.5 ml) was refluxed with stirring for 0.5 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (7:4)] yielded a product with lower polarity than **68,** which was identical with **5** (5.5 mg, 92%).

4.5.5. Deprotection of 5 with TMSI to form 69. TMSCl (26 μl, 0.206 mmol) was added to a cooled (0 °C) slurry of **5** (18 mg, 40.4 μmol) and NaI (17 mg, 0.227 mmol) in CH_3CN (2.5 ml) under an Ar atmosphere and the mixture was stirred at 0 °C for 40 min. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (1:1)] furnished **69** (15 mg, 94%) as a colorless glass.

HRMS Calcd for $C_{23}H_{31}NO_5$: 401.2200. Found: 401.2191. MS *m/z*: 401 (M^+, 15), 356 (3), 341 (6), 314 (8), 279 (10), 252 (15), 194 (12), 175 (11), 117 (16), 105 (27), 91 (44), 45 (41), 43 (100). IR ($CHCl_3$) cm^{-1}: 2230, 1725, 1705. ^1H NMR δ: 1.17–1.30 (2H, m), 1.57 (3H, s), 1.59–1.72 (1H, m), 1.78–2.15 (5H, m), 2.05 (3H, s), 2.17 (1H, s, H5), 2.21 (1H, br d, J=13 Hz), 2.32 (1H, dd, J=19, 4.5 Hz), 2.45 (1H, br d, J=19 Hz), 2.47 (1H, d, J=17.5 Hz), 2.63 (1H, d, J=17.5 Hz), 2.67–2.73 (1H, m), 3.47 (1H, ddd, J=10, 7.5, 3 Hz, OCH_2CH$_2$OH), 3.54 (1H, ddd, J=10, 4.5, 3 Hz, OCH_2CH$_2$OH), 3.58–3.69 (1H, m, changed to δ 3.63, ddd, J=10, 4.5, 3 Hz with D_2O), 3.71–3.82 (1H, m, changed to δ 3.76, ddd, J=10, 7.5, 3 Hz with D_2O), 3.97 (1H, d, J=6 Hz), 4.05 (1H, ddd, J=11.5, 7, 7 Hz), 4.18 (1H, ddd, J=11.5, 7.5, 6.5 Hz), 5.57–5.73 (2H, m). ^{13}C NMR δ: 20.2 (CH_2), 21.0 (CH_3), 27.1 (CH_2), 28.0 (CH_3, C4-methyl), 29.3 (CH_2), 32.2 (CH_2), 33.4 (C, C4), 38.4 (CH_2), 43.2 (C), 47.9 (C), 48.1 (CH), 51.6 (CH_2), 54.1 (CH), 61.5 (CH_2), 61.9 (CH_2, CH_2OH), 66.0 (CH, C5), 70.0 (CH_2, OCH_2CH_2OH), 83.2 (CH), 123.0 (C, CN), 125.7 (CH), 126.4 (CH), 170.6 (C), 205.8 (C).

4.5.6. Bromination of 69 to form 70. A solution of **69** (14 mg, 34.9 μmol), Ph_3P (56 mg, 0.214 mmol), and CBr_4 (58 mg, 0.175 mmol) in CH_2Cl_2 (4 ml) was stirred at 0 °C for 5 min and at 24 °C for 1.5 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–EtOAc (2:1)] provided **70** (15 mg, 93%) as a colorless glass. HRMS Calcd for $C_{23}H_{30}BrNO_4$: 465.1337, 463.1357. Found: 465.1355, 463.1370. MS *m/z*: 465, 463 (M^+, 6, 5), 405, 403 (3, 2), 384 (8), 378, 376 (4, 5), 279 (23), 109, 107 (36, 37), 91 (51), 43 (100). IR ($CHCl_3$) cm^{-1}: 2237, 1728, 1707. ^1H NMR δ: 1.15–1.29 (2H, m), 1.56 (3H, s), ca. 1.57–1.70 (1H, m), 1.77–1.82 (1H, m), 1.85–2.04 (2H, m), 1.88 (1H, ddd, J=14, 7.5, 6.5 Hz), 2.05 (3H, s), 2.09 (1H, ddd, J=14, 7.5, 7 Hz), 2.16 (1H, s), 2.23 (1H, dddd, J=13, 3.5, 3.5, 1.5 Hz), 2.31 (1H, dd, J=19, 5 Hz), 2.45 (1H, br d, J=19 Hz), 2.46 (1H, d, J=17.5 Hz), 2.64–2.70 (1H, m), 2.63 (1H, d, J=17.5 Hz), 3.43 (1H, ddd, J=10.5, 6.5, 6 Hz, CH_2Br), 3.48 (1H, ddd, J=10.5, 6, 6 Hz, CH_2Br), 3.63 (1H, ddd, J=10.5, 6.5, 6 Hz), 3.76 (1H, ddd, J=10.5, 6, 6 Hz), 3.93 (1H, d, J=6 Hz), 4.05 (1H, ddd, J=11, 7.5, 7 Hz), 4.17 (1H, ddd, J=11, 7.5, 6.5 Hz), 5.55–5.71 (2H, m). ^{13}C NMR δ: 20.2 (CH_2), 21.0 (CH_3), 27.0 (CH_2), 28.1 (CH_3), 29.3 (CH_2), 30.5 (CH_2, CH_2Br), 32.2 (CH_2), 33.3 (C), 38.4 (CH_2), 43.2 (C), 48.1 (C), 48.3 (CH), 51.6 (CH_2), 54.2 (CH), 61.5 (CH_2), 66.1 (CH), 69.0 (CH_2), 83.6 (CH), 122.8 (C, CN), 125.4 (CH), 126.5 (CH), 170.6 (C), 205.8 (C).

4.5.7. Zinc reduction of 70 to form 71. Zn (784 mg, 12.0 mg atom) and NH_4Cl (43 mg, 0.804 mmol) were added to a solution of **70** (37 mg, 79.7 μmol) in 2-PrOH–H_2O (14:1, 12 ml) and the mixture was refluxed with stirring for 12 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (4:3)] afforded **71** (27 mg, 95%) as colorless prisms, mp 138–139 °C (CH_2Cl_2–hexane). Anal. Calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92. Found: C, 70.49; H, 7.70; N, 3.93. HRMS Calcd for $C_{21}H_{27}NO_4$: 357.1939. Found: 357.1938. MS *m/z*: 357 (M^+, 3), 339 (3), 297 (8), 279 (8), 270 (8), 252 (11), 242 (14), 105 (28), 91 (35), 43 (100). IR (KBr) cm^{-1}: 2238, 1734, 1694. ^1H NMR δ: 1.25 (1H, ddd, J=13.5, 13.5, 3.5 Hz), 1.30 (1H, ddd, J=13, 13, 4 Hz), 1.57 (3H, s), 1.68 (1H, ddddd, J=14, 3.5, 3.5, 3.5, 3.5 Hz), 1.78–2.12 (6H, m), 2.04 (3H, s), 2.14 (1H, d, J=10 Hz, OH), 2.18 (1H, s), 2.35 (1H, dddd, J=19.5, 4.5, 3, 2 Hz), 2.45 (1H, br d, J=19.5 Hz), 2.45 (1H, d, J=17 Hz), 2.63 (1H, d, J=17 Hz), 2.68 (1H, ddd, J=6.5, 6.5, 2 Hz), 4.05 (1H, ddd, J=11.5, 7, 7 Hz), 4.18 (1H, ddd, J=11.5, 7.5, 7.5 Hz), 4.37 (1H, dd, J=10, 6.5 Hz, changed to d, J=6.5 Hz with D_2O, H20), 5.71 (1H, dddd, J=9.5, 6.5, 2, 1.5 Hz), 5.83 (1H, ddd, J=9.5, 3, 3 Hz). ^{13}C NMR δ: 20.2 (CH_2), 21.0 (CH_3), 27.3 (CH_2), 28.0 (CH_3), 29.6 (CH_2), 32.2 (CH_2), 33.3 (C), 38.4 (CH_2), 42.9 (C), 48.1 (C), 50.2 (CH), 51.4 (CH_2), 54.1 (CH), 61.4 (CH_2), 65.8 (CH), 76.0 (CH, C20), 122.7 (C, CN), 127.3 (CH), 128.0 (CH), 170.6 (C), 205.8 (C).

4.5.8. Pyrrolidine ring formation from 71 to form 74 and 75. BuLi (1.56 M in hexane, 1.44 ml, 2.26 mmol) was added to a cooled (−18 °C) solution of *i*-Pr_2NH (0.48 ml, 3.43 mmol) in THF (5 ml) under an Ar atmosphere and the mixture was stirred at the same temperature for 10 min. The mixture was then cooled to −78 °C and TMSCl (0.72 ml, 5.68 mmol) was added. A solution of **71** (20 mg, 56.0 μmol) in THF (3 ml) was added dropwise to this, and the resulting mixture was stirred at −78 °C for 30 min. Et_3N (1.56 ml, 11.2 mmol) was added to the mixture and the whole was further stirred at −78 °C for 15 min. Saturated $NaHCO_3$–H_2O was added and the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed successively with saturated $CuSO_4$–H_2O and saturated $NaHCO_3$–H_2O and then treated as usual to give a mixture of **72** and **73** (34 mg). This was dissolved in THF (8 ml), $LiAlH_4$ (106 mg, 2.79 mmol) was added, and the whole was vigorously stirred under reflux for 1.5 h under an Ar atmosphere. The reaction mixture was cooled in an ice bath and Et_2O saturated with H_2O (4 ml) was slowly added. The volatile materials were removed under reduced pressure and the residue was dried over P_2O_5 for 3 h. A slurry of the residue in CH_2Cl_2 (6 ml) and Et_3N (0.98 ml, 7.04 mmol) was cooled in an ice bath and Boc_2O (328 μl, 1.43 mmol) was added to this under an Ar atmosphere. After the mixture had been stirred at 22 °C for 7 h, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [benzene–EtOAc (6:1)] yielded **74** (7 mg, 31%) and **75** (7 mg, 25%) in order of decreasing polarity. **74**: Colorless glass. HRMS Calcd for $C_{24}H_{35}NO_4$: 401.2564. Found: 401.2578. MS *m/z*: 401 (M^+, 4), 344 (9), 317 (14), 300 (18), 261 (29), 246 (13), 244 (15), 91 (13), 57 (100), 41 (46). IR ($CHCl_3$) cm^{-1}: 1681. ^1H NMR δ: 1.13 (3H, s), 1.30–1.70 (5H, m, including OH), 1.51 (9H, s), 1.67 (1H, ddd, J=14, 7.5, 6.5 Hz, CH_2CH$_2$OH), 1.77–1.90 (2H, m), 1.95–2.00 (1H, m), 2.02 (1H, ddd, J=14, 7.5, 6.5 Hz, CH_2CH$_2$OH), 2.07 (1H, d, J=12.5 Hz, OH), 2.23–2.39 (2H, m), 2.47 (1H, ddd, J=7, 7, 1.5 Hz, H14), 2.51 (1H, d, J=3 Hz, H5), 3.13 (1H, d, J=11 Hz, H19), 3.58 (1H, d, J=11 Hz, H19), 3.71–3.86 (2H, m), 4.11 (1H, dd, J=12.5, 7 Hz, changed to d, J=7 Hz with D_2O, H20), 5.39 (1H, br s, H7), 5.58 (1H, dddd, J=9.5, 7, 2, 2 Hz, H13), 5.83 (1H, ddd, J=9.5, 3, 3 Hz, H12). ^{13}C NMR δ: 17.3 (CH_2, C2), 22.3 (CH_2, C1), 27.2 (CH_2, C11), 28.4 ($CH_3\times3$), 30.5 (CH_3, C18), 31.3 (CH_2, C3), 34.5 (C, C4), 35.2 (CH_2, CH_2CH_2OH), 45.1

(C, C8), 48.4 (C, C10), 50.9 (CH, C9), 52.7 (CH, C14), 60.9 (CH, C5), 61.0 (CH_2, CH_2OH), 64.3 (CH_2, C19), 79.2 (CH, C20), 80.4 (C, $COOCMe_3$), 110.1 (CH, C7), 126.5 (CH, C13), 129.6 (CH, C12), 139.8 (C, C6), 152.5 (C, $COOCMe_3$). **75**: Colorless glass. HRMS Calcd for $C_{29}H_{43}NO_6$: 501.3088. Found: 501.3101. MS *m*/*z*: 501 (M^+, 2), 400 (6), 344 (16), 317 (6), 261 (10), 244 (8), 200 (6), 91 (6), 57 (100), 41 (30). IR ($CHCl_3$) cm^{-1}: 1730, 1681. ^1H NMR δ: 1.12 (3H, s), 1.17–1.26 (1H, m), 1.43–1.68 (3H, m), 1.47 (9H, s), 1.51 (9H, s), 1.75 (1H, ddd, *J*=13.5, 9, 6.5 Hz), ca. 1.77–1.89 (2H, m), 1.93–1.98 (1H, m), 2.06 (1H, d, *J*=12 Hz, OH), 2.09 (1H, ddd, *J*=13.5, 9, 6.5 Hz), 2.27–2.33 (2H, m), 2.48 (1H, ddd, *J*=7, 6, 1.5 Hz), 2.50 (1H, d, *J*=3 Hz, H5), 3.13 (1H, d, *J*=11 Hz), 3.58 (1H, d, *J*=11 Hz), 4.04–4.25 (2H, m), 4.10 (1H, dd, *J*=12, 6 Hz, changed to d, *J*=6 Hz with D_2O), 5.35 (1H, br s), 5.57 (1H, dddd, *J*=9.5, 7, 2, 2 Hz), 5.82 (1H, ddd, *J*=9.5, 3, 3 Hz). ^{13}C NMR δ: 17.3 (CH_2), 22.3 (CH_2), 27.1 (CH_2), 27.7 ($CH_3\times3$), 28.4 ($CH_3\times3$), 30.5 (CH_3), 31.3 ($CH_2\times2$, C3 and CH_2CH_2OBoc), 34.5 (C, C4), 44.8 (C), 48.4 (C), 50.8 (CH), 52.6 (CH), 60.9 (CH, C5), 64.4 (CH_2, C19), 65.1 (CH_2, CH_2OBoc), 79.2 (CH), 80.4 (C, $NCOOCMe_3$), 81.6 (C, $OCOOCMe_3$), 109.3 (CH, C7), 126.3 (CH), 129.6 (CH), 140.0 (C, C6), 152.5 (C), 153.3 (C).

4.5.9. Alcoholysis of 75 to form 74. K_2CO_3 (15 mg, 0.109 mmol) was added to a solution of **75** (7 mg, 14.0 μmol) in MeOH (3 ml) and the mixture was gently refluxed with stirring for 6 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (1:1)] provided **74** (5 mg, 89%).

4.5.10. $NaBH_3CN$ reduction of 74 to form 76. $NaBH_3CN$ (22 mg, 0.349 mmol) and HCl–H_2O (2.5%, 0.50 ml, 0.342 mmol) were successively added in this order to a cooled (0 °C) solution of **74** (7 mg, 17.5 μmol) in MeOH (2 ml) and the mixture was stirred at the same temperature for 10 min and at 19 °C for 2.5 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [benzene–EtOAc (1:1)] afforded **76** (6 mg, 85%) as a colorless glass. HRMS Calcd for $C_{24}H_{37}NO_4$: 403.2721. Found: 403.2719. MS *m*/*z*: 403 (M^+, 1), 347 (5), 303 (32), 258 (11), 105 (13), 91 (15), 57 (100), 41 (44). IR ($CHCl_3$) cm^{-1}: 1674. ^1H NMR (at 50 °C) δ: 0.94 (3H, s), 1.03 (1H, ddd, *J*=13, 11.5, 4.5 Hz, H1), 1.19–1.34 (2H, m), ca. 1.45–1.55 (1H, m), 1.46 (9H, s), ca. 1.59–1.75 (2H, m), 1.60 (1H, d, *J*=7 Hz, H5), 1.76 (1H, ddd, *J*=13, 4, 4 Hz, H1), 1.85–2.05 (3H, m, including OH), 2.15 (1H, dd, *J*=16.5, 8.5 Hz, H7), 2.21–2.28 (2H, m), 2.29 (1H, br d, *J*=16.5 Hz, H7), 2.44 (1H, br dd, *J*=7, 6.5 Hz, H14), 3.10–3.24 (1H, m, H19), 3.39 (1H, d, *J*=11 Hz, H19), 3.61–3.83 (2H, m), 4.11 (1H, br dd, *J*=8.5, 7 Hz, H6), 4.46 (1H, dd, *J*=12, 6.5 Hz, changed to d, *J*=6.5 Hz with D_2O, H20), 5.64 (1H, dddd, *J*=9.5, 7, 2, 2 Hz, H13), 5.79 (1H, ddd, *J*=9.5, 3, 3 Hz, H12).

4.5.11. Benzoylation of 76 to form 77. Benzoyl chloride (44 μl, 0.379 mmol) was added to a solution of **76** (5 mg, 12.4 μmol) in pyridine (0.8 ml) and the mixture was stirred at 19 °C for 14 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (5:1)] gave **77** (6 mg, 95%) as a colorless glass. HRMS Calcd for $C_{31}H_{41}NO_5$: 507.2982. Found: 507.2987. MS *m*/*z*: 507 (M^+, 0.5), 451 (1), 423 (2), 407 (39), 389 (4), 379 (4), 325 (4), 302 (5), 285 (9), 258 (9), 185 (10), 105 (47), 77 (22), 57 (100), 41 (31). IR ($CHCl_3$) cm^{-1}: 1708, 1676. ^1H NMR (at 50 °C) δ: 0.95 (3H, s), 1.05 (1H, ddd, *J*=12.5, 11, 4 Hz), 1.23–1.35 (2H, m), ca. 1.44–1.56 (1H, m), 1.46 (9H, s), 1.62 (1H, d, *J*=7 Hz, H5), 1.66–2.20 (5H, m), 2.02 (1H, d, *J*=12 Hz, OH), 2.23 (1H, dd, *J*=16, 8.5 Hz), 2.25–2.30 (2H, m), 2.31 (1H, br d, *J*=16 Hz), 2.52 (1H, br dd, *J*=7, 6 Hz), 3.10–3.26 (1H, m), 3.40 (1H, d, *J*=11 Hz), 4.14 (1H, br dd, *J*=8.5, 7 Hz), 4.36 (1H, ddd, *J*=11, 8, 7 Hz, CH_2OBz), 4.46 (1H, ddd, *J*=11, 8, 5.5 Hz, CH_2OBz), 4.50 (1H, dd, *J*=12, 6 Hz, changed to d, *J*=6 Hz with D_2O, C20), 5.63 (1H, dddd, *J*=9.5, 7, 2, 2 Hz), 5.80 (1H, ddd, *J*=9.5, 3, 3 Hz), 7.38–7.44 (2H, m), 7.50–7.57 (1H, m), 7.99–8.04 (2H, m).

4.5.12. Completion of the synthesis of 1 from 77. CF_3COOH (0.10 ml, 1.30 mmol) was added to a cooled (0 °C) solution of **77** (5 mg, 9.86 μmol) in CH_2Cl_2 (0.9 ml) and the mixture was stirred at the temperature for 2 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up gave a residue (5 mg). The residue was dissolved in CH_2Cl_2 (3 ml) and the solution was cooled in an ice bath. Pyridine (32 μl, 0.396 mmol) and $SOCl_2$ (14 μl, 0.192 mmol) were added in this order under an Ar atmosphere and the mixture was stirred at 0 °C for 30 min and at 19 °C for 22 h. Saturated $NaHCO_3$–H_2O was added and the resulting mixture was stirred for 15 min. Extraction with CH_2Cl_2, usual work-up, and separation by Al_2O_3–PTLC (Merck-type E, 0.1% MeOH–CH_2Cl_2) provided **1** (3 mg, 78%) as a colorless glass. HRMS Calcd for $C_{26}H_{31}NO_2$: 389.2353. Found: 389.2353. MS *m*/*z*: 389 (M^+, 100), 374 (5), 284 (13), 254 (48), 240 (17), 160 (37), 105 (81), 91 (27), 77 (64), 41 (24). IR ($CHCl_3$) cm^{-1}: 1708. ^1H NMR δ: 0.96 (3H, s, H18), 1.16–1.55 (4H, m), 1.43 (1H, br s, H5), 1.56–1.66 (2H, m), 1.66–1.70 (1H, m, H9), 1.75 (1H, dd, *J*=13.5, 2.5 Hz, H7), 1.89–2.01 (2H, m, CH_2CH_2OBz), 2.01 (1H, dd, *J*=13.5, 3 Hz, H7), 2.14 (1H, br d, *J*=19 Hz, H11), 2.25 (1H, dddd, *J*=19, 4, 2.5, 2.5 Hz, H11), 2.26 (1H, br d, *J*=7 Hz, H14), 2.38 (1H, d, *J*=12.5 Hz, H19-α), 2.52 (1H, d, *J*=12.5 Hz, H19-β), 2.76 (1H, d, *J*=1 Hz, H20), 3.23–3.28 (1H, m, H6), 4.34 (1H, ddd, *J*=11, 7, 7 Hz, CH_2OBz), 4.44 (1H, ddd, *J*=11, 7.5, 6.5 Hz, CH_2OBz), 5.52 (1H, dddd, *J*=9.5, 4, 2.5, 1 Hz, H12), 5.72 (1H, dddd, *J*=9.5, 7, 2, 1.5 Hz, H13), 7.40–7.47 (2H, m), 7.52–7.58 (1H, m), 8.00–8.04 (2H, m). ^{13}C NMR δ: 19.6 (CH_2, C2), 26.3 (CH_2, C11), 28.0 (CH_2, C1), 28.7 (CH_3, C18), 30.9 (CH_2, CH_2CH_2OBz), 34.0 (CH_2, C3), 35.4 (CH_2, C7), 38.0 (C, C4), 41.6 (C, C8), 48.5 (CH, C14), 49.88 (CH, C9), 49.91 (C, C10), 59.6 (CH, C5), 62.5 ($CH_2\times2$, C19 and CH_2OBz), 65.1 (CH, C6), 72.9 (CH, C20), 125.9 (CH, C12), 128.1 (CH×2, Bz), 129.0 (CH, C13), 129.3 (CH×2, Bz), 130.2 (C, Bz), 132.6 (CH, Bz), 166.4 (C, *C*OPh).

4.5.13. Quaternization of 1 to form 78. MeI (96 μl, 1.54 mmol) was added to a solution of **1** (1.5 mg, 3.86 μmol) in MeOH (1 ml) and the mixture was stirred at 22 °C for 50 h. Volatile materials were removed and the resulting residue was subjected to PTLC (10% MeOH–CH_2Cl_2) to afford **78** (1.5 mg, 73%) as a colorless glass.

MS *m*/*z*: 389 (M^+−MeI, 56), 374 (3), 284 (9), 267 (51), 254 (28), 160 (30), 142 (58), 127 (26), 122 (40), 105 (100), 91 (26), 77 (91), 51 (35). IR ($CHCl_3$) cm^{-1}: 1710. ^1H NMR δ: 1.30 (3H, s, H18), 1.16–1.87 (6H, m), 1.96 (1H, br s, H5), 1.91–1.95 (1H, m, H9), ca. 1.96–2.15 (3H, m), 2.21 (1H, br d, J=20 Hz, H11), 2.30–2.40 (1H, m, H11), 2.37 (1H, dd, J=15, 3 Hz, H7), 2.76 (1H, dd, J=6, 1 Hz, H14), 3.47 (1H, d, J=12.5 Hz, H19-α), 3.53 (3H, s, N^+–CH_3), 3.93 (1H, d, J=12.5 Hz, H19-β), 3.98 (1H, d, J=1.5 Hz, H20), 4.32–4.37 (1H, m, H6), 4.40 (1H, ddd, J=11.5, 6.5, 6.5 Hz), 4.47 (1H, ddd, J=11.5, 6.5, 6.5 Hz), 5.77 (1H, br d, J=9.5 Hz, H12), 5.96 (1H, br dd, J=9.5, 6 Hz, H13), 7.44–7.51 (2H, m), 7.56–7.63 (1H, m), 7.98–8.03 (2H, m). ^{13}C NMR δ: 18.6 (CH_2, C2), 25.7 (CH_2, C11), 27.9 (CH_2, C1), 28.5 (CH_3, C18), 29.9 (CH_2, *C*$H_2$$CH_2$OBz), 31.2 ($CH_2$, C7), 32.9 ($CH_2$, C3), 36.6 (C, C4), 41.8 (CH_3, N^+–CH_3), 42.4 (C, C8), 43.6 (CH, C14), 49.8 (CH, C9), 50.6 (C, C10), 57.1 (CH, C5), 61.4 (CH_2, CH_2*C*H_2OBz), 70.8 (CH_2, C19), 71.7 (CH, C6), 76.7 (CH, C20), 124.8 (CH, C12), 128.4 (CH×2, Bz), 128.9 (CH, C13), 129.2 (CH×2, Bz), 129.4 (CH, Bz), 133.1 (CH, Bz), 166.2 (C, *C*OPh).

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Synthetic study of hetisine-type aconite alkaloids. Part 3: Total synthesis of (±)-nominine

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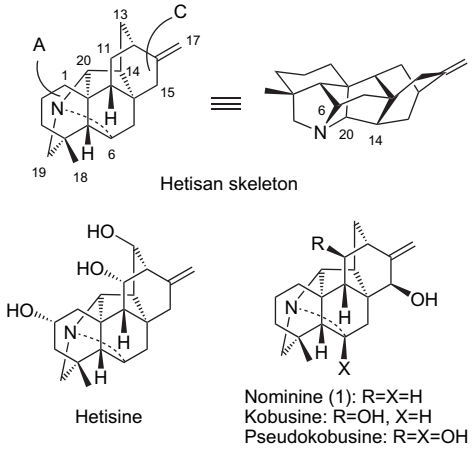
Abstract—Completion of the total synthesis of (±)-nominine (**1**) is described in detail. Based on the results of the preceding two papers, total synthesis of (±)-nominine was accomplished diverging from the intermediate **7**. Thus, following pyrrolidine ring formation through transformation from **7** to **8**, the C-ring was constructed by radical cyclization to form **10** from the enyne precursor **9**. Subsequent elaboration of the C-ring, followed by formation of the azabicyclic ring system, completed a total synthesis of (±)-**1**. Single-crystal X-ray analysis of (±)-**1** unambiguously confirmed its molecular structure and racemic crystal structure.

1. Introduction

1.1. The hetisine-type aconite alkaloid nominine

The term aconite alkaloid is applied to the diterpene alkaloids isolated from *Aconitum*, *Delphinium*, *Consolida*, *Thalictrum*, and *Spiraea*. These alkaloids are generally classified into five skeletons, atidane, veatchane, cycloveatchane, aconitane, and hetisan (the name of which is derived from hetisine) based on their fundamental frameworks.[1,2] Extensive synthetic efforts over the last 40 years have resulted in total syntheses of several alkaloids belonging to the first four of the above five groups. However, the synthesis of even the basic skeleton of the hetisine-type alkaloids, which include hetisine, nominine, kobusine, etc. had remained elusive until we recently reported a total synthesis of (±)-nominine (**1**) (Scheme 1).[3]



Scheme 1. Hetisan skeleton and representative hetisine-type aconite alkaloids.

Nominine (**1**) is structurally the simplest hetisine-type aconite alkaloid. Ochiai et al. first isolated **1** as 'Nomi-base I' from *Aconitum sanyoense* Nakai, collected at Nomi, Sakyo-ku, Kyoto prefecture, Japan in 1956.[4] Sakai et al. gave it the name nominine in 1982 and determined the absolute structure by chemical correlation with kobusine, whose structure was established unequivocally by single crystal X-ray analysis.[5] The name nominine was redundantly given to an insecticidal indole diterpene in 1989.[6]

1.2. Synthetic background

Discovery of the palladium-catalyzed intramolecular α-arylation reaction of aliphatic ketone, formyl, and nitro groups[7] triggered our synthetic studies leading toward the total synthesis of aconite alkaloids with the hetisan skeleton.[8] Our synthetic efforts culminated in a total synthesis of **1**, which was reported in a preliminary communication.[3] In the preceding two papers,[9,10] we have presented full details of the preparation of compound **2** lacking the C-ring of the hetisan framework, starting from **3** by way of the intermediates **4**–**7**. We employed the acetal ene-reaction to form **6**, stereoselective hydrocyanation to form **7**, and azabicyclic ring formation to form **2**, as well as the above Pd-catalyzed cyclization reaction (**4**→**5**), as the key reactions (Scheme 2). Here, we present full details of the synthesis of **1**, diverging from the above-mentioned intermediate **7**.

Keywords: Aconite; Alkaloid; (±)-Nominine; Hetisan; Radical cyclization; X-ray analysis.
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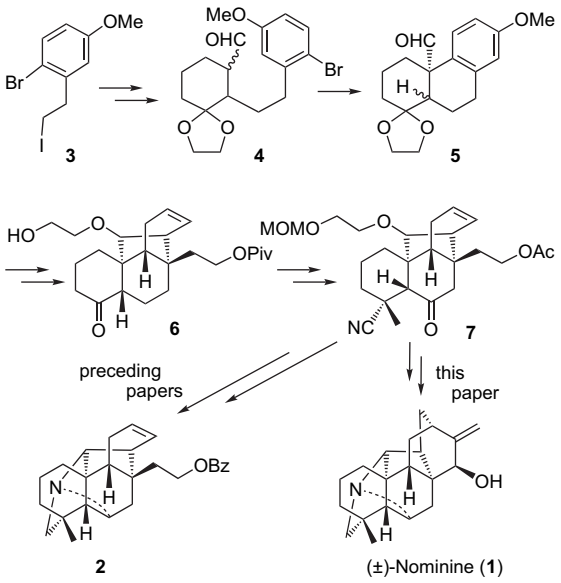
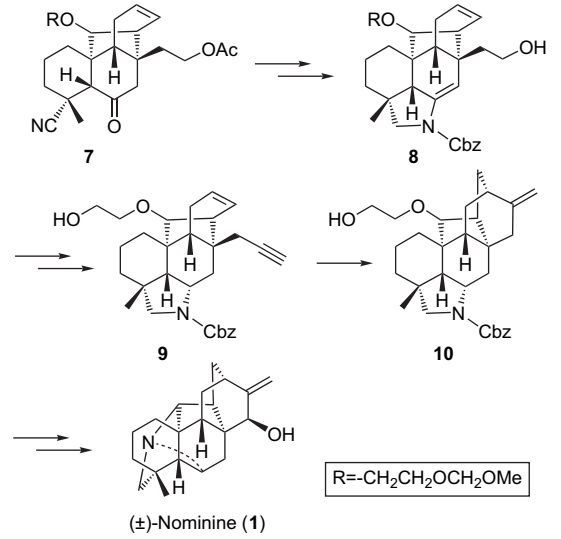


Scheme 2. Outline of the synthetic pathways in the three papers.

We had initially considered synthesizing **1** from **2** through C-ring construction followed by functionalization at C15 with a β-hydroxy substituent. However, taking into consideration that the strong basicity of **2** seriously restricts its versatility as a synthetic intermediate, we decided to construct the bicyclo[2.2.2]octane ring (C-ring) from the intermediate **7**, keeping the nitrogen protected as a carbamate, prior to the creation of the azabicyclic ring system for completion of the total synthesis of **1**.

2. Results and discussion

An outline of the reaction sequence described in this paper is shown in Scheme 3. Compound **7** was transformed to a pentacyclic intermediate **8** according to the method reported in the preceding paper.[10] Compound **8** was led to an enyne derivative **9**, which was then subjected to radical cyclization reaction to secure the hexacyclic intermediate **10**. C-ring elaboration followed by *O*- and *N*-deprotections and azabicyclic ring formation completed the total synthesis of (±)-nominine (**1**). These results constitute the first total synthesis of a hetisine-type aconite alkaloid. We present below full details of not only the reactions along Scheme 3, but also reaction procedures that were ultimately not employed for the total synthesis.

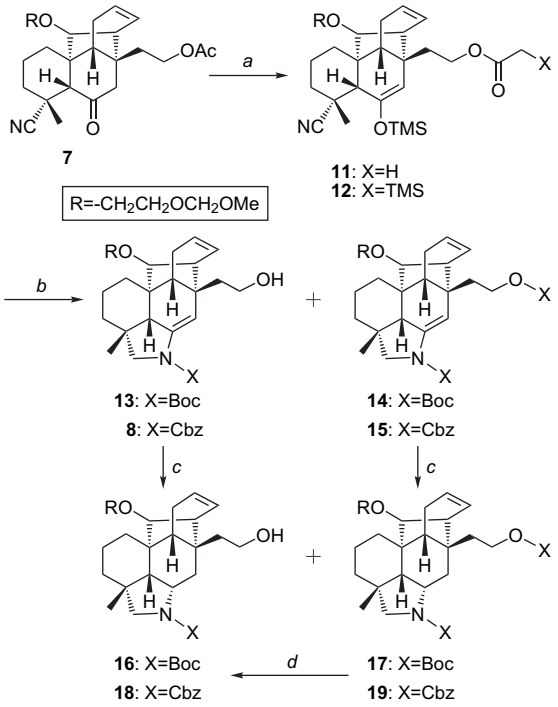


Scheme 3. Outline of the synthesis of **1** from **7**.

2.1. Preparation of pentacyclic intermediates 16 and 18 from 7 by way of 13 and 8

In the preceding paper,[10] we formed the pyrrolidine ring after deprotection of the C20 hydroxy group. In this paper, this protecting group was retained until the final stage of the synthesis with a change of the functional group at the primary hydroxy part of the 2-hydroxyethyl protecting group. The protecting group played a pivotal role in this total synthesis in that it prevented a possible retro-ene reaction with the bond fission of C14 and C20, and it was extremely stable under a wide variety of reaction conditions encountered in the synthetic procedures.

Kinetic enolate formation from **7** with lithium diisopropylamide (LDA) and simultaneous trapping with chlorotrimethylsilane (TMSCl) afforded two products, **11** and **12** (Scheme 4). The latter was a trimethylsilylated compound at the methyl carbon of the acetyl group. Compound **11** was the product derived from the corresponding O-silylated intermediate, which was hydrolyzed during extractive isolation. Of the two products, **11** was subjected, as before,[10] to



Scheme 4. Preparation of **16** and **18** from **7** by way of **13** and **8**: (a) TMSCl, LDA, THF, **11** (79%), **12** (12%); (b) $LiAlH_4$, THF, then Boc_2O or ClCbz, Et_3N, CH_2Cl_2, **13** (62%) from **11**, **14** (16%) from **11**, **13** (55%) overall from **7**, **14** (10%) overall from **7**, **8** (63%) overall from **7**, **15** (4%) overall from **7**; (c) $NaBH_3CN$, 2.5% HCl–H_2O, MeOH, **16** (91%) from **13**, **17** (93%) from **14**, **18** (90%) from **8**, **19** (91%) from **15** and (d) K_2CO_3, MeOH, **16** (quant.) from **17**, **18** (quant.) from **19**.

the lithium aluminum hydride (LAH) reduction followed by protection with di-*tert*-butyl dicarbonate (Boc_2O) to afford **13** (62%) and **14** (16%). As the desired compounds were obtained, the above three operations [enol silylation, LAH reduction, protection with Boc_2O or benzyl chloroformate (ClCbz)] were carried out on **7** without isolation of **11** and **12** to yield **13** (55%) and **14** (10%) or **8** (63%) and **15** (4%), respectively, overall from **7**. Compounds **8** and **15** with the Cbz group functioned as intermediates leading toward **1** in the event, since we could not find suitable reaction conditions to cleave the Boc group after C-ring formation (vide infra).

These four compounds **13**, **14**, **8**, and **15** were separately reduced with sodium cyanoborohydride ($NaBH_3CN$) in a weak acid solvent to afford **16**, **17**, **18**, and **19** in high yields, respectively. Then the carbonates **17** and **19** were converged to **16** and **18**, respectively, by treatment with potassium carbonate (K_2CO_3) in methanol (MeOH).

2.2. Some trials of C-ring formation from 16

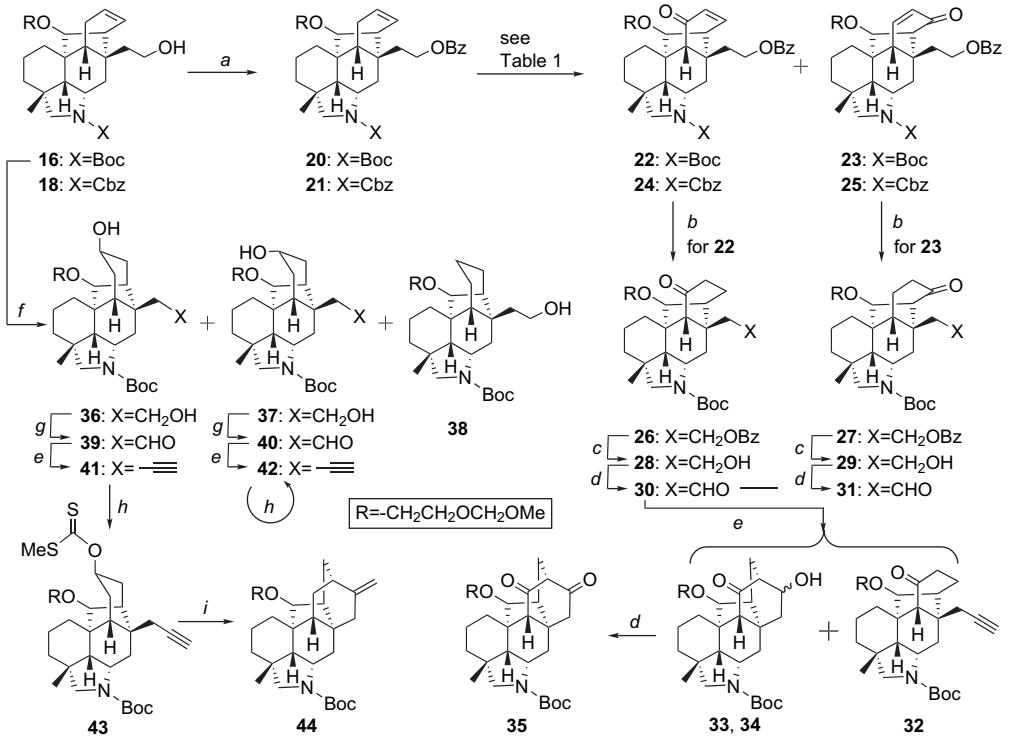
Before description of the C-ring formation by radical cyclization from the enyne precursor **9** to form **10** (Scheme 3), we report here some other attempts to achieve the C-ring formation.

2.2.1. C-ring formation by aldol reaction. Compounds **16** and **18** were led to the benzoates **20** and **21**, respectively, then oxidized with chromium oxide (CrO_3) according to the preceding paper (Scheme 5).[10] Different from the previous case, where the pyrrolidine ring had not yet been formed, the oxidation with CrO_3 and 3,5-dimethylpyrazole did not afford satisfactory results (Table 1). Various amounts of the oxidant were tested (runs a–d), and 6 equiv of CrO_3 (run b) was found to give the best yields of **22** (45%) and **23** (28%). Nevertheless, this condition could not adapted to **21** with the Cbz group, since **24** (12%) and **25** (6%) were obtained only in low yields with 50% recovery of **21** (run e). Further excess of the oxidant resulted in decreased yields of **24**, **25**, and as well as recovery of **21**. Although various conditions[11–14] for the allylic oxidation of **21** were attempted, the only condition affording better results than run e was oxidation with CrO_3 and *tert*-butyl hydroperoxide (*t*-BuOOH),[14] which still gave unsatisfactory yields of **24** (22%) and **25** (16%), along with recovery of **21** in 44% (run f). Since oxidation of **21** gave only disappointing results, we employed **22** and **23** for further transformation in this route.

Table 1. Allylic oxidation of **20** and **21** with CrO_3 (yield: %)

Run[a]	Sub.	CrO_3 (equiv)	Additive[b] (equiv)	**22**	**23**	**24**	**25**	Recovery
a	**20**	4	DP (4.3)	29	20			30
b	**20**	6	DP (6.7)	45	28			4
c	**20**	8	DP (8.7)	34	20			0
d	**20**	12	DP (13)	24	15			0
e	**21**	6.3	DP (7.4)			12	6	50
f	**21**	1.75	BH (10)			22	16	44

[a] All reactions were carried out in CH_2Cl_2.
[b] Additive, DP: 3,5-dimethylpyrazole; BH: *t*-BuOOH.



Scheme 5. Some trials for the C-ring formation: (a) BzCl, Et_3N, CH_2Cl_2, **20** (99%) from **16**, **21** (97%) from **18**; (b) H_2, Pd/C, MeOH, **26** (quant.) from **22**, **27** (quant.) from **23**; (c) K_2CO_3, MeOH, **28** (quant.) from **26**, **29** (quant.) from **27**; (d) PCC–Al_2O_3, CH_2Cl_2, **30** (90%) from **28**, **31** (75%) from **29**, **35** (quant.) from **33**, **35** (quant.) from **34**; (e) dimethyl (1-diazo-2-oxopropyl)phosphonate, K_2CO_3, MeOH, **32** (34%), **33** (33%), **34** (22%), **41** (96%) from **39**, **42** (91%) from **40**; (f) $BH_3{\cdot}SMe_2$, THF then H_2O_2, NaOH, **36** (48–17%), **37** (26–9%), **38** (18–62%); (g) TEMPO, $PhI(OAc)_2$, CH_2Cl_2, **39** (75%) from **36**, **40** (75%) from **37**; (h) NaH, imidazole, THF, then CS_2, then MeI, **43** (85%) and (i) Bu_3SnH, AIBN, toluene, **44** (85%).

The enones **22** and **23** were converted to ketoaldehydes **30** and **31** in three steps with conventional methods [(i) hydrogenation on palladium for **26** and **27**, (ii) alcoholysis with potassium carbonate (K_2CO_3) for **28** and **29**, and (iii) oxidation with pyridinium chlorochromate (PCC)–aluminum oxide (Al_2O_3) for **30** and **31**]. Treatment of **30** with dimethyl (1-diazo-2-oxopropyl)phosphonate[15] and K_2CO_3 as before[10] provided the desired compound **32**, but in only 34% yield, together with 33 and 22% yields of the two isomeric aldol products **33** and **34**, whose stereochemistry at the C16 hydroxy group remains unclear. While these aldols **33** and **34** were readily converted to diketone **35** with PCC–Al_2O_3, reaction of the compound **35** with methylenetriphenylphosphorane gave no desired product but recovery in a preliminary experiment.

2.2.2. C-ring formation by radical cyclization from xanthate. As the attempted route of allylic oxidation followed by aldol reaction proved tricky, we next explored the route by way of the radical intermediate generated from xanthate. The hydroboration–oxidation reaction of **16** provided three products, **36**, **37**, and **38**. We could not find suitable reaction conditions to prevent the formation of the dihydro derivative **38**, and its yield varied from 18 to 62% even though the same equivalent ratio of borane–dimethyl sulfide complex ($BH_3 \cdot SMe_2$) was used, with a corresponding yield variation of **36** (48–17%) and **37** (26–9%). The direction of the hydroxy group of **36** and **37** was determined by the fact that H12 of the major product **36** (equatorial OH) appeared at δ4.22 (ddd, J=10, 10, 7.5, 7.5 Hz) in the ^{1}H NMR spectrum.

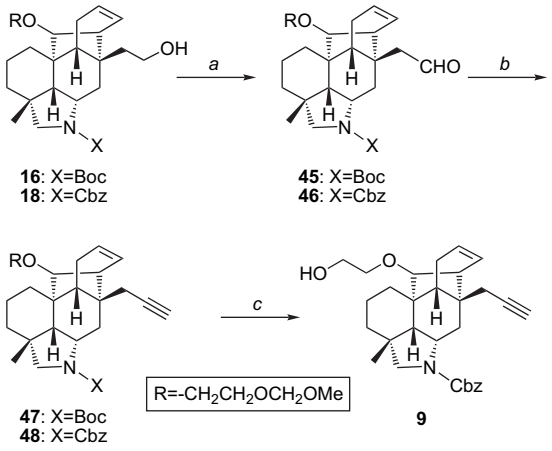
The primary alcohol of the obtained diols **36** and **37** was selectively oxidized with iodobenzene diacetate [PhI(OAc)$_2$] and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) to yield **39** and **40**, respectively.[16] These products were led to the alkynes **41** and **42** in high yields. Our choice of method to cyclize the C-ring from the alkyne-alcohols **41** and **42** was the intramolecular radical cyclization reaction via thioimidazolide or xanthate ester.[17] As the attempted formation of thioimidazolide from **41** or **42** with thiocarbonyldiimidazole in refluxing 1,2-dichloroethane resulted in an intractable mixture from the former or recovery of the starting material from the latter, we next tried to form a xanthate ester as the radical precursor. According to the literature,[18] the equatorial alcohol **41** was easily converted to the corresponding xanthate **43** by successive treatment with sodium hydride (NaH)–imidazole in boiling tetrahydrofuran (THF), carbon disulfide (CS_2), and then iodomethane (MeI). On the other hand, the axial alcohol **42** did not afford the xanthate at all under the same or modified [in THF–dimethylformamide (DMF)] conditions. Radical reaction of **43** with tributyltin hydride (Bu_3SnH) in the presence of 2,2′-azobisisobutyronitrile (AIBN) readily provided the desired hexacyclic compound **44** in a high yield. However, all attempts to remove the methoxymethyl (MOM) or Boc group from **44** failed due to the instability of the newly formed methylenebicyclo[2.2.2]octane ring of **44** under acidic conditions.

Thus, we could acquire the desired compound **44** from **16**. This route, however, suffered from the following three drawbacks: (1) the reproducibility of the hydroboration–oxidation used to form **36** from **16** was poor; (2) the xanthate could not be obtained from the axial alcohol **37**; (3) the MOM and Boc groups could not be removed after the formation of the methylenebicyclo[2.2.2]octane ring. Therefore, we decided to explore another C-ring formation method starting from **16** and **18** in a more straightforward manner.

2.3. C-ring formation from 16 and 18 by radical cyclization of enyne precursor

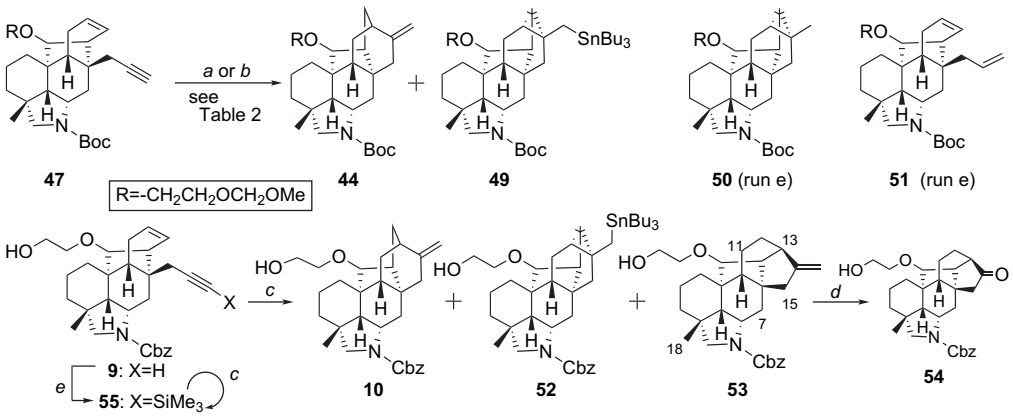
As described above, it turned out that (1) the methylenebicyclo[2.2.2]octane framework was more sensitive than expected to acidic conditions, and (2) the radical cyclization method was effective for construction of this framework, but the examined xanthate route was problematic. Taking these points into consideration, we decided to construct the C-ring by the radical cyclization of an enyne precursor prepared from **16** and **18**.

2.3.1. Preparation of enyne radical cyclization precursors. Compounds **16** and **18** were oxidized separately with PCC–Al_2O_3 as usual to get the aldehydes **45** and **46**, respectively (Scheme 6). These were led to the enyne derivatives **47** and **48**, respectively, with dimethyl (1-diazo-2-oxopropyl)-phosphonate[15] and K_2CO_3 as before. The MOM group of **48** was cleaved at this stage to yield **9**, which was finally employed as the radical cyclization precursor. This is because of the instability of the methylenebicyclo[2.2.2]octane framework under acidic conditions, as noted above. Compounds **47** and **9** were the precursors of choice for the next radical cyclization reaction.



Scheme 6. Preparation of enyne radical cyclization precursors: (a) PCC–Al_2O_3, CH_2Cl_2, **45** (91%) from **16**, **46** (84%) from **18**; (b) dimethyl (1-diazo-2-oxopropyl)phosphonate, K_2CO_3, MeOH, **47** (96%) from **45**, **48** (98%) from **46** and (c) 5% HCl, DME–H_2O (3:1), **9** (96%) from **48**.

2.3.2. C-ring formation from 47 and 9. At first, compound **47** was subjected to radical cyclization reaction (Scheme 7, Table 2). The reactions with Bu_3SnH in the presence of AIBN in refluxing benzene or toluene (runs a–d) according to the protocol of Stork[18] afforded the desired compound **44** and a stannylcyclopropane derivative **49** as a by-product after destannylation of the vinyl stannane products with silica gel. Compound **44** was identical with an authentic specimen obtained via the xanthate route from **43** (Scheme 5). The reactions with 10 equiv of Bu_3SnH gave comparable results in either benzene (run a) or toluene (run b). The



Scheme 7. C-ring formation from **47** and **9** by radical reaction or reductive palladium catalyst: (a) Bu_3SnH, AIBN (20 mol %), benzene or toluene under reflux, then SiO_2, CH_2Cl_2 (for yields see Table 2); (b) $Pd_2(dba)_3 \cdot CHCl_3$, poly(methylhydrosiloxane) (PMHS), *N*,*N*′-bis(benzylidene)-1,2-ethylenediamine, HOAc, benzene under reflux (for yields see Table 2); (c) slow addition of Bu_3SnH in toluene into dilute solution of **9** and AIBN in toluene under reflux, then SiO_2, CH_2Cl_2, **10** (57%), **52** (31%), **53** (8%); (d) cat. OsO_4, $NaIO_4$, THF–H_2O, **54** (quant.) and (e) TMSCl, $(TMS)_2NLi$, THF, then aq HCl–THF, **55** (81%).

Table 2. C-ring formation from **47** to form **44**

Run	Bu_3SnH (equiv)	Solvent	Time (h)	**44** (%)	**49** (%)	Recovery (%)
a	10	Benzene	2	40	50	—
b	10	Toluene	0.5	43	49	—
c	5	Benzene	2	33	52	—
d	1.3	Toluene	3	50	18	29
e[a]	—	Benzene	0.5	19	—	—

[a] Reaction was carried out with $Pd_2(dba)_3 \cdot CHCl_3$, PMHS, *N*,*N*′-bis(benzylidene)-1,2-ethylenediamine, and HOAc; **50** (19%) and **51** (25%) were also isolated.

reaction in benzene with 5 equiv of Bu_3SnH resulted in lowering of the yield of **44** (run c). The use of a slight excess amount (1.3 equiv) of Bu_3SnH brought about a better result, giving rise to **44** in 50% yield along with **49** in 18% yield and a recovery of **47** in 29% yield (run d). Although the palladium-catalyzed reductive cyclization[19] was adopted for **47**, **44** was obtained in only 19% yield, and the cyclopropane derivative **50** (19%) and simply reduced compound **51** (25%) were isolated as by-products (run e).

These results indicated that coexistence of an excess amount of Bu_3SnH with **47** increased the formation of the by-product **49**. With this in mind, we executed the radical cyclization of **9** as follows so as to allow the substrate **9** to react with small amounts of Bu_3SnH at a time (Scheme 7). A solution of Bu_3SnH (70 mM) in toluene was slowly added dropwise (over 1.5 h) to a solution of **9** (5.5 mM) and a catalytic amount of AIBN (22 mol %) in toluene at reflux to secure the desired compound **10** in 57% yield, together with by-products **52** (31%) and **53** (8%). The structure of **53** was confirmed by transformation to the corresponding cyclopentanone derivative **54** (IR: ν_{max}=1730 cm^{-1}) through the Lemieux oxidation. The formation of the by-product **52** was attributable to radical trapping with Bu_3SnH at C17 after two radical cyclizations, 6-*exo* (*endo*)-*trig* and 3-*exo-trig* (vide infra). Therefore **9** was led to the trimethylsilyl derivative **55**, and this was subjected to the radical reaction for the purpose of avoiding formation of **52**. This attempt, however, resulted in complete recovery of **55**, probably due to failure of formation of the initial vinyl radical.

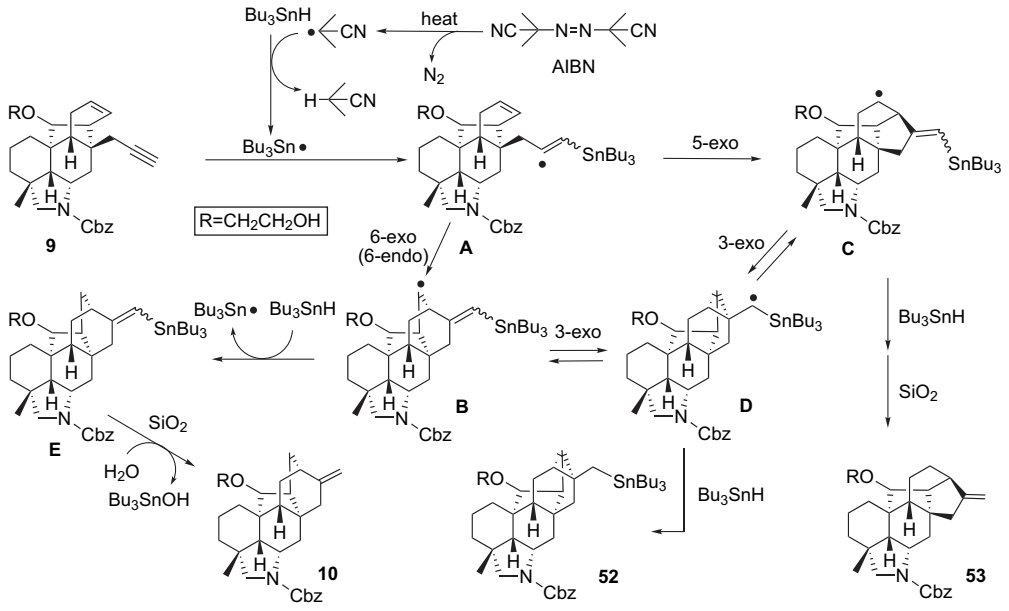
2.3.3. Reaction mechanism of the radical cyclization. A likely reaction mechanism for the radical cyclization is shown in Scheme 8, taking the reaction from **9** as an example. The reaction starts with the pyrolysis of AIBN to generate isobutyronitrile radical by elimination of molecular nitrogen. The radical abstracts hydrogen radical from the gradually dropped Bu_3SnH to give tributyltin radical. Then the tin radical adds to the alkyne of **9** to form radical intermediate **A**. There are two modes for the radical cyclization from **A**, i.e., 6-*exo-trig* mode giving radical intermediate **B** and 5-*exo-trig* mode giving **C**. The desired former mode is also conceivable as 6-*endo-trig*, as the olefin $\Delta_{12,13}$ was originally involved in a six-membered ring. Although formation of the undesired **C** is in danger of taking precedence over that of **B** in accordance with the Baldwin rule,[20] in practice, we were able to get **10** as the main product.

The intermediate **B** could plausibly be derived from **C** by way of the cyclopropane-radical intermediate **D** through homoallyl–homoallyl radical rearrangement.[21] But the facts shown in Table 2, runs a–d, suggest that the intermediate **B** is directly generated from **A**, because the compound corresponding to **53** derived from a **C**-type intermediate was not isolated in runs a–d, where **47** and AIBN was simply heated with a coexisting excess amount of Bu_3SnH from the start.

The radicals **B** and **C** were trapped by Bu_3SnH to form **10** and **53**, respectively, after destannylation from vinyl stannane by stirring with SiO_2 in CH_2Cl_2. The intermediate **D**, generated from **B** and/or **C** in 3-*exo-trig* mode, abstracts a hydrogen radical to yield the by-product **52**. There appears to be fast equilibration between **B** (thermodynamically favored) and **D** (kinetically favored), judging from the results that **49** was the main product in runs a–d (Table 2), while the desired **10** became the main product from **9** when Bu_3SnH was slowly added.

2.4. Completion of the total synthesis of (±)-nominine (1) from 10

The remaining requirements to obtain **1** from **10** are: (i) introduction of 15β-OH and (ii) construction of the azabicyclic ring system after deprotections of oxygen and nitrogen.

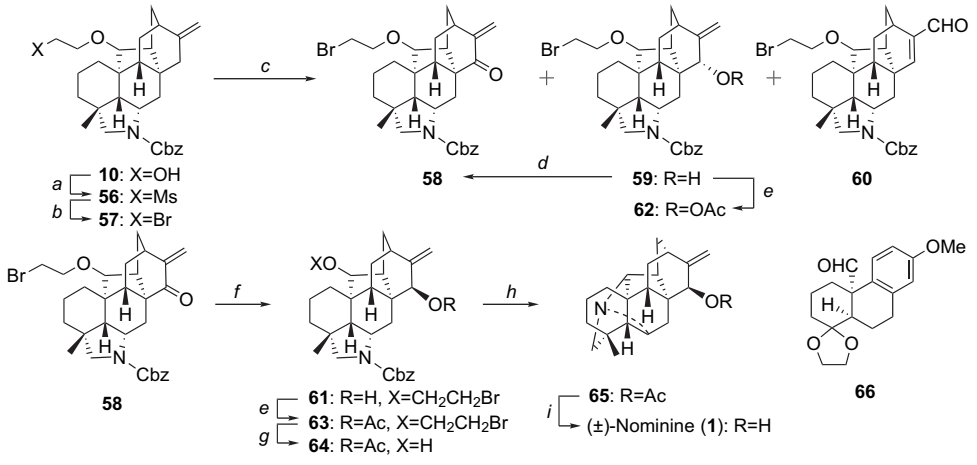


Scheme 8. Reaction mechanism for the radical cyclization of **9** to form **10**, **52**, and **53**.

2.4.1. Introduction of 15β-OH. Prior to the oxidation at C15, the hydroxy group was transformed to a bromide **57** by way of a mesylate **56** (Scheme 9). The 2-bromoethyl group is a convenient precursor for the unprotected 20-hydroxy derivative, as reported in the preceding paper.[9] Attempted direct conversion of **10** to **57** with Br_4C and Ph_3P as before, however, failed due to instability of the methylenebicyclo[2.2.2]octane framework of **10** under the slightly acidic reaction conditions.

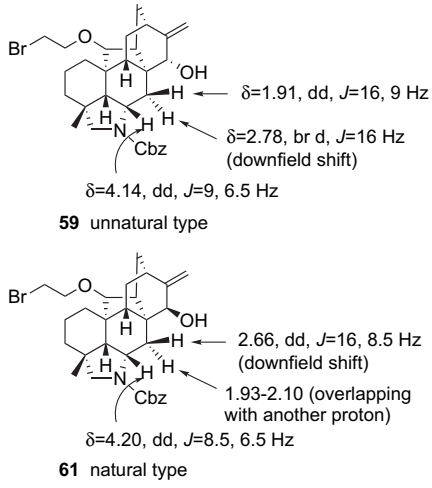
The high reactivity of the framework turned out to be favorable in the next oxidation, as follows. Exposure of **57** to *tert*-butyl hydroperoxide (*t*-BuOOH) and selenium dioxide (SeO_2) readily afforded the enone **58** (77%), allyl alcohol **59** (14%), and enal **60** (trace amount). The 15α-hydroxy compound **59**, with unnatural C15 configuration, was oxidized quantitatively to **58** with manganese dioxide (MnO_2). The desired 15β-hydroxy compound **61** was secured by the reduction of **58** with sodium borohydride ($NaBH_4$) and cerium chloride ($CeCl_3 \cdot 7H_2O$) in MeOH. The stereochemistry of **59** and **61** is described below. These results mean that both oxidizing agent for **57** and reducing agent for **58** attack C15 exclusively from the α side. We cannot give a satisfactory explanation for such stereoselectivity in spite of the symmetric nature of the surroundings of C15 of **57** and **58** in the methylenebicyclo[2.2.2]octane framework.

2.4.2. Stereoconfiguration of 59 and 61. The stereochemistries of **59** and **61** were determined on the basis of the rule reported by Kawazoe et al. (Scheme 10).[22] Thus, in the ^1H NMR spectra, the signals for 7α-H of **59** and 7β-H of **61** are shifted downfield by the influence of the vicinal *syn* 15-hydroxy group (the signals due to the 7α- and 7β-protons of **59**, **61** are easily discriminable by their *J* values with H6). Furthermore, the signals due to the corresponding hydrogen atom of the acetylated compounds **62** and **63** are shifted



Scheme 9. Completion of the total synthesis of (±)-nominine (**1**) from **10**: (a) MsCl, Et_3N, CH_2Cl_2, **56** (97%); (b) LiBr, acetone, **57** (90%); (c) *t*-BuOOH, SeO_2, CH_2Cl_2–H_2O, **58** (77%), **59** (14%), **60** (trace); (d) MnO_2, CH_2Cl_2, **58** (quant.) from **59**; (e) Ac_2O, pyridine, CH_2Cl_2, **62** (93%) from **59**, **63** (93%) from **61**; (f) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, **61** (quant.); (g) Zn, NH_4Cl in *i*-PrOH–H_2O (14:1), **64** (97%); (h) Et_3SiH, cat. $Pd(OAc)_2$, cat. Et_3N, CH_2Cl_2, then $SOCl_2$, pyridine, CH_2Cl_2, **65** (80%) overall from **64** and (i) K_2CO_3 in MeOH, (±)-nominine (**1**) (95%).

upfield in accordance with the rule [δ=2.38–2.58 ppm for 7α-H of **62** and $\delta \approx$ 2.0–2.2 ppm for 7β-H of **63** (overlapping with other signals)]. Consequently, it was clarified that the 15-hydroxy group of **59** has the unnatural α-configuration, while **61** has the natural β-configuration. In the ^{1}H NMR spectrum of **62**, the fact that a weak NOE enhancement (ca. 2.1%) was observed at δ 7.24–7.39 (phenyl protons of the Cbz group) on irradiation of the singlet at δ 1.48 (methyl protons of the acetyl group) also gave support to the above assignment.



Scheme 10. Structure assignments of **59** and **61**.

2.4.3. The final stage of the total synthesis. Now the synthesis reached its final stage. The 2-bromoethyl group of **63** was readily removed to provide **64** in a high yield by the method reported before,[9] i.e., stirring with zinc (Zn) and ammonium chloride (NH_4Cl) in refluxing 2-propanol/H_2O (14:1) (Scheme 9). The protecting group of the 20-hydroxy group, originating from ethylene glycol, had been retained for 21 steps, since it was first introduced at the ene reaction in the preceding paper.[9] The next task is removal of the Cbz group. Hydrogenation or Birch reduction, generally used for this purpose, cannot be employed for **64**, as it contains olefin and ester groups. The cleavage of the Cbz group was executed with triethylsilane (Et_3SiH) in the presence of palladium acetate [$Pd(OAc)_2$] and triethylamine (Et_3N) according to the literature method.[23] The resulting amino-alcohol was then subjected, without purification other than extractive isolation, to azabicyclo ring formation with thionyl chloride ($SOCl_2$) and pyridine[24] to furnish *O*-acetylnominine (**65**) in good yield. The target alkaloid, (±)-nominine (**1**) was easily obtained from **65** by conventional alcoholysis with K_2CO_3 in MeOH.

2.4.4. Identity with the natural alkaloid and single-crystal X-ray analysis. The spectral data (MS, IR, ^{1}H NMR, and ^{13}C NMR) of the synthesized (±)-**1** were indistinguishable with those of natural nominine (see the Section 4). However, these data do not verify the synthesized specimen to be a racemate. Therefore, we carried out a single-crystal X-ray analysis. The molecular structure of (±)-**1** was proved to be identical with that of nominine (Fig. 1). This provides a direct demonstration of the β-configuration of the 15-hydroxy group, which had been assigned by chemical correlation[5] of

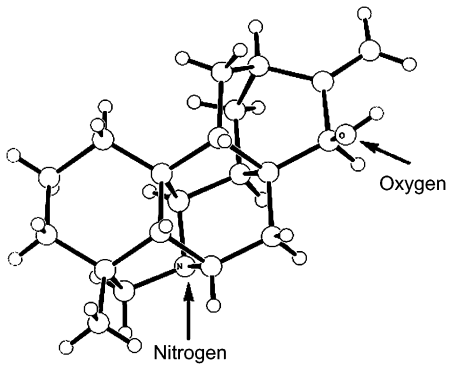


Figure 1. Molecular structure of (±)-nominine (**1**).

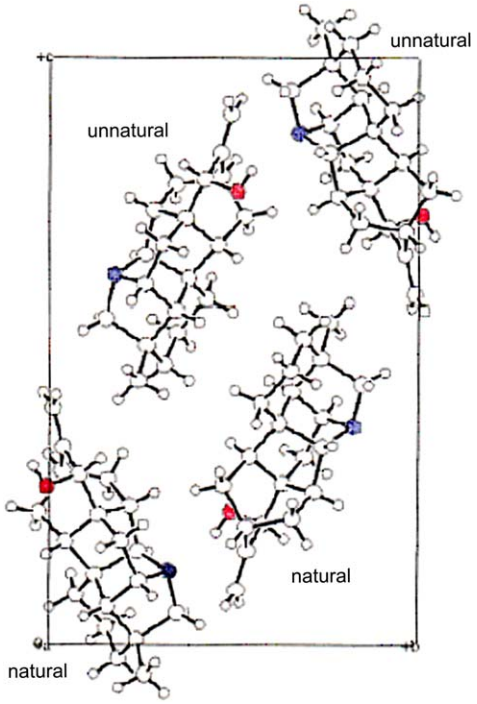


Figure 2. Crystal structure of (±)-nominine (**1**).

natural nominine with kobusine. Furthermore, the crystal structure of (±)-**1** revealed that the analyzed single crystal is racemic (Fig. 2). Thus, the X-ray analysis confirmed the validity of the total synthesis executed according to the schemes described in this and the preceding two papers.[9,10] It was fortunate that recrystallization of (±)-**1** gave a racemic crystal, since spontaneous resolution occurs occasionally during recrystallization. A single crystal of the intermediate **66**, incidentally, was proved to be optically active by X-ray analysis.[7c,9]

3. Conclusion

In summary, a synthetic route to (±)-nominine (**1**) from **7** was established, involving radical cyclization for the C-ring formation (**9**→**10**), stereoselective introduction of the 15β-hydroxy group into **10**, as well as azabicyclic ring construction to afford (±)-**1**. The work described in this and the two preceding reports[9,10] constitutes a 40-step total

synthesis of (±)-**1** starting from 1-bromo-2-(2-iodoethyl)-4-methoxybenzene in 0.15% overall yield (ca. 85% yield per step). This is the first total synthesis of a hetisine-type aconite alkaloid, of which nearly 100 have been isolated up to now. Over 60 years have elapsed since hetisine was isolated[25] as the first example of an aconite alkaloid with the hetisan skeleton, and over 40 years since its structure was elucidated by X-ray analysis.[26]

4. Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus (hot plate), and are not corrected. MS and high-resolution MS (HRMS) were recorded on a Hitachi M-80B spectrometer in direct inlet mode at an ionizing voltage of 70 eV, and figures in parentheses indicate the relative intensities. IR spectra were measured on a Shimadzu IR-460 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian Mercury 300 (300 MHz) in $CDCl_3$ unless otherwise specified, and coupling constants (J values) are rounded to the nearest 0.5 Hz. ^{13}C NMR spectra were measured on a Varian Mercury 300 (75 MHz) in $CDCl_3$ and ^{13}C multiplicities are shown in parentheses as CH_3 (primary), CH_2 (secondary), CH (tertiary), and C (quaternary). ^{13}C NMR of compound **16** and subsequently synthesized compounds with a Boc or Cbz group on the nitrogen could not be determined due to the presence of rotational isomers at ambient temperature. The NMR signals were assigned using proton decoupling techniques, as well as gCOSY, DEPT, gHSQC, gHMBC and/or NOESY spectra. Some characteristic signals for ^{1}H and ^{13}C NMR were selected and assigned as HX and CX, respectively, where X represents hetisan carbon numbering. Column chromatography was conducted on silica gel (SiO_2, Fuji Davison BW 200) or aluminum oxide (Merck, aluminum oxide 90), and the weight of SiO_2 or Al_2O_3 and the eluting solvent are indicated in parentheses. Preparative TLC (PTLC) was carried out on glass plates (20×20 cm) coated with Merck Silica gel $60PF_{254}$ (0.8 mm thick) unless otherwise specified and the developing solvent is indicated in parentheses. Usual work-up refers to washing of the organic layers with water or brine, drying over anhydrous Na_2SO_4, and evaporating off the solvents under reduced pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use.

4.1. Preparation of 16 and 18 from 7 (Scheme 4)

4.1.1. Preparation of 11 and 12 from 7. Butyl lithium (BuLi, 1.57 M in hexane, 0.56 ml, 0.879 mmol) was added to a cooled (−18 °C) solution of diisopropylamine (*i*-Pr_2NH, 164 μl, 1.17 mmol) in THF (2 ml) under an Ar atmosphere and the mixture was stirred at that temperature for 10 min. The resulting solution was cooled to −78 °C and to this were added TMSCl (0.28 ml, 2.21 mmol) and a THF (2 ml) solution of **7** (13 mg, 29.2 μmol) in this order. After the mixture had been stirred at −78 °C for 30 min, Et_3N (0.61 ml, 4.38 mmol) was added and the resulting mixture was stirred for 5 min. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $CuSO_4$–H_2O, saturated $NaHCO_3$–H_2O, and H_2O, and then treated as usual. Separation by SiO_2 column chromatography [8 g, hexane–EtOAc (3:1)] afforded **11** (12 mg, 79%) and **12** (2 mg, 12%) in order of decreasing polarity. **11**: Colorless glass. HRMS Calcd for $C_{28}H_{43}NO_6Si$: 517.2857. Found: 518.2853. MS *m/z*: 517 (M^+, 3), 472 (2), 428 (2), 351 (16), 247 (81), 148 (14), 105 (13), 89 (17), 73 (59), 45 (100). IR ($CHCl_3$) cm^{-1}: 2225, 1726, 1635. ^{1}H NMR δ: 0.30 (9H, s), 1.05 (1H, ddd, J=12.5, 12.5, 4 Hz, H1), 1.25 (1H, ddd, J=14.5, 14.5, 3.5 Hz, H3), 1.51–1.77 (3H, m), 1.60 (3H, s, H18), 1.92–2.15 (4H, m), 2.04 (3H, s), 2.21 (1H, dd, J=19, 5 Hz, H11), 2.29 (1H, d, J=2 Hz, H5), 2.30 (1H, br d, J=19 Hz, H11), 2.54–2.61 (1H, m, H14), 3.35 (3H, s), 3.48–3.75 (4H, m), 4.03–4.18 (2H, m, CH_2OAc), 4.54 (1H, d, J=6 Hz, H20), 4.63 (1H, d, J=6.5 Hz, CH_2OMe), 4.67 (1H, d, J=6.5 Hz, CH_2OMe), 4.82 (1H, d, J=2 Hz, H7), 5.47–5.62 (2H, m). ^{13}C NMR δ: 0.3 (CH_3×3, $SiMe_3$), 20.4 (CH_2, C2), 21.1 (CH_3), 26.7 (CH_2, C11), 29.6 (CH_2, C1), 30.8 (CH_3, C18), 31.2 (CH_2, CH_2CH$_2$OAc), 33.3 (C, C4), 40.5 (CH_2, C3), 41.4 (C, C8), 49.1 (CH, C14), 48.7 (C, C10), 50.9 (CH, C9), 55.0 (CH_3, CH_2OCH_3), 58.5 (CH, C5), 62.3 (CH_2, CH_2OAc), 66.6 (CH_2, CH_2OMOM), 68.4 (CH_2, CH_2CH$_2$OMOM), 83.9 (CH, C20), 96.2 (CH_2, OCH_2OMe), 111.9 (CH, C7), 124.0 (C, CN), 125.1 (CH, C12 or C13), 126.4 (CH, C12 or C13), 149.8 (C, C6), 170.8 (C, O*C*OCH$_3$). **12**: Colorless glass. HRMS Calcd for $C_{31}H_{51}NO_6Si_2$: 589.3252. Found: 589.3241. MS *m/z*: 589 (M^+, 3), 574 (1), 558 (1), 544 (1), 500 (2), 351 (22), 247 (100), 148 (13), 105 (18), 89 (19), 75 (15), 73 (87), 59 (18), 45 (81). IR ($CHCl_3$) cm^{-1}: 2230, 1705, 1638. ^{1}H NMR δ: 0.11 (9H, s, $COCH_2Si$*Me*$_3$), 0.30 (9H, s, $OSiMe_3$), 1.04 (1H, ddd, J=12.5, 12.5, 4 Hz), 1.19–1.31 (1H, m), 1.53–1.74 (3H, m), 1.88 (2H, s, $COCH_2Si$), 1.92–2.15 (4H, m), 1.60 (3H, s), 2.17–2.36 (2H, m), 2.78 (1H, d, J=2 Hz, H5), 2.54–2.60 (1H, m), 3.35 (3H, s), 3.48–3.75 (4H, m), 3.99–4.13 (2H, m), 4.53 (1H, d, J=6 Hz, H20), 4.63 (1H, d, J=6.5 Hz), 4.67 (1H, d, J=6.5 Hz), 4.81 (1H, d, J=2 Hz, H7), 5.47–5.62 (2H, m).

4.1.2. Preparation of 13 and 14 from 11. LAH (40 mg, 1.05 mmol) was added to a solution of **11** (11 mg, 21.3 μmol) in THF (4 ml) and the mixture was refluxed with stirring under an Ar atmosphere for 2 h. The mixture was allowed to cool in an ice bath, and water–saturated Et_2O (2 ml) was slowly added dropwise to it to decompose excess LAH. Volatile materials were evaporated off and the residue was dried over P_2O_5 in vacuo for 3 h. To a slurry of the residue in CH_2Cl_2 (1.5 ml) and Et_3N (0.44 ml, 3.16 mmol) was added Boc_2O (150 μl, 0.640 mmol) at 0 °C under an Ar atmosphere, and the resulting mixture was stirred at 0 °C for 10 min and at 19 °C for 18 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [benzene–EtOAc (4:1)] afforded **13** (6.5 mg, 62%), and **14** (2 mg, 16%) in order of decreasing polarity. **13**: Colorless glass. HRMS Calcd for $C_{28}H_{43}NO_6$: 489.3088. Found: 489.3079. MS *m/z*: 489 (M^+, 4), 389 (3), 344 (7), 317 (10), 300 (18), 282 (13), 261 (70), 246 (15), 57 (100), 45 (92), 41 (33). IR ($CHCl_3$) cm^{-1}: 1682. ^{1}H NMR δ: 1.12 (3H, s), 1.12–1.22 (1H, m), 1.33–1.49 (2H, m), 1.52 (9H, s), 1.53–1.64 (2H, m, including OH), 1.67 (1H, ddd, J=13.5, 7.5, 6.5 Hz, CH_2CH$_2$OH), 1.75–1.90 (1H, m), 1.94–1.99 (1H, m), ca. 1.97–2.04 (1H, m), 2.07 (1H, ddd, J=13.5, 7.5, 6 Hz, CH_2CH$_2$OH), 2.21 (1H, br dddd, J=19,

5, 2.5, 2.5 Hz, H11), 2.32 (1H, br d, *J*=19 Hz, H11), 2.42 (1H, ddd, *J*=6.5, 6, 1.5 Hz, H14), 2.49 (1H, d, *J*=3 Hz, H5), 3.09 (1H, d, *J*=11 Hz, H19), 3.29 (1H, ddd, *J*=10.5, 5.5, 5 Hz, CH_2CH$_2$OMOM), 3.36 (3H, s), 3.48 (1H, ddd, *J*=10.5, 5, 4.5 Hz, CH_2CH$_2$OMOM), 3.57 (1H, d, *J*=11 Hz, H19), ca. 3.60–3.67 (2H, m), 3.68–3.86 (2H, m), 3.77 (1H, d, *J*=6 Hz, H20), 4.62 (1H, d, *J*=6.5 Hz, OCH_2OMe), 4.65 (1H, d, *J*=6.5 Hz, OCH_2OMe), 5.37 (1H, br s, H7), 5.51 (1H, dddd, *J*=9.5, 6.5, 1.5, 1.5 Hz, H13), 5.58 (1H, ddd, *J*=9.5, 3.5, 2.5 Hz, H12). ^{13}C NMR *δ*: 17.6 (CH$_2$), 22.0 (CH$_2$), 26.7 (CH$_2$), 28.4 (CH$_3$×3), 30.6 (CH$_3$, C18), 31.4 (CH$_2$), 34.5 (C, C4), 35.2 (CH$_2$), 45.2 (C), 48.4 (C), 50.2 (CH, C14), 51.2 (CH, C9), 55.1 (CH$_3$), 61.1 (CH$_2$), 61.4 (CH), 64.4 (CH$_2$, C19), 66.9 (CH$_2$), 68.8 (CH$_2$), 80.4 (C, O*C*Me$_3$), 88.1 (CH, C20), 96.5 (CH$_2$), 110.5 (CH, C7), 125.4 (CH), 126.0 (CH), 139.8 (C, C6), 152.6 (C). **14**: Colorless glass. HRMS Calcd for $C_{33}H_{51}NO_8$: 589.3612. Found: 589.3593. MS *m*/*z*: 589 (M$^+$, 3), 489 (2), 433 (2), 417 (5), 400 (4), 344 (15), 317 (12), 282 (10), 261 (53), 57 (100), 45 (59), 41 (29). IR (CHCl$_3$) cm^{-1}: 1731, 1682. ^{1}H NMR *δ*: 1.11 (3H, s), 1.12–1.21 (1H, m), 1.30–1.63 (3H, m), 1.47 (9H, s), 1.52 (9H, s), 1.74 (1H, ddd, *J*=13.5, 9, 6 Hz), 1.79–1.89 (1H, m), 1.93–1.97 (1H, m), 1.97–2.06 (1H, m), 2.14 (1H, ddd, *J*=13.5, 9, 6.5 Hz), 2.21 (1H, br dddd, *J*=19, 5, 2.5, 2.5 Hz, H11), 2.32 (1H, br d, *J*=19 Hz, H11), 2.41 (1H, br dd, *J*=6.5, 6 Hz, H14), 2.44 (1H, d, *J*=3 Hz, H5), 3.09 (1H, d, *J*=11 Hz, H19), 3.29 (1H, ddd, *J*=10.5, 5.5, 5.5 Hz), 3.35 (3H, s), 3.47 (1H, ddd, *J*=10.5, 5, 5 Hz), 3.57 (1H, d, *J*=11 Hz, H19), 3.60–3.66 (2H, m), 3.76 (1H, d, *J*=6 Hz, H20), 4.07–4.24 (2H, m, CH_2OBoc), 4.62 (1H, d, *J*=6.5 Hz), 4.65 (1H, d, *J*=6.5 Hz), 5.34 (1H, br s, H7), 5.49 (1H, br dd, *J*=9.5, 6.5 Hz, H13), 5.57 (1H, ddd, *J*=9.5, 2.5, 2.5 Hz, H12). ^{13}C NMR *δ*: 17.6 (CH$_2$), 21.9 (CH$_2$), 26.7 (CH$_2$), 27.8 (CH$_3$×3), 28.4 (CH$_3$×3), 30.6 (CH$_3$, C18), 31.2 (CH$_2$), 31.4 (CH$_2$), 34.4 (C, C4), 45.0 (C), 48.4 (C), 50.2 (CH, C14), 51.1 (CH, C9), 55.1 (CH$_3$), 61.4 (CH), 64.4 (CH$_2$, C19), 65.3 (CH$_2$, *C*H$_2$OBoc), 66.9 (CH$_2$), 68.9 (CH$_2$), 80.4 (C, O*C*Me$_3$), 81.5 (C, O*C*Me$_3$), 88.1 (CH, C20), 96.5 (CH$_2$), 109.8 (CH, C7), 125.2 (CH), 126.1 (CH), 139.9 (C, C6), 152.6 (C, N*C*OO*t*-Bu), 153.3 (C, O*C*OO*t*-Bu).

4.1.3. Sequential preparation of 13 and 14 from 7 by way of a mixture of 11 and 12. In the same manner as described in Section 4.1.1, a mixture of **11** and **12** was prepared from **7** (52 mg, 0.117 mmol). It was allowed to react with LAH (111 mg, 2.92 mmol) as before and then treated as above. The residue was dissolved in CH_2Cl_2 (4 ml) and Et_3N (0.98 ml, 7.04 mmol) and then the solution was stirred with Boc_2O (0.41 ml, 1.75 mmol) under an Ar atmosphere at 0 °C for 1 h, and at 20 °C for 15 h. The same work-up and separation by PTLC [benzene–EtOAc (6:1)] afforded **13** (38 mg, 55% overall), and **14** (7 mg, 10% overall).

4.1.4. Sequential preparation of 8 and 15 from 7 by way of a mixture of 11 and 12. In the same manner as above (Section 4.1.3), **7** (142 mg, 0.319 mmol) was treated with LDA, TMSCl, and then with LAH to give a residue after having been dried over P_2O_5 in vacuo overnight. To the residue in CH_2Cl_2 (6 ml) and Et_3N (1.33 ml, 9.56 mmol) was added dropwise a solution of ClCbz (0.46 ml, 3.22 mmol) in CH_2Cl_2 (2 ml) during 15 min at −18 °C and the mixture was stirred under an Ar atmosphere at −18 °C for 10 min, at 0 °C for 0.5 h, and at 22 °C for 17 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [benzene–EtOAc (9:1) for **15** and then benzene–EtOAc (5:2) for **8**] afforded **15** (8 mg, 4%) and **8** (105 mg, 63%) in order of increasing polarity. **8**: Colorless glass. HRMS Calcd for $C_{31}H_{41}NO_6$: 523.2932. Found: 523.2941. MS *m*/*z*: 523 (M$^+$, 2), 479 (2), 434 (2), 417 (2), 390 (5), 351 (23), 216 (5), 91 (100), 45 (32). IR (CHCl$_3$) cm^{-1}: 1693. ^{1}H NMR (at 50 °C) *δ*: 1.11 (3H, s, H18), ca. 1.12–1.23 (1H, m, H1), 1.30–1.48 (3H, m, including OH), 1.50–1.63 (1H, m), 1.65 (1H, ddd, *J*=14, 7.5, 6.5 Hz, CH_2CH$_2$OH), 1.76–1.92 (1H, m), 1.93–1.98 (1H, m), ca. 2.00–2.09 (1H, m), 2.05 (1H, ddd, *J*=14, 7, 7 Hz, CH_2CH$_2$OH), 2.22 (1H, dddd, *J*=19, 5, 2.5, 2.5 Hz, H11), 2.31 (1H, br d, *J*=19 Hz, H11), 2.39 (1H, ddd, *J*=7, 6, 1.5 Hz, H14), 2.49 (1H, d, *J*=2.5 Hz, H5), 3.16 (1H, d, *J*=10.5 Hz, H19), 3.26 (1H, ddd, *J*=10.5, 5, 5 Hz, CH_2CH$_2$OMOM), 3.34 (3H, s), 3.46 (1H, ddd, *J*=10.5, 5, 4.5 Hz, CH_2CH$_2$OMOM), 3.59–3.64 (2H, m, CH_2OMOM), 3.67 (1H, d, *J*=10.5 Hz, H19), 3.68–3.78 (2H, m, CH_2OH), 3.75 (1H, d, *J*=6 Hz, H20), 4.60 (1H, d, *J*=6.5 Hz, CH_2OMe), 4.62 (1H, d, *J*=6.5 Hz, CH_2OMe), 5.16 (1H, d, *J*=12.5 Hz, COOCH_2Ph), 5.21 (1H, d, *J*=12.5 Hz, COOCH_2Ph), ca. 5.43–5.52 (1H, m, H7), 5.49 (1H, dddd, *J*=9.5, 7, 1.5, 1.5 Hz, H13), 5.56 (1H, ddd, *J*=9.5, 3, 2.5 Hz, H12), 7.27–7.42 (5H, m, COOCH$_2$*Ph*). *^{13}C NMR of this and subsequently synthesized compounds containing the Cbz group could not be determined due to the presence of rotational isomers*. **15**: Colorless glass. HRMS Calcd for $C_{39}H_{47}NO_8$: 657.3299. Found: 657.3316. MS *m*/*z*: 657 (M$^+$, 1), 568 (1), 524 (3), 485 (11), 333 (4), 198 (4), 91 (100), 45 (33). IR (CHCl$_3$) cm^{-1}: 1734, 1693. ^{1}H NMR *δ*: 1.11 (3H, s), 1.11–1.21 (1H, m), 1.30–1.65 (3H, m), 1.66–1.90 (2H, m), 1.92–1.98 (1H, m), 2.01 (1H, ddd, *J*=13, 11, 1 Hz, H1), 2.11–2.25 (2H, m), 2.30 (1H, br d, *J*=19 Hz, H11), 2.39 (1H, br dd, *J*=6, 6 Hz, H14), 2.48 (1H, d, *J*=3 Hz, H5), 3.17 (1H, d, *J*=11 Hz, H19), 3.25 (1H, ddd, *J*=10.5, 5, 5 Hz), 3.34 (3H, s), 3.45 (1H, ddd, *J*=10.5, 5, 4.5 Hz), 3.59–3.64 (2H, m), 3.66 (1H, d, *J*=11 Hz, H19), 3.76 (1H, d, *J*=6 Hz, H20), 4.18–4.29 (2H, m, CH_2OCbz), 4.61 (1H, d, *J*=6.5 Hz), 4.63 (1H, d, *J*=6.5 Hz), 5.15 (2H, s, OCOOCH_2Ph), 5.16 (1H, d, *J*=12 Hz), 5.21 (1H, d, *J*=12 Hz), ca. 5.36–5.50 (1H, m), 5.46 (1H, br dd, *J*=9.5, 6.5 Hz, H13), 5.55 (1H, ddd, *J*=9.5, 3, 2.5 Hz, H12), 7.30–7.42 (10H, m).

4.1.5. Reduction of 13, 14, 8, and 15 with $NaBH_3CN$. The procedure for the preparation of **16** from **13** is described as a representative example. $NaBH_3CN$ (34 mg, 0.540 mmol) and HCl–H_2O (2.5%, 0.51 ml, 0.349 mmol) were added in this order to a cooled (0 °C) solution of **13** (22 mg, 45.0 μmol) in MeOH (5 ml) and the mixture was stirred at that temperature for 30 min and at 21 °C for 1 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by purification by PTLC [benzene–EtOAc (3:2)] provided **16** (20 mg, 91%) as a colorless glass. HRMS Calcd for $C_{28}H_{45}NO_6$: 491.3244. Found: 491.3246. MS *m*/*z*: 491 (M$^+$, 2), 402 (3), 390 (12), 346 (18), 302 (25), 284 (36), 57 (100), 45 (69), 41 (26). IR (CHCl$_3$) cm^{-1}: 1673. ^{1}H NMR (at 50 °C) *δ*: 0.94 (3H, s, H18), 0.99 (1H, ddd, *J*=10, 7.5, 5.5 Hz, H1), 1.18–1.23 (1H, m, H9), 1.23–1.32 (1H, m, H3), 1.38–1.47 (1H, m,

H2), 1.47 (9H, s), 1.51 (1H, br s, OH), 1.59–1.70 (2H, m), 1.62 (1H, d, *J*=7 Hz, H5), 1.79–2.30 (7H, m), 2.39 (1H, dd, *J*=6, 5.5 Hz, H14), 3.18–3.32 (2H, m, H19×2), 3.35 (3H, s), 3.48 (1H, ddd, *J*=10.5, 6, 5 Hz, C*H*$_2$CH$_2$OMOM), 3.52 (1H, ddd, *J*=10.5, 5, 4.5 Hz, C*H*$_2$CH$_2$OMOM), 3.61–3.68 (2H, m, C*H*$_2$OMOM), ca. 3.62–3.81 (2H, m, C*H*$_2$OH), 4.02 (1H, d, *J*=6 Hz, H20), 4.06–4.16 (1H, m, H6), 4.62 (1H, d, *J*=6.5 Hz, OC*H*$_2$OMe), 4.64 (1H, d, *J*=6.5 Hz, OC*H*$_2$OMe), 5.46–5.61 (2H, m, H12 and H13). *^{13}C NMR of this and subsequently synthesized compounds containing the Boc group could not be determined due to the presence of rotational isomers*. In the same manner, **17** (13 mg, 93%) was obtained from **14** (14 mg, 23.8 μmol) after PTLC [hexane–EtOAc (3:1)] as a colorless glass. HRMS Calcd for $C_{33}H_{53}NO_8$: 591.3768. Found: 591.3761. MS *m*/*z*: 591 (M^+, 1), 502 (1), 491 (3), 434 (9), 402 (10), 390 (5), 346 (45), 284 (10), 89 (8), 57 (100), 45 (40), 41 (26). IR ($CHCl_3$) cm^{-1}: 1732, 1674. ^{1}H NMR (at 50 °C) *δ*: 0.93 (3H, s, H18), 0.98 (1H, ddd, *J*=12.5, 10, 5.5 Hz, H1), 1.23 (1H, d, *J*=4.5 Hz, H9), 1.27 (1H, ddd, *J*=13.5, 11, 5 Hz, H3), ca. 1.39–1.50 (1H, m), 1.47 (18H, s), ca. 1.60–1.70 (1H, m, H3), 1.62 (1H, d, *J*=7.5 Hz, H5), 1.74 (1H, ddd, *J*=13.5, 9, 6 Hz, C*H*$_2$CH$_2$OBoc), 1.79–1.92 (1H, m, H2), 1.92–2.24 (4H, m), 2.24 (1H, dd, *J*=16.5, 9 Hz, H7), 2.25 (1H, br d, *J*=20 Hz, H11), 2.39 (1H, br dd, *J*=6, 6 Hz, H14), ca. 3.11–3.28 (1H, m, H19), 3.28 (1H, br d, *J*=9.5 Hz, H19), 3.35 (3H, s), 3.38 (1H, dt, *J*=10, 5 Hz, C*H*$_2$CH$_2$OMOM), 3.52 (1H, dt, *J*=10, 5 Hz, C*H*$_2$CH$_2$OMOM), 3.64 (2H, dd, *J*=5, 5 Hz, C*H*$_2$OMOM), 4.03 (1H, d, *J*=6 Hz, H20), 4.01–4.23 (3H, m), 4.62 (1H, d, *J*=6.5 Hz, C*H*$_2$OMe), 4.64 (1H, d, *J*=6.5 Hz, C*H*$_2$OMe), 5.50 (1H, br dd, *J*=9.5, 3 Hz, H12), 5.55 (1H, br dd, *J*=9.5, 6 Hz, H13). In the same manner, **18** (77 mg, 90%) was obtained from **8** (85 mg, 0.163 mmol) after PTLC [hexane–EtOAc (3:2)] as a colorless glass. HRMS Calcd for $C_{31}H_{43}NO_6$: 525.3088. Found: 525.3062. MS *m*/*z*: 525 (M^+, 1), 480 (1), 436 (8), 420 (2), 392 (13), 390 (12), 300 (4), 284 (4), 91 (100), 45 (39). IR ($CHCl_3$) cm^{-1}: 1683. ^{1}H NMR (at 50 °C) *δ*: 0.94 (3H, s), 0.98 (1H, ddd, *J*=12.5, 10, 6 Hz, H1), 1.17–1.24 (1H, m), 1.28 (1H, ddd, *J*=13.5, 11, 5 Hz, H3), 1.38–1.50 (2H, m, including OH), 1.55–1.71 (2H, m), 1.64 (1H, d, *J*=7 Hz, H5), 1.80–2.07 (4H, m), 2.10–2.33 (4H, m), 2.32–2.44 (1H, m, H14), ca. 3.21–3.40 (2H, m), 3.33 (3H, s), 3.38 (1H, d, *J*=11 Hz, H19), 3.50 (1H, ddd, *J*=10.5, 5, 4.5 Hz, C*H*$_2$CH$_2$OMOM), ca. 3.55–3.80 (2H, m), 3.60–3.65 (2H, m), 4.00 (1H, d, *J*=6 Hz, H20), 4.19 (1H, dd, *J*=9, 7 Hz, H6), 4.59 (1H, d, *J*=6.5 Hz, C*H*$_2$OMe), 4.62 (1H, d, *J*=6.5 Hz, C*H*$_2$OMe), 5.09 (1H, d, *J*=12.5 Hz, OC*H*$_2$Ph), 5.19 (1H, d, *J*=12.5 Hz, OC*H*$_2$Ph), 5.48–5.60 (2H, m, H12 and H13), 7.26–7.38 (5H, m). In the same manner, **19** (5.5 mg, 91%) was obtained from **15** (6 mg, 9.13 μmol) after PTLC [hexane–EtOAc (4:1)] as a colorless glass. HRMS Calcd for $C_{39}H_{49}NO_8$: 659.3455. Found: 659.3434. MS *m*/*z*: 659 (M^+, 0.8), 614 (0.5), 570 (4), 526 (12), 524 (8), 436 (3), 91 (100), 45 (27). IR ($CHCl_3$) cm^{-1}: 1735, 1684. ^{1}H NMR (at 50 °C) *δ*: 0.94 (3H, s), 0.97 (1H, ddd, *J*=12.5, 9.5, 5.5 Hz, H1), 1.18–1.25 (1H, m), 1.27 (1H, ddd, *J*=13.5, 11, 5 Hz, H3), 1.39–1.51 (1H, m), ca. 1.60–1.71 (1H, m), 1.79–1.92 (1H, m), 1.63 (1H, d, *J*=7.5 Hz, H5), 1.98 (1H, ddd, *J*=12.5, 4.5, 4.5 Hz, H1), 2.10 (1H, ddd, *J*=13.5, 9, 6.5 Hz), 2.13–2.32 (5H, m), 2.32–2.43 (1H, m), 3.22–3.41 (3H, m), 3.32 (3H, s), 3.48 (1H, ddd, *J*=10.5, 4.5, 4.5 Hz), 3.59–3.65 (2H, m), 4.00 (1H, d, *J*=6 Hz, H20), 4.04–4.34 (3H, m, H6 and C*H*$_2$OCbz), 4.59 (1H, d, *J*=6.5 Hz), 4.61 (1H, d, *J*=6.5 Hz), 5.09 (1H, d, *J*=12.5 Hz), 5.14 (2H, s, OCOOC*H*$_2$Ph), 5.19 (1H, br d, *J*=12.5 Hz), 5.45–5.55 (2H, m), 7.27–7.40 (10H, m).

4.1.6. Alcoholysis of 17 and 19 to form 16 and 18, respectively. The procedure for the preparation of **16** from **17** was typical. A solution of **17** (6 mg, 10.2 μmol) in K_2CO_3 in MeOH (1% w/v, 2 ml) was stirred under reflux for 2.5 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [benzene–EtOAc (3:2)] gave **16** (5 mg, quant.) as a colorless glass. In the same manner, **18** (4 mg, quant.) was obtained from **19** (5 mg, 7.59 μmol).

4.2. Attempts at C-ring formation (Scheme 5)

4.2.1. Benzoylation of 16 and 18 to form 20 and 21, respectively. The procedure for the preparation of **20** from **16** is described as a representative example. Benzoyl chloride (98 μl, 0.819 mmol) was added to a cooled (0 °C) solution of **16** (20 mg, 40.7 μmol) in CH_2Cl_2 (1.5 ml) and Et_3N (0.56 ml, 4.03 mmol). Stirring was continued at 0 °C for 15 min and at 20 °C for 16 h, then the mixture was cooled again in an ice bath. MeOH (66 μl, 1.63 mmol) was added to it and the whole was stirred at that temperature for 1 h. Addition of saturated $NaHCO_3$–H_2O followed by extraction with CH_2Cl_2, usual work-up, and separation by PTLC [hexane–EtOAc (4:1)] afforded **20** (24 mg, 99%) as a colorless glass. HRMS Calcd for $C_{35}H_{49}NO_7$: 595.3506. Found: 595.3512. MS *m*/*z*: 595 (M^+, 1), 495 (7), 494 (9), 406 (45), 390 (10), 389 (10), 346 (7), 284 (17), 105 (33), 69 (31), 57 (100), 45 (62), 41 (39). IR ($CHCl_3$) cm^{-1}: 1706, 1674. ^{1}H NMR (at 50 °C) *δ*: 0.95 (3H, s), 0.95–1.05 (1H, m), 1.22–1.34 (2H, m), 1.41–1.54 (1H, m), 1.47 (9H, s), 1.61–1.70 (1H, m), 1.64 (1H, d, *J*=7 Hz, H5), 1.80–1.96 (2H, m), 2.00 (1H, ddd, *J*=13, 5, 5 Hz, H1), 2.12–2.34 (4H, m), 2.35 (1H, dd, *J*=16, 9 Hz, H7), 2.46 (1H, br dd, *J*=6, 5 Hz, H14), 3.13–3.33 (2H, m), 3.35 (3H, s), 3.35–3.43 (1H, m), 3.47–3.56 (1H, m), 3.61–3.68 (2H, m), 4.06 (1H, d, *J*=6 Hz, H20), 4.09–4.21 (1H, m), 4.35 (1H, ddd, *J*=10.5, 8.5, 7 Hz, C*H*$_2$OBz), 4.44 (1H, ddd, *J*=10.5, 8.5, 6 Hz, C*H*$_2$OBz), 4.62 (1H, d, *J*=6.5 Hz), 4.64 (1H, d, *J*=6.5 Hz), 5.48–5.62 (2H, m), 7.39–7.46 (2H, m), 7.50–7.57 (1H, m), 8.00–8.06 (2H, m). In the same manner, **21** (73 mg, 97%) was obtained from **18** (63 mg, 0.120 mmol) as a colorless glass after PTLC [hexane–EtOAc (3:1)]. HRMS Calcd for $C_{38}H_{47}NO_7$: 629.3350. Found: 629.3338. MS (*m*/*z*): 629 (M^+, 3), 540 (10), 496 (20), 494 (15), 480 (5), 404 (5), 336 (6), 105 (21), 91 (100), 59 (11), 45 (38). IR ($CHCl_3$) cm^{-1}: 1684. ^{1}H NMR (at 50 °C) *δ*: 0.95 (3H, s), 1.00 (1H, ddd, *J*=13, 10, 5.5 Hz, H1), 1.22–1.35 (2H, m), 1.39–1.52 (1H, m), 1.61–1.72 (1H, m), 1.67 (1H, d, *J*=7 Hz, H5), 1.77–1.96 (2H, m), 2.01 (1H, ddd, *J*=13, 4.5, 4.5 Hz, H1), 2.13–2.42 (5H, m), 2.39–2.51 (1H, m), ca. 3.25–3.42 (3H, m), 3.32 (3H, s), 3.50 (1H, dt, *J*=10, 5 Hz), 3.63 (2H, dd, *J*=5, 5 Hz), 4.04 (1H, d, *J*=5.5 Hz, H20), 4.23 (1H, br dd, *J*=8, 7 Hz, H6), 4.26–4.48 (2H, m, C*H*$_2$OBz), 4.60 (2H, s), 5.10 (1H, d, *J*=12.5 Hz), 5.18 (1H, d, *J*=12.5 Hz), 5.50–5.60 (2H, m), 7.26–7.38 (5H, m), 7.38–7.46 (2H, m), 7.50–7.57 (1H, m), 8.00–8.07 (2H, m).

4.2.2. Allylic oxidation of 20 and 21 for respective formation of 22, 23 and 24, 25. The procedure for the preparation of **22**, **23** from **20** (Table 1, run b) is described as a representative example. 3,5-Dimethylpyrazole (13 mg, 0.135 mmol) was added to a cooled (−18 °C) slurry of CrO_3 (12 mg, 0.120 mmol) in CH_2Cl_2 (1.5 ml) under an Ar atmosphere and the mixture was stirred at that temperature for 15 min. A CH_2Cl_2 (2 ml) solution of **20** (12 mg, 20.2 μmol) was added to it, and the resulting mixture was stirred at −18 to 23 °C for 48 h. Successive addition of saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O followed by extraction with CH_2Cl_2, usual work-up, and separation by PTLC [hexane–EtOAc (3:1)] afforded **22** (5.5 mg, 45%) and **23** (3.5 mg, 28%) in order of decreasing polarity. **22**: Colorless glass. HRMS Calcd for $C_{35}H_{47}NO_8$: 609.3299. Found: 609.3291. MS (*m*/*z*): 609 (M^+, 1), 509 (18), 420 (29), 360 (14), 105 (53), 89 (11), 77 (18), 57 (100), 45 (85), 41 (25). IR ($CHCl_3$) cm^{-1}: 1709, 1665. ^1H NMR (at 50 °C) *δ*: 0.98 (3H, s), 1.17 (1H, ddd, *J*=13, 10, 5 Hz), 1.25–1.81 (5H, m), 1.47 (9H, s), 1.82 (1H, d, *J*=7 Hz), 1.83 (1H, ddd, *J*=14, 7, 7 Hz), 2.07 (1H, ddd, *J*=14, 7, 7 Hz), 2.13 (1H, s, H9), 2.20–2.38 (2H, m), 3.08 (1H, ddd, *J*=7, 6, 1.5 Hz, H14), 3.21–3.37 (2H, m), 3.33 (3H, s), 3.47–3.58 (2H, m), 3.59–3.65 (2H, m), 4.10–4.19 (1H, m), 4.27–4.34 (2H, m), 4.41 (1H, d, *J*=6 Hz), 4.58 (1H, d, *J*=6.5 Hz), 4.60 (1H, d, *J*=6.5 Hz), 6.19 (1H, dd, *J*=9.5, 1.5 Hz, H12), 6.99 (1H, dd, *J*=9.5, 7 Hz, H13), 7.40–7.47 (2H, m), 7.51–7.58 (1H, m), 7.99–8.03 (2H, m). **23**: Colorless glass. HRMS Calcd for $C_{35}H_{47}NO_8$: 609.3299. Found: 609.3292. MS (*m*/*z*): 609 (M^+, 0.6), 509 (20), 464 (9), 420 (47), 404 (5), 298 (6), 105 (51), 89 (7), 77 (16), 57 (100), 45 (72), 41 (23). IR ($CHCl_3$) cm^{-1}: 1708, 1676. ^1H NMR (at 50 °C) *δ*: 1.00 (3H, s), 1.09 (1H, ddd, *J*=12.5, 11.5, 4 Hz, H1), 1.35 (1H, ddd, *J*=13.5, 3, 4 Hz), 1.47 (9H, s), 1.62–1.79 (3H, m), 1.75 (1H, d, *J*=6.5 Hz, H5), 1.82 (1H, dt, *J*=14, 7 Hz), 1.91 (1H, dt, *J*=14, 7 Hz), 2.01 (1H, ddd, *J*=12.5, 4, 4 Hz, H1), 2.06 (1H, dd, *J*=7, 1.5 Hz, H9), 2.20 (1H, dd, *J*=16.5, 8.5 Hz, H7), 2.39 (1H, br d, *J*=16.5 Hz, H7), 3.11 (1H, ddd, *J*=7, 1.5, 1.5 Hz, H14), 3.21 (1H, br d, *J*=11 Hz, H19), 3.32 (3H, s), 3.32–3.42 (1H, m), 3.38 (1H, d, *J*=11 Hz, H19), 3.50–3.55 (2H, m), 3.60–3.67 (1H, m), 4.07–4.16 (1H, m), 4.28 (2H, dd, *J*=7, 7 Hz), 4.40 (1H, d, *J*=7 Hz, H20), 4.55 (1H, d, *J*=6.5 Hz), 4.57 (1H, d, *J*=6.5 Hz), 6.15 (1H, dd, *J*=9.5, 1.5 Hz, H12), 6.93 (1H, dd, *J*=9.5, 7 Hz, H11), 7.39–7.46 (2H, m), 7.51–7.58 (1H, m), 7.99–8.04 (2H, m). In the same manner, **24** (1 mg, 12%) and **25** (0.5 mg, 6%) were obtained along with a recovery of unchanged starting material (4 mg, 50%) from **21** (8 mg, 12.7 μmol), in order of decreasing polarity (Table 1, run e) after separation by PTLC [hexane–EtOAc (3:1)]. **24**: Colorless glass. HRMS Calcd for $C_{38}H_{45}NO_8$: 643.3143. Found: 643.3142. MS (*m*/*z*): 643 (M^+, 8), 554 (2), 510 (15), 507 (7), 418 (3), 105 (20), 91 (100), 89 (7), 77 (6), 45 (36). IR ($CHCl_3$) cm^{-1}: 1706, 1687, 1666. ^1H NMR (at 50 °C) *δ*: 0.99 (3H, s), 1.11–1.84 (7H, m), 1.84 (1H, d, *J*=7 Hz, H5), 2.06 (1H, ddd, *J*=14, 7, 7 Hz), 2.13 (1H, s, H9), 2.24–2.38 (2H, m), 3.01–3.10 (1H, m, H14), 3.30 (3H, s), 3.30–3.69 (6H, m), 4.18–4.33 (3H, m), 4.38 (1H, d, *J*=6 Hz, H20), 4.56 (2H, s), 5.11 (1H, d, *J*=12.5 Hz), 5.19 (1H, d, *J*=12.5 Hz), 6.11 (1H, dd, *J*=9.5, 1 Hz, H12), 6.98 (1H, dd, *J*=9.5, 7 Hz, H13), 7.26–7.39 (5H, m), 7.40–7.47 (2H, m), 7.51–7.58 (1H, m), 7.98–8.04 (2H, m). **25**: Colorless glass. HRMS Calcd for $C_{38}H_{45}NO_8$: 643.3143. Found: 643.3144. MS (*m*/*z*): 643 (M^+, 3), 554 (3), 510 (18), 508 (9), 476 (6), 420 (4), 105 (27), 91 (100), 59 (7), 45 (46). IR ($CHCl_3$) cm^{-1}: 1702 (sh), 1680. ^1H NMR (at 50 °C) *δ*: 1.01 (3H, s), 1.01 (1H, ddd, *J*=12, 12, 4 Hz), 1.20–1.75 (4H, m), 1.78 (1H, d, *J*=6.5 Hz, H5), 1.81 (1H, dt, *J*=15, 6.5 Hz), 1.89 (1H, dt, *J*=15, 6.5 Hz), 2.01 (1H, br ddd, *J*=12, 3.5, 3.5 Hz), 2.06 (1H, br d, *J*=7 Hz, H9), 2.22 (1H, dd, *J*=16.5, 8.5 Hz), ca. 2.28–2.50 (1H, br m), 3.09 (1H, br d, *J*=7 Hz, H14), 3.25–3.38 (2H, m), 3.29 (3H, s), 3.44–3.53 (3H, m), 3.61 (1H, ddd, *J*=10, 4, 4 Hz), 4.19 (1H, br dd, *J*=8.5, 6.5 Hz), 4.26 (2H, dd, *J*=6.5, 6.5 Hz), 4.37 (1H, d, *J*=7 Hz), 4.54 (2H, s), 5.12 (1H, d, *J*=12.5 Hz), 5.18 (1H, d, *J*=12.5 Hz), 6.15 (1H, dd, *J*=9.5, 1.5 Hz, H12), 6.93 (1H, dd, *J*=9.5, 7 Hz, H11), 7.26–7.38 (5H, m), 7.39–7.47 (2H, m), 7.50–7.58 (1H, m), 7.99–8.05 (2H, m).

4.2.3. Allylic oxidation of 21 with CrO_3 and *t*-BuOOH (Table 1, run f). *t*-BuOOH (70%, 20 μl, 0.146 mmol) was added to a slurry of CrO_3 (2.5 mg, 25 μmol) in CH_2Cl_2 (1.5 ml) and the mixture was stirred at 25 °C for 10 min under an Ar atmosphere. The mixture was allowed to cool in an ice bath, and to this was added a CH_2Cl_2 (1.5 ml) solution of **21** (9 mg, 14.3 μmol). Stirring was continued at 0–25 °C for 42 h and the reaction was quenched by addition of saturated $NaHCO_3$–H_2O and saturated $Na_2S_2O_3$–H_2O. Extraction with CH_2Cl_2, usual work-up, and PTLC [hexane–EtOAc (3:1)] gave **24** (2 mg, 22%), **25** (1.5 mg, 16%), and recovered **21** (4 mg, 44%) in order of decreasing polarity.

4.2.4. Hydrogenation of 22 and 23 to form 26 and 27, respectively. The procedure for the preparation of **26** from **22** was typical. A slurry of **22** (6 mg, 9.85 μmol) and 10% Pd/C (1.5 mg, 1.4 μg atom) in MeOH (3 ml) was stirred under a hydrogen atmosphere (1 atm) at 21 °C for 2 h. The mixture was filtered through a Celite pad and the pad was rinsed with CH_2Cl_2. Evaporation of the combined organic layers followed by separation by PTLC [hexane–EtOAc (7:4)] provided **26** (6 mg, quantitative) as a colorless glass. HRMS Calcd for $C_{35}H_{49}NO_8$: 611.3455. Found: 611.3448. MS (*m*/*z*): 611 (M^+, 1), 510 (10), 422 (57), 377 (16), 349 (4), 328 (6), 300 (14), 105 (49), 89 (7), 77 (12), 57 (100), 45 (63), 41 (19). IR ($CHCl_3$) cm^{-1}: 1706, 1681. ^1H NMR (at 50 °C) *δ*: 0.95–1.07 (1H, m), 0.98 (3H, s), 1.31–1.42 (1H, m), 1.43–1.54 (1H, m), 1.47 (9H, s), 1.62–2.02 (7H, m), 1.64 (1H, d, *J*=7 Hz, H5), 1.84 (1H, s, H9), 2.10 (1H, dd, *J*=16, 8.5 Hz), 2.28 (1H, dd, *J*=18, 7 Hz, H12), 2.40–2.52 (2H, m), 2.50 (1H, ddd, *J*=18, 10, 10 Hz, H12), 3.28 (1H, br d, *J*=11 Hz, H19), 3.35 (3H, s), 3.37 (1H, d, *J*=11 Hz, H19), 3.52–3.62 (1H, m), 3.65–3.74 (3H, m), 4.10 (1H, dd, *J*=8.5, 7 Hz, H6), 4.25 (1H, d, *J*=6.5 Hz), 4.35 (1H, ddd, *J*=11, 9, 6 Hz), 4.46 (1H, ddd, *J*=11, 9, 6.5 Hz), 4.62 (2H, s), 7.39–7.46 (2H, m), 7.51–7.57 (1H, m), 7.99–8.04 (2H, m). In the same manner as above, **27** (4 mg, quantitative) was obtained from **23** (4 mg, 6.57 mmol) as a colorless glass after PTLC [hexane–EtOAc (2:1)]. HRMS Calcd for $C_{35}H_{49}NO_8$: 611.3455. Found: 611.3478. MS (*m*/*z*): 611 (M^+, 1), 510 (10), 466 (9), 450 (7), 422 (51), 328 (7), 300 (7), 105 (38), 57 (100), 45 (61), 41 (23). IR ($CHCl_3$) cm^{-1}: 1704, 1679. ^1H NMR (at 50 °C) *δ*: 0.99 (3H, s), 1.11 (1H, ddd, *J*=13, 11, 4.5 Hz), 1.35 (1H, ddd, *J*=13.5, 12, 5.5 Hz), 1.44–1.58 (2H, m), 1.46 (9H, s), 1.65–2.17 (7H, m), 1.68 (1H, d, *J*=6.5 Hz, H5), 2.17 (1H, dd,

J=16.5, 8.5 Hz), 2.25 (1H, dd, J=17.5, 8.5 Hz, H12), 2.33 (1H, br d, J=16.5 Hz), 2.58 (1H, ddd, J=17.5, 10.5, 10.5 Hz, H12), 2.90 (1H, d, J=7 Hz, H14), 3.22 (1H, br d, J=10.5 Hz, H19), 3.33 (3H, s), 3.36 (1H, d, J=10.5 Hz, H19), 3.38–3.46 (1H, m), 3.51–3.59 (3H, m), 4.12 (1H, dd, J=8.5, 6.5 Hz, H6), 4.28 (1H, d, J=7 Hz), 4.33 (1H, ddd, J=11, 8, 6.5 Hz), 4.43 (1H, ddd, J=11, 8, 6 Hz), 4.58 (2H, s), 7.39–7.46 (2H, m), 7.50–7.57 (1H, m), 8.01–8.07 (2H, m).

4.2.5. Alcoholysis of 26 and 27 to form 28 and 29, respectively. In a similar manner to that described for the preparation of **16** from **17** (Section 4.1.6), **26** (6 mg, 9.82 μmol) and **27** (5 mg, 8.18 μmol) were separately heated in K_2CO_3–MeOH (0.5% w/v, 2.5 ml each) for 2 h to afford, respectively, **28** (5 mg, quantitative) and **29** (4 mg, quantitative) after purification by PTLC [hexane–EtOAc (3:2)]. **28**: Colorless glass. HRMS Calcd for $C_{28}H_{45}NO_7$: 507.3193. Found: 507.3188. MS (*m*/*z*): 507 (M^+, 2), 418 (2), 406 (10), 362 (33), 345 (7), 318 (23), 300 (12), 273 (20), 270 (9), 59 (11), 57 (100), 45 (72), 41 (22). IR ($CHCl_3$) cm^{-1}: 1679. ^1H NMR (at 50 °C) δ: 0.97 (3H, s), 0.99 (1H, ddd, J=12.5, 9.5, 5 Hz, H1), 1.35 (1H, ddd, J=13, 10.5, 4.5 Hz, H3), 1.39–1.89 (8H, m, including OH), 1.49 (9H, s), 1.62 (1H, d, J=7.5 Hz, H5), 1.81 (1H, s, H9), 1.87–1.97 (1H, m), 2.05 (1H, dd, J=16, 9 Hz, H7), 2.25 (1H, dd, J=18, 7.5 Hz, H12), 2.26–2.43 (2H, m), 2.48 (1H, ddd, J=18, 10, 10 Hz, H12), 3.27 (1H, br d, J=11 Hz, H19), 3.35 (3H, s), 3.36 (1H, d, J=11 Hz, H19), 3.51–3.60 (1H, m), 3.65–3.79 (5H, m), 4.07 (1H, dd, J=9, 7.5 Hz, H6), 4.21 (1H, d, J=6.5 Hz, H20), 4.63 (2H, s). **29**: Colorless glass. HRMS Calcd for $C_{28}H_{45}NO_7$: 507.3193. Found: 507.3170. MS (*m*/*z*): 507 (M^+, 2), 434 (2), 406 (9), 362 (21), 318 (35), 300 (17), 270 (13), 57 (100), 45 (79), 41 (27). IR ($CHCl_3$) cm^{-1}: 1679. ^1H NMR (at 50 °C) δ: 0.98 (3H, s), 1.10 (1H, ddd, J=12.5, 11.5, 4.5 Hz, H1), 1.35 (1H, ddd, J=13.5, 12, 5 Hz, H3), 1.44–2.16 (10H, m, including OH), 1.47 (9H, s), 1.67 (1H, d, J=6.5 Hz, H5), 2.03 (1H, dd, J=16, 8.5 Hz, H7), 2.21 (1H, dd, J=17.5, 8.5 Hz, H12), 2.33 (1H, br d, J=16 Hz, H7), 2.57 (1H, ddd, J=17.5, 10.5, 10.5 Hz, H12), 2.90 (1H, d, J=7 Hz, H14), 3.21 (1H, br d, J=10.5 Hz, H19), 3.33 (3H, s), 3.39 (1H, d, J=10.5 Hz, H19), 3.35–3.45 (1H, m), 3.50–3.58 (3H, m), 3.68–3.76 (2H, m, CH_2OH), 4.09 (1H, dd, J=8.5, 6.5 Hz, H6), 4.25 (1H, d, J=7 Hz, H20), 4.58 (2H, s).

4.2.6. Oxidation of 28 and 29 to form 30 and 31, respectively. The procedure for the preparation of **30** from **28** was typical. PCC–Al_2O_3 (20 wt %, 32 mg, 29.7 μmol) was added in one-portion to a cooled (0 °C) solution of **28** (5 mg, 9.86 mmol) in CH_2Cl_2 (3 ml) and the mixture was stirred at 0 °C for 10 min and at 24 °C for 1 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (1:1)] provided **30** (4.5 mg, 90%) as a colorless glass. HRMS Calcd for $C_{28}H_{43}NO_7$: 505.3037. Found: 505.3042. MS (*m*/*z*): 505 (M^+, 2), 416 (2), 404 (12), 360 (31), 316 (25), 299 (16), 298 (14), 271 (17), 57 (100), 45 (75), 41 (31). IR ($CHCl_3$) cm^{-1}: 1715, 1680. ^1H NMR (at 50 °C) δ: 0.99 (3H, s), 1.02 (1H, ddd, J=13, 9.5, 5 Hz), 1.37 (1H, ddd, J=13.5, 10.5, 5 Hz), 1.44–1.54 (1H, m), 1.47 (9H, s), 1.59 (1H, d, J=7 Hz), 1.59–2.04 (5H, m), 1.93 (1H, s, H9), 2.03 (1H, dd, J=16, 9 Hz, H7), 2.25 (1H, dd, J=18, 7.5 Hz), 2.42 (2H, d, J=2 Hz, CH_2CHO), 2.53 (1H, ddd, J=18, 10, 10 Hz), ca. 2.61–2.69 (1H, m), 2.65 (1H, d, J=16 Hz, H7), 3.28 (1H, br d, J=11 Hz), 3.35 (3H, s), 3.38 (1H, d, J=11 Hz), 3.54–3.63 (1H, m), 3.66–3.74 (3H, m), 4.07 (1H, dd, J=9, 7 Hz, H6), 4.27 (1H, d, J=6.5 Hz), 4.62 (2H, s), 9.78 (1H, t, J=2 Hz, CHO). In the same manner as above, **31** (3 mg, 75%) was obtained from **29** (4 mg, 7.89 μmol) as a colorless glass after separation by PTLC [hexane–EtOAc (2:1)]. HRMS Calcd for $C_{28}H_{43}NO_7$: 505.3037. Found: 505.3034. MS (*m*/*z*): 505 (M^+, 1), 432 (2), 404 (11), 360 (28), 316 (25), 298 (13), 288 (7), 57 (100), 45 (81), 41 (28). IR ($CHCl_3$) cm^{-1}: 1714, 1680. ^1H NMR (at 50 °C) δ: 0.99 (3H, s), 1.13 (1H, ddd, J=13, 11, 4.5 Hz), 1.36 (1H, ddd, J=13.5, 12, 5 Hz), 1.46 (9H, s), ca. 1.46–1.57 (1H, m), 1.64–2.28 (7H, m), 1.72 (1H, d, J=7 Hz), 2.17 (1H, dd, J=16, 8 Hz), 2.28 (1H, dd, J=17, 2 Hz, CH_2CHO), 2.34 (1H, br d, J=16 Hz), 2.44 (1H, dd, J=17, 1.5 Hz, CH_2CHO), 2.61 (1H, ddd, J=17.5, 10.5, 10.5 Hz), 2.93 (1H, d, J=6.5 Hz), 3.22 (1H, br d, J=11 Hz), 3.33 (3H, s), 3.36 (1H, d, J=11 Hz), 3.39–3.47 (1H, m), 3.51–3.58 (3H, m), 4.29 (1H, d, J=6.5 Hz), 4.58 (2H, s), 9.76 (1H, dd, J=2, 1.5 Hz, CHO).

4.2.7. Preparation of 32, 33, and 34 from 30. K_2CO_3 (7.5 mg, 54.3 μmol) was added to a solution of **30** (4.5 mg, 8.91 μmol) and dimethyl (1-diazo-2-oxopropyl)phosphonate (15 mg, 78.1 μmol) in MeOH (1.5 ml) and the mixture was stirred at 24 °C under an Ar atmosphere for 3 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (5:1)] furnished **32** (1.5 mg, 34%) and a mixture of **33** and **34** (4 mg) in order of increasing polarity. The latter was purified by PTLC [hexane–EtOAc (1:1)] to give **33** (1.5 mg, 33%) and **34** (1 mg, 22%) in order of decreasing polarity. **32**: Colorless glass. HRMS Calcd for $C_{29}H_{43}NO_6$: 501.3088. Found: 501.3106. MS (*m*/*z*): 501 (M^+, 2), 400 (10), 356 (30), 312 (15), 295 (9), 294 (14), 267 (15), 57 (100), 45 (64), 41 (27). IR ($CHCl_3$) cm^{-1}: 2120, 1680. ^1H NMR (at 50 °C) δ: 0.95–1.06 (1H, m), 0.99 (3H, s), 1.30–1.50 (2H, m), 1.49 (9H, s), 1.61 (1H, d, J=7 Hz, H5), 1.61–1.88 (4H, m), 1.82 (1H, br s), 1.96 (1H, ddd, J=13.5, 10, 3 Hz, H13), 1.98 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.08 (1H, br dd, J=16.5, 8 Hz, H7), 2.12 (1H, dd, J=16.5, 2.5 Hz, CH_2C≡CH), 2.19 (1H, dd, J=16.5, 2.5 Hz, CH_2C≡CH), 2.25 (1H, dd, J=18, 7.5 Hz, H12), 2.45–2.52 (1H, m), 2.51 (1H, ddd, J=18, 10, 10 Hz, H12), 2.67 (1H, d, J=16.5 Hz, H7), 3.28 (1H, br d, J=11 Hz, H19), 3.35 (3H, s), 3.38 (1H, d, J=11 Hz, H19), 3.54–3.62 (1H, m), 3.66–3.74 (3H, m), 4.08 (1H, dd, J=8, 7 Hz, H6), 4.25 (1H, d, J=7 Hz, H20), 4.62 (2H, s). **33**: Colorless glass. HRMS Calcd for $C_{28}H_{43}NO_7$: 505.3037. Found: 505.3028. MS (*m*/*z*): 505 (M^+, 1), 404 (11), 360 (28), 316 (16), 299 (32), 298 (23), 271 (14), 254 (8), 59 (10), 57 (100), 45 (67), 41 (23). IR ($CHCl_3$) cm^{-1}: 1706, 1678. ^1H NMR (at 50 °C) δ: 0.99 (3H, s), 1.19–1.72 (8H, m, including OH), 1.38 (1H, d, J=14.5 Hz, H15), 1.47 (9H, s), 1.60 (1H, d, J=5.5 Hz, H5), 1.83 (1H, dd, J=16, 8 Hz, H7), 1.85–1.94 (1H, m), 1.97 (1H, dd, J=13.5, 5 Hz, H13), 2.16 (1H, dd, J=14.5, 8 Hz, H15), 2.17–2.24 (1H, m, H14), 2.32 (1H, dd, J=5, 5 Hz, H12), 2.60 (1H, br d, J=16 Hz, H7), 3.17 (1H, br d, J=11.5 Hz), 3.31–3.39 (1H, m), 3.33 (3H, s), 3.40 (1H, d, J=11.5 Hz), 3.44–3.52 (1H, m), 3.60–3.65 (2H, m), 4.03 (1H, dd, J=8, 5.5 Hz, H6), 4.04 (1H, d,

J=7 Hz, H20), 4.14 (1H, dd, J=8, 5 Hz, H16), 4.59 (2H, s). **34**: Colorless glass. HRMS Calcd for $C_{28}H_{43}NO_7$: 505.3037. Found: 505.3022. MS (*m/z*): 505 (M^+, 1), 404 (13), 360 (24), 316 (19), 299 (34), 298 (25), 271 (9), 254 (9), 59 (12), 57 (100), 45 (60), 41 (21). IR ($CHCl_3$) cm^{-1}: 1706, 1678. ^1H NMR (at 50 °C) δ: 0.99 (3H, s), 1.16–1.96 (9H, m, including OH), 1.42 (1H, d, J=1.5 Hz, H9), 1.48 (9H, s), 1.57 (1H, d, J=6.5 Hz, H5), 1.61 (1H, dd, J=14.5, 5.5 Hz, H15), 1.77 (1H, dd, J=16, 8 Hz, H7), 1.90 (1H, dd, J=14.5, 9.5 Hz, H15), 2.26–2.30 (1H, m, H12), 2.59 (1H, br d, J=16 Hz, H7), 3.18 (1H, br d, J=10.5 Hz, H19), 3.31–3.39 (1H, m), 3.33 (3H, s), 3.40 (1H, d, J=10.5 Hz, H19), 3.46–3.55 (1H, m), 3.61–3.66 (2H, m), 4.01 (1H, dd, J=8, 6.5 Hz, H6), 4.02 (1H, d, J=7 Hz, H20), 4.04–4.12 (1H, m, H16), 4.60 (2H, s).

4.2.8. Oxidation of 33 and 34 to form 35. The procedure from **33** was typical. To a cooled (0 °C) solution of **33** (1.5 mg, 2.97 μmol) in CH_2Cl_2 (2 ml) was added PCC–Al_2O_3 (20 wt %, 16 mg, 14.8 μmol) and the mixture was stirred at 0 °C for 5 min and at 25 °C for 1.5 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and purification by PTLC [hexane–EtOAc (2:1)] yielded **35** (1.5 mg, quantitative) as a colorless glass. HRMS Calcd for $C_{28}H_{41}NO_7$: 503.2881. Found: 503.2875. MS (*m/z*): 503 (M^+, 2), 447 (1), 402 (8), 358 (23), 314 (14), 297 (18), 269 (10), 57 (100), 45 (64), 41 (24). IR ($CHCl_3$) cm^{-1}: 1734, 1698, 1680. ^1H NMR (at 50 °C) δ: 1.02 (3H, s), 1.23–1.74 (6H, m), 1.49 (9H, s), 1.65 (1H, d, J=6 Hz), 1.68 (1H, dd, J=14, 9.5 Hz, H13), 1.87–1.95 (1H, m), 1.91 (1H, dd, J=16, 8 Hz), 2.16 (1H, d, J=19.5 Hz, H15), 2.39 (1H, dd, J=14, 4.5 Hz, H13), 2.51 (1H, br dd, J=9.5, 6.5 Hz, H14), 2.62 (1H, d, J=19.5 Hz, H15), 2.76 (1H, br d, J=16 Hz), 3.11 (1H, d, J=4.5 Hz, H12), 3.21 (1H, br d, J=11 Hz, H19), 3.33 (3H, s), 3.36–3.44 (1H, m), 3.42 (1H, d, J=11 Hz, H19), 3.46–3.55 (1H, m), 3.62–3.67 (2H, m), 4.09 (1H, dd, J=8, 6 Hz, H6), 4.16 (1H, d, J=6.5 Hz), 4.59 (2H, s). In the same manner, **35** (1 mg, quantitative) was obtained from **34** (1 mg, 1.98 μmol) by oxidation with PCC–Al_2O_3 (20 wt %, 11 mg, 10.2 μmol).

4.2.9. Hydroboration–oxidation of 16 to form 36, 37, and 38. As described in the text, the reproducibility of this reaction is low. The following was the best result. $BH_3 \cdot SMe_2$ (64 μl, 0.674 mmol) was added to a cooled (0 °C) solution of **16** (11 mg, 22.4 μmol) in THF (2.5 ml) under an Ar atmosphere and the mixture was stirred at 0–24 °C for 15 h. After the mixture had been cooled again in an ice bath, EtOH (76 μl, 1.35 mmol) was added and the mixture was vigorously stirred for 5 min. NaOH–H_2O (1 N, 0.90 ml, 0.90 mmol) and H_2O_2–H_2O (30%, 203 μl, 1.79 mmol) were successively added, and the whole was further stirred at 0 °C for 30 min and at 25 °C for 5 h. Saturated NH_4Cl–H_2O and saturated $Na_2S_2O_3$–H_2O were added and the whole was extracted with CH_2Cl_2. Usual work-up followed by separation by PTLC (3% MeOH–CH_2Cl_2) afforded **36** (5.5 mg, 48%), **37** (3 mg, 26%), and **38** (2 mg, 18%) in order of decreasing polarity. **36**: Colorless glass. HRMS Calcd for $C_{28}H_{47}NO_7$: 509.3350. Found: 509.3334. MS *m/z*: 509 (M^+, 1), 420 (3), 408 (13), 364 (29), 320 (19), 302 (23), 57 (100), 45 (76), 41 (27). IR ($CHCl_3$) cm^{-1}: 1674. ^1H NMR (at 50 °C) δ: 0.94 (3H, s), 0.98 (1H, ddd, J=13, 10, 4.5 Hz, H1), 1.11–1.16 (1H, m), 1.31 (1H, ddd, J=13.5, 11, 4.5 Hz, H3), 1.40–2.15 (15H, m, including OH×2), 1.46 (9H, s), 1.56 (1H, d, J=7 Hz, H5), 3.14–3.29 (1H, m), 3.30 (1H, d, J=10.5 Hz, H19), 3.36 (3H, s), 3.44–3.52 (1H, m), 3.58–3.79 (5H, m), 4.01–4.10 (1H, m, H6), 4.04 (1H, d, J=6.5 Hz, H20), 4.22 (1H, dddd, J=10, 10, 7.5, 7.5 Hz, H12), 4.63 (2H, s). **37**: Colorless glass. HRMS Calcd for $C_{28}H_{47}NO_7$: 509.3350. Found: 509.3345. MS *m/z*: 509 (M^+, 0.6), 420 (5), 408 (10), 364 (5), 320 (18), 302 (52), 284 (32), 258 (11), 57 (100), 45 (78), 41 (30). IR ($CHCl_3$) cm^{-1}: 1674. ^1H NMR (at 50 °C) δ: 0.91–1.03 (1H, m), 0.96 (3H, s), 1.08 (1H, br s), 1.33 (1H, ddd, J=13.5, 12, 5 Hz, H3), 1.43–1.87 (8H, m, including OH), 1.47 (9H, s), 1.55 (1H, d, J=6.5 Hz, H5), 2.00–2.10 (1H, m), 2.13–2.19 (1H, m), 2.18–2.28 (1H, m), 2.36 (1H, ddd, J=13.5, 4, 4 Hz, H1), 3.21 (1H, br d, J=10 Hz, H19), 3.34 (1H, d, J=10 Hz, H19), 3.36 (3H, s), 3.46–3.53 (1H, m), 3.61–3.83 (6H, m, including H12), 4.05 (1H, dd, J=8.5, 6.5 Hz, H6), 4.18 (1H, d, J=6.5 Hz, H20), 4.63 (2H, s), 4.78 (1H, J=10 Hz, CHO*H*). **38**: Colorless syrup. HRMS Calcd for $C_{28}H_{47}NO_6$: 493.3401. Found: 493.3376. MS *m/z*: 493 (M^+, 1), 404 (4), 392 (18), 348 (34), 304 (22), 287 (29), 256 (25), 57 (100), 45 (75), 41 (30). IR ($CHCl_3$) cm^{-1}: 1672. ^1H NMR (at 50 °C) δ: 0.90–1.01 (1H, m), 0.94 (3H, s), 1.03 (1H, br s, H9), 1.16 (1H, br s, OH), 1.25–1.38 (2H, m), 1.39–1.56 (3H, m), 1.46 (9H, s), 1.58–1.72 (5H, m), 1.60 (1H, d, J=7.5 Hz, H5), 1.72–1.90 (1H, m, H2), 1.93–2.16 (4H, m), 2.10 (1H, dd, J=15.5, 9.5 Hz, H7), 3.15–3.29 (1H, br m), 3.33 (1H, d, J=11 Hz, H19), 3.36 (3H, s), 3.49 (1H, ddd, J=10.5, 5, 4.5 Hz), 3.57–3.78 (5H, m), 4.03 (1H, d, J=6.5 Hz, H20), 4.64 (2H, s).

4.2.10. Respective oxidation of 36 and 37 to form 39 and 40. The procedure for the preparation of **39** from **36** is described as a representative example. To a cooled (0 °C) solution of **36** (16 mg, 31.4 μmol) in CH_2Cl_2 (2.5 ml) were added TEMPO (0.5 mg, 3.21 μmol) and $PhI(OAc)_2$ (11 mg, 34.2 μmol), and the mixture was stirred at the same temperature for 20 min and at 25 °C for 6 h. Saturated $Na_2S_2O_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $NaHCO_3$–H_2O and H_2O, then treated as usual. The resulting residue was separated by PTLC (3% MeOH–CH_2Cl_2) to afford **39** (12 mg, 75%) along with recovered **36** (1 mg, 6%) in order of increasing polarity. **39**: Colorless glass. HRMS Calcd for $C_{28}H_{45}NO_7$: 507.3193. Found: 507.3178. MS *m/z*: 507 (M^+, 0.4), 489 (2), 408 (13), 406 (7), 388 (8), 362 (17), 344 (14), 318 (13), 300 (17), 283 (16), 57 (100), 45 (76), 41 (29). IR ($CHCl_3$) cm^{-1}: 1714, 1674. ^1H NMR (at 50 °C) δ: 0.95 (3H, s), 1.01 (1H, ddd, J=13, 10, 5 Hz, H1), 1.20–2.25 (12H, m, including OH), 1.45 (9H, s), 1.60 (1H, d, J=7 Hz, H5), 2.25–2.34 (1H, m), 2.63 (1H, br d, J=16.5 Hz, CH_2CHO), 2.77 (1H, br d, J=16.5 Hz, CH_2CHO), 3.23 (1H, br d, J=10.5 Hz, H19), 3.31 (1H, d, J=10.5 Hz, H19), 3.36 (3H, s), 3.47–3.55 (1H, m), 3.59–3.71 (3H, m), 4.02–4.11 (1H, m), 4.09 (1H, d, J=6.5 Hz, H20), 4.26 (1H, dddd, J=10, 10, 7.5, 7.5 Hz, H12), 4.64 (2H, s), 9.85 (1H, dd, J=2, 2 Hz, CHO). In the same manner as above, **40** (6 mg, 75%) was obtained from **37** (8 mg, 15.7 μmol) as a colorless glass after PTLC (2% MeOH–CH_2Cl_2). HRMS Calcd for $C_{28}H_{45}NO_7$: 507.3193. Found: 507.3185. MS *m/z*: 507 (M^+, 0.7), 418 (5), 406 (11), 362 (11), 318 (24), 300 (47),

282 (23), 57 (100), 45 (74), 41 (27). IR ($CHCl_3$) cm^{-1}: 1714, 1676. ^1H NMR (at 50 °C) δ: 0.97 (3H, s), 0.99 (1H, ddd, J=13.5, 11, 4 Hz, H1), 1.29–1.33 (1H, m, H9), 1.34 (1H, ddd, J=13.5, 12.5, 4.5 Hz, H3), 1.44–1.83 (4H, m), 1.46 (9H, s), 1.59 (1H, d, J=7 Hz, H5), 1.90–2.05 (2H, m), 2.06–2.22 (2H, m), 2.32–2.46 (3H, m), 2.42 (1H, dd, J=16, 2 Hz, CH_2CHO), 2.55 (1H, dd, J=16, 2 Hz, CH_2CHO), 3.23 (1H, br d, J=11 Hz, H19), 3.35 (1H, d, J=11 Hz, H19), 3.36 (3H, s), 3.48–3.57 (1H, m), 3.64–3.81 (3H, m), 4.07 (1H, dd, J=8, 7 Hz, H6), 4.23 (1H, d, J=7 Hz, H20), 4.63 (2H, s), 4.81 (1H, d, J=10 Hz, OH), 9.85 (1H, dd, J=2, 2 Hz, CHO).

4.2.11. Conversion of 39 and 40 to 41 and 42, respectively. The procedure for the preparation of **41** from **39** was typical. K_2CO_3 (18 mg, 0.130 mmol) was added to a solution of **39** (11 mg, 21.7 μmol) and dimethyl (1-diazo-2-oxopropyl)-phosphonate (33 mg, 0.172 mmol) and the mixture was stirred under an Ar atmosphere at 25 °C for 3.5 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [benzene–EtOAc (3:1)] furnished **41** (10.5 mg, 96%) as a colorless glass. HRMS Calcd for $C_{29}H_{45}NO_6$: 503.3244. Found: 503.3260. MS m/z: 503 (M^+, 1), 414 (2), 402 (13), 358 (38), 314 (19), 297 (26), 278 (7), 57 (100), 45 (76), 41 (26). IR ($CHCl_3$) cm^{-1}: 2116, 1674. ^1H NMR (at 50 °C) δ: 0.95–1.04 (1H, m, H1), 0.96 (3H, s), 1.24–1.28 (1H, m, H9), 1.32 (1H, ddd, J=13.5, 11.5, 4.5 Hz, H3), 1.41–1.70 (5H, m, including OH), 1.47 (9H, s), 1.58 (1H, d, J=7 Hz, H5), 1.72–1.86 (1H, m), 1.86–2.06 (3H, m), 1.93 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.13–2.19 (1H, m), 2.20 (1H, br d, J=16.5 Hz, CH_2C≡CH), 2.25–2.54 (3H, m), 3.23 (1H, br d, J=10.5 Hz, H19), 3.32 (1H, d, J=10.5 Hz, H19), 3.36 (3H, s), 3.44–3.55 (1H, m), 3.57–3.70 (3H, m), 4.09 (1H, d, J=6.5 Hz, H20), 4.24 (1H, dddd, J=10, 10, 7.5, 7.5 Hz, H12), 4.63 (2H, s). In the same manner, **42** (4.5 mg, 91%) was obtained from **40** (5 mg, 9.86 μmol) as a colorless glass after separation by PTLC [hexane–EtOAc (3:2)]. HRMS Calcd for $C_{29}H_{45}NO_6$: 503.3244. Found: 503.3254. MS m/z: 503 (M^+, 2), 414 (10), 402 (14), 358 (12), 314 (22), 297 (31), 296 (72), 278 (27), 57 (100), 45 (70), 41 (27). IR ($CHCl_3$) cm^{-1}: 2112, 1675. ^1H NMR (at 50 °C) δ: 0.97 (3H, s), 0.98 (1H, ddd, J=13.5, 11.5, 4 Hz, H1), 1.16–1.22 (1H, m), 1.33 (1H, ddd, J=13.5, 12, 5 Hz, H3), 1.48 (9H, s), 1.48–1.81 (4H, m), 1.57 (1H, d, J=7 Hz, H5), 1.92–1.99 (3H, m, H11×2 and C≡CH), 2.13–2.31 (4H, m, H7, H14, and CH_2C≡CH), 2.16 (1H, ddd, J=16, 7, 3 Hz, H13), 2.36 (1H, ddd, J=13.5, 4, 4 Hz, H1), 2.39 (1H, d, J=16 Hz, H7), 3.23 (1H, br d, J=10.5 Hz, H19), 3.36 (3H, s), 3.36 (1H, d, J=10.5 Hz, H19), 3.47–3.55 (1H, m), 3.65–3.81 (4H, m), 4.09 (1H, dd, J=8, 7 Hz, H6), 4.23 (1H, d, J=6 Hz, H20), 4.63 (2H, s), 4.82 (1H, d, J=10 Hz, OH).

4.2.12. Preparation of xanthate 43 from 41. NaH (60% in mineral oil, 22 mg, 0.550 mmol) and imidazole (2 mg, 29.4 μmol) were added to a solution of **41** (11 mg, 21.9 μmol) in THF (3 ml) and the mixture was refluxed with stirring under an Ar atmosphere for 3 h. CS_2 (0.33 ml, 5.47 mmol) was added to this and the resulting solution was further refluxed for 40 min, during this time it gradually changed into a yellow-white slurry. MeI (0.41 ml, 6.58 mmol) was further added to this and the whole was heated for 40 min. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$–H_2O and then treated as usual. Separation by PTLC [hexane–EtOAc (3:1)] gave **43** (11 mg, 85%) and recovery of **41** (1 mg, 9%) in order of increasing polarity. **43**: Colorless glass. HRMS Calcd for $C_{31}H_{47}NO_6S_2$: 593.2842. Found: 593.2861. MS m/z: 593 (M^+, 0.5), 518 (1), 504 (1), 492 (4), 448 (7), 385 (37), 346 (22), 280 (39), 57 (74), 45 (100), 41 (42). IR ($CHCl_3$) cm^{-1}: 2112, 1675. ^1H NMR (at 50 °C) δ: 0.96 (3H, s), 0.99 (1H, ddd, J=13, 10.5, 4.5 Hz, H1), 1.27–1.38 (2H, m), 1.45–1.55 (1H, m, H2), 1.48 (9H, s), 1.58 (1H, d, J=7 Hz, H5), 1.62–1.75 (3H, m), 1.75–1.91 (1H, m, H2), 1.95 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.08–2.18 (1H, m), 2.13 (1H, ddd, J=13, 5, 4.5 Hz, H1), 2.19–2.53 (6H, m), 2.54 (3H, s, SCH_3), 3.23 (1H, br d, J=11 Hz, H19), 3.34 (1H, d, J=11 Hz, H19), 3.35 (3H, s), 3.51 (1H, ddd, J=10.5, 6.5, 4 Hz), 3.60 (1H, ddd, J=10.5, 5, 4 Hz), 3.68 (1H, ddd, J=11, 5, 4 Hz), 3.75 (1H, ddd, J=11, 6.5, 4 Hz), 4.05–4.15 (1H, m, H6), 4.63 (2H, s), 6.03 (1H, dddd, J=10, 10, 7.5, 7.5 Hz, H12).

4.2.13. Radical cyclization of 43 to form 44. Bu_3SnH (22 μl, 81.8 μmol) and AIBN (1 mg, 6.10 μmol) were added to a solution of **43** (5 mg, 8.43 μmol) in toluene (5 ml) and Ar gas was bubbled into the mixture for 15 min at an ambient temperature. Then the solution was stirred under reflux for 15 min under an Ar atmosphere. After evaporation of the reaction solvent in vacuo, the residue was separated by PTLC [hexane–EtOAc (4:1)] to provide **44** (3.5 mg, 85%) as a colorless glass. HRMS Calcd for $C_{29}H_{45}NO_5$: 487.3295. Found: 487.3280. MS m/z: 487 (M^+, 3), 398 (2), 386 (18), 342 (24), 298 (15), 281 (46), 69 (15), 57 (100), 45 (66), 41 (34). IR ($CHCl_3$) cm^{-1}: 1674. ^1H NMR (at 50 °C) δ: 0.81–0.93 (1H, m, H1), 0.97 (3H, s, H18), 1.11 (1H, br d, J=10 Hz, H9), 1.25–1.51 (3H, m), 1.46 (9H, s), 1.53 (1H, d, J=6.5 Hz, H5), 1.58–1.82 (5H, m), 1.72 (1H, dd, J=16, 8 Hz, H7), ca. 2.04–2.10 (1H, m, H14), 2.05 (1H, br d, J=16 Hz, H7), 2.11 (1H, br d, J=18 Hz, H15), 2.17 (1H, ddd, J=13, 4.5, 4.5 Hz, H1), 2.26–2.41 (1H, m), 2.38 (1H, ddd, J=18, 2.5, 2 Hz, H15), 3.18 (1H, br d, J=11 Hz, H19), 3.32 (1H, dt, J=10.5, 5 Hz), 3.37 (3H, s), 3.40 (1H, d, J=11 Hz, H19), 3.48 (1H, dt, J=10.5, 5 Hz), 3.68 (2H, dd, J=5, 5 Hz), 3.95 (1H, d, J=6.5 Hz, H20), 3.99 (1H, br dd, J=8, 6.5 Hz, H6), 4.48 (1H, ddd, J=2, 2, 2 Hz, H17), 4.62–4.65 (1H, m, H17), 4.65 (2H, s).

4.3. Preparation of enyne radical cyclization precursors (Scheme 6)

4.3.1. Oxidation of 16 and 18 to form 45 and 46, respectively. The procedure for the preparation of **45** from **16** is described as a representative example. PCC–Al_2O_3 (20 wt %, 348 mg, 0.323 mmol) was added to a solution of **16** (53 mg, 0.108 mmol) in CH_2Cl_2 (8 ml) and the mixture was stirred at 0 °C for 1 h. Saturated $NaHCO_3$–H_2O was added and the whole was filtered through a Celite bed. The filtrate was extracted with CH_2Cl_2. Usual work-up followed by PTLC [hexane–EtOAc (3:1)] afforded **45** (48 mg, 91%) as a colorless glass. HRMS Calcd for $C_{28}H_{43}NO_6$: 489.3088. Found: 489.3091. MS m/z: 489 (M^+, 4), 400 (4), 388 (13), 346 (33), 344 (25), 300 (25), 300 (25), 282 (28), 91 (10), 89 (11), 57 (100), 45 (76), 41 (28). IR

($CHCl_3$) cm^{-1}: 1713, 1675. ^1H NMR (at 50 °C) δ: 0.95 (3H, s), 1.01 (1H, ddd, J=12.5, 10, 5.5 Hz, H1), 1.28 (1H, ddd, J=13.5, 11, 5 Hz, H3), 1.39–1.53 (2H, m), 1.46 (9H, s), 1.66 (1H, d, J=7.5 Hz, H5), 1.66 (1H, ddd, J=13.5, 5, 5 Hz, H3), 1.80–1.96 (1H, m), 2.01 (1H, ddd, J=12.5, 4.5, 4.5 Hz, H1), 2.09 (1H, dddd, J=19.5, 5, 2, 2 Hz, H11), 2.10–2.36 (3H, m), 2.53 (1H, dd, J=6, 5.5 Hz, H14), 2.67 (2H, br s, CH_2CHO), 3.18–3.30 (1H, br m), 3.30 (1H, br d, J=10.5 Hz, H19), 3.35 (3H, s), 3.40 (1H, dt, J=10.5, 5 Hz), 3.53 (1H, dt, J=10.5, 5 Hz), 3.65 (2H, dd, J=5, 5 Hz), 4.07–4.18 (1H, m, H6), 4.08 (1H, d, J=6 Hz, H20), 4.62 (1H, d, J=6.5 Hz), 4.64 (1H, d, J=6.5 Hz), 5.52–5.63 (2H, m), 9.82 (1H, t, J=2 Hz, CHO). In the same manner, **46** (62 mg, 84%) was obtained from **18** (74 mg, 0.141 mmol) as a colorless glass after purification by PTLC [hexane–EtOAc (2:1)]. HRMS Calcd for $C_{31}H_{41}NO_6$: 523.2932. Found: 523.2918. MS m/z: 523 (M^+, 2), 480 (1), 478 (1), 434 (6), 390 (15), 388 (10), 351 (9), 298 (4), 91 (100), 89 (6), 45 (38). IR ($CHCl_3$) cm^{-1}: 1712, 1684. ^1H NMR (at 50 °C) δ: 0.95 (3H, s), 1.01 (1H, ddd, J=12.5, 10, 5.5 Hz, H1), 1.29 (1H, ddd, J=13.5, 11.5, 5 Hz, H3), 1.40–1.56 (2H, m), 1.62–1.72 (1H, m), 1.68 (1H, d, J=7 Hz, H5), 1.88 (1H, ddddd, J=12.5, 11, 9.5, 6.5, 4.5 Hz, H2), 2.01 (1H, ddd, J=12.5, 4.5, 4.5 Hz, H1), ca. 2.01–2.39 (4H, m), 2.51 (1H, dd, J=6, 5 Hz, H14), 2.65 (2H, br s, CH_2CHO), 3.18–3.42 (3H, m), 3.33 (3H, s), 3.51 (1H, ddd, J=10.5, 5, 4.5 Hz), 3.60–3.66 (2H, m), 4.06 (1H, d, J=6 Hz, H20), 4.17–4.26 (1H, m), 4.61 (2H, s), 5.10 (1H, d, J=12.5 Hz), 5.18 (1H, d, J=12.5 Hz), 5.52–5.62 (2H, m), 7.26–7.37 (5H, m), 9.79 (1H, br s, CHO).

4.3.2. Preparation of enyne derivatives 47 and 48 from 45 and 46, respectively. In the same manner as described for the preparation of **41** from **39** (Section 4.2.11), **45** (44 mg, 0.090 mmol) was stirred with dimethyl (1-diazo-2-oxopropyl)phosphonate (86 mg, 0.448 mmol) and K_2CO_3 (49 mg, 0.355 mmol) in MeOH (5 ml) at 0 °C for 5 min and at 25 °C for 4 h. Saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [hexane–EtOAc (4:1)] furnished **47** (42 mg, 96%) as a colorless glass. HRMS Calcd for $C_{29}H_{43}NO_5$: 485.3139. Found: 485.3149. MS m/z: 485 (M^+, 1), 446 (1), 384 (9), 346 (22), 340 (17), 324 (9), 296 (17), 279 (36), 57 (100), 45 (76), 41 (31). IR ($CHCl_3$) cm^{-1}: 2116, 1673. ^1H NMR (at 50 °C) δ: 0.95 (3H, s), 0.99 (1H, ddd, J=12.5, 10, 5.5 Hz, H1), 1.27 (1H, ddd, J=13.5, 11.5, 5 Hz, H3), 1.32–1.38 (1H, m, H9), 1.38–1.51 (1H, m, H2), 1.48 (9H, s), 1.61–1.71 (1H, m, H3), 1.64 (1H, d, J=7 Hz, H5), 1.79–1.95 (1H, m, H2), 1.91 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.00 (1H, ddd, J=12.5, 4.5, 4.5 Hz, H1), 2.08–2.34 (4H, m, H11×2 and CH_2C≡CH), 2.35–2.42 (1H, m, H14), 2.41–2.64 (2H, m, H7×2), 3.16–3.33 (1H, br m), 3.30 (1H, br d, J=10.5 Hz, H19), 3.35 (3H, s), 3.39 (1H, dt, J=10, 5 Hz), 3.52 (1H, dt, J=10, 5 Hz), 3.65 (2H, dd, J=5, 5 Hz), 4.09 (1H, d, J=6 Hz, H20), 4.16 (1H, br dd, J=8, 7 Hz, H6), 4.62 (1H, d, J=6.5 Hz), 4.64 (1H, d, J=6.5 Hz), 5.50–5.59 (2H, m). In the same manner, **48** (36 mg, 98%) was obtained from **46** (37 mg, 0.071 mmol) as a colorless glass after PTLC [hexane–EtOAc (3:1)]. HRMS Calcd for $C_{32}H_{41}NO_5$: 519.2982. Found: 519.2984. MS m/z: 519 (M^+, 1), 480 (2), 430 (5), 386 (11), 294 (4), 280 (4), 91 (100), 45 (38). IR ($CHCl_3$) cm^{-1}: 2105, 1683. ^1H NMR (at 50 °C) δ: 0.96 (3H, s), 0.99 (1H, ddd, J=13, 10, 5.5 Hz, H1), 1.28 (1H, ddd, J=13.5, 11, 5 Hz, H3), 1.31–1.40 (1H, m, H9), 1.45 (1H, ddddd, J=14.5, 5, 5, 5, 5 Hz, H2), 1.61–1.72 (1H, m), 1.67 (1H, d, J=7 Hz, H5), 1.80–1.96 (1H, m), 1.91 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.00 (1H, ddd, J=13, 4.5, 4.5 Hz, H1), 2.09–2.30 (4H, m, including CH_2C≡CH), 2.34–2.41 (1H, m), 2.45–2.67 (2H, m), 3.23–3.42 (3H, m), 3.33 (3H, s), 3.50 (1H, ddd, J=10.5, 4.5, 4.5 Hz), 3.60–3.65 (2H, m), 4.08 (1H, d, J=6 Hz, H20), 4.25 (1H, ddd, J=9, 7, 1 Hz, H6), 4.59 (1H, d, J=6.5 Hz), 4.62 (1H, d, J=6.5 Hz), 5.12 (1H, d, J=12.5 Hz), 5.20 (1H, d, J=12.5 Hz), 5.49–5.59 (2H, m), 7.26–7.41 (5H, m).

4.3.3. Cleavage of MOM group of 48 to form 9. HCl–H_2O (20%, 1 ml) was added to a cooled (0 °C) solution of **48** (33 mg, 63.6 μmol) in DME (3 ml) and the mixture was stirred for 10 min and at 22 °C for 9 h. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [hexane–EtOAc (7:4)] provided **9** (29 mg, 96%) as a colorless glass. HRMS Calcd for $C_{30}H_{37}NO_4$: 475.2721. Found: 475.2719. MS m/z: 475 (M^+, 3), 430 (3), 386 (8), 370 (4), 340 (9), 91 (100), 65 (5), 45 (8), 41 (4). IR ($CHCl_3$) cm^{-1}: 2120, 1684. ^1H NMR (at 50 °C) δ: 0.97 (3H, s), 1.02 (1H, ddd, J=13, 10, 5 Hz, H1), 1.31 (1H, ddd, J=13.5, 11.5, 5 Hz, H3), 1.34–1.41 (1H, m, H9), 1.44–1.56 (2H, m, including OH), 1.60–1.71 (1H, m, H3), 1.68 (1H, d, J=7 Hz, H5), 1.71–1.85 (1H, m, H2), 1.91 (1H, dd, J=2.5, 2.5 Hz, C≡CH), 2.02 (1H, ddd, J=13, 4.5, 4.5 Hz, H1), 2.14–2.32 (4H, m), 2.41 (1H, dd, J=6, 5.5 Hz, H14), 2.46–2.64 (2H, m), 3.27–3.38 (3H, m), 3.43 (1H, ddd, J=9.5, 6.5, 3.5 Hz), 3.58–3.76 (2H, m, CH_2OH), 4.10 (1H, d, J=6 Hz, H20), 4.26 (1H, dd, J=8, 7 Hz, H6), 5.13 (1H, d, J=12.5 Hz), 5.20 (1H, d, J=12.5 Hz), 5.51–5.62 (2H, m), 7.28–7.40 (5H, m).

4.4. C-ring formation from 47 and 9 (Scheme 7, Table 2)

4.4.1. Radical cyclization of 47 to form 44 and 49 (Table 2, runs a–d). The procedure for run c of Table 2 is described as a representative example among runs a–d. A benzene (3 ml) solution of **47** (9 mg, 18.6 μmol), tributyltin hydride (25 μl, 93 μmol), and azobisisobutyronitrile (0.5 mg, 3.05 μmol) was degassed by bubbling of Ar gas for 10 min at 25 °C. The solution was then refluxed with stirring for 2 h under an Ar atmosphere. The solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2 (3 ml). SiO_2 (1.0 g, well-dried prior to use) was added to this and the mixture was stirred at 25 °C for 14 h. The whole was filtered under reduced pressure and the filtered SiO_2 was rinsed with 10% MeOH–CH_2Cl_2. The solvent was evaporated off and the residue was separated by PTLC [hexane–EtOAc (11:1)] to give **44** (3 mg, 33%) and **49** (7.5 mg, 52%). The former was identical with the authentic specimen (see Section 4.2.13) by ^1H NMR and IR. **49**: Colorless glass. HRMS Calcd for $C_{41}H_{71}NO_5Sn$: 777.4349. Found: 777.4383. MS m/z: 778, 777, 776, 775, 774, 773 (M^+, 0.02, 0.11, 0.15, 0.13, 0.10, 0.10), 721, 720, 719, 718, 717, 716 (11, 27, 14, 21, 11, 11), 677, 676, 675, 674, 673, 672 (1, 2, 1, 2, 1, 1), 621, 620, 619, 618, 617, 616 (0.5, 0.9, 0.5, 1, 0.7, 1), 619, 618, 617, 616, 615, 614 (0.5, 1, 0.7, 1, 0.7, 1), 515, 514, 513, 512, 511, 510 (1, 4, 2, 3, 1, 2), 486 (8), 430 (5), 386 (21), 280 (83), 180, 179, 178, 177, 176,

175 (1, 21, 6, 19, 7, 13), 57 (100), 45 (44), 41 (27). IR ($CHCl_3$) cm^{-1}: 1675. ^1H NMR (at 50 °C) δ: 0.56 (1H, dd, J=7.5, 4 Hz, H12), 0.77 (1H, ddd, J=13, 10, 6 Hz, H1), 0.79–1.00 (10H, m), 0.89 (9H, t, J=7.5 Hz), 0.96 (3H, s, H18), 1.08 (1H, d, J=13 Hz, H17), 1.24–1.68 (16H, m), 1.38 (1H, d, J=6 Hz, H5), 1.47 (9H, s), 1.63 (1H, d, J=12 Hz, H15), 1.71 (1H, dd, J=15, 7 Hz, H7), 1.75 (1H, dd, J=14, 9.5 Hz, H11), 2.01 (1H, dd, J=14, 4 Hz, H11), 2.26 (1H, ddd, J=13, 5, 5 Hz, H1), 2.42 (1H, dd, J=6.5, 4 Hz, H14), 2.50 (1H, br d, J=15 Hz, H7), 3.12 (1H, d, J=10.5 Hz, H19), 3.37 (3H, s), 3.40 (1H, d, J=10.5 Hz, H19), 3.41–3.50 (1H, m), 3.67–3.76 (3H, m), 3.83 (1H, d, J=6.5 Hz, H20), 3.87 (1H, dd, J=7, 6 Hz, H6), 4.65 (1H, d, J=6.5 Hz), 4.68 (1H, d, J=6.5 Hz).

4.4.2. Reductive Pd-catalyzed cyclization of 47 to form 44, 50, and 51 (Table 2, run e). A benzene (2 ml) solution containing **47** (8 mg, 16.5 μmol), poly(methylhydrosiloxane) (21 μl, 0.333 mmol), tris(benzylideneacetone)dipalladium(0)-chloroform adduct (2 mg, 1.93 μmol), *N*,*N*′-bis(benzylidene)-1,2-ethylenediamine (1 mg, 4.24 μmol), and HOAc (2 μl, 35.0 μmol) was refluxed with stirring under an Ar atmosphere for 30 min. After the mixture had been cooled, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [hexane–EtOAc (12:1)] afforded crude **50** (2.5 mg), **44** (1.5 mg, 19%), and **51** (2 mg, 25%) in order of increasing polarity. As attempted purification of the crude **50** was unsuccessful, the crude product was treated with OsO_4 (0.5 mg, 1.97 μmol) and $NaIO_4$ (11 mg, 51.4 μmol) in THF–H_2O (5:1, 1.8 ml) at 0 °C for 10 min and at 25 °C for 14 h in order to isolate **50** without change; this was why the concomitant had vinyl group signals in its ^1H NMR spectrum. Saturated $Na_2S_2O_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (10:1)] gave pure **50** (1.5 mg, 19%). **50**: Colorless glass. HRMS Calcd for $C_{29}H_{45}NO_5$: 487.3295. Found: 487.3275. MS *m*/*z*: 487 (M^+, 4), 431 (6), 386 (7), 342 (5), 326 (10), 298 (10), 281 (100), 57 (99), 45 (61), 41 (29). IR ($CHCl_3$) cm^{-1}: 1674. ^1H NMR (at 50 °C) δ: 0.57 (1H, dd, J=7.5, 4 Hz, H12), 0.77 (1H, ddd, J=13, 9, 5 Hz, H1), 0.81–1.01 (3H, m), 0.96 (3H, s, H18), 1.12 (3H, s, H17), 1.24–1.70 (4H, m), 1.38 (1H, d, J=6 Hz, H5), 1.46 (9H, s), 1.67 (1H, d, J=12 Hz), 1.71 (1H, dd, J=15, 7 Hz, H7), 1.74 (1H, dd, J=14, 9.5 Hz, H11), 2.00 (1H, dd, J=14, 4 Hz, H11), 2.26 (1H, ddd, J=13, 5, 5 Hz), 2.41 (1H, dd, J=6.5, 4 Hz, H14), 2.51 (1H, br d, J=15 Hz, H7), 3.12 (1H, br d, J=11 Hz), 3.37 (3H, s), 3.41 (1H, d, J=11 Hz), 3.42–3.49 (1H, m), 3.67–3.79 (3H, m), 3.84 (1H, d, J=6.5 Hz), 3.87 (1H, dd, J=7, 6 Hz, H6), 4.65 (1H, d, J=6.5 Hz), 4.68 (1H, d, J=6.5 Hz). **51**: Colorless glass. HRMS Calcd for $C_{29}H_{45}NO_5$: 487.3295. Found: 487.3282. MS *m*/*z*: 487 (M^+, 2), 446 (2), 398 (2), 386 (9), 346 (31), 342 (18), 326 (11), 298 (13), 281 (27), 240 (7), 91 (12), 57 (100), 45 (71), 41 (38). IR ($CHCl_3$) cm^{-1}: 1673. ^1H NMR (at 50 °C) δ: 0.93 (3H, s), 0.98 (1H, ddd, J=13.5, 10, 5.5 Hz), 1.17–1.70 (4H, m), 1.47 (9H, s), 1.60 (1H, d, J=7.5 Hz), 1.79–2.04 (3H, m), 2.10–2.35 (5H, m), 2.31 (1H, br dd, J=7, 6 Hz), 3.15–3.30 (1H, br m), 3.28 (1H, br d, J=10 Hz), 3.35 (3H, s), 3.38 (1H, dt, J=10, 5 Hz), 3.52 (1H, dt, J=10, 5 Hz), 3.65 (2H, dd, J=5, 5 Hz), 4.02 (1H, d, J=6 Hz), 4.05–4.16 (1H, m), 4.62 (1H, d, J=6.5 Hz), 4.64 (1H, d, J=6.5 Hz), 4.98–5.06 (2H, m, CH=CH_2), 5.50–5.60 (2H, m, H12, H13), 5.72–5.88 (1H, m, CH=CH_2).

4.4.3. Radical cyclization of 9 to form 10, 52, and 53. A toluene (4 ml) solution of tributyltin hydride (74 μl, 0.275 mmol) was added dropwise to a refluxing solution of **9** (13 mg, 27.4 μmol) and azobisisobutyronitrile (1 mg, 6.10 μmol) in toluene (5 ml) during 1.5 h under an Ar atmosphere and then the resulting mixture was further stirred under reflux for 1.5 h. The solvent was removed in vacuo and the residue (95 mg) was dissolved in CH_2Cl_2 (5 ml). SiO_2 (1.5 g, dried in an oven prior to use) was added, and the mixture was stirred at 20 °C for 18 h. The mixture was filtered through a Celite bed and the bed was rinsed with 10% MeOH–CH_2Cl_2. The filtrate was evaporated off and the residue was purified by PTLC [hexane–EtOAc (11:2)] to provide **52** (6.5 mg, 31%), **53** (1 mg, 8%), and **10** (7.5 mg, 57%) in order of increasing polarity. **10**: Colorless glass. HRMS Calcd for $C_{30}H_{39}NO_4$: 477.2877. Found: 477.2885. MS *m*/*z*: 477 (M^+, 1), 432 (3), 388 (5), 342 (9), 298 (9), 91 (100), 65 (10), 45 (15). IR ($CHCl_3$) cm^{-1}: 1684. ^1H NMR (at 50 °C) δ: 0.90 (1H, ddd, J=13, 10, 5 Hz, H1), 0.99 (3H, s), 1.14 (1H, br d, J=10 Hz, H9), 1.29–1.85 (8H, m, including OH), 1.57 (1H, d, J=6.5 Hz, H5), 1.75 (1H, dd, J=16, 8 Hz, H7), 1.89 (1H, dd, J=13, 4.5 Hz, H13), 1.96–2.11 (2H, m, H12 and H14), 2.11 (1H, d, J=18 Hz, H15), 2.17 (1H, ddd, J=13, 5, 5 Hz, H1), ca. 2.35–2.55 (1H, m, H7), 2.36 (1H, br d, J=18 Hz, H15), 3.23 (1H, ddd, J=9.5, 6, 3.5 Hz, CH_2CH$_2$OH), 3.28 (1H, br d, J=11 Hz, H19), 3.41 (1H, ddd, J=9.5, 5, 3.5 Hz, CH_2CH$_2$OH), 3.46 (1H, d, J=11 Hz, H19), 3.67–3.80 (2H, m, CH_2OH), 3.95 (1H, d, J=6.5 Hz, H20), 4.08 (1H, dd, J=8, 6.5 Hz, H6), 4.49 (1H, ddd, J=2.5, 2, 2 Hz, H17), 4.64 (1H, ddd, J=2.5, 2, 2 Hz, H17), 5.11 (1H, d, J=13 Hz, OCH_2Ph), 5.16 (1H, br d, J=13 Hz, OCH_2Ph), 7.25–7.40 (5H, m). **52**: Colorless glass. MS *m*/*z*: 712, 711, 710, 709, 708, 707 (M^+−Bu, 2, 4, 9, 5, 7, 4), 576, 575, 574, 573, 572, 571 (1, 1, 2, 1, 2, 1), 532, 531, 530, 529, 528, 527 (1, 1, 4, 2, 3, 1), 476 (6), 432 (6), 342 (8), 280 (11), 180, 179, 178, 177, 176, 175 (1, 11, 4, 12, 4, 8), 91 (100), 65 (9), 41 (15). IR ($CHCl_3$) cm^{-1}: 1686. ^1H NMR (at 50 °C) δ: 0.56 (1H, dd, J=7.5, 4 Hz, H12), 0.71–1.03 (10H, m), 0.88 (1H, d, J=13 Hz, H17), 0.89 (9H, t, J=7.5 Hz), 0.98 (3H, s, H18), 1.10 (1H, d, J=13 Hz, H17), 1.24–1.69 (16H, m), 1.42 (1H, d, J=6 Hz, H5), 1.64 (1H, d, J=12 Hz, H15), 1.74 (1H, dd, J=15, 7 Hz, H7), 1.78 (1H, dd, J=14, 10 Hz, H11), 2.01 (1H, dd, J=14, 4 Hz, H11), 2.08 (1H, dd, J=6, 6 Hz, OH), 2.26 (1H, ddd, J=12.5, 5, 5 Hz, H1), 2.42 (1H, dd, J=6.5, 3.5 Hz, H14), 2.58 (1H, br d, J=15 Hz, H7), 3.22 (1H, d, J=11.5 Hz, H19), 3.31–3.38 (1H, m), 3.62–3.76 (3H, m), 3.68 (1H, d, J=11.5 Hz, H19), 3.83 (1H, d, J=6.5 Hz, H20), 3.96 (1H, dd, J=7, 6 Hz, H6), 5.11 (1H, d, J=13 Hz, OCH_2Ph), 5.15 (1H, d, J=13 Hz, OCH_2Ph), 7.25–7.44 (5H, m). **53**: Colorless glass. HRMS Calcd for $C_{30}H_{39}NO_4$: 477.2877. Found: 477.2868. MS *m*/*z*: 477 (M^+, 4), 432 (6), 388 (11), 342 (13), 296 (6), 280 (3), 91 (100), 65 (6), 45 (6). IR ($CHCl_3$) cm^{-1}: 1681. ^1H NMR (at 50 °C) δ: 0.92–1.05 (1H, m), 1.00 (3H, s, H18), 1.10–1.14 (1H, m, H9), 1.14–1.75 (7H, m), 1.60 (1H, d, J=7 Hz, H5), 1.79 (1H, br dd, J=6.5, 6.5 Hz, OH), 2.00–2.16 (2H, m), 2.01 (1H, dd, J=16, 8 Hz, H7), 2.11 (1H, br dd, J=7, 5 Hz, H14),

ca. 2.23–2.44 (2H, m), 2.45 (1H, br d, J=17 Hz, H15), 2.61–2.68 (1H, m, H13), 3.33 (1H, br d, J=11 Hz, H19), 3.43 (1H, ddd, J=9.5, 5, 4.5 Hz), 3.46 (1H, d, J=11 Hz, H19), 3.63 (1H, ddd, J=9.5, 4.5, 4 Hz), 3.70–3.76 (2H, m), 4.10 (1H, dd, J=8, 7 Hz, H6), 4.14 (1H, d, J=7 Hz, H20), 4.66–4.70 (1H, m, C=CH_2), 4.74–4.78 (1H, m, C=CH_2), 5.11 (1H, d, J=12.5 Hz), 5.16 (1H, d, J=12.5 Hz), 7.26–7.40 (5H, m).

4.4.4. Lemieux oxidation of 53 to form 54. $NaIO_4$ (9 mg, 42.1 μmol) and OsO_4 (0.5 mg, 1.97 μmol) were successively added to a solution of **53** (1 mg, 2.10 μmol) in THF (1.5 ml) and H_2O (0.3 ml) at 0 °C and the mixture was stirred for 10 min and then at 21 °C for 14 h. Saturated $Na_3S_2O_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and PTLC [hexane–EtOAc (3:2)] provided **54** (1 mg, quant.) as a colorless syrup. HRMS Calcd for $C_{29}H_{37}NO_5$: 479.2670. Found: 479.2672. MS m/z: 479 (M^+, 0.5), 434 (4), 390 (11), 344 (13), 298 (4), 282 (2), 91 (100), 65 (5), 45 (6). IR ($CHCl_3$) cm^{-1}: 1730, 1682. ^1H NMR (at 50 °C) δ: 0.99–1.08 (1H, m), 1.03 (3H, s), 1.19–1.80 (10H, m, including OH), 1.65 (1H, d, J=7 Hz, H5), 1.96 (1H, br d, J=19 Hz, CH_2CO), 2.08–2.18 (1H, m), 2.11 (1H, dd, J=16, 8 Hz, H7), 2.31 (1H, d, J=19 Hz, CH_2CO), 2.35–2.62 (3H, m), 3.35 (1H, d, J=11 Hz, H19), 3.45 (1H, ddd, J=10, 5, 5 Hz), 3.48 (1H, d, J=11 Hz, H19), 3.56 (1H, ddd, J=10, 4, 4 Hz), 3.67–3.79 (2H, m), 4.15 (1H, dd, J=8, 7 Hz, H6), 4.25 (1H, d, J=6 Hz, H20), 5.11 (1H, d, J=12.5 Hz), 5.18 (1H, br d, J=12.5 Hz), 7.28–7.39 (5H, m).

4.4.5. Preparation of 55 from 9. $(TMS)_2NLi$ (1 M in THF, 0.22 ml, 0.22 mmol) was added to a cooled (−78 °C) solution of **9** (7 mg, 14.7 μmol) in THF (2 ml) and the mixture was stirred under an Ar atmosphere for 1.5 h. A THF (0.5 ml) solution of TMSCl (19 μl, 0.150 mmol) was added dropwise to it, and the resulting mixture was stirred at −78 to 12 °C for 15.5 h. Saturated NH_4Cl–H_2O and saturated $NaHCO_3$–H_2O were successively added and the whole was extracted with CH_2Cl_2. Usual work-up gave a residue (12 mg) and this was dissolved in THF (3 ml). Aqueous HCl (2.5%, 0.25 ml) was added to this at 0 °C and the mixture was stirred for 10 min. Quenching with saturated $NaHCO_3$–H_2O, extraction with CH_2Cl_2, and usual work-up followed by PTLC [hexane–EtOAc (5:2)] furnished **55** (6.5 mg, 81%) along with recovered **9** (1 mg, 14%). **55**: Colorless glass. HRMS Calcd for $C_{33}H_{45}NO_4Si$: 547.3115. Found: 547.3118. MS m/z: 547 (M^+, 8), 502 (1), 486 (2), 458 (5), 442 (5), 412 (7), 368 (3), 352 (4), 294 (4), 91 (100), 73 (19). IR ($CHCl_3$) cm^{-1}: 2170, 1683. ^1H NMR (at 50 °C) δ: 0.13 (9H, s, $SiMe_3$), 0.97 (3H, s), 1.04 (1H, ddd, J=13, 10, 5.5 Hz, H1), 1.31 (1H, ddd, J=13.5, 11, 4.5 Hz, H3), ca. 1.33–1.40 (1H, m), 1.43–1.56 (3H, m, H3 and CH_2C≡CSiMe_3), 1.64 (1H, ddd, J=13.5, 5, 5 Hz, H3), 1.66 (1H, d, J=7 Hz, H5), 1.70–1.85 (1H, m), 1.04 (1H, br s, OH), 2.02 (1H, ddd, J=13, 4.5, 4.5 Hz, H1), 2.10–2.31 (2H, m), 2.36 (1H, dd, J=6, 6 Hz, H14), ca. 2.55–2.74 (1H, m), 2.59 (1H, dd, J=16, 9 Hz, H7), 3.26–3.38 (3H, m), 3.42 (1H, ddd, J=9.5, 6.5, 3.5 Hz), 3.58–3.76 (2H, m), 4.09 (1H, d, J=6 Hz, H20), 4.25 (1H, dd, J=9, 7 Hz, H6), 5.13 (1H, d, J=12 Hz), 5.19 (1H, br d, J=12 Hz), 5.49–5.58 (1H, m, H13), 5.58 (1H, br dd, J=12, 2.5 Hz, H12), 7.27–7.40 (5H, m).

4.5. Completion of the total synthesis of (±)-nominine (Scheme 9)

4.5.1. Mesylation of 10 to form 56. MsCl (10 μl, 129 μmol) was added to a solution of **10** (7.5 mg, 15.8 μmol) and Et_3N (88 μl, 0.633 mmol) in CH_2Cl_2 (2 ml) at −20 °C under an Ar atmosphere and the mixture was stirred for 30 min. Saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. The organic layer was successively washed with saturated $CuSO_4$–H_2O, saturated $NaHCO_3$–H_2O, and water and then treated as usual. Purification was carried out by PTLC [hexane–EtOAc (2:1)] to provide **56** (8.5 mg, 97%) as a colorless glass. HRMS Calcd for $C_{31}H_{41}NO_6S$: 555.2652. Found: 555.2668. MS m/z: 555 (M^+, 0.3), 432 (11), 420 (6), 388 (14), 296 (7), 123 (7), 91 (100), 79 (8). IR ($CHCl_3$) cm^{-1}: 1684. ^1H NMR (at 50 δ) δ: 0.89 (1H, ddd, J=13, 10, 5.5 Hz, H1), 0.99 (3H, s), 1.14 (1H, d, J=10.5 Hz, H9), 1.30–1.78 (7H, m), 1.57 (1H, d, J=6.5 Hz, H5), 1.75 (1H, dd, J=16, 8 Hz, H7), 1.87 (1H, dd, J=13, 4.5 Hz, H13), 1.96–2.06 (1H, m), 2.05–2.20 (2H, m), 2.11 (1H, d, J=18 Hz, H15), ca. 2.35–2.55 (1H, m, H7), 2.36 (1H, br d, J=18 Hz, H15), 3.00 (3H, s, SO_2CH_3), 3.28 (1H, br d, J=11 Hz, H19), 3.28 (1H, ddd, J=11.5, 5.5, 4.5 Hz), 3.43 (1H, d, J=11 Hz, H19), 3.55 (1H, ddd, J=11.5, 5, 4 Hz), 3.95 (1H, d, J=7 Hz, H20), 4.08 (1H, dd, J=8, 6.5 Hz, H6), 4.30–4.36 (2H, m, CH_2OMs), 4.49 (1H, ddd, J=2, 2, 2 Hz, H17), 4.65 (1H, ddd, J=2, 2, 2 Hz, H17), 5.11 (1H, d, J=12.5 Hz), 5.17 (1H, d, J=12.5 Hz), 7.25–7.41 (5H, m).

4.5.2. Preparation of bromide 57 from mesylate 56. Anhydrous LiBr (19 mg, 0.218 mmol) was added to a solution of **56** (8 mg, 14.4 μmol) in acetone (3 ml) and the mixture was stirred under reflux for 15 h. After the mixture had been cooled, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and subsequent separation by PTLC [hexane–EtOAc (5:1)] provided **57** (7 mg, 90%) as a colorless glass. HRMS Calcd for $C_{30}H_{38}BrNO_3$: 541.2014 and 539.2034. Found: 541.2002 and 539.2039. MS m/z: 541, 539 (M^+, 0.7, 0.7), 432 (14), 406, 404 (5, 5), 388 (15), 296 (8), 109, 107 (4, 4), 91 (100), 65 (6). IR ($CHCl_3$) cm^{-1}: 1683. ^1H NMR (at 50 °C) δ: 0.87 (1H, ddd, J=13, 9.5, 5 Hz, H1), 0.99 (3H, s), 1.13 (1H, br d, J=10.5 Hz, H9), 1.28–1.61 (4H, m), 1.57 (1H, d, J=6.5 Hz, H5), 1.63–1.82 (3H, m), 1.75 (1H, dd, J=16, 8.5 Hz, H7), 1.91 (1H, dd, J=13, 4.5 Hz, H13), 1.94–2.04 (1H, m, H14), 2.05–2.11 (1H, m, H12), 2.10 (1H, d, J=18 Hz, H15), 2.21 (1H, ddd, J=13.5, 4.5, 4.5 Hz, H1), ca. 2.35–2.54 (1H, m, H7), 2.36 (1H, br d, J=18 Hz, H15), 3.28 (1H, br d, J=10.5 Hz, H19), 3.38–3.50 (4H, m, H19 and CH_2CH_2Br), 3.56–3.68 (1H, m, CH_2CH_2Br), 3.94 (1H, d, J=7 Hz, H20), 4.08 (1H, dd, J=8.5, 6.5 Hz, H6), 4.49 (1H, ddd, J=2, 2, 2 Hz, H17), 4.64 (1H, ddd, J=2, 2, 2 Hz, H17), 5.11 (1H, d, J=12.5 Hz), 5.17 (1H, br d, J=12.5 Hz), 7.27–7.41 (5H, m).

4.5.3. Oxidation of 57 to form 58, 59, and 60. To a slurry of SeO_2 (6 mg, 54.1 μmol) in CH_2Cl_2 (1.5 ml) was added 70% t-BuOOH/H_2O (28 μl, 0.203 mmol) and the mixture was stirred at 0 °C for 10 min and at 22 °C for 20 min. The mixture was allowed to cool in an ice bath and a CH_2Cl_2 (2.5 ml) solution of **57** (7 mg, 13.0 μmol) was added to this. After the mixture had been stirred at 0–21 °C for 16 h, saturated

$Na_2S_2O_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by PTLC [hexane–EtOAc (5:2)] afforded **58** (5.5 mg, 77%), **60** (<1 mg, trace), and crude **59** (1.5 mg) in order of increasing polarity. The crude **59** was further purified by PTLC (0.5% MeOH–CH_2Cl_2) to give **59** (1 mg, 14%). **58**: Colorless glass. MS *m/z*: 446 ($M^+-CH_2CH_2Br$, 7), 420, 418 (3, 3), 402 (14), 310 (8), 109, 107 (6, 5), 91 (100), 65 (7). IR ($CHCl_3$) cm^{-1}: 1690, 1628. ^1H NMR (at 50 °C) δ: 0.95 (1H, ddd, *J*=13, 10, 5 Hz, H1), 1.00 (3H, s), 1.20–1.61 (5H, m), 1.57 (1H, d, *J*=7 Hz, H5), 1.65–1.86 (2H, m), 1.98 (1H, ddd, *J*=13.5, 2.5, 2.5 Hz, H11), 2.15 (1H, dd, *J*=13, 4.5 Hz, H13), 2.20–2.44 (2H, m), 2.25 (1H, ddd, *J*=13, 4.5, 4.5 Hz, H1), 2.55–2.60 (1H, m, H12), 2.68 (1H, dd, *J*=17, 8.5 Hz, H7), 3.32 (1H, br d, *J*=10.5 Hz, H19), 3.42 (1H, d, *J*=10.5 Hz, H19), 3.43–3.53 (3H, m), 3.57–3.67 (1H, m), 4.05 (1H, d, *J*=7 Hz, H20), 4.17 (1H, dd, *J*=8.5, 7 Hz, H6), 5.04 (1H, d, *J*=2 Hz, H17), 5.13 (1H, d, *J*=13 Hz), 5.17 (1H, d, *J*=13 Hz), 5.85 (1H, d, *J*=2 Hz, H17), 7.26–7.40 (5H, m). **59**: Colorless glass. HRMS Calcd for $C_{30}H_{38}BrNO_4$: 557.1963 and 555.1983. Found: 557.1988 and 555.2004. MS *m/z*: 557, 555 (M^+, 0.4, 0.7), 448 (11), 422, 420 (4, 4), 404 (13), 312 (6), 296 (4), 109, 107 (5, 5), 91 (100), 65 (7). IR ($CHCl_3$) cm^{-1}: 1681. ^1H NMR (at 50 °C) δ: 0.86–0.95 (1H, m), 0.99 (3H, s), 1.10 (1H, br d, *J*=10.5 Hz, H9), 1.28–1.82 (7H, m, including OH), 1.60 (1H, d, *J*=6.5 Hz, H5), 1.78 (1H, ddd, *J*=13.5, 3, 3 Hz, H11), 1.91 (1H, dd, *J*=16, 9 Hz, H7), 1.94 (1H, dd, *J*=13, 4.5 Hz, H13), 2.15–2.34 (3H, m), 2.78 (1H, br d, *J*=16 Hz, H7), 3.31 (1H, br d, *J*=11 Hz, H19), 3.42–3.50 (2H, m), 3.43 (1H, d, *J*=11 Hz, H19), 3.52–3.74 (2H, m), 3.95 (1H, d, *J*=7 Hz, H20), 3.98 (1H, br s, H15), 4.14 (1H, dd, *J*=9, 6.5 Hz, H6), 4.91 (1H, dd, *J*=1.5, 1 Hz, H17), 4.93 (1H, dd, *J*=1.5, 1 Hz, H17), 5.12 (1H, d, *J*=12.5 Hz), 5.18 (1H, d, *J*=12.5 Hz), 7.26–7.40 (5H, m). **60**: Colorless glass. HRMS Calcd for $C_{30}H_{36}BrNO_4$: 555.1807 and 553.1827. Found: 555.1780 and 553.1823. MS *m/z*: 555, 553 (M^+ 0.4, 0.4), 446 (12), 420, 418 (3, 3), 402 (14), 310 (5), 109, 107 (4, 4), 91 (100), 65 (5). ^1H NMR (at 50 °C) only selected signals were given δ: 1.02 (3H, s), 1.65 (1H, d, *J*=6.5 Hz, H5), 2.36 (1H, dd, *J*=16, 8.5 Hz, H7), 3.04–3.09 (1H, m), 3.33 (1H, d, *J*=10.5 Hz, H19), 3.37–3.50 (4H, m), 3.60–3.72 (1H, m), 4.04 (1H, d, *J*=6.5 Hz, H20), 4.20 (1H, dd, *J*=8.5, 6.5 Hz, H6), 5.15 (2H, s), 6.77 (1H, s, H15), 7.24–7.40 (5H, m), 9.41 (1H, s, H17).

4.5.4. Oxidation of 59 to form the enone 58. MnO_2 (12 mg, 0.138 mmol) was added to a solution of **59** (2 mg, 3.60 μmol) in CH_2Cl_2 (2 ml) and the mixture was stirred at 20 °C for 15 h. The whole was filtered through a Celite bed and the Celite was washed with CH_2Cl_2. The solvent was evaporated off and the residue was purified by PTLC [hexane–EtOAc (3:1)] to give a colorless glass (2 mg, quant.), whose ^1H NMR spectrum was identical with that of the enone **58**.

4.5.5. Reduction of 58 to form β-allyl alcohol 61. $NaBH_4$ (3 mg, 78.9 μmol) was added to a solution of **58** (5.5 mg, 9.93 μmol) and $CeCl_3\cdot 7H_2O$ (31 mg, 83.2 μmol) in MeOH (2.5 ml) at 0 °C, and the mixture was stirred for 20 min. The reaction was quenched by successive addition of saturated NH_4Cl–H_2O and saturated $NaHCO_3$–H_2O, and the mixture was extracted with CH_2Cl_2. Usual work-up followed by separation by PTLC [hexane–EtOAc (2:1)] afforded **61** (5.5 mg, quant.) as a colorless glass. HRMS Calcd for $C_{30}H_{38}BrNO_4$: 557.1963 and 555.1983. Found: 557.1971 and 555.1969. MS *m/z*: 557, 555 (M^+, 0.5, 0.6), 448 (20), 422, 420 (5, 5), 404 (16), 312 (9), 296 (4), 109, 107 (3, 4), 91 (100), 65 (2). IR ($CHCl_3$) cm^{-1}: 1682. ^1H NMR (at 50 °C) δ: 0.88 (1H, ddd, *J*=13, 10, 5 Hz, H1), 0.99 (3H, s), 1.20–1.55 (5H, m, including OH), 1.58 (1H, d, *J*=6.5 Hz, H5), 1.64–1.83 (4H, m), 1.92 (1H, dd, *J*=13.5, 4.5 Hz, H13), 1.93–2.10 (2H, m), 2.14–2.20 (1H, m, H12), 2.19 (1H, ddd, *J*=13, 4.5, 4.5 Hz, H1), 2.66 (1H, dd, *J*=16, 8.5 Hz, H7), 3.30 (1H, br d, *J*=10.5 Hz, H19), 3.40–3.49 (3H, m), 3.44 (1H, d, *J*=10.5 Hz, H19), 3.53–3.65 (1H, m), 3.97–4.04 (1H, m, H15), 4.01 (1H, d, *J*=6.5 Hz, H20), 4.20 (1H, dd, *J*=8.5, 6.5 Hz, H6), 4.91 (1H, br s, H17), 4.99 (1H, br s, H17), 5.15 (2H, s), 7.28–7.38 (5H, m).

4.5.6. Acetylation of 59 and 61 to form 62 and 63, respectively. The procedure for the preparation of **63** from **61** is described as a representative example. A solution of **61** (5 mg, 8.99 μmol), Ac_2O (0.2 ml), and pyridine (0.3 ml) in CH_2Cl_2 (1.5 ml) was stirred at 21 °C for 24 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (3:1)] gave **63** (5 mg, 93%) as a colorless glass. HRMS Calcd for $C_{32}H_{40}BrNO_5$: 599.2068 and 597.2088. Found: 599.2075 and 597.2061. MS (*m/z*): 599, 597 (M^+, 0.5, 0.5), 490 (10), 464, 462 (4, 3), 446 (10), 404 (2), 354 (4), 296 (9), 91 (100), 65 (7), 43 (26). IR ($CHCl_3$) cm^{-1}: 1726, 1683. ^1H NMR of two rotamers (ca. 4:1) at 50 °C δ: (major rotamer) 0.84–0.96 (1H, m), 0.99 (3H, s), 1.16–1.60 (6H, m), 1.59 (1H, d, *J*=6.5 Hz, H5), 1.63–1.83 (3H, m), 1.94 (1H, dd, *J*=13, 4 Hz, H13), 1.96–2.34 (4H, m), 2.09 (3H, s, $OCOCH_3$), 3.31 (1H, br d, *J*=11 Hz, H19), 3.42–3.50 (3H, m), 3.43 (1H, d, *J*=11 Hz, H19), 3.55–3.65 (1H, m), 3.99 (1H, d, *J*=7 Hz, H20), 4.06–4.17 (1H, m), 4.80 (1H, br s, H17), 4.87 (1H, br s, H17), 5.10 (1H, d, *J*=12.5 Hz), 5.16 (1H, d, *J*=12.5 Hz), 5.49 (1H, dd, *J*=2, 2 Hz, H15), 7.25–7.40 (5H, m); (minor rotamer) 3.94–3.98 (1H, m), 4.88 (1H, br s, H17), 4.90 (1H, br s, H17), 5.08 (1H, d, *J*=12.5 Hz), 5.19 (1H, d, *J*=12.5 Hz), 5.45 (1H, br s, H15). In the same manner, **59** (1 mg, 1.80 μmol) was acetylated to yield **62** (1 mg, 93%) as a colorless glass after separation by PTLC [hexane–EtOAc (3:1)]. HRMS Calcd for $C_{32}H_{40}BrNO_5$: 599.2068 and 597.2088. Found: 599.2065 and 597.2113. MS (*m/z*): 599, 597 (M^+, 0.6, 0.6), 540, 538 (0.2, 0.2), 490 (15), 464, 462 (5, 4), 446 (13), 354 (6), 296 (11), 91 (100), 43 (17). IR ($CHCl_3$) cm^{-1}: 1716, 1682. ^1H NMR of two rotamers (ca. 7:1) at 50 °C δ: (major rotamer) 0.83–0.95 (1H, m), 0.99 (3H, s), 1.16–2.06 (6H, m), 1.19 (1H, br d, *J*=10.5 Hz, H9), 1.48 (3H, s, $OCOCH_3$), 1.60 (1H, d, *J*=6.5 Hz, H5), 1.77 (1H, ddd, *J*=13.5, 3, 3 Hz, H11), 1.83 (1H, dd, *J*=16, 8.5 Hz, H7), 1.95 (1H, dd, *J*=13, 4.5 Hz, H13), 2.14–2.20 (1H, m, H12), 2.21 (1H, ddd, *J*=13, 4.5, 4.5 Hz, H1), 2.30 (1H, br dd, *J*=9, 7 Hz, H14), ca. 2.38–2.58 (1H, m), 3.31 (1H, br d, *J*=11 Hz, H19), 3.42–3.50 (3H, m), 3.43 (1H, d, *J*=11 Hz, H19), 3.58–3.70 (1H, m), 3.97 (1H, d, *J*=7 Hz, H20), 4.10 (1H, dd, *J*=8.5, 6.5 Hz, H6), 4.88 (1H, br s, H17), 4.91 (1H, br s, H17), 5.08 (1H, d, *J*=12.5 Hz), 5.19 (1H, d, *J*=12.5 Hz), 5.44 (1H, br s, H15), 7.24–7.39 (5H, m); (minor rotamer) 4.13–4.19 (1H, m, H6), 4.99 (1H, br s, H17), 5.02

(1H, br s, H17), 5.09 (1H, d, J=12.5 Hz), 5.17 (1H, d, J=12.5 Hz), 5.64 (1H, br s, H15). NOE (ca. 2.1%) was observed at δ=7.24–7.39 on irradiation at δ=1.48 (3H, s).

4.5.7. Reductive deprotection of 63 to form 64. A slurry of **63** (5 mg, 8.36 μmol), Zn powder (110 mg, 1.68 mmol), and NH_4Cl (5 mg, 93.5 μmol) in *i*-PrOH–H_2O (14:1, 3.5 ml) was refluxed with stirring for 5 h. After the mixture had been cooled, saturated NH_4Cl–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up and separation by PTLC [hexane–EtOAc (5:2)] provided **64** (4 mg, 97%) as a colorless glass. HRMS Calcd for $C_{30}H_{37}NO_5$: 491.2670. Found: 491.2657. MS (*m*/*z*): 491 (M^+, 6), 356 (37), 312 (5), 239 (5), 91 (100), 65 (8), 43 (26). IR ($CHCl_3$) cm^{-1}: 1725, 1682. ^1H NMR of two rotamers (ca. 4:1) at 50 °C δ: (major rotamer) 0.90–1.05 (1H, m), 1.00 (3H, s), 1.17–1.63 (8H, m, including OH), 1.59 (1H, d, J=6.5 Hz, H5), 1.67–1.85 (3H, m), 191–2.29 (4H, m), 2.10 (3H, s), 3.28 (1H, br d, J=11 Hz, H19), 3.46 (1H, d, J=11 Hz, H19), 4.13 (1H, dd, J=8.5, 6.5 Hz, H6), 4.49 (1H, d, J=7.5 Hz, H20), 4.77 (1H, br s, H17), 4.86 (1H, br s, H17), 5.08 (1H, d, J=12.5 Hz), 5.16 (1H, d, J=12.5 Hz), 5.51 (1H, br s, H15), 7.26–7.40 (5H, m); (minor rotamer) 4.06–4.13 (1H, m, H6), 4.44–4.50 (1H, m, H20), 4.87 (1H, br s, H17), 4.91 (1H, br s, H17), 5.05 (1H, d, J=12.5 Hz), 5.20 (1H, d, J=12.5 Hz), 5.44 (1H, br s, H15).

4.5.8. Cyclization of 64 to form *O*-acetylnominine (65). $Pd(OAc)_2$ (1 mg, 4.45 μmol) and Et_3N/CH_2Cl_2 (5% v/v, 13 μl, 4.67 μmol) were successively added to a solution of **64** (4.5 mg, 9.16 μmol) and Et_3SiH (0.22 ml, 1.38 mmol) in CH_2Cl_2 (0.2 ml) under an Ar atmosphere and the mixture was stirred at 23 °C for 3 h. Saturated $NaHCO_3$–H_2O was added and the mixture was extracted with CH_2Cl_2. Usual work-up gave a residue (37 mg, containing Et_3SiH polymer). The residue was dissolved in CH_2Cl_2 (2.5 ml) and to this were added pyridine (56 μl, 0.693 mmol) and $SOCl_2$ (20 μl, 0.274 mmol) in this order at 0 °C under an Ar atmosphere. After the mixture had been stirred at the temperature for 30 min and at 22 °C for 48 h, saturated $NaHCO_3$–H_2O was added and the whole was extracted with CH_2Cl_2. Usual work-up followed by Al_2O_3 column chromatography [20 g, benzene–DME (2:1)] afforded **65** (2.5 mg, 80%) as colorless prisms, mp: 153–155 °C (CH_2Cl_2–hexane). HRMS Calcd for $C_{22}H_{29}NO_2$: 339.2197. Found: 339.2183. MS (*m*/*z*): 339 (M^+, 100), 324 (5), 311 (4), 296 (14), 280 (17), 160 (12), 146 (32), 91 (13), 79 (14), 55 (15), 43 (62), 41 (24). IR ($CHCl_3$) cm^{-1}: 1728, 1714. ^1H NMR δ: 0.97 (3H, s, H18), 1.12 (1H, ddd, J=13, 2.5, 2.5 Hz, H13), 1.19–1.33 (2H, m), 1.42–1.50 (1H, m), 1.44 (1H, s, H5), 1.54–1.99 (7H, m), 1.66 (1H, dd, J=13, 3 Hz, H7), 1.71 (1H, dd, J=13, 3 Hz, H7), 1.94 (1H, dd, J=14, 4.5 Hz, H11), 2.07 (3H, s), 2.16–2.21 (1H, m, H12), 2.40 (1H, d, J=12 Hz, H19), 2.54 (1H, d, J=12 Hz, H19), 2.54 (1H, br s, H20), 3.20–3.24 (1H, m, H6), 4.93 (1H, s, H17), 4.97 (1H, s, H17), 5.44 (1H, s, H15). ^{13}C NMR δ: 19.6 (CH_2, C2), 21.3 (CH_3, CO*C*H3), 26.4 (CH_2, C11), 26.9 (CH_2, C1), 28.7 (CH_3, C18), 32.6 (CH_2, C7), 33.2 (CH_2, C13), 33.7 (CH, C12), 34.0 (CH_2, C3), 37.8 (C, C4), 43.4 (CH, C9 or C14), 44.1 (CH, C14 or C9), 44.8 (C, C10), 49.5 (C, C8), 60.9 (CH, C5), 62.4 (CH_2, C19), 65.0 (CH, C6), 72.8 (CH, C15), 74.6 (CH, C20), 110.6 (CH_2, C17), 144.6 (C, 16), 170.1 (C, *C*OCH_3).

4.5.9. (±)-Nominine (1). A solution of **65** (3 mg, 8.85 μmol) in 2% w/v K_2CO_3/MeOH (2 ml) was refluxed with stirring for 30 min under an Ar atmosphere. After the mixture had been cooled, water was added and the mixture was extracted with CH_2Cl_2. Usual work-up and purification by Al_2O_3 column chromatography (20 g, 2% MeOH–CH_2Cl_2) provided (±)-nominine (**1**, 2.5 mg, 95%), colorless prisms, mp: 233–236 °C (MeOH–acetone) [cf. natural nominine,[4] mp: 255–258 °C (MeOH–acetone)]. HRMS Calcd for $C_{20}H_{27}NO$: 297.2091. Found: 297.2087. MS (*m*/*z*): 297 (M^+, 100), 282 (6), 280 (5), 269 (6), 160 (9), 148 (11), 146 (30), 105 (10), 91 (18), 79 (10), 77 (12), 55 (12), 53 (10), 41 (22). IR ($CHCl_3$) cm^{-1}: 2925 (s), 2870 (w), 1648 (w), 1632 (w), 1616 (w), 1578 (w), 1558 (w), 1538 (w), 1520 (w), 1487 (w), 1455 (m), 1437 (w), 1373 (w), 1317 (w), 1130 (w), 1112 (m), 1028 (w), 1002 (m), 980 (w), 938 (w), 902 (w), 882 (w), 846 (w). ^1H NMR δ: 0.98 (3H, s, H18), 1.11 (1H, ddd, J=13, 2.5, 2.5 Hz, H13), 1.19–1.34 (2H, m, H1 and H3), 1.38–1.50 (1H, m, H3), 1.44 (1H, s, H5), 1.56 (1H, dddd, J=14, 10, 3, 1.5 Hz, H11), 1.62–1.90 (7H, m, including OH, H1, H2×2, H9, H13, and H14), 1.68 (1H, dd, J=13, 3 Hz, H7), 1.94 (1H, dd, J=14, 4.5 Hz, H11), 2.05 (1H, dd, J=13, 2.5 Hz, H7), 2.17–2.22 (1H, m, H12), 2.39 (1H, d, J=12 Hz, H19), 2.52 (1H, br s, H20), 2.54 (1H, d, J=12 Hz, H19), 3.24–3.28 (1H, m, H6), 4.01 (1H, br s, H15), 4.94 (1H, dd, J=1.5, 1 Hz, H17), 4.97 (1H, dd, J=1.5, 1 Hz, H17). ^{13}C NMR δ: 19.7 (CH_2, C2), 26.7 (CH_2, C11), 27.0 (CH_2, C1), 28.8 (CH_3, C18), 32.7 (CH_2, C7), 33.1 (CH_2, C13), 33.7 (CH, C12), 34.0 (CH_2, C3), 37.8 (C, C4), 43.5 (CH, C9 or C14), 43.9 (CH, C14 or C9), 45.5 (C, C10), 49.6 (C, C8), 60.9 (CH, C5), 62.5 (CH_2, C19), 65.3 (CH, C6), 71.7 (CH, C15), 74.7 (CH, C20), 108.3 (CH_2, C17), 156.5 (C, 16).

4.5.10. Single-crystal X-ray analysis of (±)-nominine (1). Crystal data: $C_{20}H_{27}NO$, M=297.44, monoclinic, $P2_1/n$, a=7.059(1) Å, b=11.614(1) Å, c=18.959(1) Å, β=94.44(1)°, V=1549.7(2) $Å^3$, Z=4, ρ_c=1.275 g/cm^3, $F(000)$=648, λ=1.54178 Å, T=296(1) K, μ(Cu Kα)=5.92 cm^{-1}, crystal size=0.20×0.25×0.30 mm^3, 3336 reflections (2922 independent, R_{int}=0.012) were collected on a Rigaku AFC7R diffractometer. The structure was solved by direct methods (SHELXS-97)[27] and 203 variable parameters were refined using the least-squares method on F^2. The maximum electron density residue: 0.36e^-/$Å^3$, R_1[for $I>2\sigma(I)$]=0.048 and wR=0.206 (all data) with $R_1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$ and $wR=[\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{0.5}$. Crystallographic data for (±)-nominine (**1**) reported in this paper have been deposited at the Cambridge Crystallographic Data Centre, under publication number CCDC 244252. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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Direct synthesis of tetrahydropyrans via one-pot Babier–Prins cyclization of allylbromide with carbonyl compounds promoted by RTILs BPyX/SnX′$_2$ or BBIMBr/SnBr$_2$

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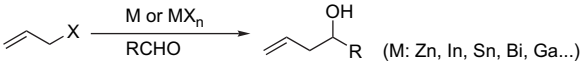
Abstract—Tetrahydropyran compounds can be directly synthesized from allylbromide and carbonyl compounds by means of one-pot Babier–Prins cyclization promoted by BPyX/SnX′$_2$ or BBIMBr/SnBr$_2$ complex (functionalized RTILs) under solvent-free conditions. 2,6-Homo-bissubstituted- and 2,6,6-trisubstituted, especially 6-(spirocycloalkyl)-, tetrahydropyran compounds can be prepared in good yields.

1. Introduction

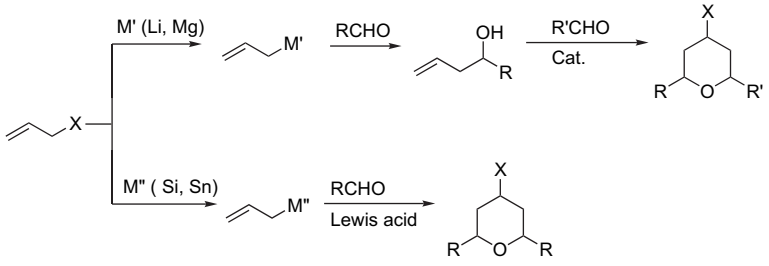
Tetrahydropyrans are common subunits in a variety of biologically important natural products such as polyether antibiotics, marine toxins, pheromones, and pharmaceutical agents.[1] The Prins cyclization[2] is one of the most effective reaction for construction of the tetrahydropyran unit. In most cases, the Prins cyclization involves the condensation of homoallylic alcohol with carbonyl compounds (Eq. 2) in the presence of protic acid[3] or Lewis acid, such as $TiBr_4$,[4] $ZrCl_4$,[5] $AlCl_3$,[6] $InCl_3$,[7] $Sc(OTf)_3$,[8] $Ce(OTf)_3 \cdot H_2O$ in ionic liquid,[9] as well as TMSCl/NaI.[10] The homoallylic alcohols can be prepared by means of Babier reaction[11] (Eq. 1), Grignard reaction (Eq. 2) or reaction of organolithium reagents with aldehydes (Eq. 2). Alternatively, allyl silyl[12] and allyltin[13] reagents can also react with aldehydes in the presence of Lewis acid to give tetrahydropyran compounds (Eq. 3). However, there are few examples of direct formation of tetrahydropyran compounds via one-pot reaction of allylhalide with carbonyl compounds. As reported by Li,[7a] during investigation of indium-mediated reaction of allylbromide with aldehyde under neat conditions, the formation of a mixture of tetrahydropyran-4-ol and 4-bromotetrahydropyran compounds was accidentally observed. It is interesting to combine Babier reaction with Prins cyclization into an one-pot tandem reaction system for direct formation of tetrahydropyran (Eq. 4). Recently, noticeable progress in the transformation under solvent-free condition has attracted considerable attention.[14] Herein, we report on the direct synthesis of tetrahydropyran compounds through one-pot Babier–Prins cyclization of allylbromide with carbonyl compounds promoted by BPyX/SnX$_2$ or BBIMBr/SnBr$_2$ complex (functionalized RTILs) without the use of organic solvent.

Babier Reaction



(1)

Prins Cyclization



(2)

(3)

Keywords: Babier–Prins cyclization; Allylbromide; Carbonyl compound; BBIMBr/SnBr$_2$; Tetrahydropyran; Direct synthesis.
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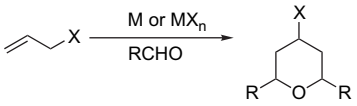
Babier-Prins Cyclization (one pot, tandem reaction)

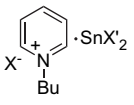


(4)

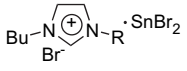
2. Results and discussion

2.1. Direct synthesis of 4-halo-2,6-homo-bissubstituted-tetrahydropyrans

$SnCl_2$-mediated Babier-type reaction of allylhalides with carbonyl compounds yield homoallylic alcohol, though requiring additional catalyst in most cases.[15] Among the catalysts suitable to this type of reaction, quaternary ammonium salts are more convenient.[16] It is also noteworthy that the combination of quaternary ammonium salts with stannous halides can generate a room temperature liquid state complex (so-called room temperature ionic liquid: RTILs).[17] Thus, it is expected that a complex of a quaternary ammonium salt with $SnCl_2$ can serve as both functionalized reagent with Lewis acidity and reaction media.



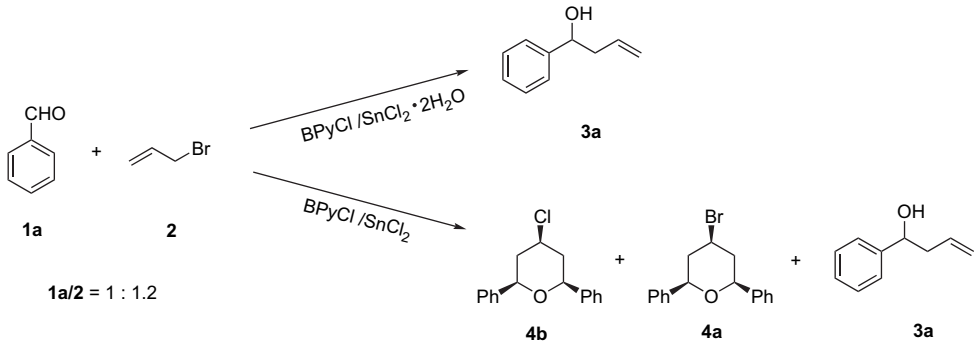
IA, X:Cl, X':Cl
IB, X:Cl, X':Br
IC, X:Br, X':Cl
ID, X:Br, X':Br
IE, X: I, X': Cl



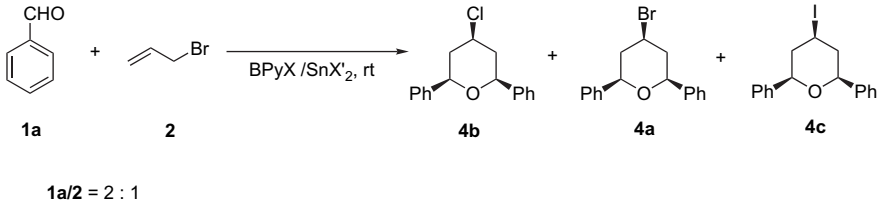
IIA, R:CH_3
IIB, R: Bu

According to the authors,[18] the complexes (**IA**–**E**) of *N*-benzylpyridine halides (BPyX) with SnX'_2 (1:2) were easily synthesized. At first, the complex **IA**·$2H_2O$ (BPyCl/$SnCl_2$·$2H_2O$) was employed in the reaction of allylbromide **2** with benzaldehyde **1a** (**IA**·$2H_2O$:**2**:**1a**=2:1.2:1) without the use of any organic solvent at room temperature for 24 h (Scheme 1). The homoallylic alcohol **3a** was obtained in 93% yield, which is consistent with the observation of Li.[19] However, it is interesting to note that if complex **IA** derived from anhydrous $SnCl_2$ and BPyCl was used under the same reaction conditions, a mixture of **3a** (22%), 4-chloro-(**4b**), and 4-bromo-2,6-diphenyltetrahydropyran (**4a**) (67%, **4a**+**4b**) produced (Scheme 1). When the ratio of **2** to **1a** was changed to 1:2, only tetrahydropyran compounds (**4a** and **4b**) were obtained in 78% yield, but no homoallylic alcohol **3a** was detected (Scheme 2, Table 1, entry 1). A series of RTILs (**IA**–**IE**) containing various tin halides were synthesized and employed in the reaction of **1a** with **2**. The results in Table 1 showed that the halide atom in the 4-position of the formed tetrahydropyran unit originates from substrate **2** and complex BPyX/SnX'_2. The use of **ID** (X and X′=Br) provided a single product **4a** in 80% yield (entry 4). However, in most cases, the reaction system became too sticky to be stirred during the course of the reaction promoted by **ID** without using organic solvent. For improving the fluidity of the complex as a reaction medium, two complexes of imidazole salts with $SnBr_2$ (1:1.5, **IIA** and **IIB**)[18] were synthesized and also employed in the reaction of **2** with **1a**. As shown in Table 1 BBIMBr/$SnBr_2$ (**IIB**) is a better reaction system than **ID** and **IIA** (entry 4 vs 7 and 6 vs 7). Moreover, for most substrates used, the reaction systems employed **IIB** were in a liquid state during the course of the reaction. If $SnBr_2$ was used alone (without quaternary ammonium salt), the reaction of **1a** with **2** did not proceed and starting materials were recovered, even after 24 h (entry 8).

Meanwhile, in order to examine solvent effects, various solvents were used as reaction media in the reaction of **1a** with **2** promoted by **IIB** (Scheme 3, Table 2). It was found that in organic solvents the reactions gave homoallylic alcohol **3a**



Scheme 1.



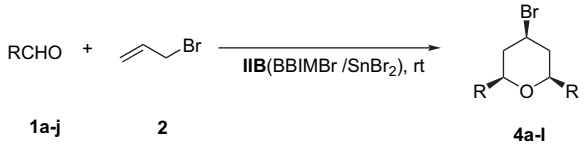
Scheme 2.

Table 1. The reaction of **1a** with **2** promoted by complex **I** or **II**[a]

Entry	Complexes (**I** and **II**)[b]	Yield[c] (%) (**4b**:**4a**:**4c**)[d]
1	**IA**	78 (5.3:1:0)
2	**IB**	90 (1:1:0)
3	**IC**	77 (3.2:1:0)
4	**ID**	80 (0:1:0)
5	**IE**	89 (1.6:1:2.5)
6	**IIA**	81 (0:1:0)
7	**IIB**	86 (0:1:0)
8	$SnBr_2$	—[e]

[a] Reaction time: 24 h at rt.
[b] BPyX/SnX'_2=1:2.
[c] Isolated yield.
[d] Determined by ^1H NMR.
[e] Starting materials were recovered.

solely or a mixture of **3a** and tetrahydropyran compound **4a**. Only under solvent-free conditions the single product, tetrahydropyran compound **4a** was obtained in 86% yield (Table 2, entry 5).



Scheme 3.

Table 2. The reaction of **1a** with **2**[a]

Entry	Solvent[b]	Yield[c] (%) (**3a**:**4a**)
1	THF	44 (44:0)
2	DMF	72 (72:0)
3	CH_3CN	67 (13:54)
4	CH_2Cl_2	73 (8:65)
5	None	86 (0:86)

[a] **1a**:**2**:**IIB**=2:1:2, 24 h at rt.
[b] All solvents are anhydrate.
[c] Isolated yield.

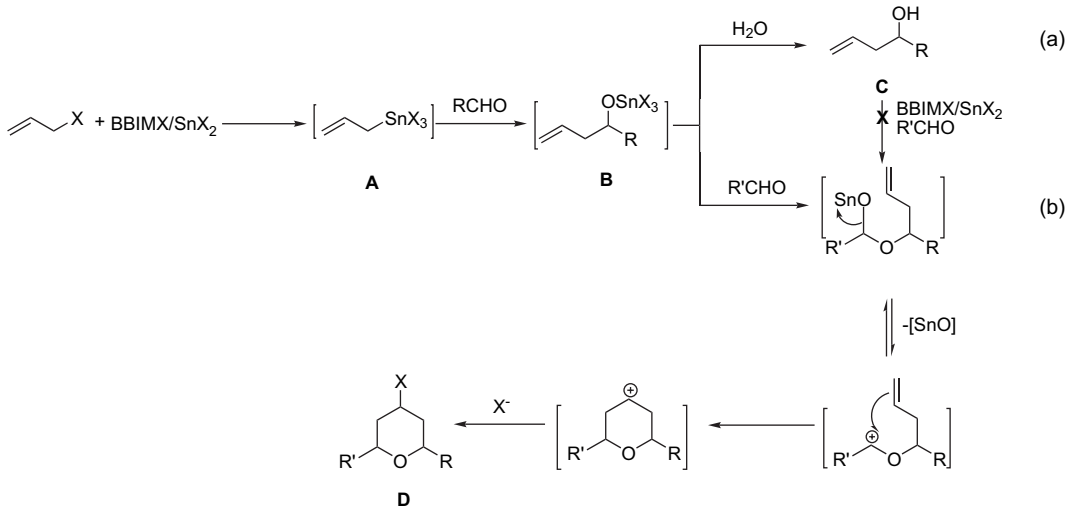
On the basis of the above experimental results, a variety of aldehydes were employed in the reactions with allylbromide **2** promoted by **IIB** without the use of organic solvent, affording the products of 4-bromo-2,6-homo-bissubstituted-tetrahydropyrans (Scheme 3, Table 3). As shown in Table 3 both aromatic and aliphatic aldehydes are able to react with **2** to directly generate tetrahydropyran compounds **4a–l** in moderate to good yields (52–86%). The stereochemistry of 2,4,6-trisubstituents of the tetrahydropyran ring was found to be all-cis in all cases. The assignment was based on the coupling constants of the protons at the C-2, C-4, and C-6 positions. The coupling constants of 11.0–12.7 Hz were observed for all such protons, indicating the presence of axial–axial coupling. The three substituents are therefore all in equatorial positions, in agreement with an all-cis stereochemistry.[12b]

2.2. A plausible reaction mechanism

In the mechanism for the present reaction, it is assumed that at the first is a Babier-type reaction of allylbromide with $SnBr_2$ in the presence of quaternary ammonium salt to produce allyltin compound **A**,[15d] which subsequently undergoes reaction with aldehyde to generate the reactive intermediate **B**. This intermediate exhibits high reactivity to give two different products (**C** and **D**) by two pathways (Scheme 4, path a and b). In pathway (a), **B** could be hydrolyzed by the water in the reaction system or during work-up procedure, giving homoallylic alcohol **C** as product. However, under strict anhydrous conditions the intermediate **B** was sufficiently activated to react with another aldehyde molecule via pathway (b) affording a Prins cyclization product, tetrahydropyran compound **D**.[8] It is interesting to note that when homoallylic alcohol **C** was mixed with aldehyde in the presence of **IIB**, no reaction product **D** was detected.

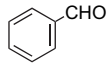
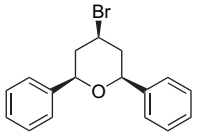
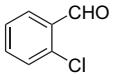
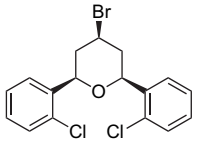
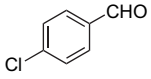
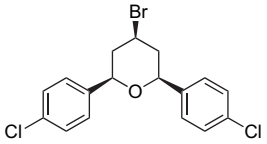
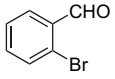
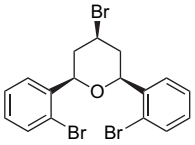
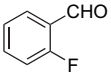
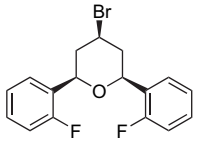
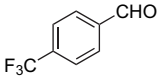
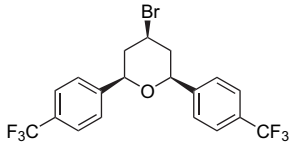
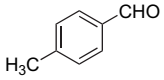
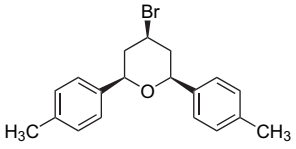
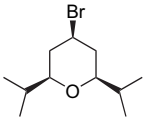
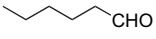
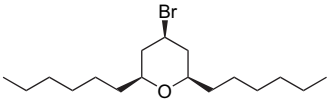
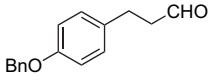
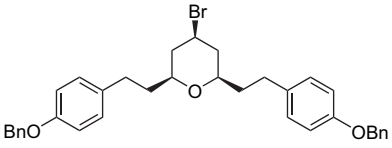
2.3. Direct synthesis of 4-bromo-2,6,6-trisubstituted-tetrahydropyran

For preparing 2,6-cross-substituted-tetrahydropyran, two different aromatic aldehydes were used in the reaction; a mixture of the products containing 2,6-homo- and 2,6-cross-bissubstituted-tetrahydropyrans was obtained. In view



Scheme 4.

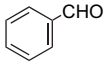
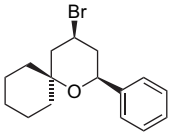
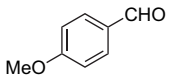
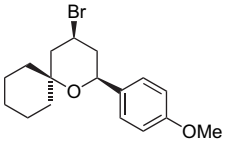
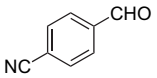
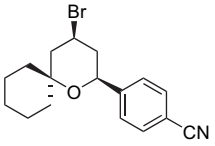
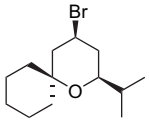
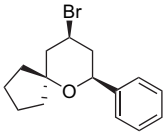
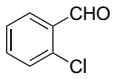
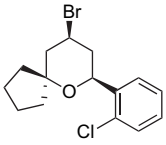
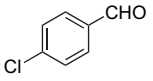
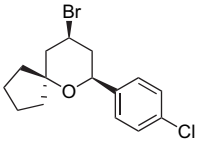
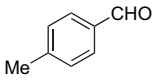
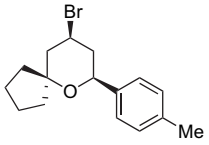
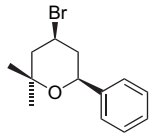
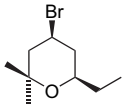
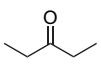
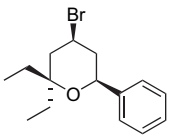
Table 3. Direct synthesis of **4** via reaction of **2** with **1** promoted by **IIB**[a]

Entry	Aldehyde	Product	Yield (%)[b]
1	**1a** 	**4a** 	86
2	**1b** 	**4d** 	78
3	**1c** 	**4e** 	78
4	**1d** 	**4f** 	82
5	**1e** 	**4g** 	75
6	**1f** 	**4h** 	54
7	**1g** 	**4i** 	60
8	**1h** 	**4j** 	82
9	**1i** 	**4k** 	72
10	**1j** 	**4l** 	79

[a] Reaction time: 24 h at rt, **1**:**2**=2:1.
[b] Isolated yield.

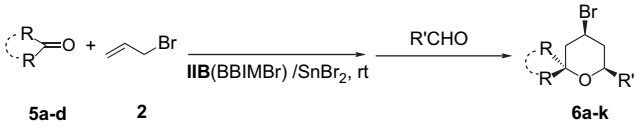
of the plausible reaction mechanism discussed above, formation of 2,6-homo-bissubstituted product may be retarded by use of a relatively less reactive carbonyl compound in the first step. Thus, the reaction could stop at intermediate **B** and not go on to generate homo-bissubstituted-tetrahydropyran compound. Then, more active aldehyde may be added to continue the reaction with **B** to give 2,6-cross-bissubstituted-tetrahydropyran. Interestingly, when cyclic ketones with less reactivity were used, 6-(spirocycloalkyl)-2-substituted-tetrahydropyran compounds were obtained

Table 4. Direct synthesis of cross-bissubstituted tetrahydropyrans **6** via reaction of **2** with carbonyl compounds

Entry	Ketone	Time[a] (h)	Aldehyde	Time[b] (h)	Product	Yield[c] (%)
1	**5a** 	6	**1a** 	10	**6a** 	85
2		6	**1k** 	10	**6b** 	76
3		6	**1l** 	10	**6c** 	78
4		6	**1h** 	10	**6d** 	72
5	**5b** 	5	**1a**	10	**6e** 	74
6		5	**1b** 	10	**6f** 	68
7		5	**1c** 	10	**6g** 	70
8		5	**1g** 	10	**6h** 	64
9	**5c** 	8	**1a**	10	**6i** 	67
10		8	**1i** 	10	**6j** 	54
11	**5d** 	8	**1a**	10	**6k** 	64

[a] Reaction time with ketone in the first step.
[b] Reaction time with aldehyde in the second step.
[c] Isolated yield.

(Scheme 5). For example, cyclohexanone **5a** was allowed to react with allylbromide **2** in the presence of **IIB** for 6 h. Then, benzaldehyde **1a** was added, followed by stirring for 10 h to give the product, 4-bromo-2-phenyl-6-(spirocyclohexyl)tetrahydropyran **6a** in 85% yield (Table 4, entry 1). No 2,6-diphenyltetrahydropyran **4a** and 2,6-bis(spirocyclohexyl)tetrahydropyran were observed in the reaction mixture. It is indicated in Table 4 that either cyclic or acyclic ketones, and aromatic or aliphatic aldehydes can be employed in the one-pot Babier–Prins cyclization, affording the products of 4-bromo-2,6,6-trisubstituted-tetrahydropyrans **6a–i** in good yields of 64–85%. The stereochemistry of the substituents at C-4 and C-2 positions was deduced as cis in consideration of the coupling constants for the protons (J∼11 Hz). The method of one-pot Babier–Prins cyclization provided a direct and convenient synthesis of 2,6,6-trisubstituted-tetrahydropyran compounds, especially for constructing a structural unit of 6-(spirocycloalkyl)-2-substituted-tetrahydropyran, which is of importance in organic synthesis and not easily prepared by conventional methods.[20]



Scheme 5.

3. Conclusion

Babier and Prins reactions can be combined into an one-pot tandem reaction system promoted by functionalized RTILs derived from quaternary ammonium salt and Sn(II) halides. By utilizing this method, either 2,6-homo-bissubstituted or 2,6,6-trisubstituted-tetrahydropyran, especially 6-(spirocycloalkyl)-2-substituted-tetrahydropyran compounds can be directly synthesized from allylbromide and carbonyl compounds. The study on scope of the reaction and application in the synthesis of natural compounds is in process.

4. Experimental

4.1. General

IR spectra were recorded on a Bruker Tensor 27 infra-red spectrometer. ^{1}H and ^{13}C NMR spectra were measured by Bruker AV-300 spectrometers in $CDCl_3$ with tetramethylsilane as an internal standard. Mass spectra were recorded on a GCT–MS Micromass spectrometer. Elemental analyses were performed on a Carlo Flash 1112 Element Analysis instrument. Melting points were measured by a Beijing-Tike X-4 apparatus and were uncorrected. Common reagents and materials were purchased from commercial sources and purified before used. Complexes **IA–IE** and **IIA–IIB** were synthesized according to literature procedures.[18]

4.2. Typical experimental procedure for the synthesis of 4-bromo-2,6-homo-bissubstituted-tetrahydropyrans

A mixture of benzaldehyde (**1a**, 212 mg, 2.0 mmol), allylbromide (**2**, 120 mg, 1.0 mmol) and complex **IIB** derived from BBIMBr (522 mg, 2.0 mmol), and $SnCl_2$ (1100 mg, 4.0 mmol) was stirred at ambient temperature for 24 h. The reaction mixture was extracted with diethyl ether. The combined ether phases were washed by 2 mL aqueous HCl, and dried over Na_2SO_4. The solvent was removed in vacuum and the crude product was purified by flash chromatography on silica gel (eluent: ethyl acetate/petroleum ether, 1:40) to afford a white solid **4a** (273 mg, 86%).

4.3. Typical experimental procedure for the synthesis of 4-bromo-2,6,6-trisubstituted-tetrahydropyrans

A mixture of cyclohexanone (294 mg, 3.0 mmol), allylbromide (120 mg, 1.0 mmol) and complex **IIB** derived from BBIMBr (522 mg, 2.0 mmol), and $SnBr_2$ (840 mg, 3.0 mmol) was stirred at ambient temperature for 6 h. Then benzaldehyde **1a** (106 mg, 1.0 mmol) was added, followed by stirring for 10 h. The reaction mixture was extracted with diethyl ether. The combined ether phases were washed by 2 M aqueous HCl, and dried over Na_2SO_4. The solvent was removed in vacuum and the crude product was purified by flash chromatography on silica gel (eluent: ethyl acetate/petroleum ether, 1:40) to afford a pale-yellow oil **6a** (260 mg, 85%).

4.3.1. 2-Phenyl-4-bromo-1-oxaspiro[5.5]undecane 6a. ^{1}H NMR ($CDCl_3$) δ 7.28–7.58 (m, 5H), 4.65 (d, J=10.1 Hz, 1H), 4.45–4.58 (m, 1H), 2.49–2.58 (dm, J=10.7 Hz, 1H), 2.26–2.36 (dm, J=11.8 Hz, 1H), 1.73–2.10 (m, 5H), 1.37–1.57 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 142.2, 128.4, 127.5, 125.8, 75.2, 71.6, 47.1, 45.6, 45.5, 40.0, 30.3, 26.0, 21.7, 21.3. FTIR (film): 2931, 1494, 1447, 1331, 1172, 1060 cm^{-1}. HRMS (EI): m/z calcd for $C_{16}H_{21}OBr$: 308.0775, found: 308.0776.

4.3.2. 2-(4′-Methoxyphenyl)-4-bromo-1-oxaspiro[5.5]undecane 6b. A yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.33 (d, J=8.7 Hz, 2H), 6.9 (d, J=8.8 Hz, 2H), 4.46–4.58 (m, 2H), 3.82 (s, 3H), 2.43–2.51 (dm, J=11.4 Hz, 1H), 2.23–2.32 (dm, 12.6 Hz, 1H), 1.99–2.10 (m, 1H), 1.60–1.90 (m, 3H), 1.31–1.59 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 158.9, 134.3, 127.1, 113.7, 75.2, 71.2, 55.3, 45.6, 45.3, 40.0, 30.3, 26.0, 21.7, 21.3. FTIR (film): 2932, 2856, 1613, 1513, 1446, 1246, 987, 829 cm^{-1}. HRMS (EI): m/z calcd for $C_{17}H_{23}O_2Br$: 338.0881, found: 338.0887.

4.3.3. 2-(4′-Nitrilphenyl)-4-bromo-1-oxaspiro[5.5]undecane 6c. A yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.60 (d, J=7.9 Hz, 2H), 7.52 (d, J=7.9 Hz, 2H), 4.63 (d, J=11.2 Hz, 1H), 4.39–4.54 (m, 1H), 2.45–2.55 (dm, J=11.9 Hz, 1H), 2.24–2.33 (dm, J=12.0 Hz, 1H), 1.99–2.10 (m, 1H), 1.67–1.84 (m, 4H), 1.36–1.52 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 147.4, 132.2, 126.4, 118.8, 111.1, 75.5, 70.9, 46.8, 45.1, 44.4, 39.8, 30.1, 25.8, 21.6, 21.2. FTIR (film): 2932, 2851, 2227, 1609, 1447, 1172, 1071, 986, 831 cm^{-1}. HRMS (EI): m/z calcd for $C_{17}H_{20}OBr$: 333.0728, found: 333.0726.

4.3.4. 2-(Isopropyl)-4-bromo-1-oxaspiro[5.5]undecane 6d. A light-yellow oil. ^{1}H NMR ($CDCl_3$) δ 4.31–4.42 (m, 1H), 3.17–3.08 (m, 1H), 2.34–2.25 (m, 1H), 2.17–2.10 (m, 1H), 1.99–2.07 (m, 1H), 1.58–1.76 (m, 7H); ^{13}C NMR

($CDCl_3$) δ 74.8, 74.1, 48.0, 46.7, 41.4, 39.9, 33.5, 33.1, 30.0, 26.0, 21.6, 20.9, 18.8, 18.5. FTIR (film): 2933, 2857, 1447, 1384, 1167, 1065 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{13}H_{23}OBr$: 274.0932, found: 274.0929.

4.3.5. 2-Phenyl-4-bromo-1-oxaspiro[5.4]decane 6e. A yellow oil. ^1H NMR ($CDCl_3$) δ 7.25–7.45 (m, 5H), 4.5 (d, J=11.4 Hz, 1H), 4.33–4.45 (m, 1H), 2.43–2.52 (dm, J=12.7 Hz, 1H), 2.11–2.23 (m, 2H), 1.90–2.04 (m, 3H), 1.27–1.69 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 141.9, 128.4, 127.6, 125.9, 85.8, 73.9, 46.6, 46.1, 45.3, 41.5, 33.0, 24.6, 23.1. FTIR (film): 2958, 2868, 1448, 1335, 1059, 995, 753, 699 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{15}H_{19}OBr$: 294.0619, found: 294.0615.

4.3.6. 2-(2′-Chlorophenyl)-4-bromo-1-oxaspiro[5.4]-decane 6f. A yellow oil. ^1H NMR ($CDCl_3$) δ 7.69 (d, J=7.2 Hz, 1H), 7.35–7.21 (m, 3H), 4.90 (d, J=11.2 Hz, 1H), 4.45–4.34 (m, 1H), 2.63–2.55 (dm, J=12.6 Hz, 1H), 2.24–2.10 (m, 2H), 1.94–1.83 (m, 2H), 1.60–1.26 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 139.3, 131.6, 129.2, 128.5, 127.4, 127.2, 86.0, 70.7, 46.7, 45.7, 43.5, 41.6, 32.9, 24.7, 23.1. FTIR (film): 2956, 2870, 1474, 1438, 1067, 994 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{15}H_{18}OClBr$: 328.0230, found: 328.0234.

4.3.7. 2-(4′-Chlorophenyl)-4-bromo-1-oxaspiro[5.4]-decane 6g. A yellow oil. ^1H NMR ($CDCl_3$) δ 7.28–7.34 (m, 5H), 4.51 (d, J=11.4 Hz, 1H), 4.32–4.44 (m, 1H), 2.40–2.50 (dm, J=12.7 Hz, 1H), 2.15–2.21 (m, 2H), 1.90–1.98 (m, 3H), 1.57–1.77 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 140.4, 133.2, 128.5, 127.3, 85.9, 73.2, 46.5, 45.6, 45.3, 41.4, 32.9, 24.6, 23.0. FTIR (film): 2959, 2868, 1490, 1335, 1207, 1066, 822, 729 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{15}H_{18}OClBr$: 328.0230, found: 328.0233.

4.3.8. 2-(4′-Methylphenyl)-4-bromo-1-oxaspiro[5.4]-decane 6h. A yellow oil. ^1H NMR ($CDCl_3$)n δ 7.23 (d, J=7.1 Hz, 2H), 7.13 (d, J=7.7 Hz, 2H), 4.48 (d, J=11.4 Hz, 1H), 4.31–4.43 (m, 1H), 2.39–2.48 (dm, J=12.6 Hz, 1H), 2.17 (s, 3H), 1.98–2.04 (m, 2H), 1.75–1.87 (m, 3H), 1.39–1.52 (m, 6H). FTIR (film): 2957, 2867, 1514, 1445, 1288, 1063, 996, 812 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{16}H_{21}OBr$: 308.0776, found: 308.0777.

4.3.9. 2-Phenyl-6,6-dimethyl-tetrahydropyran 6i. A yellow oil. ^1H NMR ($CDCl_3$) δ 7.21–7.55 (m, 5H), 4.59 (d, J=11.2 Hz, 1H), 4.39–4.55 (m, 1H), 2.40–2.50 (dm, J=12.7 Hz, 1H), 2.18–2.28 (dm, J=12.7 Hz, 1H), 1.87–2.02 (m, 2H), 1.30 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 141.9, 128.5, 127.8, 126.0, 79.7, 74.4, 73.3, 47.8, 45.4, 31.6, 22.4. FTIR (film): 2975, 2924, 2876, 1450, 1190, 1061, 973, 754 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{13}H_{17}OBr$: 268.0464, found: 268.0463.

4.3.10. 2-Ethyl-6,6-dimethyl-tetrahydropyran 6j. A light-yellow oil. ^1H NMR ($CDCl_3$) δ 4.28–4.38 (m, 1H), 3.42–3.46 (m, 1H), 2.16–2.22 (dm, J=10.6 Hz, 1H), 1.82 (t, J=12.5 Hz, 1H), 1.46–1.65 (m, 4H), 1.24 (s, 3H), 1.18 (s, 3H), 0.94 (t, J=7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 73.6, 72.2, 48.0, 46.0, 43.5, 31.4, 29.1, 22.3, 9.7. FTIR (film): 2974, 2877, 1464, 1381, 1195, 1084 cm^{-1}. HRMS (EI): *m/z* calcd for $C_9H_{17}BrO$: 220.0460, found: 220.0463.

4.3.11. 2-Phenyl-6,6-diethyl-tetrahydropyran 6k. A yellow oil. ^1H NMR ($CDCl_3$) δ 7.28–7.41 (m, 5H), 4.50–4.60 (m, 2H), 2.42–2.50 (dm, J=12.6 Hz, 1H), 2.17–2.26 (dm, J=12.9 Hz, 1H), 1.87–2.02 (m, 3H), 1.55–1.64 (m, 3H), 0.92 (t, J=7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 142.1, 128.4, 127.6, 125.9, 78.5, 72.4, 45.9, 45.5, 43.5, 31.8, 24.0, 7.5, 7.1. FTIR (film): 2968, 2936, 2878, 1452, 1335, 1058, 987, 757, 699 cm^{-1}. HRMS: *m/z* calcd for $C_{15}H_{21}OBr$: 296.0776, found: 296.0772.

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Reactions of some anellated 2-aminothiophenes with electron poor acetylenes

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Dedicated to the memory of Dr. Emmanuel Nyiondi-Bonguen

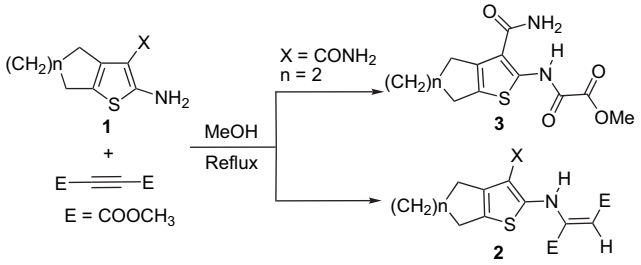
Abstract—The reactivity of 2-aminothiophenes in two different anellations: (a) [*b*]-anellation to a saturated carbocycle and (b) [3,4-*c*]-anellation to benzopyrans, towards typical acetylenic dienophiles has been investigated. Because of the absence of conjugation, the thiophenes of type (a) do not undergo [4+2]-cycloaddition with acetylenic dienophiles. Instead, the *N*-vinylated products **2** and **3** were obtained with dimethyl acetylene dicarboxylate (DMAD). Electron poor alkynes react with the thiophenes of type (b) in three main ways: DMAD reacts in a [4+2]-mode in dioxane to give the products **7**, **8** and **14**; a Michael addition type reaction also takes place at the doubly vinylene homologous carbon atoms (C-1 in the starting materials **4**, **9** and **10**) in dioxane, methanol or ethanol. Methyl propiolate reacts in a similar way. The doubly *N*-vinylated product **26** was obtained from **10** in toluene and the C-1 vinylated products **24B** and **27** were obtained from **9** in dioxane and **10** in methanol. The reaction of **10** with phenyl ethyl propiolate in dimethylformamide gave no addition product, instead a dimer of the acetylenic reagent was the isolated product. The accuracy of the assigned structures **5**, **12** and **13a** could be achieved on the basis of a single-crystal X-ray structure analysis of compound **13a**. The reaction mechanism and the nature of the isolated products are dependent on the nature of the solvent. No addition reaction was observed between **17** and DMAD. The influence of the N-substitution on the nature of the addition (Michael or Diels–Alder) could be settled through the reactions of **18** and **21** with DMAD, which gave **19** and **14** (via **22**), respectively as the only isolable products.

1. Introduction

In previous studies,[1–3a] the reactivity of compound **4** towards a variety of 1,3-dicarbonyl compounds (β-ketoesters and β-diesters) and dienophiles like dimethyl maleate (or dimethyl fumarate) and dimethyl acetylene dicarboxylate (DMAD) was reported. We here report our investigations on the reactivity of 2-aminothiophenes **1a–g**, **4**, **9**, **10**[3b] and **17**[3b] towards electron poor alkynes (DMAD, ethyl propiolate and ethyl phenylpropiolate).

2. Results

The starting compounds were prepared using either the one-pot (**1a–g** and **17**) or the two-step procedures (**4**, **9** and **10**) of the Gewald method. Compounds **1a–d** react with DMAD under reflux in methanol to afford the *N*-vinylated products **2a–d** (Scheme 1). Substrate **1e** under similar reaction conditions gave the product **3**. However, with **1f**,**g**, no product could be obtained under similar reaction conditions.



1,2	a	b	c	d	e	f	g
n	1	2	2	2	2	3	3
X	CO_2CH_3	CN	CO_3CH_3	COC_2H_5	$CONH_2$	CN	$CONH_2$

Scheme 1.

The suggested structures agreed with the analytical and spectroscopic data. The formation of compound **3** can well be attributed to the polyfunctionality of substrate **1e**. The course of the reaction involves the formation of intermediates **3A** and (after addition of water) **3B**. The determining

Keywords: 2-Aminothiophenes; Angular anellation; Diels–Alder addition; Electron poor acetylenes; Michael addition; *retro*-Aldol-reaction.
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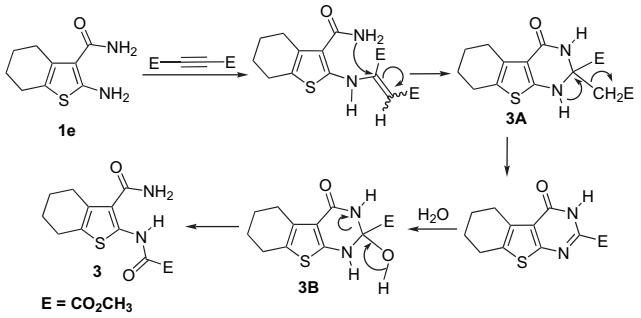
step is a Michael type addition followed by a *retro*-aldol like elimination of methyl acetate (Scheme 2).



Scheme 2.

In refluxing methanol or ethanol, compound **4** reacts with DMAD to give the 1:3 addition product **5** (Scheme 3) as a mixture (1:1) of two stereoisomers in 44% yield.

When compound **4** was treated with excess of DMAD under reflux in dioxane, compound **5** was obtained in 21% yield after cooling in liquid nitrogen. The silica-gel chromatography of the resulting filtrate gave 27% of compound **8**[3b] and 10% of compound **7**. Compound **8** can be quantitatively prepared by reacting **9** with dimethyl maleate (or dimethyl fumarate). Derivative **7** is conceivable from both precursors **6** and **8** (Scheme 3).

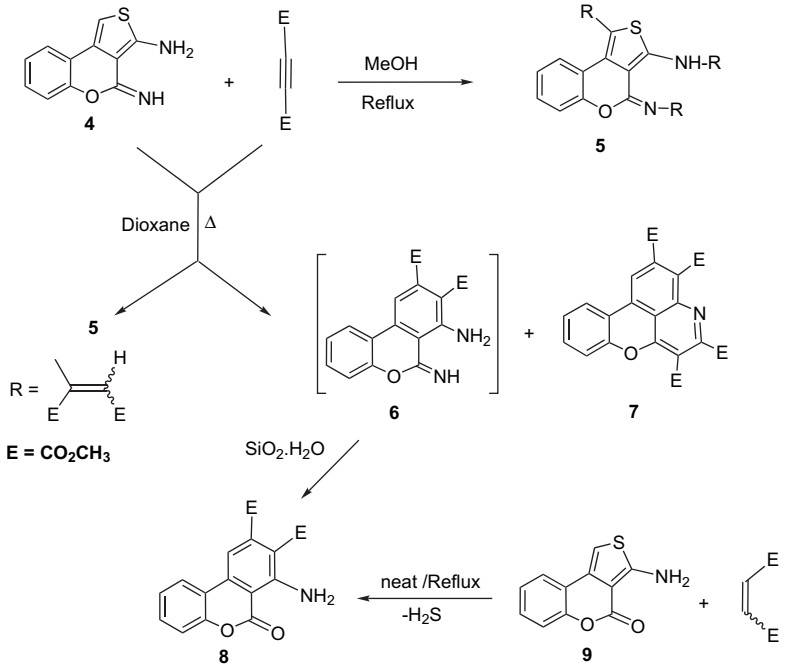
The key steps in both reaction routes could be considered as 6π-electrocyclizations of the intermediate 1:1 Michael adducts. Under the same reaction conditions as with **4**, the substrates **9** and **10**, respectively, react with DMAD to afford compounds **8** and **12** or **13a** and **14**, respectively (Scheme 4).

In the widest sense, the compounds **5**, **12** and **13a** can be considered as Michael addition products. These structures were further supported by a single-crystal X-ray structure analysis of **13a** (Fig. 1). Although the compound exists in a stable helical conformation in the crystal, the existence of stable enantiomers in solution could not be proven (HPLC using various chiral stationary phases). Rotation of the fumarate moiety relative to the thiophene ring may cause rapid interconversion of enantiomers.

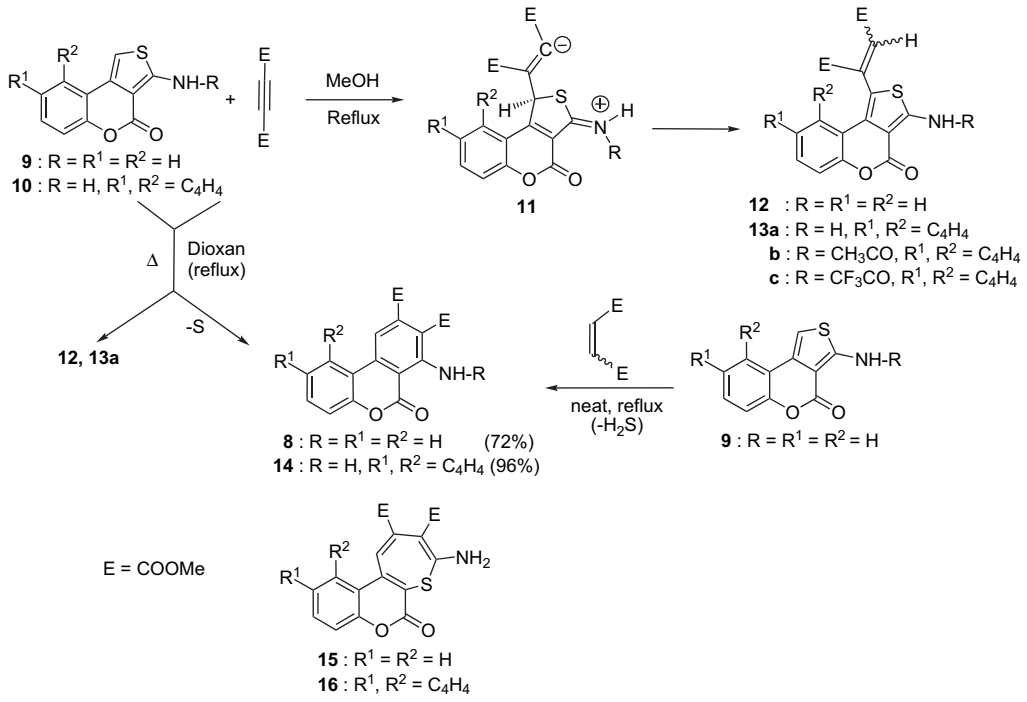
Compound **13a** was further characterized by the preparation of its *N*-acyl derivatives **13b**,**c**. The ^{1}H-NOESY experiment[3b] with **13b** provided more supporting evidence for the suggested structures **5**, **12** and **13a**. The observation of ^{1}H-NOESY cross-peaks between the singlets at δ=3.36 ppm (assigned to $COOCH_3$ at C-3 in the fumarate moiety), the singlet at δ=6.97 ppm (3-H), the doublet at δ=7.86 ppm (6′-H) and the overlapped ddd at 7.46 ppm (9′-H and 10′-H), points to the torsional flexibility of the fumarate 4-methoxy group in solution and to the proximity of the fumarate moiety to C-9′ and C-10′.

On the other hand, only one cross-peak is observed between the singlet at δ=3.52 ppm (assigned to $COOCH_3$ at C-2 in the fumarate moiety) and the singlet at δ=6.97 ppm (3-H). This is in agreement with the apparent remoteness of the carbomethoxy group at C-2 from the naphthalene ring system. Besides a cross-peak between δ=2.41 ppm (s, $COCH_3$) and δ=11.03 ppm (br s, –NH–), another cross-peak is observed between δ=2.41 ppm and δ=6.97 ppm (s, 3-H). This observation suggests a conformation in solution in which the methyl protons are close to 3-H of the fumarate fragment. Similarly all the conceivable isomeric thiepin derivatives (e.g. **15** and **16**) were ruled out on the basis of their ^{1}H and ^{13}C(^{1}H) NMR experimental and simulated spectral data.[3b]

By reacting compound **9** with DMAD under reflux for 4 h, Elnagdi et al.[4] recently isolated a substance to which they



Scheme 3.



Scheme 4.

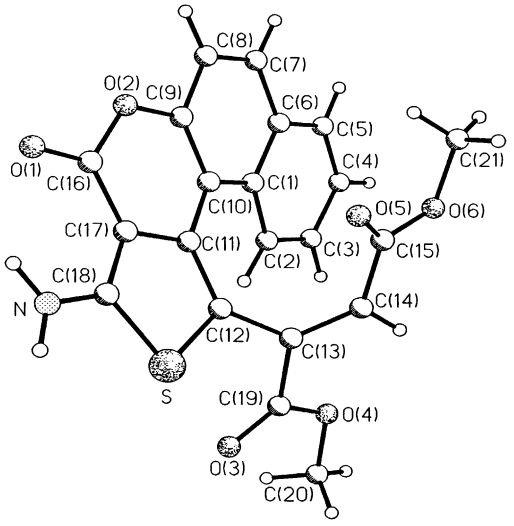
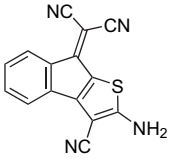


Figure 1. Single-crystal X-ray structure analysis of compound **13a**. The crystallographic numbering does not reflect the systematic IUPAC numbering.

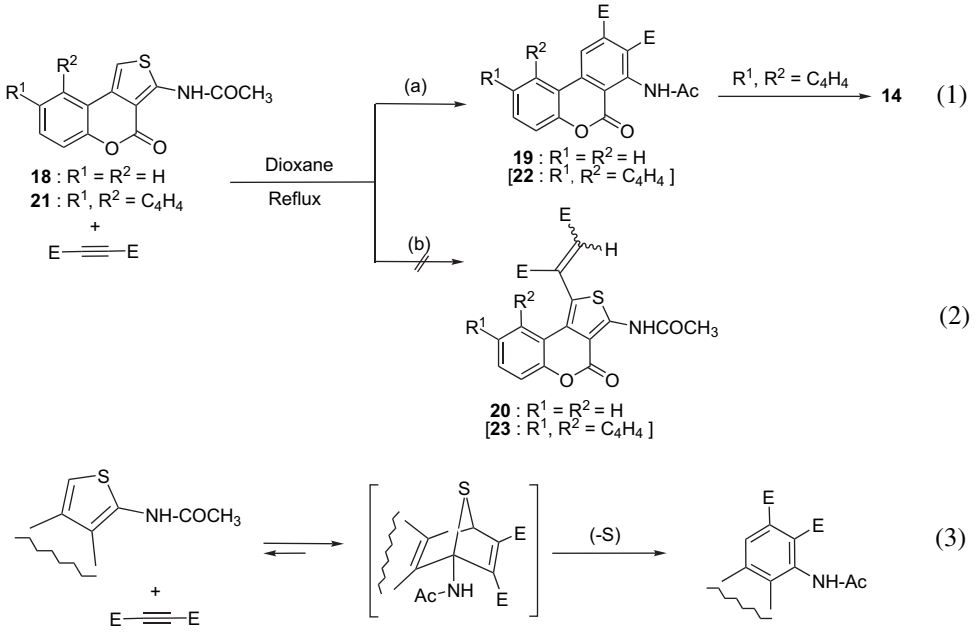
assigned the structure of the thiepin **15**, probably on the basis of former reasoning.[1,2,3a] With a melting point of 162 °C[4] from ethanol (171–173 °C from methanol for **12**) and almost identical analytical and spectroscopic data, we assume that this compound is identical to **12** (Scheme 4). The formation of compound **5** is the most complicated case and the consequence of the polyfunctionality of the starting material **4**: both amino and imino groups can add to the electron poor triple bond. The fastest reaction is obviously the addition of DMAD at the position 1 in **4**, **9** and **10**. It can be rationalized in terms of attack on the doubly vinylene-homologous position to the amino group. The known compounds **8** and **7** are nevertheless products of a Diels–Alder addition of the acetylene diester on the thiophene ring of **4** or **9** (Scheme 3). It is also conceivable that the intermediate **12** cyclizes to give such a [4+2]-adduct. In any case the newly constructed benzene ring results from the extrusion of elemental sulfur from this primary [4+2]-adduct (Scheme 4).



17

Numerous attempts to induce a reaction between compound **17** and DMAD so far remain unsuccessful. The sluggishness of the reaction of substrate **17** compared to other substrates could partly be attributed to the push–pull effect, which leads to the great stability of this substrate, and hence its unreactivity towards dienophiles.

The assumption that the Michael type addition at C-1 in **4**, **9** and **10** is favoured by the free NH_2-group, prompted us to study the reactions of the *N*-acylated products **18** and **21** towards DMAD. As anticipated, no Michael adduct was observed in these reactions in refluxing DMF. The Diels–Alder adducts **19** and **14** (through **22**), respectively, were the only isolable reaction products from substrates **18** and **21** in 42 and 11% yield (Scheme 5). The formation of compound **14** certainly results from subsequent hydrolysis of the acetamido group of the primarily formed but not isolable cycloadduct **22**. The low yields of **14** and **19** (<50% in both cases) observed in these reactions suggests that *N*-acylation as in **18** and **21** drastically decreases the vinylation at C-1 and allows the otherwise less competitive Diels–Alder addition to gain importance.

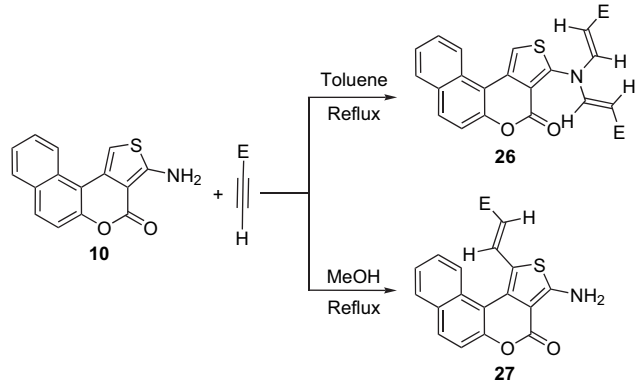


Scheme 5.

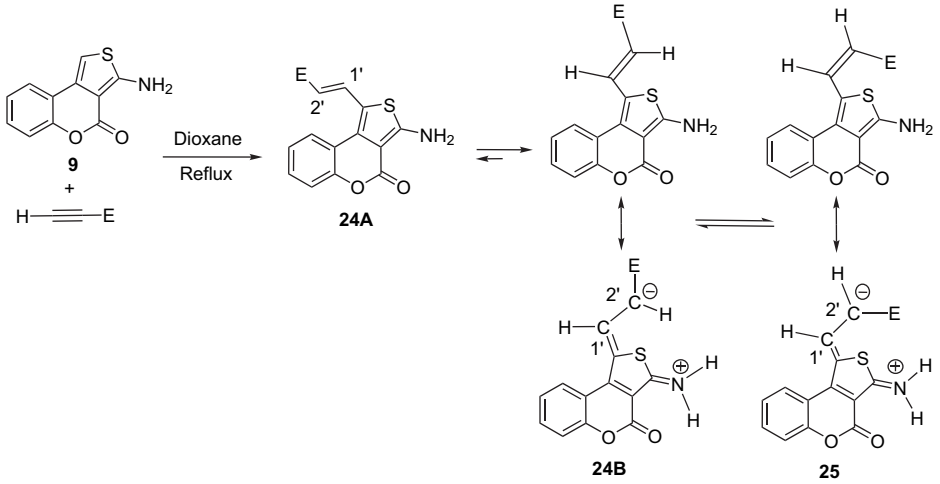
Also the reaction of substrate **9** with methyl propiolate under reflux in dioxane gave 73% yield the Michael type 1:1 adducts **24** and **25** (Scheme 6) as a 13:1 mixture.

By reacting compound **10** with methyl propiolate in refluxing toluene, the *N,N*-divinylation product **26** (Scheme 7) was the isolated product in 70% yield. The structure of **26** resulted from analytical and spectroscopic data.

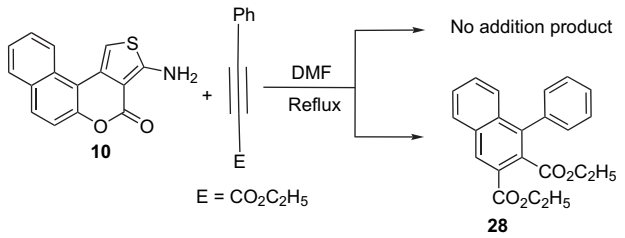
When the reaction was carried out in refluxing methanol, a yellow substance melting at 312–314 °C was isolated, to which structure **27** (Scheme 7) was assigned on the basis of IR- and mass-spectroscopic data. The reaction of **10** with ethyl phenylpropiolate gave neither a cycloaddition, nor a Michael addition product. The dimerization product **28**



Scheme 7.



Scheme 6.

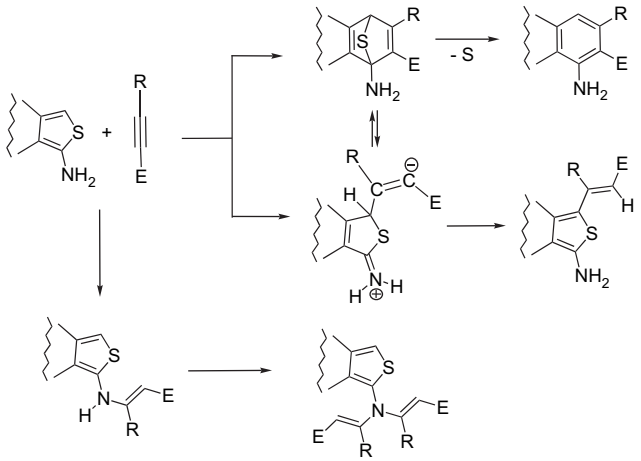


Scheme 8.

(Scheme 8) of the acetylene was instead the isolated compound from this reaction.

3. Conclusions

The reactions of 2-aminothiophenes with electron poor alkynes described in this work are of following types: (i) conjugate additions to the amino- or imino groups; (ii) conjugate additions at C-1 of the thienocoumarin derivatives **4**, **9** and **10**; (iii) formation of an anellated benzene ring across the 3,4-double bond of the coumarin derivative, very probably through a [4+2]-cycloaddition with subsequent desulfuration. One could also, in principle, envisage that the formal [4+2]-cycloaddition can also take place stepwise. The cases found by Elnagdi et al.[4] also fit in Scheme 9. The successful cycloadditions encountered in this work are normal[5] Diels–Alder reactions, in which the diene HOMO/dienophile-LUMO is the predominant interaction. They can therefore be rationalized by perturbation theory.[5,6]



Scheme 9.

4. Experimental

All elemental and spectroscopic analyses were performed in the Chemistry Department Analytical Center of Gerhard-Mercaptor-Universität Duisburg, Duisburg (Germany). All melting points were determined with a Reichert Thermovar microscope and are uncorrected. The IR and the UV spectra were measured with Perkin–Elmer 983 and 554 spectrophotometers, respectively. ^{1}H and ^{13}C(^{1}H) NMR spectra were recorded on Bruker WM 300 and DRX 500 instruments, with TMS as internal standard. Coupling constants J are reported in Hertz. Mass spectra were obtained on Varian MAT 311A and AMD 604 instruments by electron impact ionization (EI) at 18 eV or 70 eV, using a direct inlet system. Combustion analyses were carried out with a CHN+O/S elemental analyzer 'CARLO ERBA' Model 1106. Simulated ^{1}H and ^{13}C(^{1}H) NMR spectra were performed with an ACD NMR spectra simulation programme.

4.1. Gewald synthesis of 2-aminothiophenes

4.1.1. Variant A (one-pot procedure)[7–11] general procedure. To an equimolar (if not otherwise stated) mixture of ketone, nitrile and finely powdered elemental sulfur in ethanol (if not otherwise stated) the indicated amount of diethylamine, piperidine or morpholine is slowly added with magnetic stirring, so that the temperature does not exceed 50 °C. By warming, sulfur progressively dissolves. For a slower reaction one should warm periodically up to 40–50 °C (water bath). Continued warming bears the risk of the formation of the disulfide.[7–11] The reaction lasts for 4–6 h in total. The reaction mixture is kept in the refrigerator for crystallization for several hours, after which the precipitate is collected and worked up as indicated. If not otherwise stated, the given yields are based on the inputs of ketone.

4.1.1.1. Methyl 2-amino-5,6-dihydro-4*H*-cyclopenta-[*b*]thiophene-3-carboxylate (1a). From cyclopentanone (33.60 g, 0.4 mol), methyl cyanoacetate (19.80 g, 0.2 mol), powdered sulfur (3.2 g, 0.1 mol) and piperidine (8.50 g, 0.1 mol), the reaction lasted for 8 h. Crystallization from methanol gave the *title compound* **1a** (13.91 g, 35% based on the nitrile) as yellow powder, mp 181–183 °C [Found: C, 54.73; H, 5.54; N, 7.05; S, 16.45. $C_9H_{11}NO_2S$ requires C, 54.82; H, 5.58; N, 7,11; S, 16.24%]; ν_{max} (potassium bromide) 3410, 3293, 3163, 3076, 2967, 2944, 2857, 1654 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.86 (2H, br s, D_2O-exchangeable, N*H*$_2$), 3.78 (3H, s, O*Me*), 2.83–2.77 (2H, m, 4-H$_2$), 2.74–2.68 (2H, m, 6-H$_2$), 2.35–2.26 (2H, m, 5-H$_2$); δ_C (75 MHz, $CDCl_3$) 166.5, 166.1, 142.6, 121.4, 102.8, 50.6, 30.7, 28.8, 27.3; *m*/*z* (EI) 198 (5), 197 (49, M$^+$), 167 (5), 166 (15), 165 (100), 164 (12%).

4.1.1.2. 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-carbonitrile (1b). From cyclohexanone (29.4 g, 0.3 mol), malononitrile (13.20 g, 0.2 mol), powdered sulfur (6.4 g, 0.2 mol) and diethylamine (7.3 g, 0.1 mol) in ethanol, the reaction was conducted for 2 h. Crystallization from 50% aqueous ethanol gave the *title compound* **1b** (29.94 g, 84%) as yellow powder, mp 144–146 °C (lit.,[10] 147–148 °C from ethanol) [Found: C, 60.68; H, 5.64; N, 15.67; S, 17.96. $C_9H_{10}N_2S$ requires C, 60.67; H, 5.64; N, 15.67; S, 17.96%]; ν_{max} (potassium bromide) 3446, 3330, 3207, 2956, 2932, 2911, 2854, 2838, 2744, 2667, 2649, 2198 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.41 (2H, br s, D_2O-exchangeable, N*H*$_2$), 2.55–2.43 (4H, m, 4-H$_2$ and 7-H$_2$), 1.86–1.72 (4H, m, 5-H$_2$ and 6-H$_2$); δ_C (75 MHz, $CDCl_3$) 160.0, 132.4, 120.7, 115.5, 88.8, 24.6, 24.2, 23.4, 22.2; *m*/*z* (EI) 179 (6), 178 (49, M$^+$), 177 (15), 151(10), 150 (100%).

4.1.1.3. Methyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (1c). From cyclohexanone (29.4 g, 0.3 mol), methyl cyanoacetate (19.18 g, 0.2 mol), sulfur (6.40 g, 0.2 mol) and diethylamine (8.50 g, 0.1 mol), the reaction was conducted for 9.5 h. The precipitate was

crystallized from methanol/water (75:25) to give the *title compound* **1c** (29.34 g, 70%) as yellow powder; mp 128–130 °C (lit.,[12] 127–128 °C from methanol) [Found: C, 56.92; H, 6.20; N, 6.62; S, 15.27. $C_{10}H_{13}NO_2S$ requires C, 56.87; H, 6.16; N, 6.64; S, 15.17%]; ν_{max} (potassium bromide) 3421, 3315, 3157, 3013, 2929, 2837, 1653 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.95 (2H, br s, D_2O-exchangeable, NH_2), 3.78 (3H, s, O*Me*), 2.70–2.63 (2H, m, 7-H_2), 2.52–2.46 (2H, m, 4-H_2), 1.81–1.68 (4H, m, 6-H_2 and 5-H_2); δ_C (75 MHz, $CDCl_3$) 166.5, 161.9, 132.5, 117.7, 105.7, 50.6, 26.9, 24.6, 23.3, 22.8; *m/z* (EI) 212 (7), 211 (52, M^+), 183 (3), 181 (6), 180 (16), 179 (100%).

4.1.1.4. Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (1d). From cyclohexanone (29.4 g, 0.3 mol) ethylcyanoacetate (11.30 g, 0.1 mol), sulfur (3.20 g, 0.1 mol) and piperidine (4.25 g, 0.05 mol), the reaction lasted for 9 h and the precipitate was crystallized from 50% aqueous ethanol to give the *title compound* **1d** (15.76 g, 70%) as yellow powder, mp 117–118 °C (lit.,[10] 115 °C from methanol) [Found: C, 58.63; H, 6.60; N, 6.30; S, 14.35. $C_{11}H_{15}NO_2S$ requires C, 58.67; H, 6.67; N, 6.22; S, 14.22%]; ν_{max} (potassium bromide) 3405, 3300, 3230, 3167, 3077, 2986, 2939, 2886, 2855, 2841, 1643 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.97 (2H, br s, D_2O-exchangeable, NH_2), 4.24 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 2.71–2.66 (2H, m, 7-H_2), 2.49–2.45 (2H, m, 4-H_2), 1.79–1.67 (4H, m, 6-H_2 and 5-H_2), 1.37 (3H, t, *J* 7.1, OCH_2CH_3); δ_C (75 MHz, $CDCl_3$) 166.2, 161.8, 132.5, 117.6, 105.8, 59.4, 27.0, 24.6, 23.32, 22.9, 14.5; *m/z* (EI) 227 (5), 226 (11), 225 (70, M^+), 197 (3), 181(10), 180 (29), 179 (100%).

4.1.1.5. 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (1e). From cyclohexanone (3.92 g, 40 mmol), cyanoacetamide (3.36 g, 80 mmol), sulfur (1.28 g, 40 mmol) and piperidine (2.92 g, 35 mmol), the reaction was carried out for 7 h and the precipitate was crystallized from 50% aqueous ethanol to give the *title compound* **1e** (3.54 g, 45% based on the nitrile) as yellow powder, mp 180–181 °C (lit.,[10] 189–190 °C from methanol) [Found: C, 55.04; H, 6.08; N, 14.15; S, 16.40. $C_9H_{12}N_2OS$ requires C, 55.10; H, 6.12; N, 14.27; S, 16.34%]; ν_{max} (potassium bromide) 3482, 3387, 3305, 3153, 2939, 1642, 1311, 1281, 1258, 1185 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.55 (4H, br s, D_2O-exchangeable, NH_2), 2.39–2.33 (4H, m, 4-H_2 and 7-H_2), 2.28–2.26 (4H, m, 5-H_2 and 6-H_2); δ_C (75 MHz, $CDCl_3$) 168.4, 160.6, 129.2, 117.7, 107.1, 26.8, 24.4, 22.8, 22.8; *m/z* (EI) 198 (4), 197 (9), 196 (67, M^+), 181 (8), 180 (17), 179 (100%).

4.1.1.6. 2-Amino-5,6,7,8-tetrahydro-4*H*-cyclohepta-[*b*]thiophene-3-carbonitrile (1f). From cycloheptanone (16.8 g, 150 mmol), malononitrile (6.6 g, 100 mmol), sulfur (3.2 g, 100 mmol) and piperidine (4.25 g, 50 mmol) in ethanol, the reaction lasted for 6 h. Crystallization from 50% aqueous ethanol gave the *title compound* **1f** (12.09 g, 67% based on the nitrile) as brown powder, mp 115–117 °C (lit.,[13] 114 °C from ethanol) [Found: C, 62.38; H, 6.21; N, 14.39; S, 16.86. $C_{10}H_{12}N_2S$ requires C, 62.50; H, 6.25; N, 14.58; S, 16.67%]; ν_{max} (potassium bromide) 3445, 3310, 3208, 2927, 2840, 2205 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 4.57 (2H, br s, D_2O-exchangeable, NH_2), 2.68–2.60 (4H, m, 8-H_2), 2.59–2.51 (2H, m, 4-H_2), 1.86–1.78 (1H, m, 7-H_2), 1.68–1.65 (2H, m, 5-H_2), 1.64–1.60 (2H, m, 6-H_2); δ_C (75 MHz, $CDCl_3$) 158.1, 136.9, 123.8, 115.9, 91.9, 31.9, 29.4, 29.2, 28.1, 27.3; *m/z* (EI) 194 (5), 193 (15), 192 (100%, M^+).

4.1.1.7. 2-Amino-5,6,7,8-tetrahydro-4*H*-cyclohepta-[*b*]thiophene-3-carboxamide (1g). From cycloheptanone (5.60 g, 50 mmol), cyanoacetamide (4.2 g, 50 mmol), sulfur (1.6 g, 50 mmol) and piperidine (4.25 g, 50 mmol) in ethanol, the reaction was conducted for 5 h and the resulting precipitate was crystallized from 50% aqueous ethanol to give the *title compound* **1g** (5.01 g, 48% based on the nitrile) as red powder, mp 154–156 °C (lit.,[14] 183–186 °C from ethanol); ν_{max} (potassium bromide) 3375, 3198, 2915, 2844, 1630 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 6.79 (2H, br s, D_2O-exchangeable, CONH_2), 6.02 (2H, br s, D_2O-exchangeable, NH_2), 2.68–2.48 (4H, m, 4-H_2 and 8-H_2), 1.73–1.50 (6H, m, 5-H_2, 6-H_2 and 7-H_2); δ_C ($CDCl_3$, 75 MHz) 167.9, 153.6, 136.4, 120.1, 113.5, 31.7, 28.6, 28.3, 27.8, 27.2; *m/z* (EI) 210 (44, M^+), 194 (14), 193 (100), 192 (7), 178 (4), 172 (4), 166 (4), 165 (19), 164 (11), 45 (4), 44 (4%).

4.1.1.8. 3-Amino-4*H*-thieno[3,4-*c*](2*H*)chromen-4-one (9). A mixture of *o*-hydroxyacetophenone (27.2 g, 200 mmol), sulfur (6.4 g, 200 mmol) and ethylcyanoacetate (22.6 g, 200 mmol), or methyl cyanoacetate (19.8 g, 200 mmol), respectively in methanol (200 mL) in the presence of morpholine or diethylamine, was stirred for 24 h. The precipitate was crystallized from benzene to give the *title compound* **9** (16 g, 37%) as yellow powder, mp 197–199 °C (lit.,[15a,b] 198–199 °C from benzene) [Found: C, 61.17; H, 3.36; N, 6.49; S, 14.45. $C_{11}H_7NO_2S$ requires C, 60.83; H, 3.23; N, 6.45; S, 14.75%]; ν_{max} (potassium bromide) 3449, 3407, 3343, 3101, 1687 cm^{-1}; δ_H (DMSO-d_6, 300 MHz) 7.86 (6-H, dd, *J* 8.0, 1.5 Hz, 1H), 7.78 (2H, br s, D_2O-exchangeable, NH_2), 7.35 (1H, ddd, *J* 8.0, 7.4, 1.8 Hz, 7-H), 7.21 (1H, d, *J* 7.0 Hz, 9-H), 7.19 (1H, ddd, *J* 8.2, 7.6, 1.2 Hz, 8-H), 6.88 (1H, s, 1-H); δ_C (DMSO-d_6, 75 MHz) 166.6 (C-3), 158.9 (C-2), 151.0, 130.9, 129.3, 124.4, 123.9, 118.1, 117.1, 98.1, 97.54; *m/z* (EI) 219 (6), 218 (13), 217 (100%, M^+).

4.1.1.9. 3-Acetamido-4*H*-thieno[3,4-*c*](2*H*)chromen-4-one (18). From **9** (2.17 g, 10 mmol) and acetic acid anhydride (20 mL) in pyridine at room temperature, the reaction lasted for 48 h and the precipitate was crystallized from pyridine to give the *title compound* **18** (2.21 g, 85%) as yellow crystals, mp 268–270 °C [Found: C, 60.19; H, 3.50; N, 5.57; S, 12.44. $C_{13}H_9NO_3S$ requires C, 60.23; H, 3.47; N, 5.41; S, 12.36%]; ν_{max} (potassium bromide) 3465, 3299, 3100, 1678 cm^{-1}; δ_H (DMSO-d_6, 300 MHz) 10.9 (1H, br s, D_2O-exchangeable, N*H*), 8.03 (1H, dd, *J* 7.7, 1.6 Hz, 6-H), 7.69 (1H, s, 1-H), 7.44 (1H, ddd, *J* 7.0, 6.9, 1.6 Hz, 7-H), 7.34 (1H, dd, *J* 7.9, 1.2 Hz, 9-H), 7.32 (1H, ddd, *J* 7.5, 7.2, 1.4 Hz, 8-H), 2.33 (3H, s, CH_3); δ_C (DMSO-d_6, 75 MHz) 168.8, 158.6, 150.2, 149.9, 130.1, 129.8, 125.1, 124.3, 117.3, 117.1, 108.6, 105.8, 23.2; *m/z* (EI) 260 (4), 259 (25, M^+), 219 (6), 218 (14), 217 (100), 189 (4), 44 (12), 43 (23%).

4.1.1.10. 2-Amino-4-(dicyanomethylene)-4*H*-indeno[2,3-b]thiophene-1-carbonitrile (17). From indan-1,3-dione (1.46 g, 10 mmol), malononitrile (1.32 g, 20 mmol), sulfur (0.32 g, 20 mmol) and diethylamine (1.46 g, 20 mmol) in dioxane, the reaction was conducted for 6 h and the resulting

precipitate was crystallized from dioxane to give the *title compound* **17** (1.84 g, 67%) as deep blue powder, mp>350 °C [Found: C, 65.53; H, 2.24; N, 20.30; S, 11.73. $C_{15}H_6N_4S$ requires C, 65.69; H, 2.19; N, 20.44; S, 11.68%]; ν_{max} (potassium bromide) 3365, 3182, 2951, 2855, 2219 cm^{-1}; λ_{max} (dioxane) (log ε) 240 (4.32), 280 (4.14), 380 (4.14), 540 nm (3.78); δ_H (DMSO-d_6, 300 MHz) 9.24 (2H, br s, D_2O-exchangeable, N*H*$_2$), 7.71 (1H, d, *J* 6.9 Hz, 5-H), 7.35 (1H, ddd, *J* 7.4, 7.3, 1.1 Hz, 6-H), 7.29 (1H, dd, *J* 7.54, 1.25 Hz, 8-H), 7.23 (1H, ddd, *J* 7.7, 7.0, 0.7 Hz, 7-H); δ_C (DMSO-d_6, 75 MHz) 176.2, 154.9, 153.9, 137.6, 134.8, 132.1, 129.5, 123.8, 120.1, 114.9, 114.1, 113.9, 113.0, 80.3, 63.8; *m*/*z* (EI) 275 (35, MH^+), 274 (100%, M^+).

4.1.2. Variant B (two-step procedure)[7–11]. Compounds **4**,[15a,b] **9**[15a,b] and **10** were prepared according to the two-step procedure of the Gewald method, in the reported yields.

4.1.2.1. 3-Amino-4-imino-4*H*-thieno[3,4-*c*](2*H*)chromene (4). Yellowish powder from benzene, mp 158–160 °C, from xylene (lit.,[15a,b] 152 °C from xylene) [Found: C, 61.07; H, 3.74; N, 12.97; S, 14.79. $C_{11}H_8N_2OS$ requires C, 61.09; H, 3.73; N, 12.95; S, 14.83%]; ν_{max} (potassium bromide) 3382, 3276, 3108, 3057, 1630, 1589, 1475, 1355, 1292, 1179, 1099, 932, 877, 792 cm^{-1}; λ_{max} (dioxane) (log ε) 200 (4.88), 228 (5.31), 267 (5.37), 305 nm (4.80); δ_H (DMSO-d_6, 300 MHz) 7.87 (1H, br s, D_2O-exchangeable, =N*H*), 7.77 (1H, dd, *J* 7.7, 1.6 Hz, 9-H), 7.69 (2H, br s, D_2O-exchangeable, N*H*$_2$), 7.26 (1H, ddd, *J* 8.1, 7.0, 1.6 Hz, 7-H), 7.1 (1H, ddd, *J* 14.4, 8.2, 1.3 Hz, 8-H), 7.1 (1H, dd, *J* 8.1, 1.1 Hz, 6-H), 6.8 (1H, s, 1-H); δ_C (DMSO-d_6, 75 MHz) 160.7, 155.1, 150.7, 129.7, 129.0, 123.9, 123.5, 118.3, 116.3, 99.7, 97.2; *m*/*z* (EI) 218 (6), 217 (16), 216 (M^+, 100), 215 (33), 199 (5), 77 (7%).

4.1.2.2. 3-Amino-4*H*-benzo[*f*]thieno[3,4-*c*](2*H*)chromen-4-one (10). Yellow prisms from benzene, mp 235–237 °C [Found: C, 67.36, H, 3.43; N, 5.28; S, 11.90. $C_{15}H_9NO_2S$ requires C, 67.42; H, 3.37; N, 5.24; S, 11.99%]; ν_{max} (potassium bromide) 3439, 3335, 3137, 1690 cm^{-1}; δ_H (DMSO-d_6, 300 MHz) 8.69 (1H, d, *J* 8.5 Hz, 6-H), 8.33 (2H, br dd, *J* 8.1, 1.2 Hz, H-8 and H-11), 7.93 (2H, br s, D_2O-exchangeable, N*H*$_2$), 7.68 (1H, ddd, *J* 8.5, 7.0, 1.5 Hz, 10-H), 7.55 (1H, ddd, *J* 7.5, 7.4, 0.8 Hz, 9-H), 7.40 (1H, d, *J* 8.9 Hz, 7-H), 7.33 (1H, s, 1-H); δ_C (DMSO-d_6, 75 MHz) 165.8, 159.0, 150.4, 130.8, 130.5, 129.6, 129.4, 129.3, 128.3, 125.3, 124.5, 117.9, 111.6, 101.2, 99.4; *m*/*z* (EI) 269 (6, MH_2^+), 268 (17, MH^+), 267 (100%, M^+).

4.1.2.3. 3-Acetamido-4*H*-benzo[*f*]thieno[3,4-*c*](2*H*)-chromen-4-one (21). Compound **10** (0.224 g, 0.84 mmol) was treated with acetic acid anhydride (3.5 mL) in pyridine at room temperature for 48 h and gave the *title compound* **21** after crystallization from pyridine (0.13 g, 85%) as yellow prisms, mp 286–288 °C [Found: C, 66.17; H, 3.62; S, 4.53; S, 10.26. $C_{17}H_{11}NO_3S$ requires C, 66.02; H, 3.56; N, 4.53; S, 10.36%]; ν_{max} (potassium bromide) 3445, 3272, 1696, 1671 cm^{-1}; δ_H (DMSO-d_6, 300 MHz) 10.98 (1H, br s, D_2O-exchangeable, N*H*), 8.82 (1H, d, *J* 8.42 Hz, 6-H), 8.0 (2 H, br d, *J* 8.5 Hz, 8-H and 11-H), 7.75 (1H, dd, *J* 7.8, 7.7 Hz, 10-H), 7.61 (1H, dd, *J* 7.4, 7.3 Hz, 9-H), 7.51 (1H, d, *J* 8.8 Hz, 7-H), 8.09 (1H, s, 1-H), 2.36 (3H, s, CO*Me*); δ_C (DMSO-d_6, 75 MHz) 168.2, 158.3, 149.2, 149.2, 130.8, 130.6, 129.1, 129.0, 128.4, 128.2, 125.3, 124.2, 117.4, 111.2, 110.6, 106.6, 22.9; *m*/*z* (EI) 311 (4), 310 (11), 309 (M^+, 54), 269 (6), 268 (19), 267 (100), 44 (6%).

4.1.3. Reactions of 2-aminothiophenes with acetylenic reagents general procedure. An equimolar amount (1–2.5 mmol, if not specified otherwise) of the 2-aminothiophene and the electron poor acetylene in the given solvent was refluxed with magnetic stirring for 3–10 h (if not specified otherwise). The reaction mixture was worked up as usual and the product was purified as described for each particular substance. The unoptimized yields are based on the amounts of reacted 2-aminothiophene.

4.1.3.1. Dimethyl (*E*,*Z*){*N*-[2-(3-methoxycarbonyl-5,6-dihydro-4*H*-cyclopenta[*b*]thienyl)]amino}butenedioate (2a). From a mixture of **1a** (0.50 g, 2.5 mmol) and DMAD, (1.42 g, 10 mmol) in methanol, the reaction was conducted for 9.5 h and the precipitate was crystallized from methanol to give the *title compound* **2a** (700 mg, 81%) as yellow needles, mp 94–96 °C. The remaining oily fraction was discarded analyzed [Found: C, 53.01; H, 4.90; N, 3.98; S, 9.30. $C_{15}H_{17}NO_6S$ requires C, 53.10; H, 5.01; N, 4.13; S, 9.44%]; ν_{max} (potassium bromide) 3444, 3150, 3023, 2947, 2860, 1731, 1697, 1685 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 11.56 (1H, br s, D_2O-exchangeable, N*H*), 5.44 (1H, s, =C*H*), 3.87 (3H, s, O*Me*), 3.86 (3H, s, O*Me*), 3.78 (3H, s, O*Me*), 2.91–2.81 (2H, m, 4′-H_2), 2.79–2.76 (2H, m, 6′-H_2), 2.39–2.29 (2H, m, 5′-H_2); δ_C ($CDCl_3$, 75 MHz) 168.3, 164.8, 164.1, 154.8, 144.6, 143.4, 128.4, 110.1, 96.8, 53.2, 51.6, 51.5, 30.6, 29.1, 27.4; *m*/*z* (EI) 342 (7), 340 (18), 339 (100, M^+), 44 (3%).

4.1.3.2. Dimethyl (*E*,*Z*)-2{*N*-[2-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thienyl)]amino}butenedioate (2b). From **1b** (450 mg, 2.5 mmol) and DMAD (1.42 g, 10 mmol) in methanol, the reaction was carried out for 9 h. The reaction mixture was concentrated in vacuo and the resulting oily residue was kept in the refrigerator for few days. Crystallization of the solid from methanol gave the *title compound* **2b** (273 mg, 34%) as yellow needles, mp 117–118 °C. The mother liquor was discarded [Found: C, 56.14; H, 4.92; N, 8.67; S, 10.25. $C_{15}H_{16}N_2O_4S$ requires C, 56.25; H, 5.00; N, 8.75; S, 10.00%]; ν_{max} (potassium bromide) 3447, 3187, 3095, 2950, 2935, 2859, 2212, 1734, 1672 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 9.90 (1H, br s, D_2O-exchangeable, N*H*), 5.69 (1H, s, (COOMe)C*H*=), 3.83 (3H, s, COO*Me*), 3.77 (3H, s, COO*Me*), 2.63–2.57 (4H, m, 4′-H_2 and 7′-H_2), 1.87–1.78 (4H, m, 5′-H_2 and 6′-H_2); δ_C ($CDCl_3$, 75 MHz): 169.5, 162.8, 150.3, 146.1, 133.8, 130.2, 102.0, 97.5, 53.2, 51.7, 24.5, 24.4, 23.0, 22.0; *m*/*z* (EI) 322 (4), 321 (9), 320 (51, M^+), 289 (10), 288 (44), 262 (10), 261 (37), 260 (100), 59 (5), 45 (3), 32 (46%).

4.1.3.3. Dimethyl (*E*,*Z*)-2-{*N*-[2-(3-methoxycarbonyl-4,5,6,7-tetrahydrobenzo[*l*]thienyl)]amino}butenedioate (2c). From a mixture of **1c** (0.53 g, 2.5 mmol) and DMAD (1.42 g, 10 mmol) in methanol, the reaction lasted for 10 h and the resulting precipitate was crystallized from aqueous methanol to afford the *title compound* **2c** (742 mg, 84%) as yellow needles, mp 107–109 °C. The mother liquor was discarded. [Found: C, 54.35; H, 5.42; N, 3.95; S, 9.34. $C_{10}H_{13}NO_2S$ requires C, 54.39; H, 5.38; N, 3.97; S,

9.07%]; ν_{max} (potassium bromide) 3450, 3269, 3130, 3034, 3000, 2948, 2844, 1735, 1681 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 11.62 (1H, br s, D_2O-exchangeable, N*H*), 5.43 (1H, s, 3-H), 3.88 (3H, s, O*Me*), 3.87 (3H, s, O*Me*), 3.78 (3H, s, O*Me*), 2.77–2.63 (2H, m, 7′-H_2), 2.59–2.55 (2H, m, 4′-H_2), 1.83–1.71 (4H, m, 6′-H_2 and 5′-H_2); δ_C ($CDCl_3$, 75 MHz) 168.3, 165.0, 164.3, 150.4, 145.0, 133.4, 124.5, 113.2, 97.1, 53.3, 51.6, 51.4, 26.6, 24.7, 23.0, 22.6; *m/z* (EI) 353 (64, M^+), 321 (32), 293 (21), 234 (100), 44 (4%).

4.1.3.4. (*E* or *Z*) Dimethyl 2-{*N*-[2-(3-ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[*b*]thienyl)]amino}butenedioate (2d). A mixture of **1d** (0.56 g, 2.5 mmol) and DMAD (1.42 g, 10 mmol) in methanol gave after 10 h of reaction a precipitate, which was crystallized from methanol to give the *title compound* **2d** (3.43 g, 38%) as yellow needles, mp 82–84 °C. The oily mother liquor was discarded. [Found: C, 55.60; H, 5.60; N, 3.84; S, 9.07. $C_{17}H_{21}NO_6S$ requires C, 55.59; H, 5.72; N, 3.80; S, 8.72%]; ν_{max} (potassium bromide) 3450, 3232, 3029–2843, 1736, 1673 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 11.56 (1H, br s, D_2O-exchangeable, N*H*), 5.41 (1H, s, =C*H*), 4.35 (2H, q, *J* 7.1 Hz, OC$H_2$$CH_3$), 3.84 (3H, s, O*Me*), 3.75 (3H, s, O*Me*), 2.75–2.57 (2H, m, 7′-H_2), 2.56–2.53 (2H, m, 4′-H_2), 1.80–1.71 (4H, m, 6′-H_2 and 5′-H_2), 1.35 (3H, t, *J* 7.1 Hz, $OCH_2C$$H_3$); δ_C ($CDCl_3$, 75 MHz) 168.2, 164.6, 164.2, 150.3, 144.9, 133.3, 124.5, 113.5, 97.1, 60.3, 53.2, 51.5, 26.6, 24.6, 22.9, 22.6, 14.3; *m/z* (EI) 367 (95, M^+), 335 (37), 307 (25), 276 (15), 262 (100%).

4.1.3.5. Methyl {*N*-[2-(3-aminocarbonyl-4,5,6,7-tetrahydrobenzo[*b*]thienyl)]}oxamate (3). From a mixture of **1e** (0.50 g, 2.5 mmol) and DMAD (1.42 g, 10 mmol) in methanol, the reaction was carried out for 9 h and the resulting precipitate was crystallized from methanol to give the *title compound* **3** (95 mg, 13%) as yellowish powder, mp 218–220 °C. The remaining oilish mixture was not further analyzed [Found: C, 51.12; H, 4.98; N, 9.83; S, 11.50. $C_{12}H_{14}N_2O_4S$ requires C, 51.06; H, 4.96; N, 9.93; S, 11.35%]; ν_{max} (potassium bromide) 3499, 3331, 3265, 3211, 2953, 2836, 1729, 1693 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 12.81 (1H, br s, D_2O-exchangeable, N*H*), 7.65 (1H, br s, D_2O-exchangeable, N*H*), 7.05 (1H, br s, D_2O-exchangeable, N*H*), 3.84 (3H, s, COO*Me*), 2.72 (2H, m, 8′-H_2), 2.64 (2H, m, 5′-H_2), 1.73 (4H, m, 6′-H_2 and 7′-H_2); δ_C ($CDCl_3$, 75 MHz) 167.2, 167.2, 159.7, 152.6, 140.9, 129.7, 128.1, 118.1, 53.7, 25.3, 24.1, 22.5, 22.4; *m/z* (EI) 283 (6, MH^+), 282 (43, M^+), 266 (9), 265 (61), 59 (6%).

4.1.4. Reactions with 1f,g. From 2.5 mmol of **1f** (or **1g**, respectively) and 1.42 g (10 mmol) of DMAD, the reactions in methanol gave after 9 h no new products.

4.1.4.1. Dimethyl 2-{3-[1,2-di(methoxycarbonyl)vinyl]-amino-4-[1,2-di(methoxycarbonyl)vinyl]imino-4*H*-thieno-[3,4-*c*](2*H*)chromen-1-yl}butenedioate (5, *E*,*Z*-mixture). From a mixture of **4** (0.54 g, 2.5 mmol) and DMAD (3 mL, excess) in methanol, the reaction was conducted for 9 h. The solvent was evaporated in vacuo to give a crude material, which was kept in the freezer for few days. The resulting precipitate was crystallized from methanol to give the *title compound* **5** (704 mg, 44%) as orange powder, mp 165–167 °C [Found: C, 54.20; H, 4.01; N, 4.38; S, 5.04. $C_{29}H_{26}N_2O_{13}S$ requires C, 54.21; H, 4.05; N, 4.36; S, 4.98%]; ν_{max} (potassium bromide) 3433, 2953, 1725, 1661 cm^{-1}; λ_{max} (THF) (log ε) 252 (3.28), 292 (3.19), 322 (3.11), 368 (3.06), 400 nm (3.01); δ_H (500 MHz, $CDCl_3$) 12.16 (1H, br s, N*H*, D_2O-exchangeable), 11.81 (1H, br s, N*H*, D_2O-exchangeable), 7.46 (1H, dd, *J* 7.9, 1.3 Hz, 9-H), 7.40 (1H, ddd, *J* 8.0, 7.9, 1.5 Hz, 7-H), 7.35 (1H, s, olefinic H), 7.29 (1H, ddd, *J* 11.7, 8.9, 1.4 Hz, 8-H), 7.28 (1H, s, olefinic H), 7.25 (1H, dd, *J* 7.8, 1.1 Hz, 6-H), 7.12 (1H, ddd, *J* 7.7, 7.6, 1.0 Hz, 7-H), 7.08 (1H, dd, *J* 6.9, 1.0 Hz, 9-H), 7.05 (1H, dd, *J* 9.2, 2.1 Hz, 6H), 7.06 (1H, ddd, *J* 7.0, 6.2, 1.8 Hz, 8-H), 6.45 (1H, s, olefinic H), 6.35 (1H, s, olefinic H), 5.83 (1H, s, olefinic H), 5.66 (1H, s, olefinic H), 3.95 (3H, s, O*Me*), 3.90 (3H, s, O*Me*), 3.84 (3H, s, O*Me*), 3.75 (3H, s, O*Me*), 3.73 (s, 3H, OMe), 3.71 (3H, s, O*Me*), 3.70 (3H, s, O*Me*), 3.692 (3H, s, O*Me*), 3.689 (3H, s, O*Me*), 3.65 (3H, s, O*Me*); δ_C (125 MHz, $CDCl_3$) 166.7, 166.3, 165.6, 165.5, 165.4, 164.7, 164.5, 164.3, 164.2, 163.8, 151.7, 150.5, 150.4, 150.3, 150.1, 148.9, 144.9, 144.6, 143.3, 142.2, 136.4, 136.3, 134.2, 133.6, 129.7, 129.3, 124.6, 124.5, 124.2, 116.9, 116.8, 112.8, 112.1, 101.8, 97.2, 128.1, 127.7, 117.9, 117.6, 112.4, 111.7, 107.9, 107.8, 53.6, 53.5, 53.3, 53.3, 52.9, 52.9, 52.5, 52.3, 51.7, 51.6, 51.5, 51.4; *m/z* (EI) 642 (27, M^+), 585 (28), 583 (70), 552 (17), 551 (26), 425 (89), 366 (49), 334 (12), 307 (26), 248 (10), 154 (100), 59 (67), 44 (16%).

4.1.4.2. Tetramethyl 4-aza-7-oxabenz[*m*,*n*]anthracene-2,3,5,6-tetracarboxylate (7). The reaction of **4** (0.54 g, 2.5 mmol) and DMAD (3 mL, excess) in dioxane for 10 h, gave a mixture from which compound **5** (325 mg, 21%) gently separated on ice-cooling (freezer), for few days. The mother liquor residue was subjected to plc (solvent: hexane/ethyl acetate 7:3) to afford compound **8** (220 mg, 27%) as red needles (mp 201–203 °C from methanol); the *title compound* **7** (110 mg, 10%) as yellow needles (mp 268–270 °C, from aqueous DMF); compound **9** as yellow powder (mp 197–199 °C, from benzene) and the non-reacted DMAD [Found: C, 62.09; H, 4.13; N, 4.30. $C_{23}H_{17}NO_9$ requires C, 62.39; H, 3.98; N, 4.28%]; ν_{max} (potassium bromide) 3005, 2953, 1746, 1726 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 8.48 (1H, s, 1-H), 8.33 (1H, dd, *J* 8.5, 1.6 Hz, 8-H), 7.61 (1H, ddd, *J* 8.3, 7.3, 1.5 Hz, 9-H), 7.43 (1H, ddd, *J* 7.4, 7.3, 1.0 Hz, 10-H), 7.42 (1H, dd, *J* 9.4, 1.1 Hz, 11-H), 3.97 (3H, s, COO*Me*), 3.96 (3H, s, COO*Me*), 3.93 (3H, s, COO*Me*), 3.92 (3H, s, COO*Me*); δ_C (125 MHz, DMSO-d_6) 166.1, 164.7, 164.6, 163.3, 156.3, 151.0, 149.8, 144.8, 132.2, 132.0, 131.0, 128.7, 125.8, 124.1, 118.4, 117.9, 117.6, 115.8, 109.7, 53.0, 52.9, 52.6, 52.3; *m/z* (EI) 451 (83, M^+), 421 (10), 420 (39), 406 (8), 393 (4), 392 (13), 374 (6), 335 (100%).

4.1.4.3. Dimethyl 7-amino-6-oxo-6*H*-benzo[*c*](2*H*)-chromen-8,9-dicarboxylate (8). [Found: C, 62.02; H, 3.98; N, 4.22. $C_{17}H_{13}NO_6$ requires C, 62.39; H, 3.98; N, 4.28%]; ν_{max} (potassium bromide) 3415, 3311, 2958, 1740, 1710 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 8.30 (1H, dd, *J* 8.1, 1.5 Hz, 4-H), 8.19 (2H, br s, D_2O-exchangeable, NH_2), 7.60 (1H, ddd, *J* 9.5, 8.0, 1.5 Hz, 3-H), 7.59 (1H, dd, *J* 6.9, 1.8 Hz, 1-H), 7.39 (s, 1H, 10-H), 7.37 (1H, ddd, *J* 7.9, 7.2, 1.1 Hz, 2-H), 3.86 (3H, s, COO*Me*), 3.80 (3H, s,

COO*Me*); δ_C (75 MHz, DMSO-d_6) 168.1, 166.6, 161.4, 152.1, 151.3, 141.1, 139.8, 132.4, 125.2, 124.9, 117.2, 117.1, 110.0, 107.4, 104.9, 53.0, 52.8; *m/z* (EI) 328 (19), 327 (100, M^+), 238 (22), 44 (5%).

4.1.4.4. Dimethyl 2-(3-amino-4-oxo-4*H*-thieno[3,4-*c*](2*H*)chromen-1-yl)butenedioate (12). Reacting **9** (0.54 g, 2.5 mmol) with DMAD (4 mL, excess) in methanol for 5 h, gave after concentration in vacuo, a crude material, which was crystallized from methanol to afford the *title compound* **12** (570 mg, 64%) as red prisms, mp 154–156 °C. When the same reaction was conducted with the same inputs of starting materials in dioxane for 8 h, compound **12** separated from the reaction concentrate on ice-cooling (freezer), to give the *title compound* **12** (159 mg, 21%) as red prisms, mp 155–157 °C (from ethanol). [Found: C, 56.73; H, 3.68; N, 3.88; S, 8.93. $C_{17}H_{13}NO_6S$ requires C, 56.82; H, 3.62; N, 3.90; S, 8.91%]; ν_{max} (potassium bromide) 3438, 3331, 2956, 1722, 1704, 1617, 1584, 1482, 1384, 1264, 1129, 943, 888, 793 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 7.98 (2H, br s, D_2O-exchangeable, N*H*$_2$), 7.38 (1H, dd, *J* 8.03, 1.69 Hz, 6′-H), 7.37 (1H, ddd, *J* 7.2, 6.4, 1.7 Hz, 7′-H), 7.25 (1H, dd, *J* 8.6, 1.3 Hz, 9′-H), 7.16 (1H, ddd, *J* 8.2, 6.9, 1.5 Hz, 8′-H), 7.16 (1H, s, 3-H), 3.68 (3H, s, COO*Me*), 3.56 (3H, s, COO*Me*); δ_C (75 MHz, DMSO-d_6) 166.3, 165.5, 158.8, 151.4, 136.1, 133.2, 129.7, 128.7, 124.5, 124.4, 118.1, 117.5, 105.1, 98.8, 53.5, 52.3; *m/z* (EI) 361 (7, MH_2^+), 359 (33, M^+), 301 (14), 300 (60), 299 (100), 285 (8), 268 (20), 257 (12), 242 (14), 241 (81), 240 (13), 186 (19), 59 (9), 44 (5%). On further cooling of the mother liquor in liquid nitrogen a precipitate was collected and crystallized from methanol to afford compound **8** (see above, 354 mg, 44%).

4.1.4.5. Dimethyl 2-(3-amino-4-oxo-4*H*-benzo[*f*]-thieno[3,4-*c*](2*H*)chromen-1-yl)fumarate (13a). On reacting **10** (0.814 g, 3 mmol) with DMAD (5 mL, excess) in methanol for 10 h, a precipitate was obtained from the reaction concentrate after ice-cooling (freezer) for several days. Recrystallization from methanol gave the *title compound* **13a** (777 mg, 62%) as red prisms, mp 171–173 °C. When the same quantities of **10** and DMAD were reacted for 8 hours in dioxane, compound **13a** crystallized on cooling (upon storage in a freezer) the reaction concentrate after a few days. This crop was crystallited from ethanol to give 108 mg (26%) of red prisms, mp 174–176 °C [Found: C, 61.43; H, 3.75; N, 3.34; S, 7.84. $C_{21}H_{15}NO_6S$ requires C, 61.61; H, 3.67; N, 3.42; S, 7.82%]; ν_{max} (potassium bromide) 3440, 3334, 3055, 2939, 1723, 1705, 1592, 1427, 1332, 1263, 1113, 1079, 942, 874, 792 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 8.20 (2H, br s, D_2O-exchangeable, NH_2), 7.99 (1H, d, *J* 8.84 Hz, 6′-H), 7.93 (1H, dd, *J* 7.5, 1.9 Hz, 11′-H), 7.88 (1H, dd, *J* 8.3, 1.4 Hz, 8′-H), 7.46 (2H, br ddd, *J* 9.6, 6.9, 2.1 Hz, 9′-H and 10′-H), 7.43 (1H, dd, *J* 8.8, 1.3 Hz, 7′-H), 6.61 (1H, s, 3-H), 3.38 (3H, s, COO*Me*), 3.26 (3H, s, COO*Me*); δ_C (75 MHz, DMSO-d_6) 167.2, 165.4, 164.9, 158.7, 150.3, 137.0, 132.4, 131.0, 130.6, 128.4, 128.1, 126.7, 126.5, 125.8, 125.4, 117.2, 112.6, 106.6, 101.1, 52.9, 51.6; *m/z* (EI) 409 (14, M^+), 377 (17), 351 (15), 350 (53), 349 (100), 335 (11), 318 (18), 292 (19), 291 (90), 175 (18), 44 (9%). Further cooling of the mother liquor in liquid nitrogen and crystallization of the precipitate from ethanol gave 220 mg (58%) of compound **14** (see below) as yellow prisms, mp 187–189 °C. Analytical HPLC of **13a** using a Merck-Hitachi chromatograph, column length 250 mm, column diameter 5 mm, flow rate 1 mL/min, on chiral stationary phases Merck Chiraspher NT (5 μm) with heptane/THF 50:50, 70:30, and 80:20 as well as Merck Whelk-01 (5 μm) with hexane/2-propanol 95:5, 80:20 and 70:30, gave no separation.

Crystal structure analysis of **13a**: The crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 602732. Details will be published separately.

4.2. Acylations of 13a

4.2.1. Reaction with acetic acid anhydride.

4.2.1.1. Dimethyl 2-(3-acetamino-4-oxo-4*H*-benzo[*f*]-thieno[3,4-*c*](2*H*)chromen-1-yl)fumarate (13b). A mixture of **13a** (52 mg, 0.13 mmol) and acetic acid anhydride (3 mL) was treated with pyridine (4 mL) for 48 h at room temperature. Evaporation of the solvent in vacuo gave a crude material, which was crystallized from ethyl acetate to afford the *title compound* **13b** (56 mg, 98%) as yellow powder, mp 241–243 °C [Found: C, 60.98; H, 3.85; N, 3.10; S, 7.33. $C_{23}H_{17}NO_7S$ requires C, 61.20; H, 3.77; N, 3.10; S, 7.10%]; ν_{max} (potassium bromide) 3436, 3267, 2953, 1723, 1708, 1682 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 11.03 (1H, br s, D_2O-exchangeable, N*H*), 8.10 (1H, dd, *J* 7.4, 1.5 Hz, 11′-H), 7.86 (1H, d, *J* 8.7 Hz, 6′-H), 7.83 (1H, dd, *J* 7.3, 2.2 Hz, 8′-H), 7.46 (2H, br ddd, *J* 9.8, 7.7, 1.0 Hz, 9′-H and 10′-H), 7.43 (1H, d, *J* 8.8 Hz, 7′-H), 6.97 (1H, s, 3-H), 3.52 (3H, s, COO*Me*), 3.36 (3H, s, COO*Me*), 2.41 (3H, s, CO*Me*); δ_C (125 MHz, DMSO-d_6) 167.7, 165.0, 164.9, 160.2, 151.1, 149.5, 137.9, 130.9, 130.8, 130.3, 128.7, 128.4, 128.1, 126.6, 126.1, 125.6, 117.8, 117.2, 112.9, 108.8, 52.8, 52.0, 23.4; *m/z* (EI) 452 (4), 451 (17, M^+), 421 (3), 420 (8), 419 (27), 409 (14), 378 (5), 377 (17), 393 (40), 391(86), 352 (6), 349 (100), 290 (17), 44 (6%).

4.2.2. Reaction with trifluoroacetanhydride.

4.2.2.1. Dimethyl 2-(3-trifluoroacetylamino-4-oxo-4*H*-benzo[*f*]thieno[3,4-*c*](2*H*)chromen-1-yl)fumarate (13c). A stirred mixture of **13a** (52 mg, 0.13 mmol) and trifluoroacetic acid anhydride (3 mL) was heated to reflux for 24 h. After evaporating the solvent to dryness in vacuo, the resulting crude material was purified by plc (hexane/ethyl acetate 7:3) and crystallized from ethyl acetate to yield the *title compound* **13c** (52 mg, 83%) as yellow powder, mp 154–156 °C [Found: C, 54.50; H, 2.70; N, 2.76; S, 6.41. $C_{23}H_{14}NO_7SF_3$ requires C, 54.65; H, 2.77; N, 2.77; S, 6.34%]; ν_{max} (potassium bromide) 3438, 1727, 1700 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 11.91 (1H, br s, D_2O-exchangeable, N*H*COCF$_3$), 8.05 (1H, dd, *J* 9.2, 1.0 Hz, 11′-H), 7.91 (1H, d, *J* 8.8 Hz, 6′-H), 7.86 (1H, dd, *J* 8.8, 1.2 Hz, 8′-H), 7.49 (2H, br ddd, *J* 8.4, 7.0, 1.3 Hz, 9′-H and 10′-H), 7.47 (1H, d, *J* 8.9 Hz, 7′-H), 7.04 (1H, s, 3-H), 3.52 (3H, s, COO*Me*), 3.41 (3H, s, COO*Me*); δ_C (125 MHz, $CDCl_3$) 165.0, 164.8, 160.0, 149.9, 147.6, 137.5, 131.6, 131.2, 131.2, 129.7, 128.7, 128.5, 127.1, 126.3, 126.1, 120.5, 117.4, 112.7, 112.0, 53.3, 52.3; *m/z* (EI) 505 (6, M^+), 473 (11), 447(10), 446 (31), 445 (100), 387(30), 377 (17), 393 (40), 69 (6), 59 (4%).

4.2.3. Attempted reaction of 2-amino-4-(dicyanomethylene)-4*H*-indeno[2,3-*b*]thiophen-1-carbonitrile (17) with DMAD in methanol, ethanol or dioxane. Stirred mixtures of compound **17** (0.28 g, 1 mmol) and DMAD (3 mL) were successively heated to reflux in methanol, ethanol and dioxane for 9 h. Concentration in vacuo and subsequent crystallization of the resulting precipitates from dioxane gave deep blue powders (mp>350 °C), which were identified to be the starting material **17**.

4.2.3.1. Dimethyl 7-(acetylamino)-5-oxobenz[*c*](2*H*)-chromen-8,9-dicarboxylate (19). From a mixture of compound **18** (0.26 g, 1 mmol) and DMAD (3 mL) in dioxane, the reaction was carried out for 8.5 h. On cooling, the precipitate was crystallized from ethanol to afford the *title compound* **19** (155 mg, 42%) as yellow needles, mp 204–206 °C [Found: C, 61.61; H, 4.15; N, 3.72. $C_{19}H_{15}NO_7$ requires C, 61.79; H, 4.07; N, 3.79%]; ν_{max} (potassium bromide) 3431, 3273, 3066, 2953, 1740, 1725, 1671 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 10.50 (1H, br s, D_2O-exchangeable, N*H*), 8.55 (1H, s, 10-H), 8.43 (1H, dd, *J* 8.6, 1.4 Hz, 4-H), 7.63 (1H, ddd, *J* 8.0, 7.5, 1.3 Hz, 3-H), 7.44 (1H, dd, *J* 8.1, 1.2 Hz, 1-H), 7.31 (1H, ddd, *J* 7.4, 7.3, 1.2 Hz, 2-H), 3.90 (3H, s, O*Me*), 3.75 (3H, s, O*Me*), 2.09 (3H, s, CO*Me*); δ_C (75 MHz, DMSO-d_6) 169.4, 158.6, 166.3, 158.5, 151.0, 138.6, 137.5, 132.3, 129.1, 125.3, 125.0, 124.7, 117.3, 119.6, 117.1, 116.7, 108.5, 53.4, 52.7, 23.6; *m/z* (EI) 369 (26, M^+), 338 (18), 327 (100), 311 (16), 310 (80), 43 (13%).

4.2.3.2. Dimethyl 4-amino-5-oxo-5*H*-dibenzo[*c*,*f*]-(2*H*)chromen-2,3-dicarboxylate (14). From a mixture of compound **21** (0.31 g, 1 mmol) and DMAD (3 mL) in dioxane, the reaction was conducted for 8.5 h. Cooling to room temperature and subsequent crystallization of the resulting solid material from benzene afforded yellow crystals (160 mg), mp 284–286 °C, identified in all respects to the starting compound **21**. The resulting mother liquor was diluted with acetone and separated by plc using hexane/ethyl acetate 3:2 to afford the unreacted excess of DMAD and a brown amorphous substance, which was crystallized from ethanol to give 43 mg (11%) of yellow prisms (mp 180–182 °C), identified in all respects to the *title compound* **14**, mp 183–185 °C from ethanol [Found: C, 66.65; H, 4.02; N, 3.69. $C_{21}H_{15}NO_6$ requires C, 66.84; H, 3.98; N, 3.71%]; ν_{max} (potassium bromide): 3443, 3339, 2953, 1735, 1709 cm^{-1}; δ_H (500 MHz, DMSO-d_6) 8.58 (1H, d, *J* 8.59 Hz, 12-H), 8.16 (1H, dd, *J* 8.8 Hz, 7-H), 8.11 (2H, br s, D_2O-exchangeable, NH_2), 8.09 (1H, br dd, *J* 8.1, 1.2 Hz, 9-H), 7.74 (1H, ddd, *J* 8.6, 7.1, 1.5 Hz, 11-H), 7.76 (1H, d, *J* 0.5 Hz, 1-H), 7.62 (1H, ddd, *J* 7.9, 7.0, 0.9 Hz, 10-H), 7.52 (1H, d, *J* 8.83 Hz, 8-H), 3.85 (3H, s, COO*Me*), 3.82 (3H, s, COO*Me*); δ_C (125 MHz, DMSO-d_6) 167.7, 166.5, 160.9, 151.3, 150.6, 139.6, 139.6, 133.5, 131.3, 129.6, 128.7, 128.4, 125.8, 124.4, 116.8, 111.8, 111.5, 110.2, 105.8, 53.1, 52.7; *m/z* (EI) 378 (24), 377 (100, M^+), 346 (24), 288 (12).

4.2.3.3. (*E*,*Z*) Methyl 3-[1-(3-amino-4-oxo-4*H*-thieno-[3,4-*c*](2*H*)chromenyl]propoate (24). From **9** (0.54 g, 2.5 mmol) and methyl propiolate (3 mL) in dioxane, the reaction was carried out for 5.5 h. Concentration in vacuo of the resulting solution to half of its volume gave a precipitate, which was crystallized from dioxane/ethyl acetate to afford the *title compound* **24** (550 mg, 73%) as yellow powder, mp 302–304 °C [Found: C, 59.67; H, 3.72; N, 4.68; S, 10.67. $C_{15}H_{11}NO_4S$ requires C, 59.80; H, 3.65; N, 4.65; S, 10.63%]; ν_{max} (potassium bromide) 3387, 3281, 1717, 1696 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 8.37 (2H, br s, D_2O-exchangeable, NH_2), 8.21 (1H, dd, *J* 15.0, 3.3 Hz, 2-H), 7.91 (1H, d, *J* 8.1 Hz, 6′-H), 7.48 (1H, dd, *J* 7.9, 7.6 Hz, 7′-H), 7.36 (1H, d, *J* 7.9 Hz, 9′-H), 7.29 (1H, dd, *J* 8.2, 7.7 Hz, 8′-H), 5.87 (1H, dd, *J* 15.0, 3.1 Hz, 3-H), 3.72 (3H, s, O*Me*); δ_C (75 MHz, DMSO-d_6) 166.4, 166.1, 158.3, 151.9, 135.4, 133.0, 130.4, 125.4, 124.8, 117.8, 117.6, 113.4, 111.6, 100.4, 51.2; *m/z* (EI) 303 (8), 302 (21), 301 (100, M^+), 270 (32), 243 (22), 242 (96), 241 (87%).

4.2.3.4. Dimethyl 3,3′-[3-(4-oxo-4*H*-benzo[*f*]thieno-[3,4-*c*](2*H*)chromenyl)]aminodipropoate (*E*,*Z*-mixture) (26). The reaction in dioxane of a mixture of **10** (0.67 g, 2.5 mmol) with methyl propiolate (3 mL) gave after 7 h refluxing no new product. The starting compound was instead recovered after the usual work-up. From a mixture of **10** (0.54 g, 2 mmol) and methyl propiolate (3 mL) in toluene, the reaction was carried out for 8 h. Cooling to room temperature and subsequent crystallization of the solid from 10% aqueous DMF afforded the *title compound* **26** (617 mg, 70%) as yellow powder, mp 291–293 °C [Found: C, 63.45; H, 3.91; N, 3.22; S, 7.36. $C_{23}H_{17}NO_6S$ requires C, 63.44; H, 3.94; N, 3.22; S, 7.36%]; ν_{max} (potassium bromide) 3445, 3061, 2957, 1727, 1661 cm^{-1}; δ_H (300 MHz, DMSO-d_6) 8.97 (1H, s, 1″-H), 8.88 (1H, d, *J* 8.31 Hz, 6″-H), 8.20 (2H, d, *J* 13.5 Hz, 3-H and 3′-H), 8.09 (2H, br d, 8″-H and 11″-H); 7.82 (1H, ddd, 10″-H, *J* 8.5, 6.9, 1.4 Hz), 7.65 (1H, dd, *J* 8.7, 7.2, 7.1 Hz, 9″-H), 7.53 (1H, d, *J* 8.9 Hz, 7″-H), 4.84 (2H, d, *J* 13.5 Hz, 2-H and 2′-H), 3.59 (6H, s, 2×COO*Me*); δ_C (75 MHz, DMSO-d_6) 166.4, 153.7, 149.9, 148.6, 143.8, 133.1, 131.1, 130.8, 129.3, 128.9, 128.6, 125.5, 124.1, 121.8, 121.6, 117.4, 110.5, 98.7, 50.7; *m/z* (EI) 437 (9), 436 (27), 435 (100, M^+), 404 (11), 403 (12), 376 (27), 44 (7%).

4.2.3.5. (*E* or *Z*) Methyl 3-[1-(3-amino-4-oxo-4*H*-benzo[*f*]thieno[3,4-*c*](2*H*)chromenyl)]propenoate (27). From a mixture of **10** (401 g, 1.5 mmol) and methyl propiolate (3 mL) in methanol, the reaction was conducted for 7 h. Cooling at room temperature followed by crystallization of the precipitate from ethyl acetate gave the *title compound* **27** (35 mg, 7%) as yellow powder, mp 312–314 °C. The compound was not soluble enough in DMSO-d_6 to afford exploitable information from the NMR experiments. ν_{max} (potassium bromide) 3406, 3289, 2957, 1710, 1681 cm^{-1}; *m/z* (EI) 351 (49, M^+), 320 (6), 318 (4), 297 (7), 293 (21), 292 (100), 291 (36), 290 (3%).

4.2.3.6. Dimethyl 1-phenylnaphthalene-2,3-dicarboxylate (ethyl phenylethynecarboxylate dimer″) (28). The reaction in methanol of a mixture of **10** (401 mg, 1.5 mmol) and ethyl phenylethynecarboxylate (3 mL) was successively conducted for 54 and 48 h, respectively, in methanol and toluene and gave no new product, but rather the starting material **10**. From a mixture of **10** (270 mg, 1 mmol) and ethyl phenylpropiolate (2 mL) in DMF, the reaction was carried out for 7 h. The reaction mixture was then concentrated in vacuo to half of its volume. The resulting precipitate was crystallized from 10% aqueous DMF to

afford the *title compound* **28** (103 mg, 30%) as green-yellowish powder, mp 124–126 °C (lit.,[16–18] 127–128 °C from petroleum ether). The mother liquor was discarded; ν_{max} (potassium bromide) 3064, 2977, 2929, 2903, 1715, 1661 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.60 (1H, s, 7-H), 8.01–7.26 (9H, m, aromatic H), 4.42 (2H, q, *J* 7.1 Hz, OCH_2), 4.06 (2H, q, OCH_2, *J* 7.2 Hz), 1.42 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 0.97 (3H, t, OCH_2CH_3, *J* 7.1 Hz); δ_C (75 MHz, $CDCl_3$) 168.8, 166.0, 138.6, 136.8, 134.1, 132.4, 131.4, 131.3, 130.4, 129.3, 128.9, 128.0, 127.4, 127.0, 61.6, 61.1, 14.3, 13.7; *m/z* (EI) 348 (97, M^+), 320 (6), 304 (9), 290 (4), 277 (3), 276 (24), 275 (100, M–COOEt), 274 (4).

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Synthesis of (tetrahydrofuran-2-yl)acetates based on a 'cyclization/hydrogenation/enzymatic kinetic resolution' strategy

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Abstract—A variety of (tetrahydrofuran-2-yl)acetates and (pyrrolidin-2-yl)acetates have been prepared by hydrogenation of 2-alkylidenetetrahydrofurans and 2-alkylidenepyrrolidines, which are readily available by cyclization reactions of 1,3-dicarbonyl dianions ('free dianions') or 1,3-bis-silyl enol ethers ('masked dianions') with 1,2-dielectrophiles. The enzymatic kinetic resolution of (tetrahydrofuran-2-yl)acetates with recombinant esterase Est56 proceeded with excellent enantioselectivities ($E>100$).

1. Introduction

Functionalized tetrahydrofurans occur in a variety of pharmacologically relevant natural products.[1–4,6–8] (Tetrahydrofuran-2-yl)acetates are present, for example, in the polyether antibiotics lasalocid A (Fig. 1),[2] ferensimycin A, B, and lysocellin (Fig. 2).[3] They also represent versatile synthetic building blocks and have been used, for example, during the synthesis of the natural acetogenin solamin isolated from *Annonaceous*.[4] Many acetogenins exhibit remarkable cytotoxic, antitumor, antimalarial, immunosuppressive, pesticidal, and antifeedant activities.[1]

2-Alkylidenetetrahydrofurans[5,6] represent important synthetic building blocks that have been used for the synthesis of natural products. Numerous synthetic transformations of 2-alkylidenetetrahydrofurans have been reported, which include, for example, cycloadditions,[5a–d] nucleophilic additions,[5e–f] cyclopropanations,[5g] oxidative carbonylations,[5h–j] and codimerizations.[5k] The hydrogenation[5m,6h–i] of 2-alkylidenetetrahydrofurans has been applied to the synthesis of natural products, such as methyl nonactate, which represents a building block of nonactin.[6,7] The spiroketal chalcogran has been prepared from a bicyclic 2-alkylidenetetrahydrofuran.[8] In recent years, we and others have reported a number of one-pot syntheses of 2-alkylidenetetrahydrofurans by cyclization of 1,3-dicarbonyl dianions or 1,3-bis-silyl enol ethers with 1,2-dielectrophiles,[9] and also by other methods.[10c–f] 2-Alkylidenetetrahydrofurans have been functionalized by lithiation and subsequent alkylations;[10a,b] in addition, the bromination of the exocyclic double bond and subsequent cross-coupling reactions have been reported.[10g,h] Recently, we have reported the synthesis of 6-bromo-3-oxoalkanoates and functionalized benzofurans by reaction of 2-alkylidenetetrahydrofurans with boron tribromide (BBr_3).[11] In addition, furans and benzofurans have been prepared by sequential '[3+2] cyclization/dehydrogenation'[12] and '[3+2] cyclization/elimination'[13] reactions. Herein, we report a convenient approach to racemic (tetrahydrofuran-2-yl)acetates[14] based on a '[3+2] cyclization/hydrogenation'

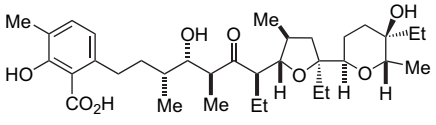

Figure 1. Lasalocid A.

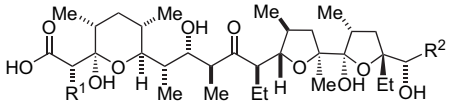

Figure 2. Ferensimycin A, B, and lysocellin.

Keywords: Cyclizations; Tetrahydrofurans; Pyrrolidines; Enzymatic kinetic resolution; Hydrogenation.
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strategy. In addition, we report the enzymatic kinetic resolution of (tetrahydrofuran-2-yl)acetates. The novel recombinant esterase Est56, extracted from soil samples using the metagenome approach, and supplied by BRAIN AG (Zwingenberg, Germany) has been used for these transformations.[15,16]

In the present manuscript, we also report the synthesis of an ethyl (pyrrolidin-2-yl)acetate by diastereoselective hydrogenation of an ethyl (4-methoxypyrrolidin-2-ylidene)acetate. 2-Alkylidenepyrrolidines[17,18] represent direct precursors for the stereoselective synthesis of pyrrolidine substructures[18] by reduction[18f] of the exocyclic double bond. Pyrrolidines are present in a variety of alkaloids, such as hygrine, hygroline or cuskhygrin, and in non-natural products used in the clinic (e.g., the vasodilator buflomedil).[18] They are ubiquitous structural motifs in drugs and drug candidates displaying antidepressant,[19a,b] antihypertensive,[19a,c] anti-arthritic,[19d,e] antibacterial,[19f–h] antithrombotic,[19i–k] and analgesic[19l,m] activities. Recent drug development incorporating the pyrrolidine motif has identified candidates with promising anti-HIV[19n,o] and antibacterial adjunct[19p,q] activities. Numerous applications in the synthesis of natural products have been reported.[20] Recently, we have reported[21a] the synthesis of 2-alkylidene-4-methoxypyrrolidines by condensation of 1,3-bis-silyl enol ethers with 1-azido-2,2-dimethoxyethane, and cyclization by Staudinger–aza-Wittig reaction.[21b]

2. Results and discussion

2.1. Synthesis of (tetrahydrofuran-2-yl)acetates based on cyclizations of free dianions with 1-bromo-2-chloroethane (*method A*)

The Pd/C catalyzed hydrogenation of the known 2-alkylidenetetrahydrofurans **3a–d**, prepared by cyclization of dilithiated methyl, ethyl, *iso*-propyl and *tert*-butyl acetoacetate (**1a–d**) with 1-bromo-2-chloroethane,[10a,b] afforded the (tetrahydrofuran-2-yl)acetates **5a–d** (Scheme 1, Table 1). 3-Methyl- and 3-ethyl-(tetrahydrofuran-2-yl)acetates **5e** and **5f** were prepared by hydrogenation of the known 2-alkylidenetetrahydrofurans **3e** and **3f**, respectively. The latter are again available by cyclization of 1,3-dicarbonyl dianions **1e,f** with 1-bromo-2-chloroethane.[10a,b] Tetrahydrofurans **5e**,**f** were isolated as inseparable 6:5 mixtures of diastereomers. The hydrogenation of tetrahydro[2,3′]bifuranyliden-2′-one **3g**,[10b] prepared by cyclization of dilithiated α-acetyl-γ-butyrolactone (**1g**) with 1-bromo-2-chloroethane, gave the octahydro[2,3′]bifuranyl-2′-one **5g** as an inseparable 3:2 mixture of diastereomers.

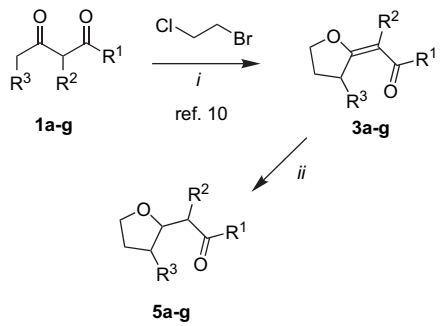
Table 1. Synthesis of (tetrahydrofuran-2-yl)acetates (**5a–g**)

Substrate (%)[a]	5	R^1	R^2	R^3	% (5)[a]	dr[b]
3a (86)[c]	**a**	OMe	H	H	97	—
3b (79)[c]	**b**	OEt	H	H	100	—
3c (77)[d]	**c**	O^{i}Pr	H	H	100	—
3d (77)[c]	**d**	O^{t}Bu	H	H	83	—
3e (72)[c]	**e**	OMe	H	Me	89	6:5
3f (82)[c]	**f**	OEt	H	Et	95	6:5
3g (58)[c]	**g**	OCH_2CH_2		H	86	3:2

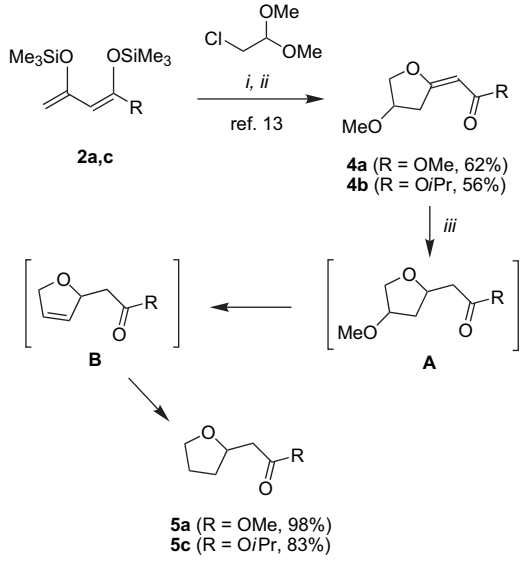
[a] Isolated yields.
[b] Diastereomeric ratio, assignment arbitrary.
[c] Known compounds (Ref. 10a,b).
[d] Combined yield (Ref. 12).

2.2. Synthesis of (tetrahydrofuran-2-yl)acetates based on cyclizations of masked dianions with 1-chloro-2,2-dimethoxyethane (*method B*)

The 4-methoxy-2-alkylidenetetrahydrofurans **4a**,**b** were prepared, following our recently reported procedure,[13] by TMSOTf catalyzed condensation of 1,3-bis-silyl enol ethers **2a**,**c** (available from **1a**,**c** in two steps) with 1-chloro-2,2-dimethoxyethane and subsequent DBU-mediated cyclization (Scheme 2). The hydrogenation of **4a**,**b** afforded the (tetrahydrofuran-2-yl)acetates **5a**,**c** by elimination of methanol and subsequent hydrogenation of the double bond thus formed (*method B*). For the synthesis of **5a**,**c**, *method A* is superior to *method B* because of the higher yields (*method A*: 83 and 77%; *method B*: 61 and 47%) and because less synthetic steps are required (*method A*: two steps; *method B*: five steps).



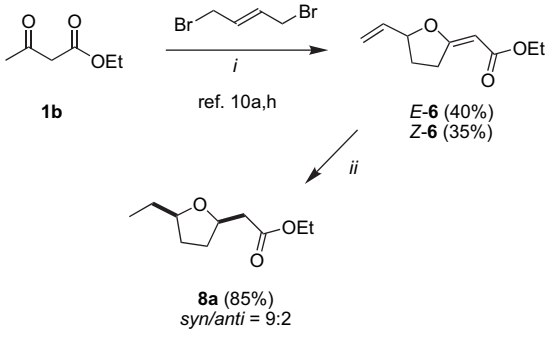
Scheme 1. Synthesis of (tetrahydrofuran-2-yl)acetates **5a–g**: (i) (1) LDA (2.3 equiv), THF, 0 °C, 1 h, (2) $Br(CH_2)_2Cl$, −78→20 °C, 14 h, then reflux, 12 h; (ii) H_2, Pd/C (0.5 equiv; for **5a**: 0.3 equiv), MeOH (or EtOH), 20 °C, 48 h.



Scheme 2. Synthesis of (tetrahydrofuran-2-yl)acetates **5a**,**c**: (i) $ClCH_2CH(OMe)_2$, Me_3SiOTf (0.5 equiv), CH_2Cl_2, −78→20 °C, 14 h, then at 20 °C, 2 h; (ii) DBU (2.0 equiv), THF, 20 °C, 6 h; (iii) H_2, Pd/C (0.5 equiv), MeOH, 20 °C, 48 h.

2.3. Synthesis of (tetrahydrofuran-2-yl)acetates based on cyclizations of free dianions with 1,4-dibromobut-2-ene (*method C*)

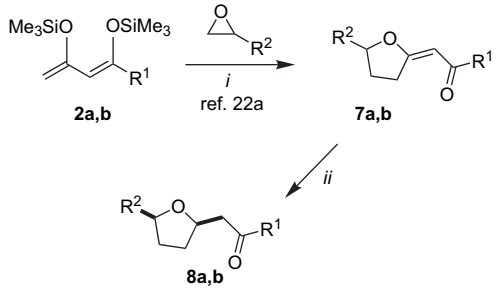
The cyclization of dilithiated ethyl acetoacetate (**1b**) with 1,4-dibromobut-2-ene afforded, following a known procedure,[10a] (5-vinyldihydrofuran-2(3*H*)-ylidene)acetate **6** as a separable mixture of *E*/*Z* isomers (Scheme 3).[10h] The hydrogenation of **6** afforded the (5-ethyltetrahydrofuran-2-yl)acetate **8a** by hydrogenation of the vinyl group and the exocyclic double bond.



Scheme 3. Synthesis of **8a**: (i) (1) LDA (2.3 equiv), THF, 0 °C, 1 h, (2) $BrCH_2CH{=}CHCH_2Br$, −78→20 °C, 14 h, then at 20 °C, 24 h; (ii) H_2, Pd/C (0.5 equiv), EtOH, 20 °C, 48 h.

2.4. Synthesis of (tetrahydrofuran-2-yl)acetates based on cyclizations of masked dianions with epoxides (*method D*)

2-Alkylidenetetrahydrofurans **7a**,**b** were prepared, following a known procedure,[22a] by $TiCl_4$ mediated cyclization of 1,3-bis-silyl enol ethers **2a**,**b** with 1,2-epoxybutane and epichlorohydrin, respectively (Scheme 4, Table 2). The hydrogenation of **7a** afforded the tetrahydrofuran **8a** with moderate diastereoselectivity. The hydrogenation of **7b** afforded (5-chloromethyltetrahydrofuran-2-yl)acetate **8b** with very good diastereoselectivity.



Scheme 4. Synthesis of 5-alkyl-(tetrahydrofuran-2-yl)acetates **8a**,**b**: (i) $TiCl_4$ (2 equiv), 4 Å MS, CH_2Cl_2, −78→20 °C, 14 h, then at 20 °C, 3 h; (ii) H_2, Pd/C (0.5 equiv), MeOH (or EtOH), 20 °C, 48 h.

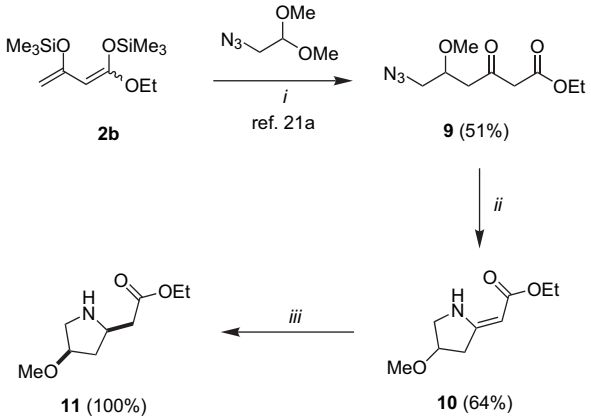
Table 2. Synthesis of 5-alkyl-(tetrahydrofuran-2-yl)acetates (**8a**,**b**)

Substrate (%)[a]	**8**	R^1	R^2	% (**8**)[a]	*syn*/*anti*[b]
7a (62)[c]	**a**	OEt	Et	70	3:1
7b (66)	**b**	OMe	CH_2Cl	100	>10:1

[a] Isolated yields.
[b] Diastereoselectivity (by ^1H NMR).
[c] Known compound (Ref. 22a).

2.5. Synthesis of (pyrrolidin-2-yl)acetates based on [3+2] cyclizations of 1,3-bis-silyl enol ethers with 1-azido-2,2-dimethoxyethane

The ethyl (4-methoxypyrrolidin-2-ylidene)acetate **10** was prepared, following our recently reported procedure,[21a] by TMSOTf catalyzed condensation of 1,3-bis-silyl enol ether **2b**, with 1-azido-2,2-dimethoxyethane and subsequent cyclization of **9** by Staudinger–aza-Wittig reaction. The palladium-catalyzed hydrogenation of **10** afforded ethyl (4-methoxypyrrolidin-2-yl)acetate (**11**) with 10:1 diastereoselectivity (Scheme 5).



Scheme 5. Synthesis of **11**: (i) $N_3CH_2CH(OMe)_2$, Me_3SiOTf (0.5 equiv), CH_2Cl_2, −78→20 °C; (ii) PPh_3, THF, 45 °C, 6 h; (iii) H_2, Pd/C (0.5 equiv), EtOH, 20 °C, 24 h.

2.6. Enzymatic kinetic resolution of (tetrahydrofuran-2-yl)acetates

To monitor the enantioselectivity in the enzymatic kinetic resolution of the (tetrahydrofuran-2-yl)acetates, suitable conditions for gas chromatographic (GC) analysis were developed (Table 3).

The enzymatic kinetic resolutions of these (tetrahydrofuran-2-yl)acetates were next studied (Table 4). The analytical scale enzyme reactions of methyl and ethyl (tetrahydrofuran-2-yl)acetates (**5a**,**b**) were carried out with esterases[15] (Est56, Est63, Est8) and lipases[15] (CAL-B, BCL). For **5a**,**b**, the best resolution results were achieved with the recombinant esterase Est56.

Enzymatic kinetic resolution of **5a** with recombinant esterase Est56 gave (−)-**5a** (40%, >99% ee) and the hydrolysis product (−)-**12** (49%, 87% ee) (Scheme 6, Table 5, entry 4). The preparative scale reaction of **5a** with Est56 was repeated

Table 3. GC analysis of (tetrahydrofuran-2-yl)acetates

Substrate	Temperature (°C)	Retention time (min)[a]			
		Substrate (ester)		Product (acid)[b,c]	
5a	70	18.2	18.8	18.2	18.8
5b	70	29.7	30.5	18.2	18.8

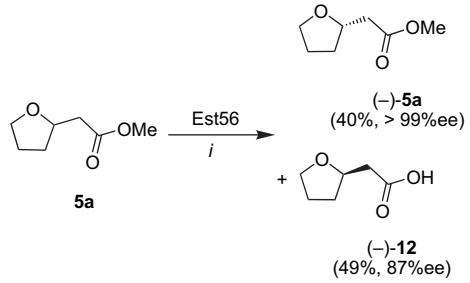
[a] Chiral column: Hydrodex®-β-3P, [Heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin].
[b] Product of enzyme reactions.
[c] Retention times for methyl ester of hydrolysis products [acid+diazomethane→methyl ester].

Table 4. Analytical scale enzymatic kinetic resolution reactions

Enzyme	Substrate	Conversion (%)	Reaction time (h)	eeS (%)[a,b]	eeP (%)[a,c]	*E*-value
Est56	**5a**	59	49	>99	70	48
Est56	**5b**	60	53	88	67	15
Est63	**5a**	30	49	33	75	9
Est8	**5a**	18	48	3	2	1
Est8	**5b**	5	48	<1	11	1
CAL-B	**5a**	95	1	—	—	1
CAL-B	**5b**	95	1	—	—	1
BCL	**5a**	35	48	19	35	2
BCL	**5b**	40	48	23	33	2

eeS: enantiomeric excess of substrate; eeP: enantiomeric excess of product.
[a] Calculated from gas chromatograms (GC).
[b] For (−)-**5a**.
[c] For (−)-**12**.

five times and optimized, which finally led to conditions in which excellent enantioselectivities ($E>100$) at high conversions and short reaction times were obtained. The resolution reaction of racemic **5a** with Est56 afforded *both* products with negative (−) optical rotation values. Unfortunately, the absolute configurations of (−)-**5a** and (−)-**12** could *not* be determined by comparison with literature results: Laxmi and Iyengar reported[23] that the lipase (*Candida cylindracea*) mediated enantioselective hydrolysis of **5a** afforded (*S*)-(+)-**5a** and (*R*)-(−)-**12**, which were used for the synthesis of the natural product (*R*)-(+)-α-lipoic acid. Co-injection (GC) of (−)-**5a** and (−)-**12** proved that the products have different configurations.



Scheme 6. Enzymatic kinetic resolution of **5a**: (i) (Table 5, entry 4) recombinant esterase Est56, phosphate buffer (50 mM, pH 7.5), 37 °C, 5 h, the assignment of the absolute configuration is arbitrary.

In conclusion, a variety of (tetrahydrofuran-2-yl)acetates have been prepared by hydrogenation of 2-alkylidenetetrahydrofurans readily available by [3+2] cyclizations of 1,3-dicarbonyl dianions ('free dianions') or 1,3-bis-silyl enol ethers ('masked dianions') with various 1,2-dielectrophiles. Similarly, (4-methoxypyrrolidin-2-yl)acetate has been prepared from 2-alkylidenepyrrolidin. Enzymatic kinetic resolution of (tetrahydrofuran-2-yl)acetates by recombinant esterase Est56 proceeded with excellent enantioselectivity affording these compounds in optically pure form.

Table 5. Preparative scale enzymatic reactions

Entry	Est56[a] (mL)	**5a** (mg)	Conversion (%)	Time (h)	eeS (%)[b,c]	eeP (%)[b,d]	*E*-value
1	2	200	35	216	38	71	9
2	1	4×20	39	187	47	75	11
3	2	50	54	22	>99	83	73
4	1.5	50	54	5	>99	87	>100
5	2	50	54	3	>99	84	100

eeS: enantiomeric excess of substrate; eeP: enantiomeric excess of product.
[a] Crude extract with 50 U/mL (based on a *p*-nitrophenyl acetate assay).
[b] For isolated products.
[c] For (−)-**5a**.
[d] For (−)-**12**.

3. Experimental

3.1. General comments

All solvents were dried by standard methods and all reactions were carried out under an inert atmosphere. For the ^{1}H and ^{13}C NMR spectra the deuterated solvents indicated were used. The NMR spectra were measured on 300 and 200 MHz instruments. Mass spectral data (MS) were obtained by electron ionization (EI, 70 eV), chemical ionization (CI, H_2O or DCI, NH_3) or electrospray ionization (ESI). For preparative scale chromatography silica gel (60–200 mesh) was used. Chiral column used for GC: Hydrodex®-β-3P, [Heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin] (25 m, 0.25 mm).

3.2. General procedure for the reaction of 1,3-bis-silyl enol ethers with epoxides

To a CH_2Cl_2 solution (4 mL/mmol) of 1,3-bis-silyl enol ether **2** (1.0 equiv) and the epoxide (1.2 equiv), in the presence of molecular sieves (4 Å), was added $TiCl_4$ (2.0 equiv) at −78 °C. The solution was stirred for 4 h at −78 °C; subsequently, the temperature was allowed to rise to 20 °C during 14 h and the solution was stirred for 3 h at 20 °C. The molecular sieves were filtered-off and washed with CH_2Cl_2. To the solution was added a saturated aqueous solution of $NaHCO_3$, the organic layer was separated and the aqueous layer was repeatedly extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/EtOAc) to give **7**. The synthesis of **7a** has been previously reported.[22a]

3.2.1. Methyl (5-chloromethyldihydrofuran-2(3*H*)-ylidene) acetate (7b). Starting with **2a** (7.814 g, 30 mmol), epichlorohydrin (2.82 mL, 36 mmol) and $TiCl_4$ (6.6 mL, 60 mmol) in CH_2Cl_2 (250 mL, 4 Å molecular sieves), **7b** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellowish oil (3.764 g, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.97–2.06 (m, 1H, CH_2), 2.23–2.32 (m, 1H, CH_2), 3.00–3.13 (m, 1H, CH_2), 3.25–3.38 (m, 1H, CH_2), 3.64 (d, J=5.1 Hz, 2H, CH_2–Cl), 3.67 (s, 3H, OCH_3), 4.62–4.70 (m, 1H, OCH), 5.35 (t, J=1.8 Hz, 1H, CH=C). IR (neat, cm^{-1}): $\tilde{\nu}$=2987 (w), 2954 (m), 1704 (s), 1642 (s), 1439 (s), 1365 (s), 1295 (m), 1246 (m), 1189 (s), 1121 (s), 1047 (s), 942 (w), 880 (w), 828 (m), 727 (w). MS (EI, 70 eV): *m*/*z* (%)=192 (M^+ [^{37}Cl], 5), 190 (M^+ [^{35}Cl], 17), 161 (16), 159 (49), 155 (2), 141 (4), 123 (10), 109 (5), 69 (100). HRMS (ESI): Calcd for $C_8H_{11}ClO_3$ [M^+]: 192.9885 (^{37}Cl), 190.0391 (^{35}Cl); found: 192.9883 (^{37}Cl), 190.0387 (^{35}Cl).

3.3. General procedure for the hydrogenation of 2-alkylidenetetrahydrofurans

To a H_2 concentrated suspension of Pd/C (0.3–0.5 equiv, 10% Pd on charcoal) in methanol (or ethanol) (5–10 mL/mmol)

was added 2-alkylidenetetrahydrofuran (**3**, **6**, **7** or **10**) (1.0 equiv). The reaction mixture was concentrated with H_2 and stirred under H_2 atmosphere at 20 °C for 48 h. Then the reaction mixture was filtered through Celite, washed with dichloromethane (4×15 mL/mmol), and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/EtOAc) to give (tetrahydrofuran-2-yl)acetate (**5**, **8** or **11**). Notably, the (tetrahydrofuran-2-yl)acetates were not UV active (neither at short nor at long wavelength); to detect the products on TLC, the following solution was used as a dying agent: MnO_2 (0.3 g/mL) in ethanol.

3.3.1. Methyl (tetrahydrofuran-2-yl)acetate (5a).[23] *Method A*: Starting with **3a** (0.500 g, 3.52 mmol) and Pd/C (10% Pd, 1.123 g, 1.06 mmol) in methanol (20 mL), **5a** was isolated without further purification as a slightly yellowish oil (0.491 g, 97%). *Method B*: Starting with **4a** (0.300 g, 1.74 mmol) and Pd/C (10% Pd, 0.556 g, 0.52 mmol) in methanol (15 mL), **5a** was isolated without further purification as a slightly yellowish oil (0.245 g, 98%). GC (chiral column, 70 °C): retention times (min)=18.2, 18.8. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.50–1.61 (m, 1H, CH_2), 1.87–1.96 (m, 2H, CH_2), 2.04–2.15 (m, 1H, CH_2), 2.49 (dd, J=15.2, 5.8 Hz, 1H, CH_2), 2.61 (dd, J=15.2, 7.2 Hz, 1H, CH_2), 3.70 (s, 3H, OCH_3), 3.72–3.79 (m, 1H, OCH_2), 3.85–3.92 (m, 1H, OCH_2), 4.26 (quint, J=7.2 Hz, 1H, OCH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=25.0, 30.6, 39.8 (CH_2), 51.1 (OCH_3), 67.4 (OCH_2), 74.6 (OCH), 171.0 (O═C–O). IR (neat, cm^{-1}): $\tilde{\nu}$=2957 (s), 2875 (m), 1741 (s), 1646 (w), 1438 (m), 1417 (w), 1382 (w), 1361 (w), 1322 (w), 1302 (m), 1261 (m), 1201 (m), 1164 (s), 1119 (s), 1100 (m), 1066 (s), 1019 (m), 875 (w), 823 (w), 806 (w). MS (EI, 70 eV): *m*/*z* (%)=143 (M^+, 76), 129 (20), 112 (14), 95 (34), 83 (75), 70 (100). HRMS (ESI): Calcd for $C_7H_{12}O_3$ ([M+1]$^+$): 145.08647; found: 145.08567.

3.3.2. Ethyl (tetrahydrofuran-2-yl)acetate (5b). Starting with **3b** (1.000 g, 6.4 mmol) and Pd/C (10% Pd, 3.407 g, 3.2 mmol) in ethanol (25 mL), **5b** was isolated without further purification as a yellowish oil (1.012 g, 100%). GC (chiral column, 70 °C): retention times (min)=29.7, 30.5. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.15–1.31 (m, 3H, CH_3), 1.55–1.69 (m, 1H, CH_2), 1.88–1.97 (m, 1H, CH_2), 2.05–2.11 (m, 1H, CH_2), 2.28–2.49 (m, 1H, CH_2), 2.54–2.79 (m, 2H, CH_2), 3.43–3.61 (m, 2H, OCH_2), 3.76–3.88 (m, 1H, OCH), 4.12–4.24 (m, 2H, OCH_2). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=14.2 (CH_3), 26.0, 31.5, 39.7 (CH_2), 61.4, 64.5 (OCH_2), 75 (OCH), 166.7 (O═C–O). IR (neat, cm^{-1}): $\tilde{\nu}$=2966 (s), 2875 (w), 1738 (s), 1651 (w), 1446 (m), 1412 (m), 1374 (m), 1304 (m), 1260 (s), 1182 (s), 1164 (s), 1096 (s), 1067 (s), 1030 (s). MS (EI, 70 eV): *m*/*z* (%)=158 (M^+, 2), 144 (13), 130 (51), 114 (31), 97 (13), 84 (21), 71 (100).

3.3.3. *iso*-Propyl (tetrahydrofuran-2-yl)acetate (5c). *Method A*: Starting with **3c** (0.150 g, 0.88 mmol) and Pd/C (10% Pd, 0.469 g, 0.44 mmol) in ethanol (10 mL), **5c** was isolated without further purification as a slightly yellowish oil (0.152 g, 100%). *Method B*: Starting with **4b** (0.250 g, 1.25 mmol) and Pd/C (0.664 g, 10% Pd, 0.62 mmol) in methanol (15 mL), **5c** was isolated without further purification as a slightly yellowish oil (0.179 g, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.24 (d, J=6.3 Hz, 6H, 2×CH_3), 1.50–1.61 (m, 1H, CH_2), 1.85–1.96 (m, 2H, CH_2), 2.03–2.12 (m, 1H, CH_2), 2.43 (dd, J=15.1, 6.3 Hz, 1H, CH_2), 2.58 (dd, J=15.1, 7.0 Hz, 1H, CH_2), 3.70–3.79 (m, 1H, OCH_2), 3.84–3.92 (m, 1H, OCH_2), 4.24 (quint, J=6.8 Hz, 1H, OCH), 5.04 (quint, J=6.3 Hz, 1H, OCH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=21.1 (2C, CH_3), 24.9, 30.5, 40.3 (CH_2), 66.9 (OCH), 67.4 (OCH_2), 74.6 (OCH), 169.9 (O═C–O). IR (neat, cm^{-1}): $\tilde{\nu}$=2962 (s), 2934 (s), 2874 (m), 1731 (s), 1649 (w), 1457 (m), 1447 (m), 1408 (w), 1379 (m), 1262 (s), 1232 (m), 1180 (s), 1108 (s), 1070 (s), 1022 (m), 967 (w), 801 (m). MS (EI, 70 eV): *m*/*z* (%)=172 (M^+, 1), 157 (3), 142 (3), 129 (11), 112 (8), 102 (28), 97 (4), 89 (48), 84 (16), 71 (100). HRMS (ESI): Calcd for $C_9H_{16}O_3$ ([M+Na]$^+$): 195.09972; found: 195.09889.

3.3.4. *tert*-Butyl (tetrahydrofuran-2-yl)acetate (5d). Starting with **3d** (0.200 g, 1.09 mmol) and Pd/C (10% Pd, 0.580 g, 0.5 mmol) in ethanol (10 mL), **5d** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→10:1) as a colorless oil (0.170 g, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.45 (s, 9H, O^{t}Bu), 1.51–1.61 (m, 1H, CH_2), 1.84–1.94 (m, 2H, CH_2), 2.01–2.10 (m, 1H, CH_2), 2.36 (dd, J=15.1, 6.7 Hz, 1H, CH_2), 2.53 (dd, J=15.1, 6.7 Hz, 1H, CH_2), 3.70–3.78 (m, 1H, OCH_2), 3.83–3.91 (m, 1H, OCH_2), 4.20 (quint, J=6.7 Hz, 1H, OCH). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C=25.5 (CH_2), 28.0 (O^{t}Bu), 31.1, 41.8 (CH_2), 68.9 (OCH_2), 75.4 (OCH), 80.4 (O^{t}Bu), 170.6 (O═C–O). IR (neat, cm^{-1}): $\tilde{\nu}$=2976 (m), 2946 (w), 2936 (w), 2874 (w), 1730 (s), 1455 (w), 1390 (w), 1368 (m), 1300 (w), 1288 (w), 1257 (m), 1208 (w), 1154 (s), 1102 (w), 1068 (m), 1020 (w). MS (EI, 70 eV): *m*/*z* (%)=186 (M^+, 1), 157 (12), 141 (4), 129 (49), 114 (17), 102 (16), 73 (10), 72 (96), 70 (45), 57 (100). MS (DCI, NH_3): *m*/*z* (%)=390 ([2×M+NH_4]$^+$, 23), 360 (15), 334 (13), 204 ([M+NH_4]$^+$, 47), 165 (28), 148 (100). The exact molecular mass *m*/*z*=186.1256±2 ppm [M^+] for $C_{10}H_{18}O_3$ was confirmed by HRMS (EI, 70 eV). Anal. Calcd for $C_{10}H_{18}O_3$ (186.248): C 64.49, H 9.74; found: C 64.52, H 9.75.

3.3.5. Methyl (3-methyltetrahydrofuran-2-yl)acetate (5e). Starting with **3e** (0.350 g, 2.24 mmol) and Pd/C (10% Pd, 1.192 g, 1.12 mmol) in methanol (15 mL), **5e** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→10:1) as a colorless oil (0.316 g, 89%, an inseparable 6:5 mixture of diastereomers). ^{1}H NMR ($CDCl_3$, 200 MHz, for both diastereomers): δ=0.92 (d, J=7.0 Hz, 3H, CH_3), 1.05 (d, J=6.5 Hz, 3H, CH_3), 1.48–1.67 (m, 2H, CH_2), 1.79–1.96 (m, 1H, CH), 2.01–2.19 (m, 2H, CH_2), 2.28–2.39 (m, 1H, CH), 2.42–2.61 (m, 4H, 2×CH_2), 3.70 (s, 6H, 2×OCH_3), 3.71–3.98 (m, 5H, 2×OCH_2, OCH), 4.19–4.29 (m, 1H, OCH). ^{13}C NMR ($CDCl_3$, 50 MHz): Major diastereomer: δ_C=16.8 (CH_3), 34.3 (CH_2), 39.0 (CH), 39.2 (CH_2), 51.6 (OCH_3), 67.0 (OCH_2), 81.7 (OCH), 171.9 (O═C–O). Minor diastereomer: δ_C=14.2, 33.5, 35.3, 36.0, 51.6, 66.4, 77.6, 172.1. IR (neat, cm^{-1}): $\tilde{\nu}$=2961 (s), 2876 (m), 1741 (s), 1455 (m), 1438 (m), 1381 (w), 1325 (w), 1303 (w), 1282 (m), 1257 (w), 1198 (m), 1169 (s), 1128 (w), 1107 (w), 1087 (m), 1043 (w), 1016 (m). MS (EI, 70 eV): *m*/*z* (%)=158 (M^+, 3), 143 (2), 130 (30), 127 (6), 98 (17), 85 (100), 72 (21). The exact molecular mass *m*/*z*=158.0943±2 ppm [M^+] for $C_8H_{14}O_3$ was confirmed by HRMS (EI, 70 eV).

3.3.6. Ethyl (3-ethyltetrahydrofuran-2-yl)acetate (5f). Starting with **3f** (0.280 g, 1.52 mmol) and Pd/C (10% Pd, 0.809 g, 0.76 mmol) in ethanol (15 mL), **5f** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→10:1) as slightly yellowish oil (0.268 g, 95%, inseparable 6:5 mixture of diastereomers). ^{1}H NMR ($CDCl_3$, 300 MHz, for both diastereomers): δ=0.94 (dt, J=7.2, 4.8 Hz, 6H, 2×CH_3), 1.27 (t, J=7.2 Hz, 6H, 2×OCH_2CH_3), 1.32–1.48 (m, 1H, CH), 1.50–1.66 (m, 4H, 2×CH_2), 1.70–1.82 (m, 1H, CH), 2 00–2.22 (m, 4H, 2×CH_2), 2.36–2.43 (m, 2H, CH_2), 2.45–2.56 (m, 2H, CH_2), 3.73 (q, J=7.8 Hz, 1H, OCH), 3.80 (t, J=6.0 Hz, 2H, OCH_2), 3.84–3.97 (m, 2H, OCH_2), 4.17 (q, J=7.2 Hz, 4H, 2×OCH_2CH_3), 4.37 (q, J=6.6 Hz, 1H, OCH). ^{13}C NMR ($CDCl_3$, 50 MHz): Major diastereomer: δ_C=12.6, 14.1 (CH_3), 25.5, 32.0, 40.1 (CH_2), 46.2 (CH), 60.4 (OCH_2CH_3), 67.1 (OCH_2), 80.3 (OCH), 171.8 (O=C–O). Minor diastereomer: δ_C=12.8, 14.1, 21.9, 30.5, 36.2, 43.3, 60.4, 66.6, 77.4, 171.5. IR (neat, cm^{-1}): $\tilde{\nu}$=2965 (s), 2934 (m), 2876 (m), 1738 (s), 1459 (w), 1377 (w), 1309 (m), 1261 (m), 1172 (s), 1141 (w), 1082 (m), 1036 (s). MS (EI, 70 eV): *m*/*z* (%)=186 (M^+, 1), 158 (44), 143 (22), 130 (5), 116 (10), 110 (13), 99 (100), 84 (16), 70 (31). The exact molecular mass *m*/*z*=186.1256±2 ppm [M^+] for $C_{10}H_{18}O_3$ was confirmed by HRMS (EI, 70 eV).

3.3.7. Octahydro-[2,3′]bifuranyl-2′-one (5g). Starting with **3g** (0.300 g, 1.95 mmol) and Pd/C (10% Pd, 0.500 g, 0.47 mmol) in ethanol (10 mL), **5g** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=50:1→1:1) as a colorless oil (0.261 g, 86%, an inseparable 3:2 mixture of diastereomers). ^{1}H NMR ($CDCl_3$, 300 MHz, for both diastereomers): δ=1.66–1.77 (m, 1H, CH_2), 1.84–2.07 (m, 6H, 3×CH_2), 2.11–2.46 (m, 5H, 3×CH_2), 2.64–7.72 (m, 1H, CH of minor diastereomer), 2.82–2.89 (m, 1H, CH of major diastereomer), 3.72–3.82 (m, 2H, OCH_2), 3.84–3.92 (m, 2H, OCH_2), 4.13–4.20 (m, 2H, OCH_2), 4.21–4.29 (m, 2H, OCH_2), 4.34–4.43 (m, 2H, 2×OCH). ^{13}C NMR ($CDCl_3$, 50 MHz): Major diastereomer: δ_C=25.8, 26.1, 28.0 (CH_2), 42.7 (CH), 67.0, 68.7 (OCH_2), 78.8 (OCH), 177.1 (O=C–O). Minor diastereomer: δ_C=23.9, 25.8, 29.9, 44.0, 66.9, 68.4, 77.4, 177.5. IR (neat, cm^{-1}): $\tilde{\nu}$=2078 (m), 2915 (w), 2876 (m), 1768 (s), 1455 (w), 1380 (m), 1218 (m), 1167 (s), 1133 (w), 1070 (m), 1026 (s), 956 (w). MS (EI, 70 eV): *m*/*z* (%)=155 (M^+, 27), 141 (7), 128 (19), 112 (9), 97 (5), 86 (24), 72 (77), 70 (53), 57 (48), 55 (43), 41 (100). Anal. Calcd for $C_8H_{12}O_3$ (156.181): C 61.52, H 7.74; found: C 61.46, H 7.94.

3.3.8. Ethyl (5-ethyltetrahydrofuran-2-yl)acetate (8a). *Method C*: Starting with **6** (0.500 g, 2.74 mmol) and Pd/C (10% Pd, 1.460 g, 1.37 mmol) in ethanol (30 mL), **8a** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→3:1) as a colorless oil (0.435 g, 85%, an inseparable 9:2 [*syn*/*anti*] mixture of diastereomers). *Method D*: Starting with **7a** (0.16 g, 0.87 mmol) and Pd/C (10% Pd, 0.469 g, 0.44 mmol) in ethanol (30 mL), **8a** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→3:1) as a colorless oil (0.11 g, 70%, an inseparable 3:1 [*syn*/*anti*] mixture of diastereomers). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.91 (t, J=7.2 Hz, 3H, CH_3), 1.26 (t, J=7.2 Hz, 3H, CH_3), 1.42–1.52 (m, 2H, CH_2), 1.53–1.65 (m, 2H, CH_2), 1.91–2.11 (m, 2H, CH_2), 2.44 (dd, J=15.0, 6.6 Hz, 1H, CH_2), 2.63 (dd, J=15.0, 6.6 Hz, 1H, CH_2), 3.79 (quint, J=6.6 Hz, 1H, OCH), 4.15 (q, J=7.2 Hz, 2H, OCH_2), 4.24 (quint, J=6.6 Hz, 1H, OCH). ^{13}C NMR ($CDCl_3$, 50 MHz): Major diastereomer: δ_C=9.8, 13.8 (CH_3), 28.5, 30.0, 30.7, 40.9 (CH_2), 59.9 (OCH_2), 74.9, 80.6 (CH), 170.9 (O=C–O). Minor diastereomer: δ_C=9.8, 13.8, 28.3, 30.9, 31.5, 40.6, 59.9, 74.5, 79.9, 170.8. IR (neat, cm^{-1}): $\tilde{\nu}$=2969 (s), 2936 (m), 2877 (m), 1737 (s), 1462 (m), 1376 (m), 1299 (m), 1252 (m), 1193 (s), 1165 (s), 1079 (s), 1036 (s), 957 (w). MS (EI, 70 eV): *m*/*z* (%)=186 (M^+, 4), 171 (4), 157 (64), 141 (6), 130 (78), 126 (5), 114 (27), 110 (100), 99 (78), 83 (54), 70 (76). MS (DCI, NH_3): *m*/*z* (%)=204 ([M+NH_4]$^+$, 100), 192 (43), 187 (M^+, 7), 151 (43), 134 (35), 108 (10). HRMS (ESI): Calcd for $C_{10}H_{18}O_3$ ([M+1]$^+$): 187.13342; found: 187.13281. Anal. Calcd for $C_{10}H_{18}O_3$ (186.248): C 64.49, H 9.74; found: C 64.46, H 9.78.

3.3.9. Methyl (5-chloromethyltetrahydrofuran-2-yl)acetate (8b). Starting with **7b** (0.500 g, 2.62 mmol) and Pd/C (10% Pd, 1.396 g, 1.31 mmol) in methanol (30 mL), **8b** was isolated without further purification as a slightly yellowish oil (0.505 g, 100%, inseparable 10:1 [*syn*/*anti*] mixture of diastereomers). ^{1}H NMR ($CDCl_3$, 300 MHz): Major diastereomer: δ=1.56–1.73 (m, 1H, CH_2), 1.84–1.89 (m, 1H, CH_2), 2.04–2.10 (m, 2H, CH_2), 2.52 (dd, J=15.3, 2.7 Hz, 1H, CH_2), 2.67 (dd, J=15.3, 3.0 Hz, 1H, CH_2), 3.45–3.63 (m, 2H, CH_2–Cl), 3.70 (s, 3H, OCH_3), 4.19 (m, 1H, OCH), 4.34 (m, 1H, OCH). ^{13}C NMR ($CDCl_3$, 75 MHz): Major diastereomer: δ_C=27.8, 29.8, 39.4 (CH_2), 46.0 (CH_2–Cl), 51.0 (OCH_3), 75.1, 77.5 (OCH), 170.1 (O=C–O). IR (neat, cm^{-1}): $\tilde{\nu}$=2955 (m), 2927 (w), 2879 (w), 1739 (s), 1649 (w), 1439 (m), 1390 (w), 1377 (w), 1352 (w), 1320 (w), 1298 (w), 1278 (w), 1260 (w), 1201 (m), 1177 (m), 1093 (m), 1065 (m), 1012 (w), 901 (w), 883 (w), 796 (w), 746 (w). MS (EI, 70 eV): *m*/*z* (%)=192 (M^+, 1), 163 (1), 161 (5), 157 (17), 143 (100), 132 (4), 127 (1), 125 (5), 121 (19), 119 (60), 116 (36), 110 (71), 103 (2), 101 (21), 85 (6), 83 (65), 77 (5), 75 (11), 72 (8), 70 (19). HRMS (ESI): Calcd for $C_8H_{13}ClO_3$ [M^+]: 193.0440 (^{37}Cl), 191.0469 (^{35}Cl); found: 193.0440 (^{37}Cl), 191.0466 (^{35}Cl).

3.3.10. Ethyl (4-methoxypyrrolidin-2-yl)acetate (11). Starting with **10**[21a] (0.200 g, 1.08 mmol) and Pd/C (0.345 g, 10% Pd, 0.32 mmol) in ethanol (10 mL), **11** was isolated without further purification as a yellowish oil (0.202 g, 100%, inseparable 10:1 mixture of diastereomers). ^{1}H NMR ($CDCl_3$, 300 MHz): Major diastereomer: δ=1.26 (dt, J=7.2, 1.8 Hz, 3H, CH_3), 1.94 (dt, J=10.6, 3.4 Hz, 1H, CH_2), 1.92–1.98 (m, 1H, CH_2), 2.87 (dd, J=17.3, 7.4 Hz, 1H, CH_2), 3.22 (dd, J=17.3, 7.2 Hz, 1H, CH_2), 3.33 (s, 3H, OCH_3), 3.40 (dd, J=12.5, 4.9 Hz, 1H, CH_2–NH), 3.54 (d, J=12.5 Hz, 1H, CH_2–NH), 3.71 (q, J=7.04 Hz, 1H, CH–NH), 4.07–4.11 (m, 1H, OCH), 4.18 (dq, J=7.2, 1.8 Hz, 2H, OCH_2), 8.00 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75 MHz): Major diastereomer: δ_C=13.9 (CH_3), 35.8, 37.0 (CH_2), 49.1 (CH_2–NH), 54.4 (CH–NH), 56.7 (OCH_3), 60.9 (OCH_2), 78.4 (OCH), 170.2 (O=C–O). IR (neat, cm^{-1}): $\tilde{\nu}$=3419 (m), 2982 (s), 2937 (s), 2831 (m), 2747 (m), 1732 (s), 1640 (w), 1445 (w), 1403 (m), 1381 (m), 1347 (w), 1324 (m), 1259 (m), 1202 (s), 1106 (s), 1068 (m), 1028 (m). MS (EI, 70 eV): *m*/*z* (%)=187 (M^+, 2), 159 (5), 156 (7), 142 (4), 128 (54), 111 (7), 100 (86), 96 (39), 84 (25), 69 (100). HRMS (ESI): Calcd for $C_9H_{17}NO_3$ ([M+1]$^+$): 188.12867; found: 188.12782.

3.4. General procedure for the enzymatic kinetic resolution of (tetrahydrofuran-2-yl)acetates

3.4.1. Analytical scale. For small-scale reactions, (tetrahydrofuran-2yl)acetate (**5a**,**b**) (0.025–0.035 mmol) and recombinant esterase Est56 solution (100 μl) were dissolved in phosphate buffer (ad 1000 μL, 50 mM, pH 7.5) and toluene (10% v/v). The mixture was shaken in a thermoshaker at 37 °C and 1400 rpm. After certain time intervals, to the sample (100 μL) taken, distilled water (100 μL) was added. The sample was acidified by HCl (aq, 1 N) addition, and extracted with diethylether (3×200 μL). The combined organic extracts were dried (Na_2SO_4) and from this solution, enantiomeric excess and conversions were determined by GC analysis. For **5a**,**b**, first substrate (ester) was extracted at neutral to basic pH, and then after acidification, the free acid, which was produced during the enzymatic hydrolysis, was extracted separately.

3.4.2. Preparative scale. The substrate (**5a**) (0.25–0.35 mmol) was added to a solution of recombinant esterase Est56 (crude extract with 50 U/mL, based on a *p*-nitrophenyl acetate assay, 1.0 mL) in phosphate buffer (50 mM, pH 7.5, 9 mL) and the mixture was stirred at 37 °C until 50% conversion (determined by GC analysis) was reached. *Method 1*: To the reaction mixture was added HCl (aq, 10%, 10 mL) and the resulting acidic mixture was extracted with dichloromethane (4×20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/EtOAc) to give the enantiomerically pure ester and hydrolysis product (acid), respectively. *Method 2*: To the reaction mixture were added water (10 mL) and aq Na_2CO_3 solution (concd, 1 mL), and the resulting basic mixture was extracted with dichloromethane (or diethylether) (4×20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo to give the enantiomerically pure ester without further purification. The aqueous layer was acidified by HCl addition (aq, 10%) and extracted with dichloromethane (4×20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo to give the enantiomerically pure hydrolysis product (acid) without further purification. In some cases, the residues were purified by column chromatography (silica gel, *n*-hexane/EtOAc) for better rotation values. Purity and structure of compounds were confirmed by ^{1}H NMR. For reaction details see Table 5.

3.4.3. Resolution of (−)-5a and (−)-12. Table 5, entry 4 (*Method 2*) Starting with racemic **5a** (50 mg, 0.35 mmol) and Est56 (1.5 mL), (−)-**5a** (20 mg, 40%) and (−)-**12** (22 mg, 49%) were isolated without further purification as slightly yellowish and colorless oils, respectively. Column chromatography is not recommended due to the invisibility of products on TLC.

3.4.4. Methyl (tetrahydrofuran-2-yl)acetate [(−)-5a].[23] GC (chiral column, 70 °C): retention time (min)=18.6, 99% ee. Rotation ($CDCl_3$): $[\alpha]_D^{20}$ −3.6. Spectral data is the same as given above for racemic **5a**.

3.4.5. (−)-2-(Tetrahydrofuran-2-yl)acetic acid [(−)-12].[23] GC (chiral column, after conversion to the methyl ester by using diazomethane, 70 °C): retention time (min)=18.7, 86.3% ee. Rotation ($CDCl_3$): $[\alpha]_D^{20}$ −6.9. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.52–1.64 (m, 1H, CH_2), 1.89–2.00 (m, 2H, CH_2), 2.08–2.18 (m, 1H, CH_2), 2.58 (d, J=0.9 Hz, 1H, CH_2), 2.60 (d, J=2.1 Hz, 1H, CH_2), 3.77–3.84 (m, 1H, OCH_2), 3.90–3.97 (m, 1H, OCH_2), 4.26 (quint, J=6.9 Hz, 1H, OCH), 9.93 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=25.5, 31.2, 40.0 (CH_2), 68.2 (OCH_2), 75.0 (OCH), 174.7 (O═C–OH). IR (neat, cm^{-1}): $\tilde{\nu}$=3404 (br), 2926 (m), 2861 (w), 1722 (s), 1433 (w), 1276 (w), 1261 (w), 1255 (w), 1252 (w), 1201 (w), 1171 (w), 1099 (w), 1058 (m). MS (EI, 70 eV): *m*/*z* (%)=130 (M^+, 14), 83 (1), 72 (1), 55 (3), 44 (100), 28 (97). MS (EI, 70 eV): *m*/*z* (%)=130 (M^+, 14), 83 (1), 72 (1), 55 (3), 44 (100), 28 (97).

3.4.6. Co-injection of (−)-5a and (−)-12. GC (chiral column, after conversion of the acid to the methyl ester by using diazomethane, 70 °C): retention times (min)=18.8 and 19.4.

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