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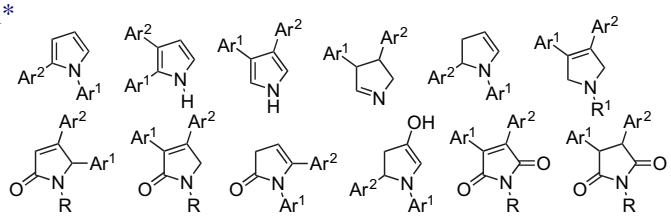
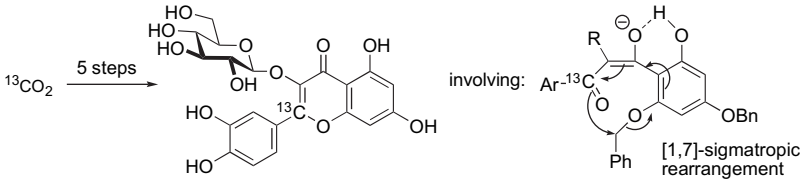
Contents

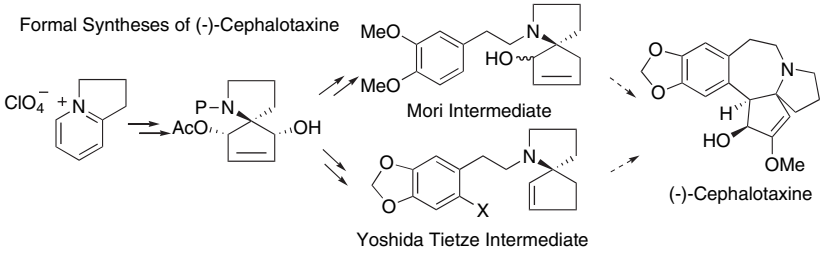
REPORT

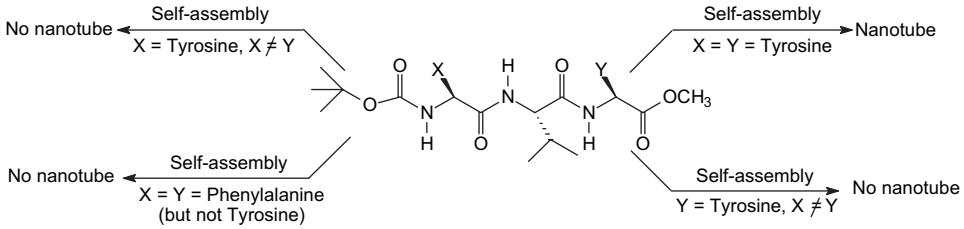


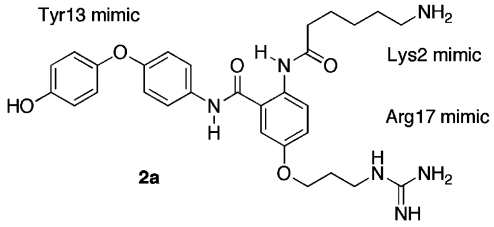
The methods used for the synthesis of vicinal diaryl-substituted pyrrole, pyrroline and pyrrolidine derivatives are reviewed. The report which contains 461 references also summarizes the bioactivity data of some of these compounds.

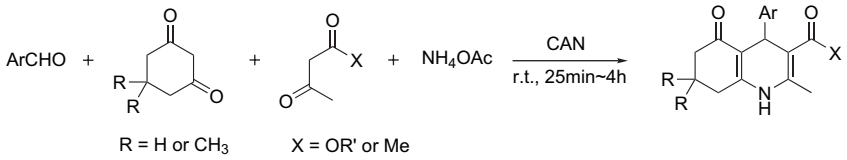
ARTICLES

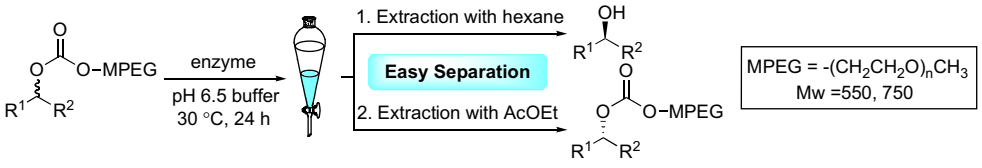


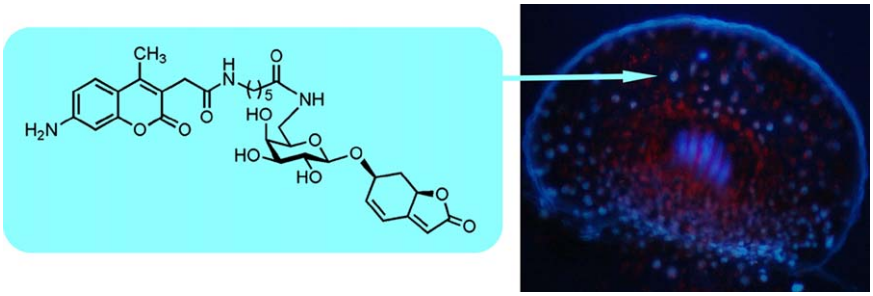


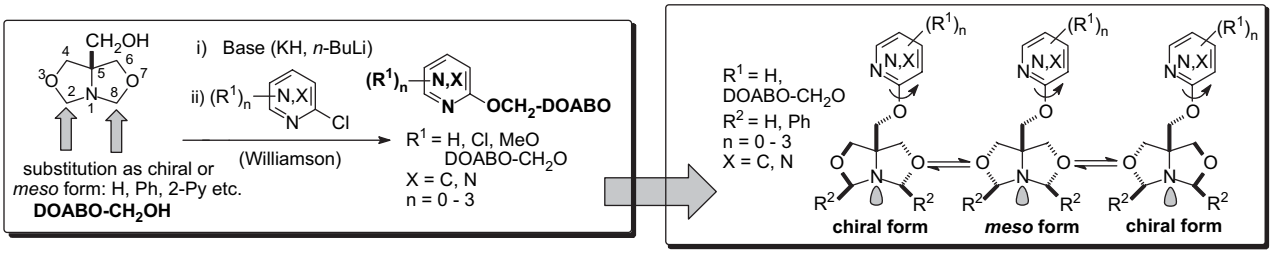


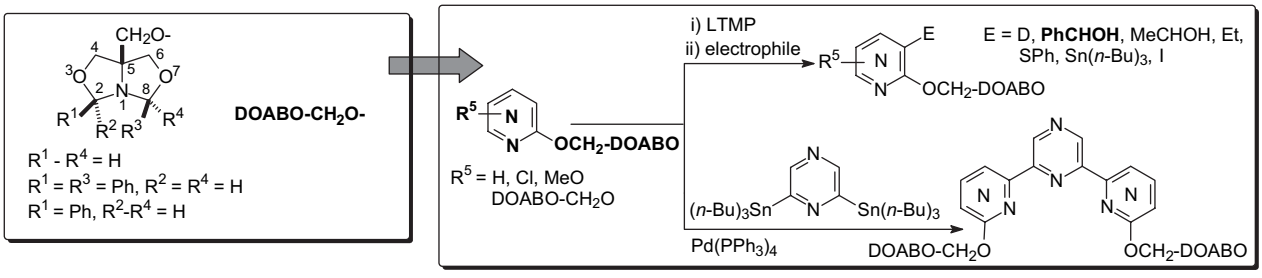


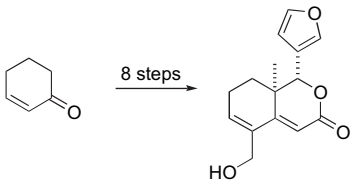


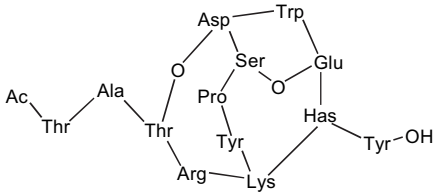




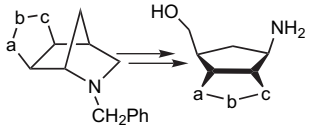




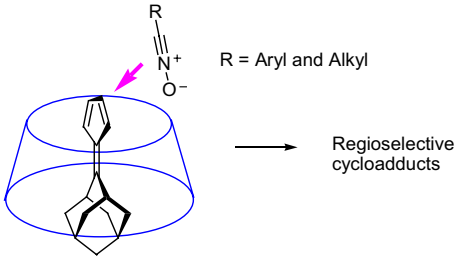


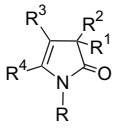


Microviridin SD1634

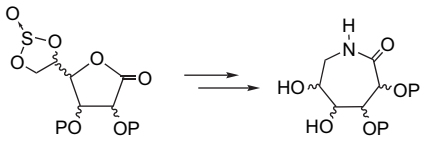


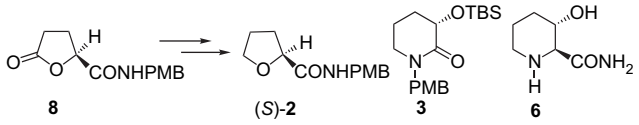
A novel approach to useful aminols for the synthesis of carbocyclic nucleosides is reported starting from a convenient source, the 2-azanorborn-5-enes. These are readily available through the Grieco cycloaddition of cyclopentadiene with iminium salts and are reactive dipolarophiles toward nitrile oxides. The prolific elaboration of the isoxazoline cycloadducts allowed preparation of the target aminols through the unmasking of the hydroxymethylene group at the C3 level of the azanorbornene structure.

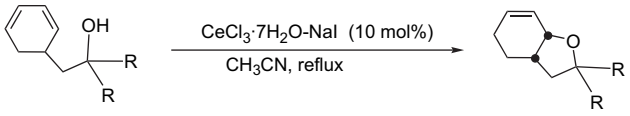


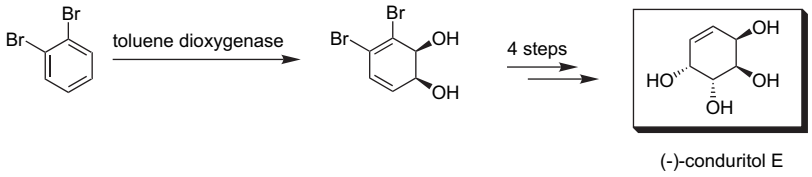


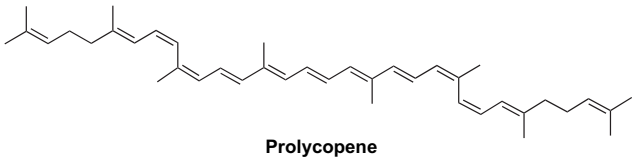
Atorvastatin undergoes a self-sensitized photooxygenation by sunlight in water. The main photoproducts, isolated by chromatographic techniques, have been identified by spectroscopic means. They present a pyrrol-2(3*H*)-one system arising from an oxidation of pyrrole ring and an alkyl/aryl shift. A mechanism involving singlet oxygen addition and an epoxide intermediate is suggested.



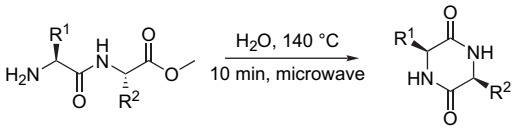








Prolycopene



63-97% yield

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

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Synthesis and biological activity of pyrrole, pyrroline and pyrrolidine derivatives with two aryl groups on adjacent positions

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Contents

1. Introduction

Five-membered heterocycle derivatives with two aryl groups on adjacent positions include several classes of natural and unnatural compounds that exhibit a variety of biological and biomedical properties.[1–10] Some excellent reviews concerning these properties have been published[11] but, to the best of our knowledge, no review has been devoted so far

Keywords: Pyrroles; Pyrrolines; Pyrrolidines; Synthesis; Bioactivity; Natural products.
Abbreviations: Ac, acetyl; Ar, aryl; Bn, benzyl; Bt, benzotriazol-1-yl; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, dimethylformamide; DMPA, *N*,*N*-dimethylaminopyridine; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; DOPA, 3,4-dihydroxyphenylalanine; GSK-3β, glycogen synthase-3β; HIV-1, human immunodeficiency virus type 1; HMG-CoA, hydroxymethylglutaryl-coenzyme A; HMPA, hexamethylphosphoric triamide; LHMDS, lithium hexamethyldisilazane; LTB_4, leukotriene-B_4; MDR, multidrug resistance; Me, methyl; NBS, *N*-bromosuccinimide; PKC, protein kinase C; SEM, 2-(trimethylsilyl)ethoxymethyl; TBAF, tetrabutylammonium fluoride; TBS, *tert*-butyldimethylsilyl; TIPS, triisopropylsilyl; TMEDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; TOSMIC, tosylmethyl isocyanide; Ts, *p*-toluenesulfonyl.
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to exhaustively summarizing and commenting on the procedures used for the synthesis of single classes of these diaryl-substituted heterocycles.

This review has the aim of covering the literature up to the end of September 2005 on the use of classical or improved methods and the design and development of new procedures for preparing pyrrole, pyrroline and pyrrolidine derivatives with two aryl groups on adjacent positions. It also aims to critically complete the picture of the studies summarized in several available reviews on the synthesis of substituted pyrroles[12] and to summarize several data concerning the biological properties of these vicinal diaryl-substituted heterocycles. In fact, these substances include unnatural compounds and a wide variety of substances isolated from natural sources (e.g., lamellarins,[1] lukianols,[13] ningalins,[14] storniamides,[15] arcyriarubins,[16] polycitones[17–19] and polycitrins[17]) that exhibit remarkable biological properties such as hypolipidemic,[20,21] antimicrobial,[22,23] anti-inflammatory[24] and antitumour activity[25,26] and are able to inhibit retroviral reverse transcriptases [i.e., human immunodeficiency virus type 1 (HIV-1)],[27] cellular DNA polymerases[27] and protein kinases.[28–31] Furthermore, some of these compounds are useful intermediates in the synthesis of biologically important naturally occurring alkaloids[32–47] and unnatural heterocycle derivatives.[48]

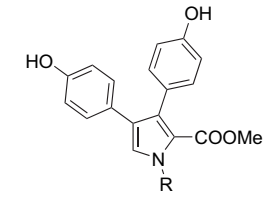
The topics that are covered in the review include (i) the description of the structures and properties of biologically active natural and unnatural pyrrole derivatives with two aryl groups on adjacent positions; (ii) a critical summary of the methods reported in the literature for the synthesis of 1,2-, 2,3- and 3,4-diaryl-1*H*-pyrroles, 1,2,3- (1,4,5-), 1,2,5-, 1,3,4-, 2,3,4- (3,4,5-) and 2,3,5-triaryl-1*H*-pyrroles, 1,2,3,5- and 2,3,4,5-tetraaryl-1*H*-pyrroles, 1,2,3,4,5-pentaaryl-1*H*-pyrroles and 2,3,3-triaryl-3*H*-pyrroles; (iii) a survey of the literature data on the biological properties and the methods used to prepare 1-, 2- and 3-pyrrolines, pyrrolin-2-ones, 2,3-dihydropyrrole-2,3-diones, pyrrolidines, 2-pyrrolidinones, 3-hydroxy-3-pyrrolin-2-ones and pyrrolidine-2,4-diones with two aryl groups on adjacent positions; (iv) a description of the structures and biological properties of naturally occurring symmetrical and unsymmetrical 2,3-diarylmaleimides (3,4-diaryl-pyrrolidine-2,5-diones); and (v) a critical summary of the methods designed and developed for preparing symmetrical and unsymmetrical 2,3-diarylmaleimides and 2,3-diarylsuccinimides (3,4-diarylpyrrolidine-2,5-diones).

This review does not cover the biological properties and the methods used to prepare vicinal diaryl-substituted nitrogen five-membered heterocycles fused with other rings such as indoles, indolizines, indolo[2,3-*a*]carbazoles, [1]pyrano[3,4-*b*]-pyrroles, 5,6-dihydropyrrolo[2,1-*a*]isoquinolines and pyrrolo[2,1-*a*]isoquinolines with two aryl groups on adjacent positions. These topics will, however, occasionally be tackled.

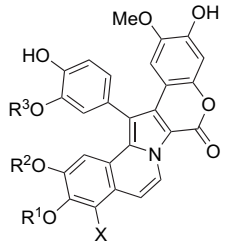
2. Pyrrole derivatives with two aryl groups on adjacent positions

2.1. Biologically active natural and unnatural pyrrole derivatives with two aryl groups on adjacent positions

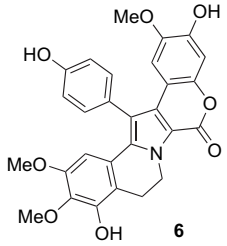
Pyrrole derivatives with two aryl groups on adjacent positions include important classes of marine natural products, some of which display remarkable biological and pharmacological properties. Thus, lamellarins O (**1**),[1,49] P (**2**),[1,49] Q (**3**)[1,50] and R (**4**)[1] are 3,4-diarylpyrrole-2-carboxylic acid esters, which belong to a large group of DOPA-[1-amino-3-(3′,4′-dihydroxyphenyl)propionic acid]-derived pyrrole alkaloids first isolated from the prosobranch mollusc *Lamellaria* sp.[12,51] and later obtained from the ascidian *Didemnum* sp.,[1,52–57] the Australian sponge *Dendrilla cactus*,[1,49,50] and an unidentified ascidian collected from the Arabian sea.[58]



1 : R = 4-(MeO)$C_6H_4COCH_2$
2 : R = 2-(HO),4-(MeO)$C_6H_3COCH_2$
3 : R = H
4 : R = 4-(HO)C_6H_4



5 : R^1 = X = H; R^2 = R^3 = Me
7 : R^1 = R^2 = R^3 = Me; X = OH
8 : R^1 = R^2 = R^3 = X = H



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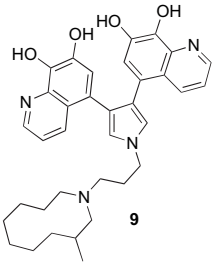
Virtually all of the lamellarins have been found to be cytotoxic to a wide range of cancer cell lines and the most potent of these compounds, i.e., lamellarins D (**5**), K (**6**) and M (**7**), have been shown to exhibit cytotoxicity values in the mid-to-high nanomolar range (38–110 nM).[59] Interestingly, lamellarins are also single-digit micromolar inhibitors of P-glycoprotein (P-gp) responsible for the multidrug resistance (MDR) effect and even at noncytotoxic concentrations they reverse MDR by inhibiting P-gp-mediated drug efflux.[32,59] Lamellarin D (**5**) is also a potent inhibitor of human topoisomerase I[60] and lamellarin H (**8**) is a potent inhibitor of both *Molluscum contagiosum* virus topoisomerase and HIV-1 integrase.[61] On the other hand, lamellarins O (**1**) and P (**2**) demonstrated antibiotic activity[49] and lamellarin D (**5**) caused inhibition of cell division.[51]

Other marine natural products possessing a 3,4-di(hetero)-aryl-substituted pyrrole ring as a common structural subunit include halitulin (**9**), which is a strongly cytotoxic pyrrole alkaloid isolated from the sponge *Haliclona tulearensis*,[26] lukianols A (**10**) and B (**11**), which have been found in an unidentified encrusting tunicate collected in the lagoon of the Palmyra atoll,[13] polycitones A (**12**) and B (**13**),[18] which have been isolated from the Indo-Pacific ascidian *Polycitor* sp.,[17,18] storniamides A (**14**), B (**15**), C (**16**) and D (**17**), which are alkaloids isolated from marine sponges of the genus *Clona*,[15] dictyodendrins A (**18**) and B (**19**), which are the first telomerase inhibitory marine natural products isolated from the Japanese marine sponge *Dictyodendrilla verongiformis*,[62] and ningalins A (**20**) and B (**21**), 3,4-diaryl-pyrrole derivatives bearing a 2-carboxylate group, which

have been isolated from the ascidian of the genus *Didemnum* collected in Western Australia near Ningaloo Reef.[14]



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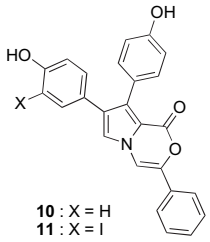
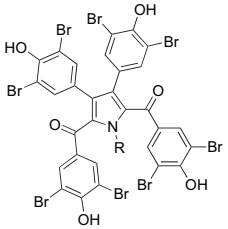
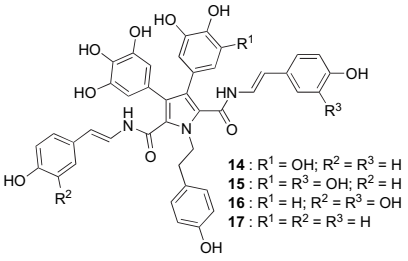


10 : X = H
11 : X = I

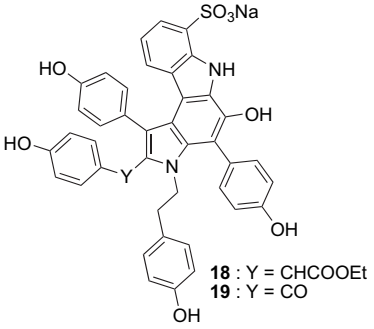


12 : R = CH_2-C_6H_4-4-OH
13 : R = H



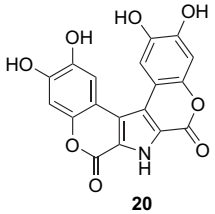
14 : R^1 = OH; R^2 = R^3 = H
15 : R^1 = R^3 = OH; R^2 = H
16 : R^1 = H; R^2 = R^3 = OH
17 : R^1 = R^2 = R^3 = H

Interestingly, halitulin (**9**), which incorporates the 3,4-bis(7′,8′-dihydroxyquinolin-5′-yl)-1*H*-pyrrole unit as key motif, was found to be cytotoxic against several tumour cell lines (e.g., P-388, A-549, HT-29 and MEL-28) with IC_{50} values in the 12–25 ng/mL range.[26]



18 : Y = CHCOOEt
19 : Y = CO

Such properties, coupled with the unique structure of this marine alkaloid, prompted a patent filing[63] claiming 3,4-bis(7′,8′-dihydroxyquinolin-5-yl)-1*H*-pyrroles as antitumour agents. On the other hand, lukianol A (**10**) was shown to exhibit cytotoxic activity against a cell line derived from human epidermatoid carcinoma (KB)[13] and afforded excellent cytotoxicity in the murine L1210 lymphoid leukaemia cell line and some human leukaemia cells with ED_{50} values less than 20 μM, which compared well with the clinical antineoplastic standards.[64] Storniamides A–D (**14**–**17**) showed antibiotic activity against several Gram-positive bacteria[15] and permethyl storniamide A (**22**), which lacks inherent cytotoxic properties, was shown to potently reverse MDR, resensitizing a resistant human colon cancer cell line (HCT 116–VM46) to vinblastine and doxorubicin.[32] Polycitone A (**12**) was found to be a potent inhibitor of retroviral transcriptases and cellular DNA polymerases,[27] while its penta-*O*-methyl derivative was found to inhibit the growth of SV40 transformed fibroblast cells at concentrations of 10 μg mL^{-1}.[17] Ningalin A (**20**), similar to lamellarin O (**1**), was found to lack cytotoxic activity, but proved to effectively reverse MDR.[31] Recently, compound **23**, which is a synthetic analogue of ningalins, was shown to be a potent MDR reversal agent that hypersensitizes P-gp-resistant tumour cell lines to front-line conventional therapeutic agents.[65]



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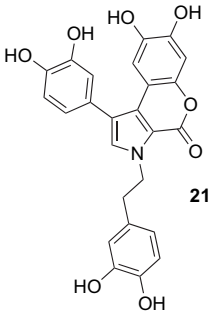

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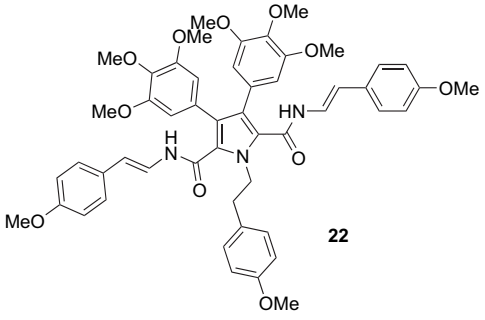
In this regard, it is worth mentioning that some literature data indicate that exhaustive O-methylation of the lateral hydroxyl groups of marine alkaloids consisting of a pyrrole core surrounded by a periphery of polyoxygenated phenyl rings significantly reduces the cytotoxicity of these compounds, but leaves the capacity of MDR reversal virtually unchanged.[32,36,66] Moreover, for the storniamide A core structure **24**, it has been demonstrated that this chemical modification goes hand in hand with a complete loss of the DNA-cleaving capacity of the alkaloid.[66]



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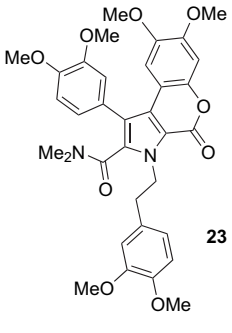

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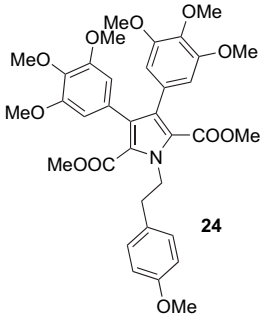


24

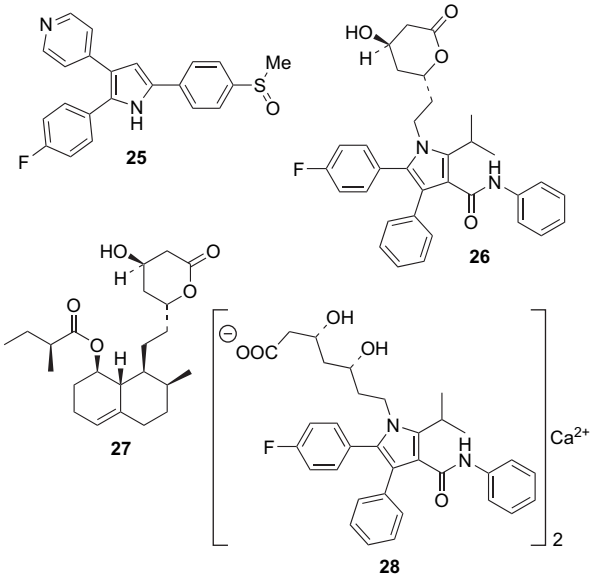
Several synthetic pyrrole derivatives with two (hetero)aryl groups on adjacent positions have also been shown to possess interesting biological and/or biomedical properties. Thus, 3-(4-pyridyl)-2-(4-fluorophenyl)-5-(4-methylsulfinylphenyl)-1*H*-pyrrole (**25**) was reported to be a potent, orally bioactive inhibitor of p38 mitogen-activated protein (MAP) kinase,[67] a family of serine/protein kinases that participate

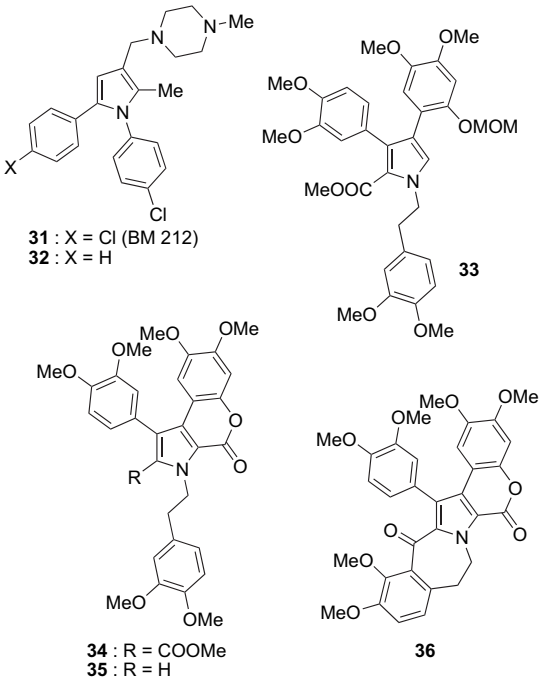
in signal transduction pathways controlling intracellular events, also involved in immunological and inflammatory disorders,[68–71a,72] including rheumatoid arthritis, inflammatory bowel disease, septic shock and osteoporosis, and that have recently been implicated in other disease states including Alzheimer's disease,[71b,c] cancer,[71d,e] asthma[73] and cardiovascular disease.[71f] 4,5-Diaryl-1*H*-pyrrole **26** is a hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitor fivefold more potent than the fungal metabolite compactin (mevastatin) (**27**) in vitro[74] and atorvastatin (**28**) is another hypolipidemic agent (CI-981)[20,21] currently marketed in the United States as LIPITOR. This last chiral 4,5-diarylpyrrole, in addition to its effect on lipoprotein profile, reduces triglycerides to a greater extent than other HMG-CoA reductase inhibitors.[21]

The pyridyl diaryl-1*H*-pyrrole **29**[75] has been reported to be a glucagon receptor antagonist, i.e., a substance able to block glucose production, and compounds **30a–c**[76] have also demonstrated good sugar-lowering activity. Thus, all these pyrrole derivatives might be used in a therapeutic approach to the treatment of diabetes.[77,78]

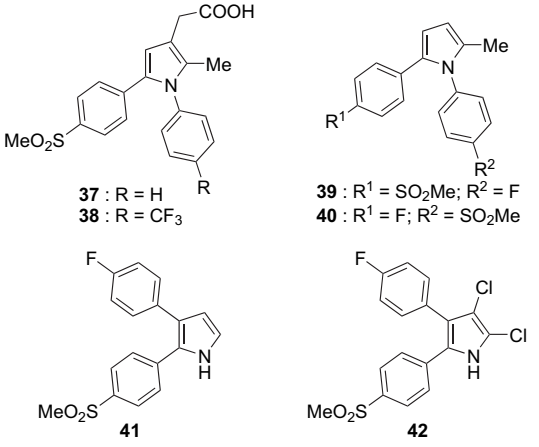
Compounds **31** (BM-212)[22] and **32**[23] have been described as antimicrobial agents. On the other hand, permethyl storniamide A (**22**),[32] and compounds **34**,[32] **33**,[36] **35**[36] and **36**,[36] like lamellarin D (**5**),[79] have been shown to be modulators of P-gp-mediated MDR.

Finally, 1,2-diaryl-1*H*-pyrroles **37**,[80] **38**,[80] **39**[81] and **40**[81] and 2,3-diaryl-1*H*-pyrroles **41**[82,83] and **42**[83] have been identified as cyclooxygenase-2 (COX-2)-selective inhibitors.[84]





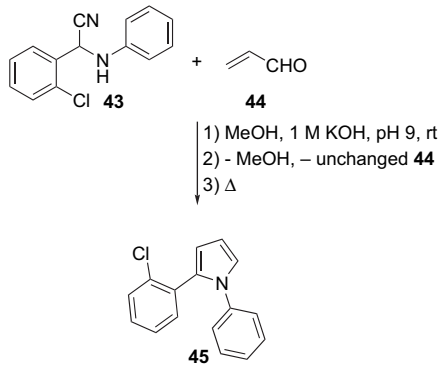
Interestingly, a correlation was found between the energy of the highest-occupied molecular orbital (E_{HOMO}) of anti-inflammatory 1,2-diaryl-1*H*-pyrroles and their COX-2 inhibition.[85] No correlation was, however, observed between E_{HOMO} and the inhibition efficiency of COX-1, the constitutively expressed enzyme, protective to organisms.[85] This result suggests that the inhibition of the two isomeric forms follows different molecular mechanisms.



2.2. Synthesis of pyrrole derivatives with two aryl groups on adjacent positions

2.2.1. Synthesis of 1,2-diaryl-1*H*-pyrroles. Several approaches have been described in the literature to the synthesis of 1,2-diaryl-1*H*-pyrroles. Thus, a low yielding procedure[86] involving the base-catalyzed condensation of a 2-(*N*-arylamino)-2-arylacetonitrile **43** with acrolein (**44**) was used to prepare 2-(2′-chlorophenyl)-1-phenyl-1*H*-pyrrole (**45**) (Scheme 1).[87]

Several other 1,2-diaryl-1*H*-pyrrole derivatives have been cleanly and more conveniently prepared in modest to high

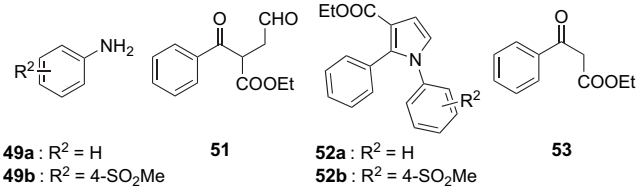


Scheme 1. Synthesis of 1,2-diaryl-1*H*-pyrrole **45**.

yields by Paal–Knorr condensation of 1,4-dicarbonyl compounds with appropriate arylamines.[23,80,81,88–90] The requisite 1,4-dicarbonyl compounds have often been obtained by the Stetter reaction,[91] which typically involves the reaction of aryl aldehydes with α,β-unsaturated ketones under cyanide or thiazolium salt catalysis. This approach was used for the efficient synthesis of a series of 1,2-diaryl-1*H*-pyrroles **50** having an alkyl group at position 5, which have been shown to be potent (IC_{50}=15–100 nM) and selective inhibitors of COX-2 (Scheme 2).[81]

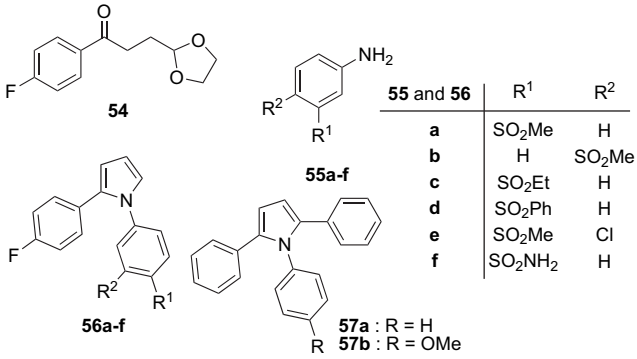
In particular, pyrroles **50** were prepared by condensation of arylamines **49** with 1,4-diketones **48** obtained by Stetter reaction of aryl aldehydes **46** with α,β-unsaturated ketones **47**.

Recently, ethyl 1,2-diaryl-1*H*-pyrrole carboxylates **52a** and **52b** have been analogously prepared by reaction of 3-ethoxycarbonyl-4-oxo-4-phenylbutanal (**51**) with anilines **49a** and **49b**, respectively, in ethanol in the presence of acetic acid.[90] The key synthon **51** was obtained by a three-step procedure starting from β-ketoester **53**.[90]



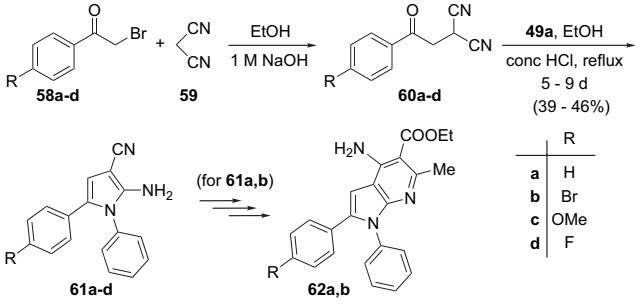
On the other hand, a Paal–Knorr-type reaction of 1,4-ketoacetal **54** with anilines **55a–f** in toluene in the presence of *p*-toluenesulfonic acid was used to synthesize some 1-(4-fluorophenyl)-2-aryl-1*H*-pyrroles **56a–f** in 50–70% yield.[81]

In 2005, the 5-alkyl-1,2-diaryl-1*H*-pyrroles **50a** (R=H) and **50b** (R=OMe) and the 1-aryl-2,5-diphenyl-1*H*-pyrroles **57a**,**b** have been prepared by Banik and co-workers using a modification of the Paal–Knorr reaction in which an arylamine is reacted with a 1,4-diketone in CH_2Cl_2 at room temperature in the presence of 5 mol % bismuth nitrate.[92]

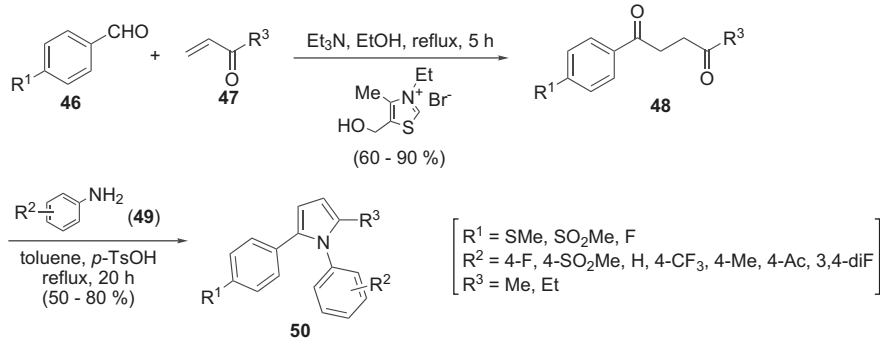


This modification, which requires very mild experimental conditions, has been shown to be much superior to the strong Lewis acid- or other strong acid-mediated syntheses of pyrroles in terms of the yields of the products. Moreover, unlike many other procedures, it does not require an extra energy source, like microwave irradiation or ultrasound.

In 2004, 1,2-diaryl-1*H*-pyrroles **61a–d**, which contain amino and cyano groups, have been synthesized in 39–46% yield by the reaction of the corresponding phenacylmalononitrile derivatives **60a–d** with aniline (**49a**) in absolute ethanol in the presence of catalytic amounts of concd HCl (Scheme 3).[93] Compounds **60a–d** have been prepared by treatment of phenacyl bromides **58a–d** with malononitrile (**59**) in ethanol in the presence of 1 M NaOH.[93,94] The pyrrole derivatives **61a** and **61b** have been used as precursors to two new pyrrolo[2,3-*b*]pyridines **62a** and **62b**, respectively, which are potent inhibitors of tumour necrosis factor.[93]

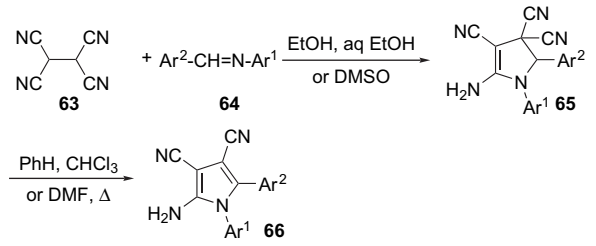


Scheme 3. Synthesis of compounds **61a–d** and **62a**,**b**.



Scheme 2. Synthesis of 5-substituted 1,2-diaryl-1*H*-pyrroles **50**.

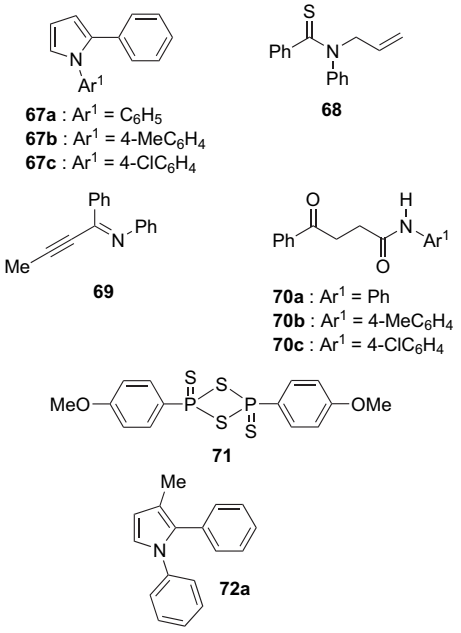
The synthesis of a great number of pyrroles of general formula **66**, which is similar to compounds **61** contain amino and cyano groups, had been previously performed in 88–98% yield by the reaction of tetracyanoethane (**63**) with the Schiff bases **64** in ethanol, aqueous ethanol or DMSO. The reaction produces tricyanodihydropyrroles **65** as intermediates, which lose hydrogen cyanide on heating in benzene, $CHCl_3$ or DMF to give the required pyrroles (Scheme 4).[95,96]



[Ar^1 = Ph, 4-HOC_6H_4, 4-ClC_6H_4, 2-pyridyl; Ar^2 = Ph, 2-HOC_6H_4, 2-furyl]

Scheme 4. Synthesis of compounds **66**.

In 1995, a two-step procedure involving the S-methylation of *N*-allyl-*N*-phenylthiobenzamide (**68**), followed by treatment of the resulting thioamidate salt with LHMDS was developed to prepare 1,2-diphenyl-1*H*-pyrrole (**67a**).[97]

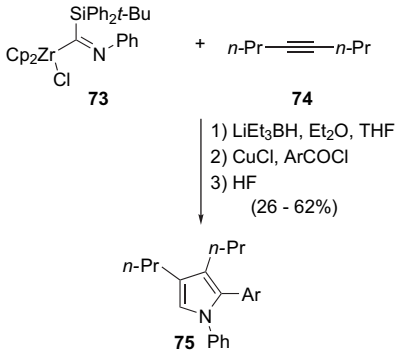


This compound was subsequently prepared in 86% yield by CuI-assisted cycloisomerization of the readily available imine **69**.[98] This procedure, which entirely satisfies the atom economy requisite, was also applied to the preparation of other 1,2-disubstituted and some 1,2,5-trisubstituted 1*H*-pyrroles.[98]

In 1998, Nishio reported that 1,2-diaryl-1*H*-pyrroles **67a–c** could be obtained in modest yields (35–48%) by reaction of the corresponding 4-ketoamides **70a–c** with an equimolar amount of Lawesson's reagent **71** in toluene at reflux temperature under argon.[99]

More recently, 3-methyl-1,2-diphenyl-1*H*-pyrrole (**72a**) and other polysubstituted 1*H*-pyrroles have been prepared in 50–91% yield by hydrogenolysis of the aldol products formed by reaction between α-(*N*-benzyl or *N*-Cbz)amino aldehydes and lithium enolates of various ketones.[100]

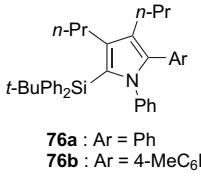
On the other hand, four tetrasubstituted 2-aryl-1-phenyl-1*H*-pyrroles of general formula **75** and some pentasubstituted pyrrole derivatives have been synthesized in low to modest yield via a CuCl-mediated one-pot reaction of acyl halides with the azazirconacyclopentene prepared from the iminosilacyl complex **73** and 4-octyne (**74**) (Scheme 5).[101]



[Ar = Ph, 4-MeC_6H_4, 4-$MeOC_6H_4$, 4-ClC_6H_4]

Scheme 5. One-pot synthesis of compounds **75**.

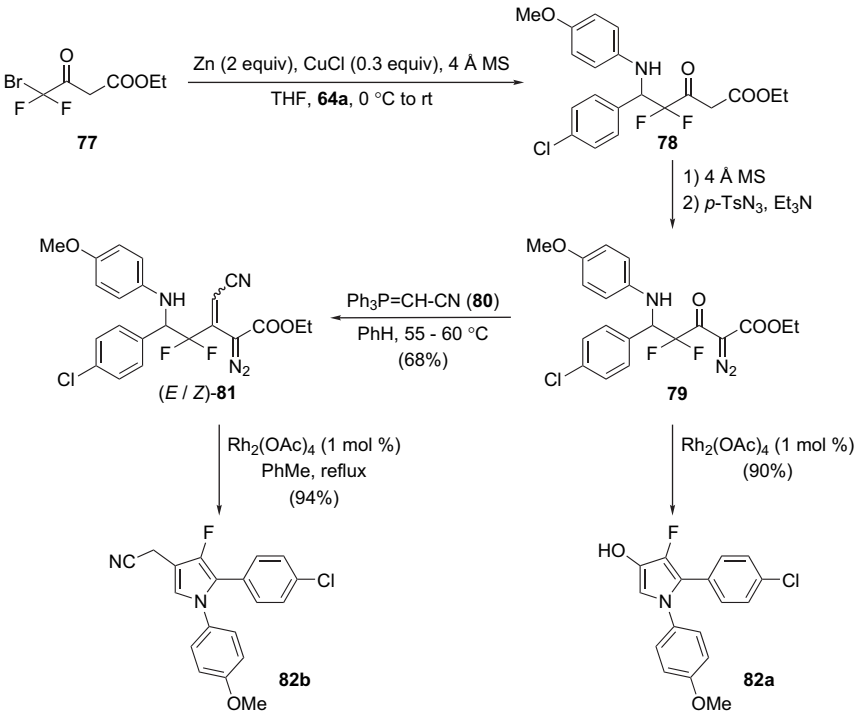
Interestingly, a catalytic amount of CuCl was found to be effective for this reaction.[101] Moreover, it was observed that, when the crude reaction mixture was treated with silica gel instead of HF, compounds **76a** and **76b** could be obtained in 50 and 55% yield, respectively.[101]



76a : Ar = Ph
76b : Ar = 4-MeC_6H_4

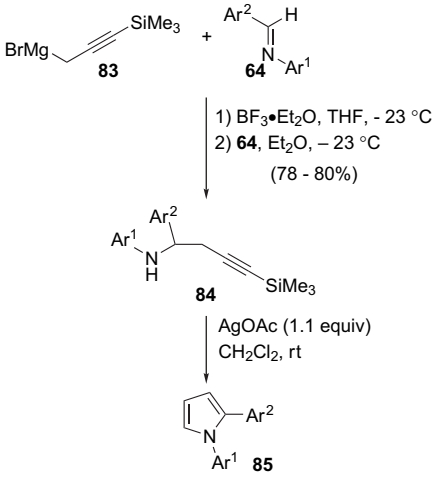
Wang and Zhu prepared the 1,2-diaryl-3-fluoro-1*H*-pyrroles **82a** and **82b** by $Rh_2(OAc)_4$-catalyzed intramolecular NH insertion of α-diazo-β-ketoester **79** and vinyldiazomethane **81**.[102] In particular, compound **82a** was synthesized in 94% yield by intramolecular NH insertion of vinyldiazomethane **79**, obtained, in good yield, by diazo transfer of compound **78** with tosyl azide and triethylamine. Compound **78** was, in turn, obtained from β-ketoester **77** and aldimine **64a** (Ar^1=4-MeC_6H_4; Ar^2=4-ClC_6H_4) (Scheme 6).[102] Compound **82b** was obtained by a Wittig reaction of the α-diazo-β-ketoester **79** with triphenylphosphoranylideneacetonitrile **80** to give the vinyldiazomethane **81** and a subsequent Rh(II)-catalyzed NH insertion (Scheme 6). Other polyfunctionalized 1,2-di(hetero)aryl-1*H*-pyrroles were obtained by similar reaction sequences from **77** and suitably substituted aldimines **64**.[102]

In 2004, Agarwal and Knölker[103] reported a novel procedure for pyrrole annulation via silver(I)-promoted oxidative cyclization of homopropargylamines **84**. This procedure, in which compounds **84** are easily prepared by addition of a propargyl Grignard reagent to the appropriate Schiff bases, has been used for the synthesis in high yields of



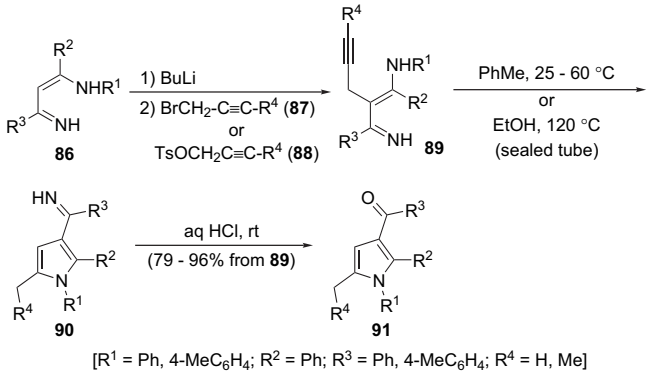
Scheme 6. Synthesis of compounds **82a–b**.

1,2-diarylpyrroles **85** starting from the Grignard reagent **83** and aldimines **64** (Scheme 7).[103]



Scheme 7. Synthesis of 1,2-diaryl-1*H*-pyrroles **85** from compounds **64** and **83**.

Previously, Barluenga and co-workers[104] had developed an efficient approach to 3-functionalized pyrroles **91** via propargylation/cycloamination of propargylazadienes **89** obtained in multigram quantities by metalation of azadienes **86**[105] with *n*-butyllithium, followed by C-alkylation with propargyl bromide **87** (R^4=H) or 2-butynyl *p*-toluenesulfonate (**88**) (R^4=Me) (Scheme 8). Interestingly, the primary cycloamination products **90** could not be isolated in pure form because of their easy hydrolysis when subjected to purification by column chromatography.[104] Their imine function could, however, be acylated or reduced in situ.[104]



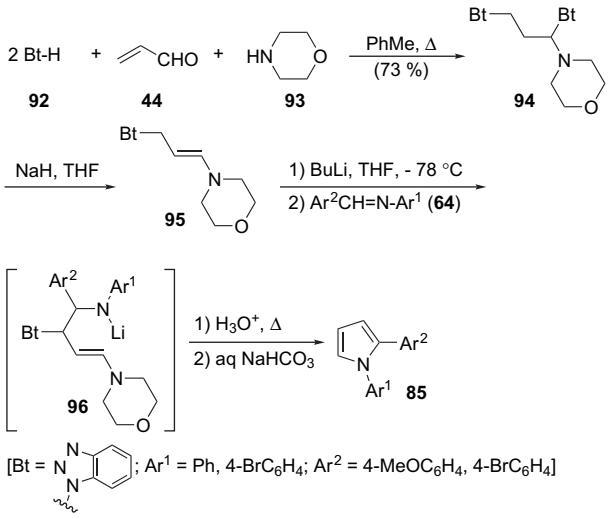
Scheme 8. Synthesis of 1,2-diaryl-1*H*-pyrroles **91**.

Some interesting procedures for the synthesis of a series of 1,2-diaryl-1*H*-pyrroles, which are based on the strong electron-withdrawing ability and nucleofugicity of the benzotriazolyl (Bt) group of benzotriazole derivatives, were developed by Katritzky and co-workers.[106–109] Despite the fact that these procedures are lengthy and do not fulfil the atom economy requisite, they were shown to be quite versatile and allowed the preparation of compounds not easily and conveniently available by other synthetic approaches.

In particular, in 1995, Katritzky and co-workers reported that 1-(3-morpholinoprop-2-enyl)benzotriazole (**95**), which can be prepared in quantity by a two-step procedure involving treatment of acrolein (**44**) with 2 equiv of benzotriazole (**92**) and 1 equiv of morpholine (**92**) and subsequent elimination of one benzotriazole moiety from **94** on treatment with NaH, is a valuable precursor of 1,2-diaryl-1*H*-pyrroles **85**.[106]

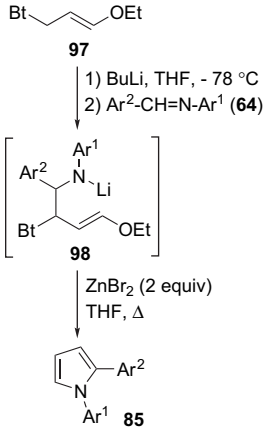
In fact, reaction of **95** with butyllithium, followed by addition of diarylimines **64** and brief heating in the presence of

a catalytic amount of sulfuric acid, provided the required pyrroles in 60–68% total yield via **96** (Scheme 9).[106]



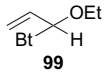
Scheme 9. Synthesis of 1,2-diaryl-1*H*-pyrroles **85** from compounds **44**, **92** and **93**.

In the same year, the same group expanded the synthetic applications of the benzotriazole derivatives and described that 1,2-diaryl-1*H*-pyrroles **85** can also be prepared regioselectively and in satisfactory yields by an approach involving treatment of 3-(benzotriazol-1-yl)-1-ethoxyprop-1-ene (**97**) with butyllithium at −78 °C, followed by addition of diarylimines **64** and heating of the resulting compounds **98** in the presence of $ZnBr_2$ (Scheme 10).[107]



Scheme 10. Synthesis of 1,2-diaryl-1*H*-pyrroles **85** from compounds **64** and **97**.

Compound **97**, which was the C_3 fragment in this [3+2] pyrrole synthesis, was prepared in 95% yield by reaction of 3-(benzotriazol-1-yl)-3-ethoxyprop-1-ene (**99**) with 1 equiv of $ZnBr_2$ in THF at room temperature.[107]

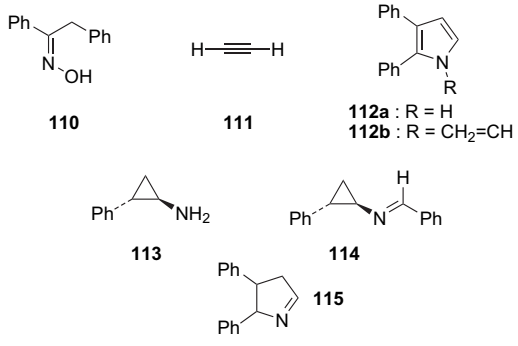


More recently, the synthesis of numerous compounds of general formula **85** and 1,2-diaryl-3-methyl-1*H*-pyrroles **72a**,**b** has been accomplished by a two-step procedure from imines **64** and *N*-allylbenzotriazole (**100**) and 2-(buten-3-yl)benzotriazole (**102**), respectively, via Pd(II)-catalyzed intramolecular oxidative cyclization (Scheme 11).[108] In particular, 1,2-diaryl-1*H*-pyrroles **85a–l** were prepared by oxidative cyclization of compounds **101** obtained by lithiation of **100** followed by treatment with imines **64**. On the other hand, 1,2-diaryl-3-methyl-1*H*-pyrroles **72a**,**b** were synthesized by intramolecular oxidative cyclization of compounds **103** prepared by lithiation of 2-(buten-3-yl)benzotriazole (**102**) and subsequent reaction with aldimines **64**. Interestingly, the yields of compounds **85a–l** were found to depend dramatically on the nature of the substituents in both the aromatic rings of imines **64**. In fact, a halogen in the *para*- or *meta*-position of these rings facilitated significantly the reaction, and the presence of electron-donor substituents, e.g., MeO, caused the opposite effect. On the contrary, the electron-donating or electron-withdrawing properties of a heterocyclic moiety did not have a significant effect on the yield of the resulting pyrrole derivatives.[108]

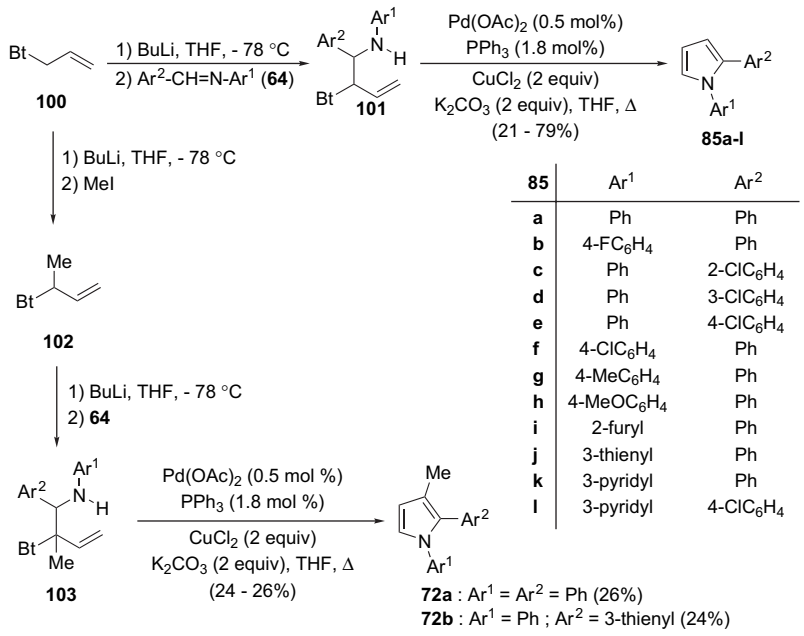
It should be noted that the benzotriazole synthetic methodology had also been previously used for the synthesis of the tetrasubstituted 1,2-diaryl-1*H*-pyrroles **109a** and **109b** from the acetylene dicarboxylates **108** and the 1,3-diaryl-2-(benzotriazol-1-yl)aziridines **106a** and **106b**, respectively, presumably via formation of azomethines **107a**,**b** (Scheme 12).[109]

Compounds **106a**,**b** were obtained in high yield by the reaction of 1-chloromethylbenzotriazole (**104**) with LHMDS in THF/HMPA at −20 °C, followed by treatment with imines **105**.[109]

2.2.2. Synthesis of 2,3-(4,5-)diaryl-1*H*-pyrroles. In 1978, 2,3-diphenyl-1*H*-pyrrole (**112a**) was prepared in 78% yield by the Trofimov reaction between oxime **110** and acetylene (**111**) under atmospheric pressure at 100 °C in DMSO in the presence of KOH.[110–112] When the initial pressure of **111** was 10–14 atm, however, the reaction furnished 2,3-diphenyl-1-vinyl-1*H*-pyrrole (**112b**) in 73% yield.[110,111]

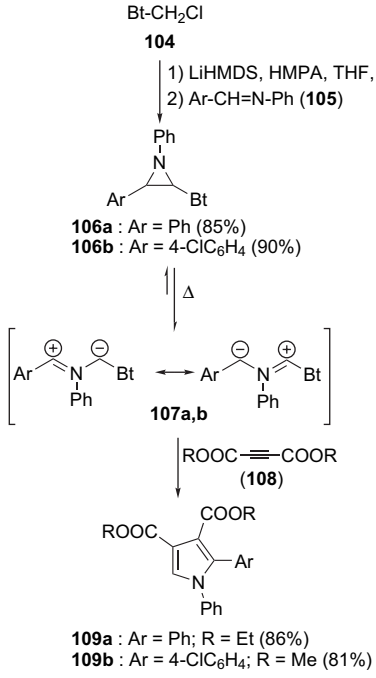


More recently, **112a** has been synthesized in 65% overall yield via photochemical rearrangement of *N*-cyclopropylimine **114**, followed by oxidation during the workup of the resulting crude 1-pyrroline **115**.[112] Imine **114** was obtained by reaction of commercially available *trans*-2-phenylcyclopropylamine (**113**) with benzaldehyde in refluxing toluene with occasional addition of molecular sieves.[112]

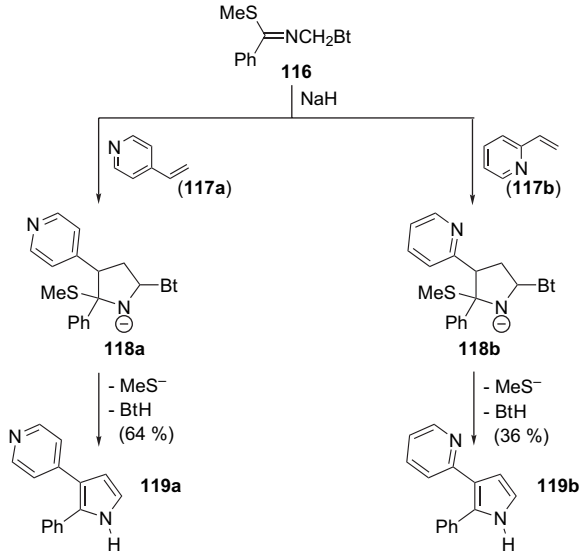


85	Ar^1	Ar^2
a	Ph	Ph
b	4-FC_6H_4	Ph
c	Ph	2-ClC_6H_4
d	Ph	3-ClC_6H_4
e	Ph	4-ClC_6H_4
f	4-ClC_6H_4	Ph
g	4-MeC_6H_4	Ph
h	4-$MeOC_6H_4$	Ph
i	2-furyl	Ph
j	3-thienyl	Ph
k	3-pyridyl	Ph
l	3-pyridyl	4-ClC_6H_4

Scheme 11. Synthesis of 1,2-diaryl-1*H*-pyrroles **85** and 1,2-diaryl-3-methyl-1*H*-pyrroles **72** from *N*-allylbenzotriazole **100**.



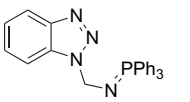
Scheme 12. Synthesis of compounds **109a** and **109b**.

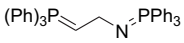


Scheme 13. Synthesis of compounds **119a** and **119b**.

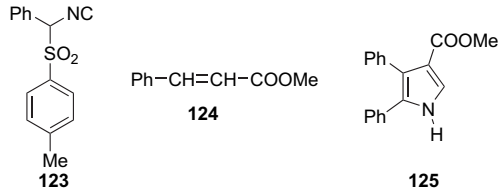
2-Phenyl-3-(pyridin-4-yl)-1*H*-pyrrole (**119a**) and 2-phenyl-3-(pyridin-2-yl)-1*H*-pyrrole (**119b**) had been previously synthesized in 64 and 36% yield, respectively, by [3+2] cycloaddition reactions of *S*-methyl *N*-(benzotriazol-1-ylmethyl)thioimidate (**116**) with the vinylpyridines **117a** and **117b**, followed by spontaneous elimination of benzotriazole and the thioalkoxy group (Scheme 13).[113]

On the other hand, 1,2-diphenyl-1*H*-pyrrole (**112a**), 2,3-bis(4-methoxyphenyl)-1*H*-pyrrole (**122a**) and 2,3-di(2-pyridin-2-yl)-1*H*-pyrrole (**122b**) were conveniently prepared in 67, 75 and 58% yield, respectively, by a Wittig/aza-Wittig reaction of the required 1,2-diketones with 1-aza-1,3-bis(triphenylphosphoranylidene)propane (**121**). This compound was synthesized in situ by treatment of 1-{[(triphenylphosphoranylidene)amino]methyl}-benzotriazole (**120**) with methylidenetriphenylphosphorane, followed by reaction with butyllithium.[114a,b]

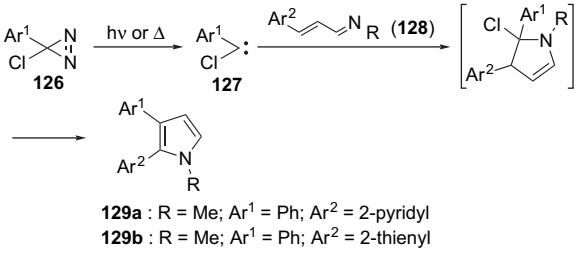




The synthesis of disubstituted 2,3-diaryl-1*H*-pyrroles different from **112a**, **119a**,**b** and **122a**,**b** has not been explored. On the contrary, since 1972, the development of efficient and/or simple protocols for the preparation of trisubstituted 2,3-diaryl-1*H*-pyrroles has received great attention. Specifically, 2,3-diphenyl-4-(methoxycarbonyl)-1*H*-pyrrole (**125**) was synthesized in 23% yield by an elegant approach based on van Leusen's chemistry, which involves treatment of α-tosylbenzyl isocyanide (**123**)[115] with the α,β-unsaturated ester **124** in Et_2O/DMSO in the presence of 1.2 equiv of NaH.[116]



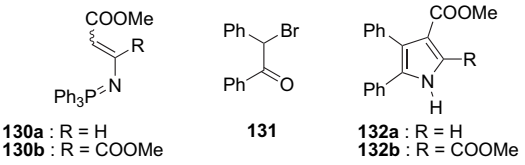
On the other hand, some 1,2,3-trisubstituted-1*H*-pyrroles that included 1-methyl- and 1-benzyl-2,3-diaryl-1*H*-pyrrole were synthesized in satisfactory yields by a method involving the reaction of arylchlorocarbenes with 1-azabuta-1,3-dienes.[117,118] In particular, arylchlorocarbenes **127**, generated by photolysis or thermolysis of arylchlorodiazirines **126**, were reacted with 1-azabuta-1,3-dienes **128** to give pyrroles **129** via, presumably, the dihydropyrrole derivatives (Scheme 14).[117] 3,4-Diaryl-1*H*-pyrroles **129** were prepared in 40–65% total yield via thermolysis of **126** and in 30–50% total yield via photolysis of these three-membered heterocycles.[117]



Scheme 14. Synthesis of compounds **129**.

The 2-aryl-3-heteroaryl-1-methyl-1*H*-pyrroles **129a**,**b** were, however, obtained only in 14–15% yield by flash photolysis of the corresponding heteroarylchlorodiazirines in the presence of the required 1-azabuta-1,3-dienes.[118]

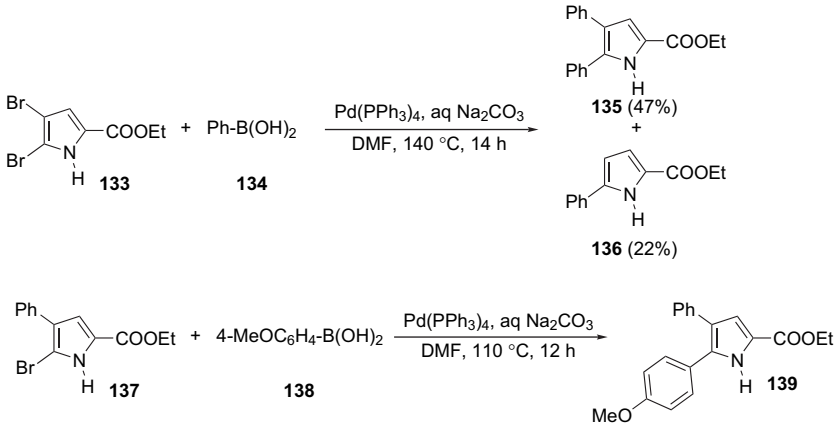
In 2002, the trisubstituted 2,3-diaryl-1*H*-pyrrole **132a** and the tetrasubstituted 2,3-diaryl-1*H*-pyrrole **132b** were synthesized in 43 and 57%, respectively, by reaction of *N*-vinylic phosphazenes **130a** and **130b** with α-bromoketone **131** in toluene at 110 °C in the presence of Et_3N.[119] This procedure was also used for the synthesis of three disubstituted 1*H*-pyrroles in satisfactory yields.[119]



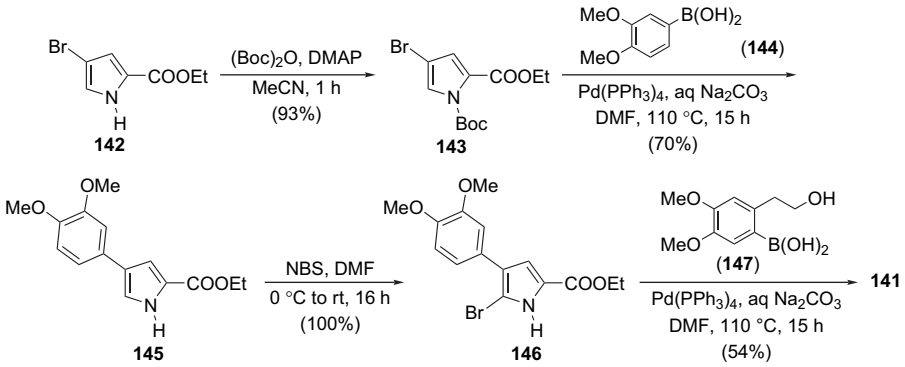
Sometimes, Pd-catalyzed cross-coupling reactions involving organometallic compounds and halopyrroles have also been used to access 2,3- (4,5-)diaryl-1*H*-pyrrole derivatives. Thus, Pd-catalyzed Suzuki-type reactions have been used for the synthesis of the trisubstituted 4,5-diaryl-1*H*-pyrroles **135** and **139** from dibromopyrrole **133** and bromopyrrole **137**, respectively (Scheme 15).[120] Interestingly, the cross-coupling reaction between **133** and phenylboronic acid (**134**) provided **135** along with a significant amount of the monoarylated pyrrole **136**.[120] This last compound most likely derived from a Suzuki-type reaction involving 2-bromo-5-ethoxycarbonyl-1*H*-pyrrole formed by selective dehalogenation of **133** during the Pd-catalyzed cross-coupling reaction.

On the other hand, the Pd-catalyzed reaction between **137** and arylboronic acid **138** furnished cleanly the diaryl-1*H*-pyrrole derivative **139** in 74% yield.

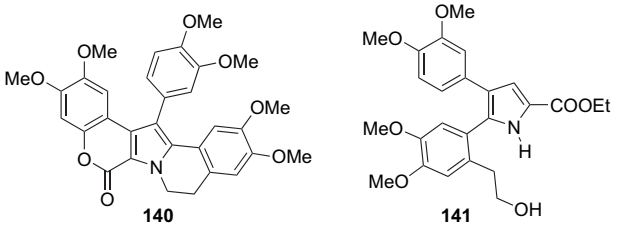
In 2004, a 4,5-diaryl-1*H*-pyrrole 2-carboxylic acid ethyl ester **141** was prepared via regioselective halogenation/Pd-catalyzed cross-coupling reactions in the course of a study concerning the total synthesis of lamellarin G trimethyl ether (**140**) (Scheme 16).[121]



Scheme 15. Synthesis of compounds **135**, **136** and **139**.



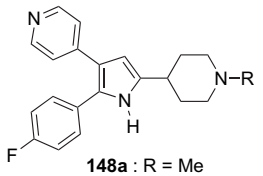
Scheme 16. Synthesis of compound **141**.



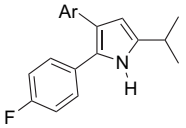
Specifically, the bromopyrrole ester **142**, prepared in three steps from pyrrole,[121] was protected as the corresponding *tert*-butyl carbamate to give **143** in 93% yield. It was necessary to perform this reaction prior to a Pd-catalyzed Suzuki-type reaction, since it had been previously found that the nitrogen of **142** must be protected to avoid extensive dehalogenation during the cross-coupling reaction.[121] In fact, the Suzuki-type reaction of **143** with 2–3 equiv of boronic acid **144** proceeded cleanly to give **145** in 70% yield. Treatment of this compound with an equimolar amount of *N*-bromosuccinimide led cleanly to the 5-bromo derivative **146**, which was finally coupled with boronic acid **147** under standard Suzuki-coupling conditions to give **141** in 54% yield (Scheme 16).[121]

Several 2-aryl-3-(4-pyridyl)-5-(*N*-substituted)piperidyl-1*H*-pyrroles have been synthesized by reaction of the corresponding 1,4-dicarbonyl compounds with ammonium acetate in acetic acid at 110 °C.[122] More recently, these trisubstituted pyrrole derivatives have been evaluated as inhibitors of *Eimeria tenella* cGMP-dependent protein kinase and in vivo anticoccial assays and, among these substances, compounds **148a** and **148b** have been shown to be the most potent and have demonstrated a broad spectrum of activity.[123]

Previously, in the context of a study concerning the development of novel potent inhibitors of HMG-CoA reductase, a Paal–Knorr condensation had been used to prepare the trisubstituted pyrroles **149a–c**, free of the corresponding regioisomers.[124,125]

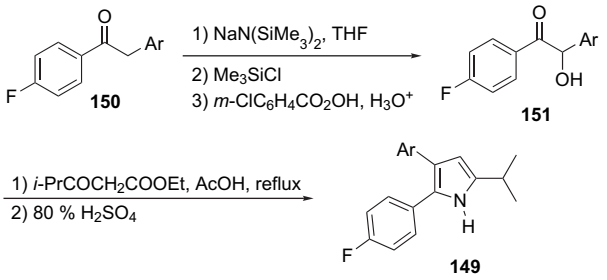


148a : R = Me
148b : R = Et



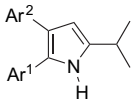
149a : Ar = 4-pyridyl
149b : Ar = 2-pyridyl
149c : Ar = 4-pyridyl-*N*-oxide
149d : Ar = 4-FC_6H_4

Some compounds of general formula **149** were alternatively obtained by hydroxylation of 4-fluorophenyl ketones **150**, followed by cyclocondensation of the resulting benzoins **151** with ethyl isobutyrylacetate in the presence of ammonium acetate in refluxing acetic acid (Scheme 17).[125] Compound **149d** was so prepared in % yield from **151a** (Ar=4-F–C_6H_4).



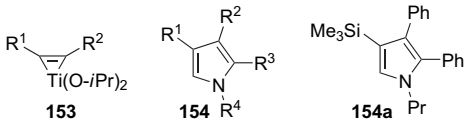
Scheme 17. Synthesis of compounds **149**.

When unsymmetrical benzoins were, however, used, both 5- and 4-(fluorophenyl)-1*H*-pyrroles **152a** and **152b** were formed in an approximate ratio of 9:1.[125]

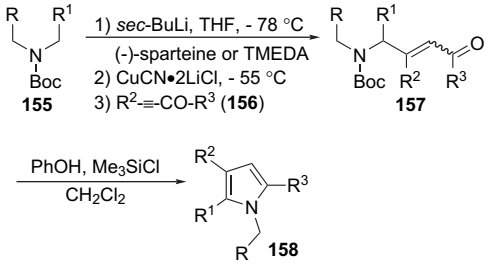


152a : Ar^1 = 4-FC_6H_4; Ar^2 = 4-pyridyl, 2-pyridyl, 4-pyridyl-*N*-oxide
152b : Ar^1 = 4-pyridyl, 2-pyridyl, 4-pyridyl-*N*-oxide; Ar^2 = 4-FC_6H_4

To the best of our knowledge, only four methods have been reported in the literature for the synthesis of tetrasubstituted 2,3-diaryl-1*H*-pyrroles.[119,126–128] The first method, which was used for the preparation of **132b**, has been previously discussed. The second method was developed in 1996 by Sato and co-workers[126] in the context of the one-pot synthesis of substituted pyrroles **154**, which included the tetrasubstituted 2,3-diaryl-1*H*-pyrrole **154a**. This convenient method allowed the preparation of the required compounds in good-to-excellent yields from the titanium–acetylene complexes **153**.[126]

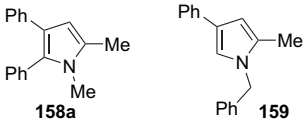


The third method was subsequently designed and developed by Dieter and Yu, who synthesized some polysubstituted pyrroles **158** by conjugate addition of *N*-protected α-aminoalkylcuprates derived from amines **155** to alkynyl ketones **156**, followed by amine deprotection and cyclization of the resulting adducts **157** (Scheme 18).[127]



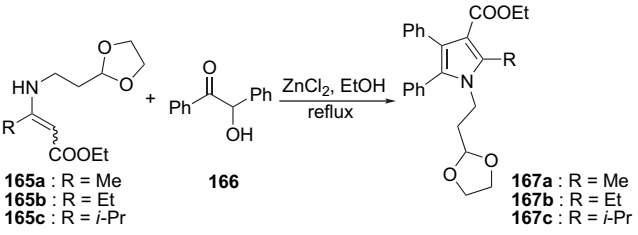
Scheme 18. Synthesis of polysubstituted pyrroles **158**.

This protocol, which exhibits a broad scope, was also used to prepare in 50% yield a mixture of the tetrasubstituted pyrrole **158a** and the trisubstituted pyrrole **159** in which **158a** was the major component.[127]

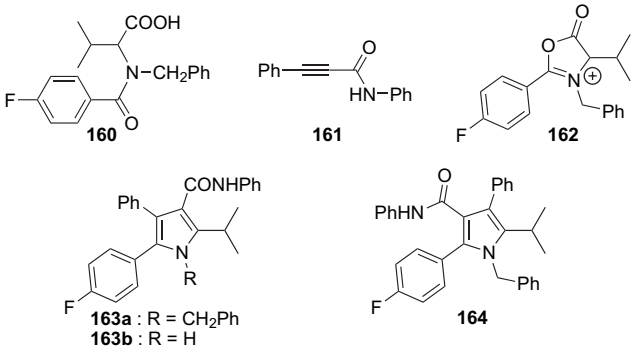


In 2004, Pandey and Rao developed the fourth method for the synthesis of tetrasubstituted 2,3-diaryl-1*H*-pyrroles.[128] These authors prepared efficiently and economically compound **163b**, which was used as a key intermediate for the synthesis of the HMG-CoA reductase inhibitor atorvastatin (**28**).[128] A key step of this method was the 1,3-dipolar cycloaddition reaction of mesoionic münchnone (1,3-oxazolium-5-olate) **162**, derived from cyclodehydration of **160**, with *N*-1,3-diphenyl-2-propynamide (**161**). This reaction furnished in 80% yield a mixture of the regioisomers **163a** and **164** in a 1:1 ratio, which were easily separated by crystallization. Regioisomerically pure **163a** could be easily debenzylated using sodium in liquid ammonia in the presence of *t*-BuOH at −78 °C to give **163b** in 83% yield.[128]

On the contrary, the development of methods useful for the synthesis of pentasubstituted 2,3-(4,5-)diaryl-1*H*-pyrroles has received little attention. In 1991, two of these compounds containing an ester group at position 4, i.e., **167a** and **167b**, were synthesized in 75 and 36% yield, respectively, by $ZnCl_2$-catalyzed condensation of benzoin (**166**) with enamines **165a** and **165b** (Scheme 19).[129]

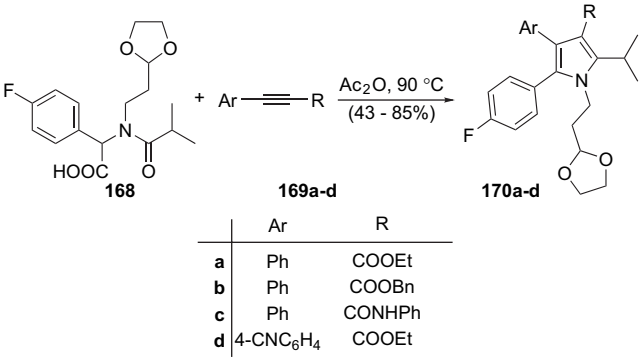


Scheme 19. Synthesis of pentasubstituted pyrroles **167a–c**.



These last compounds were prepared from (2-aminoethyl)-1,3-dioxolane[130] and the requisite β-ketoesters. The procedure used to prepare **167a** and **167b**, however, proved to be ineffective for the synthesis of the more sterically hindered pyrrole **167c**, which was obtained from **165c** in 4% yield (Scheme 19).[129]

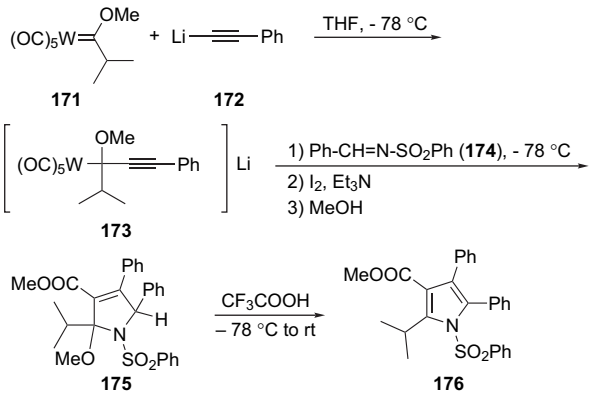
Four additional pentasubstituted 2,3-diaryl-1*H*-pyrroles containing an ester or an amide group at position 4, i.e., compounds **170a–d**, were regioselectively synthesized by [3+2] cycloaddition of the readily available amidoacid **168** with acetylenes **169a–d** (Scheme 20).[129] Compounds **167a–c** and **170a–d** were then elaborated at their position 1 to give derivatives able to inhibit the enzyme HMG-CoA reductase.[129]



	Ar	R
a	Ph	COOEt
b	Ph	COOBn
c	Ph	CONHPh
d	4-CNC_6H_4	COOEt

Scheme 20. Synthesis of pentasubstituted pyrroles **170a–d**.

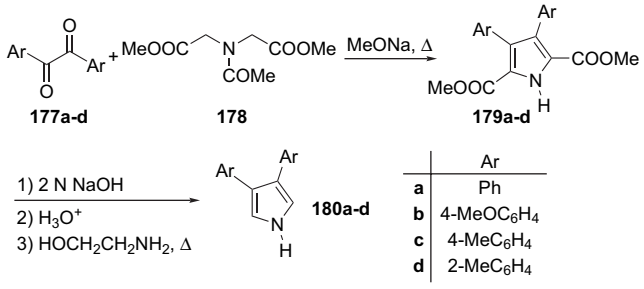
More recently, the pentasubstituted 2,3-diaryl-1*H*-pyrrole **176** has been prepared in 70% yield using a highly efficient method for the synthesis of fully substituted five-membered heterocycles from tungsten carbene complexes.[131] Specifically, complex **171** was first reacted with 1-lithium-1-alkyne **172** at −78 °C and the resulting compound **173** was then treated with sulfonylimine **174**. The iodine oxidation of the resulting crude reaction product gave dihydropyrrole **175**, which was then treated with trifluoroacetic acid to produce pyrrole **176** (Scheme 21).[131]



Scheme 21. Synthesis of compound **176**.

2.2.3. Synthesis of 3,4-diaryl-1*H*-pyrroles. 3,4-Diaryl-1*H*-pyrrole moieties appear frequently in naturally occurring compounds, such as lamellarins,[12] lukianols,[13] ningalins,[14] storniamides[15] and their congeners, that elicit important biological responses. The biological activities of 3,4-diaryl-1*H*-pyrrole derivatives have made them popular synthetic targets and numerous methods for the synthesis of these heterocycle derivatives have been developed.

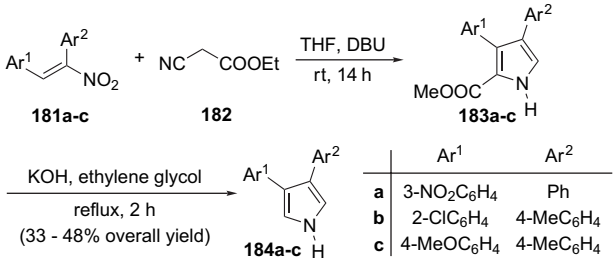
Several years ago, compounds **180a–d** were prepared in 45, 57, 41 and 2.5% overall yield by reaction of dimethyl *N*-acetyliminodiacetate (**178**) with benzyls **177a–d** in the presence of sodium methoxide, followed by hydrolysis and decarboxylation of the resulting pyrrole dicarboxylic acid esters **179a–d** (Scheme 22).[132a] Notable is that two 2,5-diamidopyrroles synthesized from diester **179a** have recently been shown to function as effective receptors for oxoanions.[132b]



Scheme 22. Synthesis of 3,4-diaryl-1*H*-pyrroles **180a–d**.

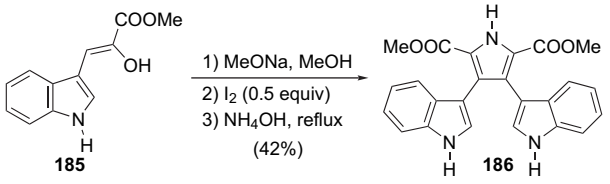
Compound **180a** was also subsequently prepared by the reaction of β-nitrostyrene (**181a**) with aqueous $TiCl_3$.[133,134] Although THF was used as the solvent in the original literature, in 1988 it was reported that replacement of THF with dioxane increases the yield of **180a** from 25 to 50%.[134] This modified procedure was used to prepare several other 3,4-diaryl-1*H*-pyrroles in 19–50% yield from the corresponding β-nitrostyrenes.

3,4-Diaryl-1*H*-pyrroles **184a–c** were prepared in low to modest yields from the α-nitrostyrenes **181a–c** and ethyl isocyanoacetate (**182**) by the Barton–Zard pyrrole synthesis and treatment of the resulting pyrrole carboxylic methyl esters **183a–c** with KOH in refluxing ethylene glycol (Scheme 23).[134–136]



Scheme 23. Synthesis of unsymmetrical 3,4-diaryl-1*H*-pyrroles **184a–c**.

The synthesis of lycogalic acid A dimethyl ester (**186**), also named lycogarubin C, which is a metabolite isolated from the fruit bodies of the myxomycete *Lycogala epidendrum*,[137] was accomplished by a one-pot reaction involving the oxidative coupling of two molecules of methyl 3-(indol-3-yl)-pyruvate (**185**) and the Paal–Knorr condensation of the resulting crude 1,4-diketone with ammonium hydroxide (Scheme 24).[138]

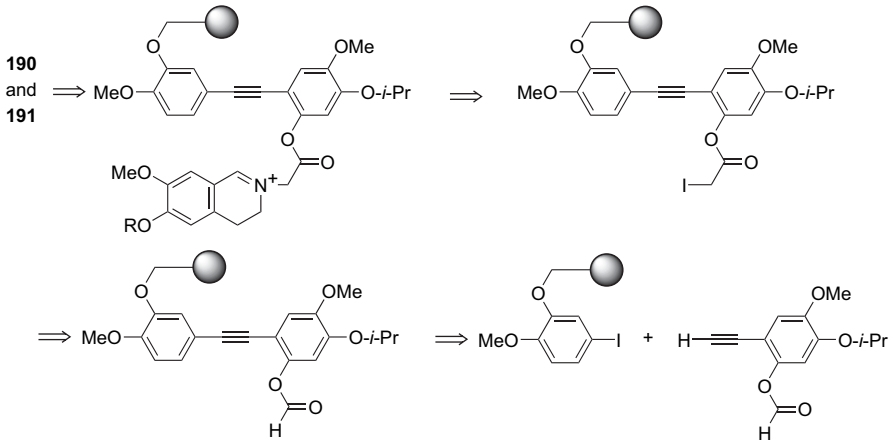


Scheme 24. Synthesis of lycogalic acid A dimethyl ester (**186**).

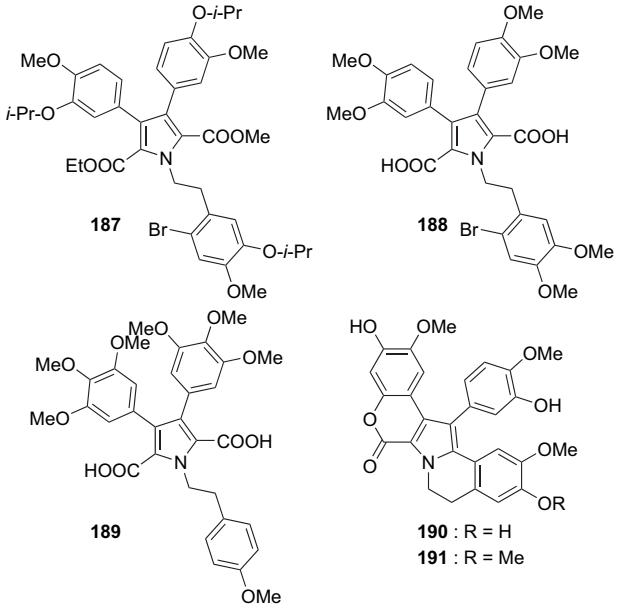
The first total synthesis of ningalin A (**20**),[139] a biomimetic synthesis of some 3,4-diaryl-1-pyrrole-2,5-dicarboxylic acids[140] and the preparation of compounds **187**,[35] **188**[141] and **189**[142] were analogously performed by oxidative dimerization of the required arylpyruvic acids, followed by condensation of the resulting 1,4-dicarbonyl compounds with the suitable 2-arylethylamines. The pyrrole derivatives **187**, **188** and **189**, prepared in 53, 62 and 56% yield, respectively, were then used as precursors to lamellarin L (**190**),[35] lamellarin G trimethyl ether (**140**)[141] and storniamide A nonamethyl ether (**22**),[142] respectively.

More recently, pentacyclic lamellarins L (**190**) and U (**191**) have been synthesized in the solid phase on the basis of a retrosynthetic analysis (Scheme 25) in which an intramolecular [3+2] cycloaddition of a 3,4-dihydroisoquinolinium salt over a triple C–C bond was a key step.[143,144]

In 2002, Smith and co-workers[145] performed an efficient one-pot synthesis of symmetrical and unsymmetrical 3,4-diaryl-1*H*-pyrroles of general formula **180** and **184**, which consisted of the reaction between symmetrical and unsymmetrical (*E*)-1,2-diarylethenes **192** and **193**, respectively, with a molar excess of tosylmethyl isocyanide (TOSMIC) (**194**)[146] in DMSO at 25–80 °C in the presence of 2 equiv of *t*-BuONa.

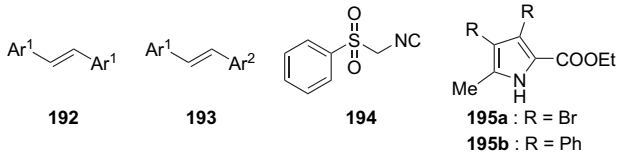


Scheme 25. Retrosynthetic analysis for the preparation of compounds **190** and **191**.



The protocol was particularly efficient (yields >65%) when electron-poor aryl groups were present in the alkene.[145]

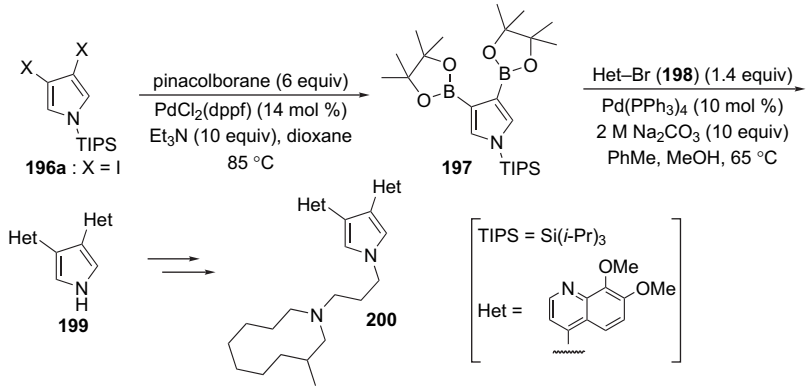
In recent years, 3,4-diaryl-1*H*-pyrroles, which include precursors to natural products and their congeners, have also been frequently prepared by Pd-catalyzed cross-coupling reactions of 3,4-(pseudo)halo-1*H*-pyrroles. Thus, ethyl 3,4-diphenyl-5-methyl-1*H*-pyrrole-2-carboxylate (**195b**) was synthesized in 95% yield by the reaction of dibromopyrrole **195a** with phenylboronic acid in DMF in the presence of aqueous Na_2CO_3 and 3.5 mol % $Pd(PPh_3)_4$.[147]



Similarly, compound **199**, which was employed as a precursor to the tetra-*O*-methyl ether derivative **200** of the strongly cytotoxic marine alkaloid halitulin (**9**), was prepared by a Suzuki reaction of the bromoquinoline derivative **198** with the organoboron derivative **197**, obtained by treatment of 3,4-diiodopyrrole (**196a**) with pinacolborane in the presence of a catalytic quantity of $PdCl_2$(dppf) (Scheme 26).[148]

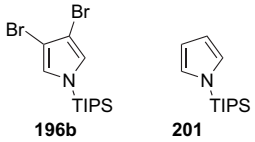
In 2003, Steglich and co-workers[149] used a very similar reaction sequence in a total synthesis of halitulin (**9**). Moreover, Alvarez and co-workers[144f] very recently performed a total synthesis of lamellarin D (**5**) in which the two aryl groups of this marine alkaloid were introduced on the pyrrole ring by a sequential and regioselective bromination/Suzuki cross-coupling procedure.

A methodology involving Stille- and Suzuki-type reactions has been used in the key steps of convergent syntheses of



Scheme 26. Synthesis of compounds **199** and **200** via Pd-catalyzed reactions.

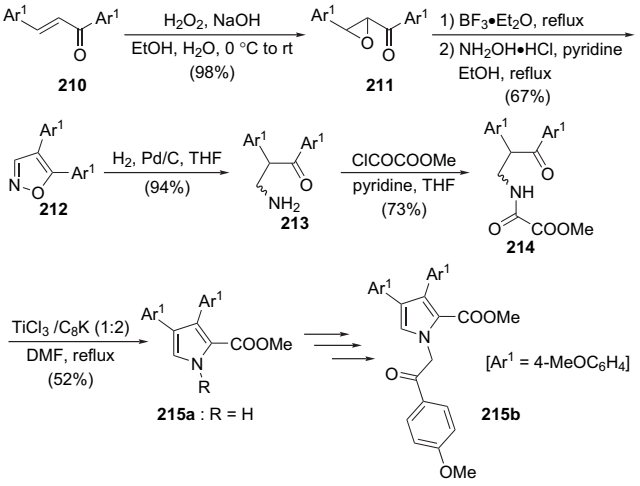
the marine natural products lamellarin O (**1**), lamellarin Q (**3**) and lukianol A (**10**).[150] The pivotal dibromopyrrole **196b** required for these syntheses was prepared from 1-triisopropylsilyl-1*H*-pyrrole (**201**) using procedures developed by Muchowski and co-workers.[151]



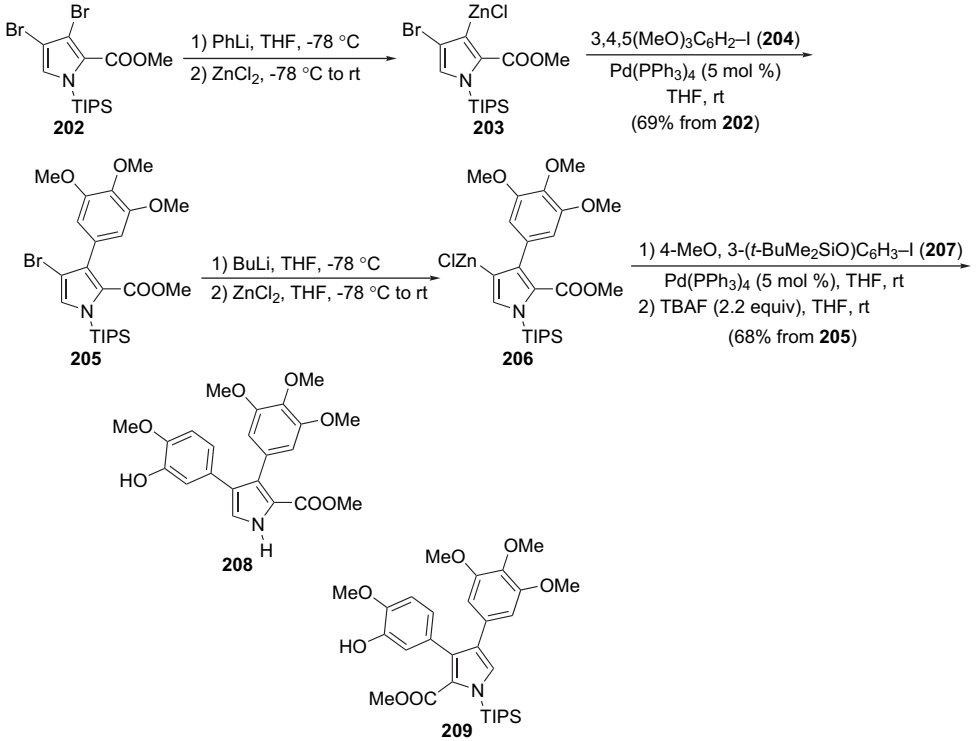
A different strategy was used to prepare the unsymmetrical 3,4-diaryl-1*H*-pyrroles **208** and **209**, which are configurationally stable structural hybrids of the powerful antimitotic agents combretastatin A-4[152] and colchicine.[153] In fact, the Stille- and Suzuki-type reactions used to prepare the naturally occurring compounds **1**, **3** and **10** proved to be unsuitable for providing access to unsymmetrical 3,4-diaryl-1*H*-pyrroles.[150] Thus, dibromopyrrole **202** was regioselectively converted into the organozinc derivative **203**, which underwent a Negishi cross-coupling reaction with aryl iodide **204** to give the monoarylated bromopyrrole **205**. This last compound was then subjected to halogen/metal exchange followed by transmetalation and the resulting organozinc derivative **206** was cross coupled with iodide **207** to give the target pyrrole **208** after desilylation.[150] The unsymmetrical 3,4-diaryl-1*H*-pyrrole **209** was next prepared from **202** via a similar reaction sequence (Scheme 27).[150]

In 2004, Marfil, Albericio and Álvarez used Pd-catalyzed Negishi- and Suzuki-type reactions for a solid-phase synthesis of lamellarins O (**1**) and Q (**3**) in which a 4-iodophenoxy resin and compound **203** were key reagents.[154]

Compound **215a**, used by Banwell and co-workers as an intermediate in the synthesis of lukianol A (**10**),[151] was employed by Fürstner in the first total synthesis of **10** and lamellarin *O*-dimethyl ether (**215b**).[155] In this synthesis, chalcone **210** was employed as the starting material, isoxazole **212** was used a surrogate of the labile keto–enamine **213** and the pyrrole ring of the required alkaloids was regio- and chemoselectively formed by a Ti-mediated oxo–amide coupling reaction of keto–enamide **214** bearing three different carbonyl groups (Scheme 28).[155] Isoxazole **212** was prepared by the reaction of hydroxylamine with the crude 1,3-keto-aldehyde obtained by the BF_3-mediated rearrangement of (*E*)-2,3-epoxy-1,3-bis(4-methoxyphenyl)propanone (**211**).

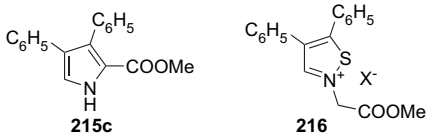


Scheme 28. Synthesis of compounds **215a** and **215b**.

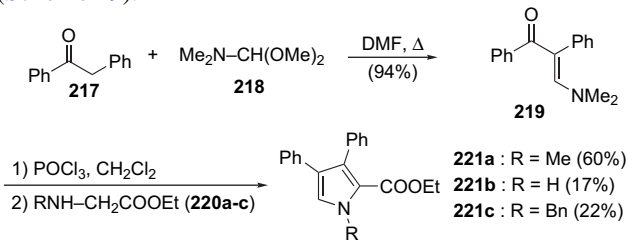


Scheme 27. Synthesis of the unsymmetrical 3,4-disubstituted-1*H*-pyrrole derivatives **208** and **209**.

Compound **215c**, which is an analogue of **215a**, had been previously synthesized in 87% yield by ring transformation of a thiazolium salt **216**.[156]



In 1998, the 3,4-diphenyl-1*H*-pyrrole derivatives **221a–c** were prepared by treatment of 3-dimethylamino-1,2-diphenyl-prop-2-enone (**219**) with $POCl_3$ in CH_2Cl_2, followed by condensation of the resulting chloropropeniminium salt with glycinates **220a–c** in DMF in the presence of NaH (Scheme 29).[157]



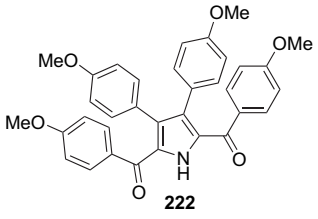
Scheme 29. Synthesis of compounds **221a–c**.

Compound **219** was readily obtained by the reaction of ketone **217** with *N*,*N*-dimethylformamide dimethylacetal (**218**) in refluxing DMF.[157]

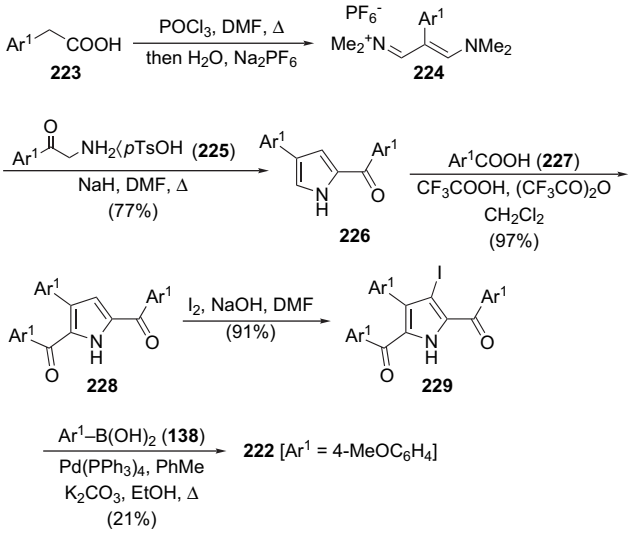
The synthetic methodology used for the synthesis of **221a–c** was subsequently employed to prepare the Fürstner intermediate **215a**[158] and ningalin B (**21**).[159]

In 2002, the Steglich group performed the total synthesis of the marine alkaloid polycitones A (**12**) and B (**13**) employing an elegant approach that included the synthesis of compound **222** by a Paal–Knorr reaction of the appropriate 1,4-diketone with ammonia.[160]

Very recently, compound **222** has been prepared from the vinamidinium salt **224** using two different approaches.[161] In the first of these, **224**, prepared from arylacetic acid **223**, was reacted with aminoketone **225** under base-mediated conditions to give **226** in 77% yield (Scheme 30). This pyrrole derivative was then acylated with carboxylic acid **227** and compound **228**, obtained in 97% yield, was converted in high yield into the iodo derivative **229**.

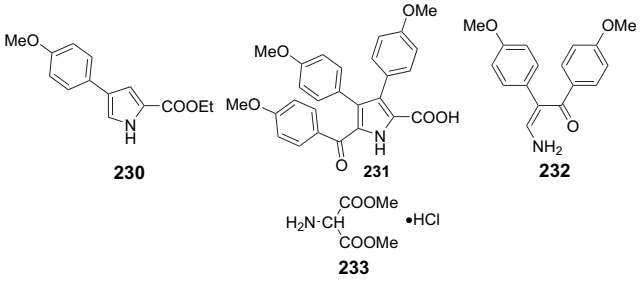


This compound was then subjected to standard Suzuki cross-coupling conditions with arylboronic acid **138** to furnish the Steglich synthon **222** in 21% yield (Scheme 30).[161] Nevertheless, when the Pd-catalyzed reaction of **229** with **138** was performed under microwave irradiation, compound **222** was obtained in 64% yield.[161]

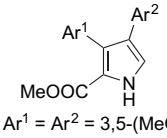


Scheme 30. Synthesis of compound **222** from arylacetic acid **223**.

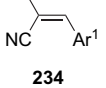
The second method for the synthesis of **222** was based on the conversion of **224** into 2-carbethoxy-4-(4-methoxyphenyl)-1*H*-pyrrole (**230**) and the subsequent preparation of the tetrasubstituted pyrrole **231** by the application of a series of reactions analogous to those reported in Scheme 30 for the preparation of **222** from **226**.[160] This method furnished **222** in 33% total yield from **224**.



In 2001, the procedure, pioneered by the Gupton group to prepare the Fürstner intermediate **215a** utilizing a vinylogous iminium salt derivative prepared from the vinylogous amide **232**,[158] was modified by Kim and co-workers who synthesized **215a** through a cyclocondensation reaction of **232** with dimethyl aminomalonate hydrochloride (**233**) in acetic acid.[162] This modified procedure was also employed to prepare 2-carbomethoxy-3,4-diaryl-1*H*-pyrroles **183d** and **183e** from the corresponding α-aryl ketones in 47 and 39% overall yield, respectively.[162] More recently, a large variety of unsymmetrical 3,4-diaryl-1*H*-pyrroles of general formula **183** have been regioselectively prepared in 51–60% yield, regardless of the electron-withdrawing or electron-releasing substituents in each aromatic ring, by [2+3] cycloaddition of ethyl isocyanoacetate (**182**) to α,β-unsaturated nitriles **234** in the presence of *t*-BuOH.[163]



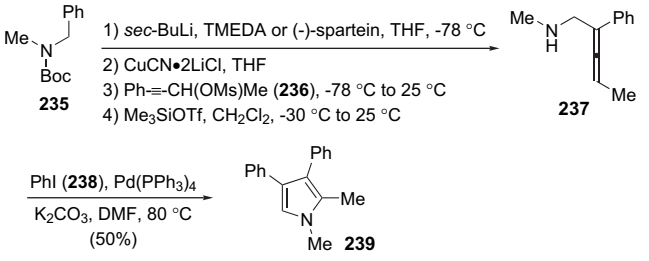
183d : $Ar^1 = Ar^2 = 3,5\text{-}(MeO)_2C_6H_3$
183e : $Ar^1 = 3,5\text{-}(MeO)_2C_6H_3$; $Ar^2 = 4\text{-}MeOC_6H_4$



234

This methodology, which represents a valuable complement to other procedures for the regioselective synthesis of unsymmetrical 3,4-diaryl-1*H*-pyrroles,[131,150,162] was employed to prepare **215a**, which is a key intermediate for the synthesis of the marine natural products lukianol A (**10**), lamellarin O (**1**) and lamellarin Q (**3**), and to perform a high yield total synthesis of ningalin B (**21**).[163]

Several procedures have also been devised for the synthesis of tetrasubstituted 3,4-diaryl-1*H*-pyrroles. In 2001, the pyrrole derivative **239** was synthesized in 50% yield by a Pd-catalyzed reaction of iodobenzene (**238**) with aminoallene **237**, which was available via reaction of the α-(*N*-carbamoyl)alkylcuprate, derived from the *N*-protected amine **235**, with the propargyl mesylate **236** (Scheme 31).[165]



Scheme 31. Synthesis of compound **239**.

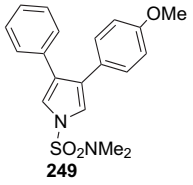
Presumably, formation of **239** involved initial formation of the corresponding 3-pyrroline, followed by Pd-promoted dehydrogenation.[164]

In 1999, permethyl storniamide A (**22**) and the marine natural products ningalin A (**20**), lamellarin O (**1**) and lukianol A (**10**) were synthesized using a concise approach[32] in which the 3,4-diaryl-1*H*-pyrrole derivatives **243a–c**, employed as precursors to these substances, were obtained by a heteroaromatic azadiene Diels–Alder reaction of compounds **240** with tetrazine **241**,[165,166] followed by a reductive ring-contraction reaction of the resulting 1,2-diazines **242a–c** (Scheme 32).

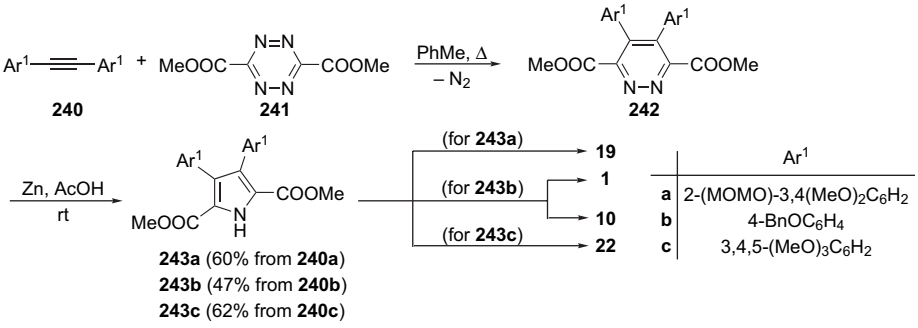
A similar strategy has recently been used by the Boger group for a concise and effective total synthesis of ningalins B (**21**)[36] and D.[167]

In 2000, the synthesis of the tetrasubstituted pyrrole derivative **248** was achieved in high yield starting from *N*,*N*-dimethyl-2-methoxycarbonyl-3,4-bis(trimethylsilyl)-1*H*-pyrrole-1-sulfonamide (**244**) through stepwise and repeated iodination and Pd-catalyzed Suzuki-type reactions (Scheme 33).[168] Iodopyrroles **245** and **247**, which were used as intermediates in this synthesis, were prepared by *ipso*-iodination of compounds **244** and **246**, respectively. Interestingly, the preferred position for iodination of **244** proved to be the 4-position. Compound **248** was then used in a formal total synthesis of lukianol A (**10**).[168]

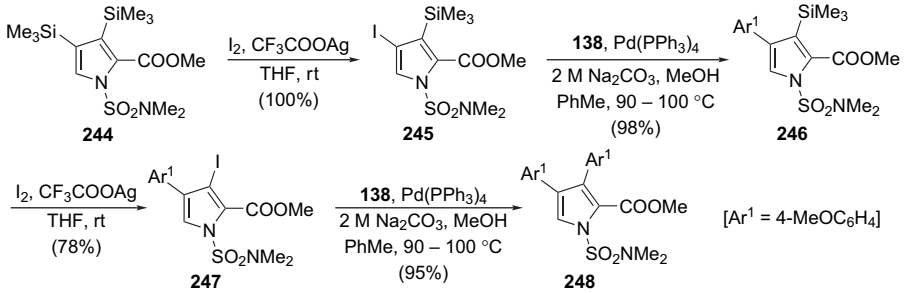
In the same year, a similar protocol was used to prepare the unsymmetrical 3,4-diaryl-1*H*-pyrrole derivative **249**.[169]



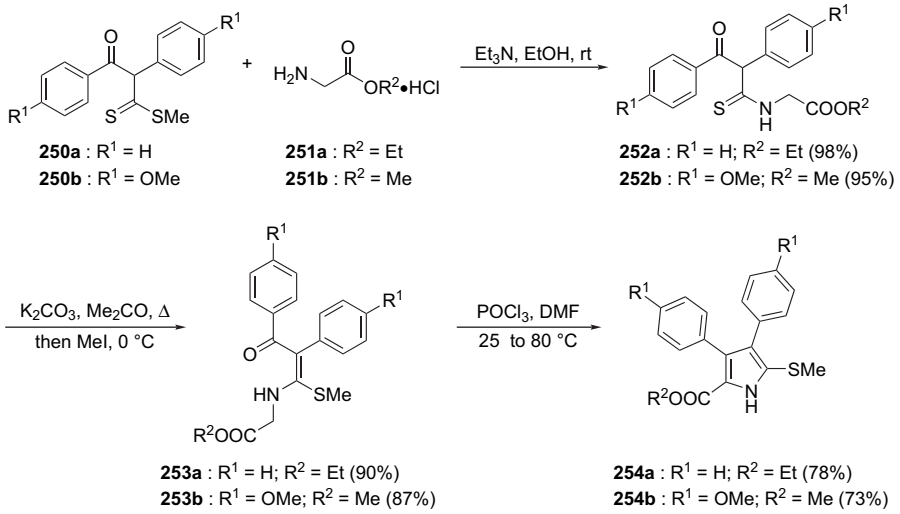
Finally, very recently, it has been reported that treatment of the dithiocarboxylates **250a**,**b** with alkyl glycinates **251a**,**b** followed by alkylation of the resulting β-oxothioamides



Scheme 32. Synthesis of compounds **1**, **10**, **19** and **22** via a heteroaromatic azadiene Diels–Alder reaction.



Scheme 33. Synthesis of compound **248**.

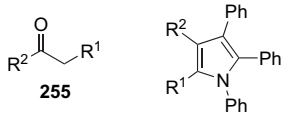


Scheme 34. Synthesis of compounds **254a** and **254b**.

252a,**b** gives ketene *N*,*S*-acetals **253a**,**b**, which are able to undergo smooth cyclization to afford the tetrasubstituted 3,4-diaryl-1*H*-pyrroles **254a**,**b** in good yields under Vilsmeier–Haack conditions (Scheme 34).[170]

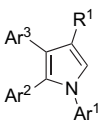
Compound **254b**, prepared in this way, has then been converted into the Fürstner intermediate **215a** by reductive removal of the alkylsulfamyl group using Raney Ni.[170]

2.2.4. Synthesis of 1,2,3- (1,4,5-), 1,2,4-, 1,2,5-, 1,3,4-, 2,3,4- (3,4,5-) and 2,3,5-triaryl-1*H*-pyrroles, 1,2,3,5- and 2,3,4,5-tetraaryl-1*H*-pyrroles, 1,2,3,4,5-pentaaryl-1*H*-pyrroles and 2,3,3-triaryl-3*H*-pyrroles. 1,2,3-Triphenyl-1*H*-pyrroles of general formula **256** have been synthesized in 20–70% yield by condensation of aniline (**49a**), benzoin (**166**) and carbonyl compounds **255**.[171] Methyl 1,2,3-triphenyl-4-carboxylate (**257a**) had been previously prepared in 70% yield by condensation of desylaniline (**258**) and methyl propiolate (**259**) in the presence of sodium acetate.[172]

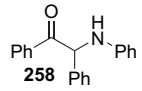


255

256 : R^1 = H, Me, Ph
R^2 = H, Me, Ph



257a : $Ar^1 = Ar^2 = Ar^3$ = Ph; R^1 = COOMe
257b : $Ar^1 = Ar^2 = Ar^3$ = Ph; R^1 = H
257c : Ar^1 = 4-$MeOC_6H_4$; $Ar^2 = Ar^3$ = Ph; R^1 = H
257d : $Ar^1 = Ar^2$ = Ph; Ar^3 = 2-MeC_6H_4; R^1 = H
257e : $Ar^1 = Ar^2$ = Ph; Ar^3 = 3-MeC_6H_4; R^1 = H
257f : $Ar^1 = Ar^2$ = Ph; Ar^3 = 4-MeC_6H_4; R^1 = H
257g : $Ar^1 = Ar^2$ = Ph; Ar^3 = 4-ClC_6H_4; R^1 = H



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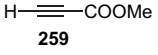

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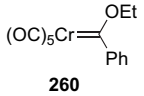

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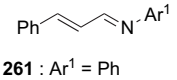
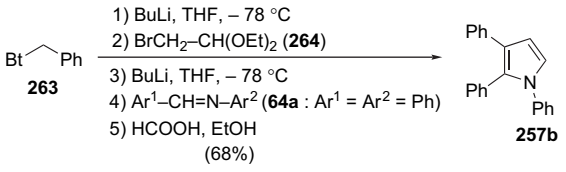


261 : Ar^1 = Ph
262 : Ar^1 = 4-$MeOC_6H_4$

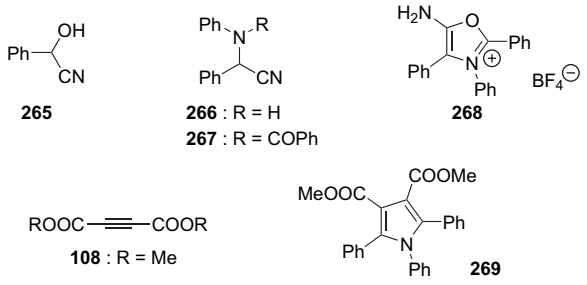
Danks and Velo-Rego have reported that thermolysis of the chromium carbene complex **260** with 1-azadienes **261** and **262** provides 1,2,3-triphenyl-1*H*-pyrrole (**257b**) and the 1,2,3-triaryl-1*H*-pyrrole **257c** in 50 and 60% yield, respectively.[173] Compound **257b** could also be obtained by hydrolysis of **257a**, followed by decarboxylation in quinoline with a copper chromite catalyst.[172]

Moreover, this pyrrole derivative **257b** could be efficiently synthesized by sequential lithiation and alkylation of 1-benzylbenzotriazole (**263**) with 2-bromoacetaldehyde diethylacetal (**264**) and *N*-benzylideneaniline (**64**, Ar^1=Ar^2=Ph), followed by treatment with formic acid in ethanol (Scheme 35).[174] This last versatile procedure was also used to prepare compounds **257d**–**g** in good yields.[174]



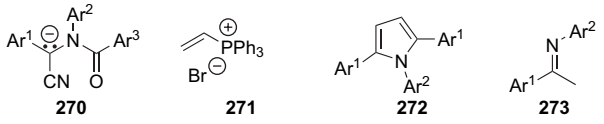
Scheme 35. Synthesis of 1,2,3-triphenyl-1*H*-pyrrole (**257b**) from 1-benzylbenzotriazole (**263**).

To the best of our knowledge, no data have been reported in the literature on the synthesis of 1,2,4-triaryl-1*H*-pyrroles. On the contrary, although 1,2,5-triaryl-1*H*-pyrrole derivatives do not include substances with significant biological activities, great attention has been given in the literature to the design and development of efficient procedures for the synthesis of this class of heterocycles.[175–181] In 1980, dimethyl 1,2,5-triphenyl-1*H*-pyrrole-3,4-dicarboxylate (**269**) was prepared in 80% yield by treatment of dimethyl acetylenedicarboxylate (**108**, R=Me) with 5-imino-2,3,4-triphenyl-1,3-oxazolinium tetrafluoroborate (**268**) at 110 °C for 12 h.[175] This last compound was readily available by the reaction of cyanohydrin **265** with aniline (**49a**), followed by acylation of the resulting aminonitrile **266** with benzoyl chloride and reaction of the open-chain analogue **267** of the resulting Reissert analogues with fluoroboric acid in glacial acetic acid.[175]

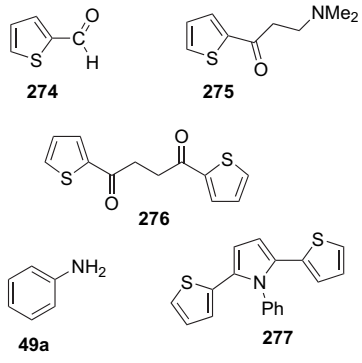


Subsequently, Cooney and McEwen prepared several 1,2,5-triaryl-1*H*-pyrroles of general formula **272** in 65–100% yield by addition of the conjugate bases **270** of open-chain analogues of Reissert analogues to vinyltriphenylphosphonium bromide (**271**).[176]

In 1999, pyrroles **272** were prepared by a simple and convenient one-pot process, which favourably compares with the method of Cooney and McEwen,[176] and consists of the reaction of CH_2Cl_2 solutions of ketimines **273** with 2 equiv of Et_3N and 2 equiv of $TiCl_4$ at 0–25 °C.[178] The compounds **272** were obtained in 63–90% yield.

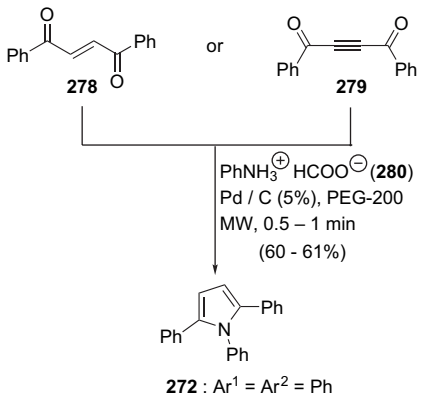


On the other hand, the Paal–Knorr condensation of aniline (**49a**) with 1,4-diketone **276** in the presence of acetic acid under azeotropic conditions was used to synthesize 1-phenyl-2,5-di(2-thienyl)-1*H*-pyrrole (**277**).[177] Compound **276** was obtained by a Stetter reaction of 2-thiophenecarbaldehyde (**274**) with the Mannich base **275**.[177]



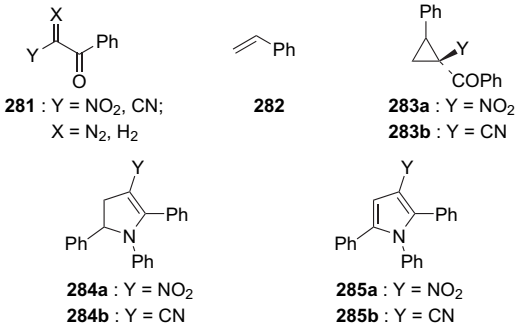
Recently, 1,2,5-triphenyl-1*H*-pyrrole (**272a**) has been conveniently prepared by a microwave-mediated one-pot reaction of ene–dione **278** or yne–dione 2**79** with anilinium formate (**280**) and Pd/C in PEG-200 (Scheme 36).[179] This interesting procedure, which conveniently combines a reduction reaction with an amination–cyclization step, was also used to prepare several other polyaryl-1*H*-pyrrole derivatives.[179]

More recently, tetrasubstituted 1,2,5-triaryl-1*H*-pyrroles **285a**,**b** have been synthesized by oxidation of the corresponding 2-pyrrolines **284a**,**b** with DDQ in refluxing toluene.[180] These pyrrolines were obtained in high yields by

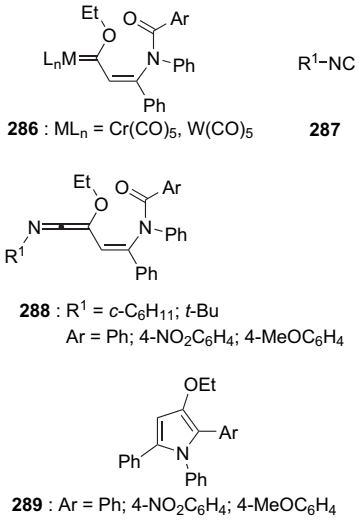


Scheme 36. Synthesis of pyrrole **272a** from compounds **278** or **279**.

a two-step sequence involving a Rh(II)-catalyzed cyclopropanation reaction of styrene (**282**) either with α-nitro- or α-cyano-α-diazoketones **281** or in situ-generated phenyliodonium ylides derived from compounds **281** (X=H_2), followed by reaction of the obtained 1-nitro- or 1-cyano-1-cyclopropyl ketones **283a**,**b** with aniline.[180]



Tetrasubstituted 2-aryl-4-ethoxy-1,5-diphenyl-1*H*-pyrroles **289** had previously been synthesized in excellent yields by thermolysis of (*Z*)-[2-(acylamino)ethenyl]ketene imines **288**, prepared in 63–95% yield by reaction of the carbene complexes **286** with 2 equiv of aryl isocyanides **287**.[181]

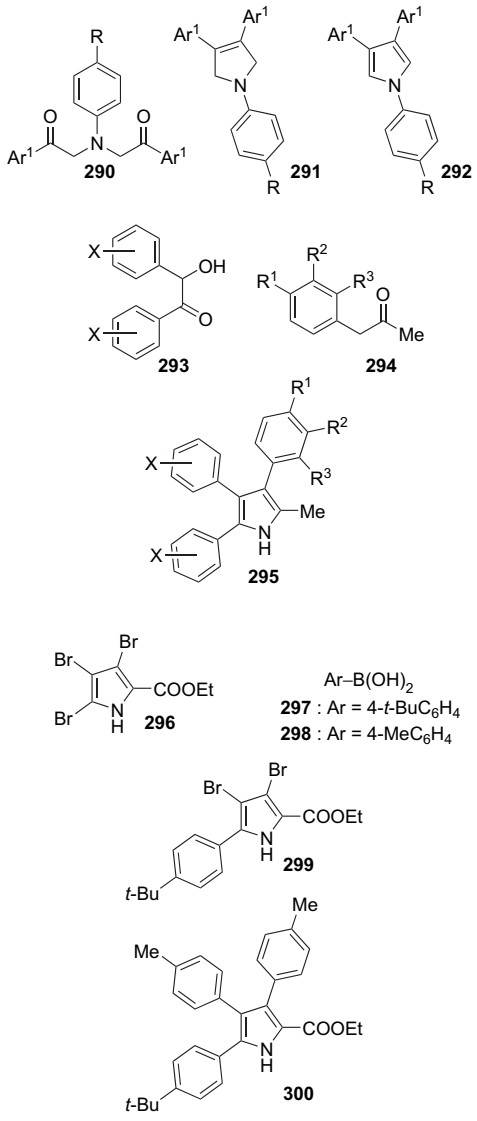


Despite the numerous known syntheses of substituted pyrroles, it is surprising that only one protocol has been

described so far for the synthesis of 1,3,4-triaryl-1*H*-pyrroles.[182] Specifically, some symmetrical pyrroles of general formula **292** have been synthesized in 65–89% yield by irradiation with a high-pressure Hg lamp of CH_2Cl_2 solutions of the corresponding 1,3,4-triaryl-2,5-dihydropyrroles **291**, prepared efficiently by McMurry coupling of dicarbonyl compounds **290** with $TiCl_4$/Zn.[182]

On the other hand, a classical method involving the reaction of a benzoin **293** with a benzyl methyl ketone **294** and anhydrous ammonium acetate in refluxing acetic acid has been used to prepare various 2-methyl-3,4,5-triaryl-1*H*-pyrroles **295**.[76] It should be noted that three of the heterocycles prepared showed a significant inhibition of post-prandial hyperglycemia in normal rats post-sucrose loaded.[76]

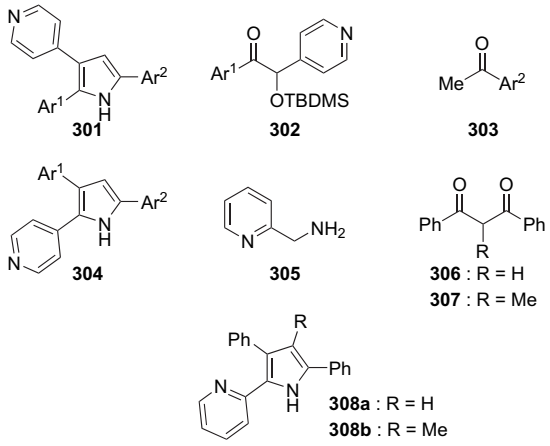
Very recently, the 3,4,5-triaryl-1*H*-pyrrole derivative **300** has been synthesized in 58.8% yield by a regioselective Pd-catalyzed Suzuki cross-coupling reaction of the tribromopyrrole derivative **296** with 1.2 equiv of arylboronic acid **297** and a subsequent cross-coupling at positions C-3 and C-4 of the resulting 5-aryl-3,4-dibromopyrrole carboxylate **299** with 4 equiv of boronic acid **298**.[183]



Recently, much attention has been directed to the preparation of 2,3,5-triaryl-1*H*-pyrroles.[67,75,184–190] Thus, de Laszlo and co-workers have utilized a Paal–Knorr condensation of 1,4-dicarbonyl compounds with ammonium acetate in refluxing acetic acid for the preparation of several 3-(4-pyridyl)-2,5-diaryl-1*H*-pyrroles **301** that include compound **25**, which is a potent orally bioactive inhibitor of p38 kinase.[67]

On the other hand, several 2-(4-pyridyl)-3,5-diaryl-1*H*-pyrroles **304**, which include a potent selective antagonist of glucagon, have been prepared in low yields in a one-pot reaction involving condensation of a silyl acyloin **302** with acetophenones (**303**) or, alternatively, in satisfactory yields via a Paal–Knorr condensation of 1,4-dicarbonyl compounds with ammonium acetate.[75]

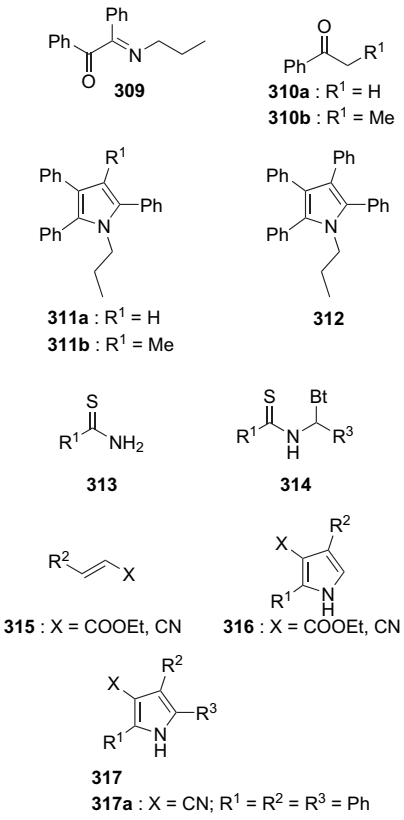
3,5-Diphenyl-2-(2-pyridyl)-1*H*-pyrroles **308a** and **308b** have been synthesized by McNeill and co-workers in 69 and 52% yield, respectively, by condensation of amine **305** with 1,3-diones **306** and **307** in xylenes at 170 °C in the presence of 0.1 equiv of *p*-toluenesulfonic acid and molecular sieves.[184]



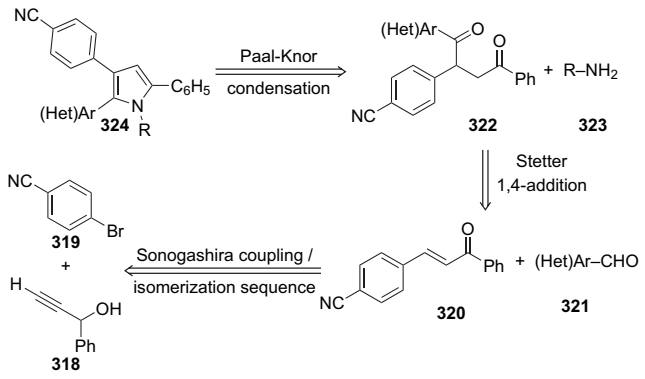
In 2001, the 2,3,5-triphenyl-1*H*-pyrroles **311a** and **311b** were prepared in good yields by the samarium(II) iodide-mediated reaction of α-iminoketone **309** with a molar excess of ketones **310a** and **310b**, respectively.[185] Moreover, similar samarium(II) iodide-mediated reactions were used to prepare efficiently some 2,3-diaryl-1*H*-pyrroles and the pentasubstituted pyrrole **312**.[185]

The year before, in continuation of their concentrated and fruitful activity on the synthetic applications of benzotriazole reagents,[106–110,113,114,174] Katritzky and co-workers had reported that a variety of tri- and tetrasubstituted 1*H*-pyrroles of general formula **316** and **317**, respectively, which include the 2,3,5-triphenyl-1*H*-pyrrole derivative **317a**, could be synthesized in moderate to good yields by a one-pot procedure involving the conversion of a thioamide **313** into the benzotriazole derivative **314**, followed by treatment with *t*-BuOH in THF and subsequent reaction with an activated olefin **315** in the presence of an additional 3 equiv of *t*-BuOH.[186]

In 2001, a variety of 2,3,5-triaryl-1*H*-pyrroles of general formula **324** were prepared in good yields by the Müller

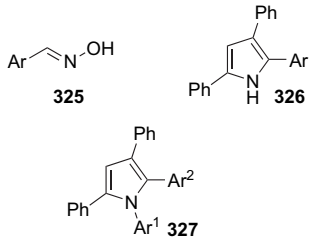


group[187] using a very interesting and convenient one-pot, three-step, four-component process.[191] This process, which used the electron-poor aryl halide **319**, the propargyl alcohol **318**, a (hetero)aryl aldehyde **321** and a primary amine **323** as starting materials, involved a Sonogashira coupling–isomerization–Stetter reaction–Paal–Knorr condensation sequence. Scheme 37 illustrates the retrosynthetic concept of this four-component synthesis.

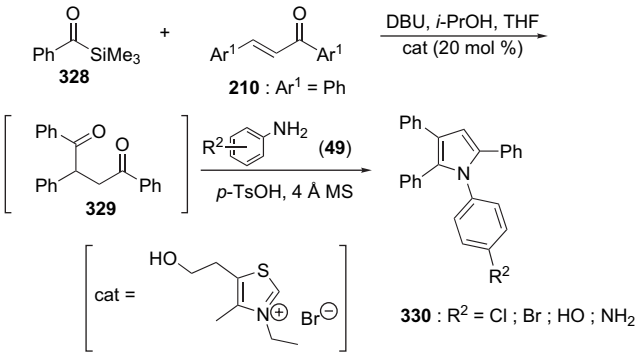


Scheme 37. Retrosynthetic analysis for the preparation of compound **324**.

More recently, some 2-aryl-3,5-diphenyl-1*H*-pyrroles **326** have been concisely and efficiently prepared by a coupling reaction of 1,3-diketone **306** with oximes **325**, which was promoted by low valent titanium prepared from $TiCl_4$ and Zn powder in anhydrous THF.[189] Some 1,2-diaryl-3,5-diphenyl-1*H*-pyrroles **327** have been similarly synthesized in good yields from **306** and imines **64**.[189]



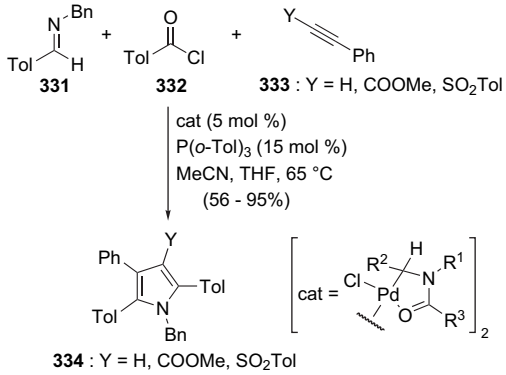
Recently, Bharadwaj and Scheidt have disclosed a novel three-component approach to the synthesis of 1-alkyl-2,3,5-triphenyl-1*H*-pyrroles and 1-aryl-2,3,5-triphenyl-1*H*-pyrroles **330**, which is based on the combination of a new variant of the Stetter reaction with a Paal–Knorr condensation (Scheme 38).[188]



Scheme 38. Synthesis of compounds **330**.

The procedure involves a thiazolium-catalyzed reaction of acylsilane **328** with the α,β-unsaturated ketone **210** (Ar^1=Ph) in the presence of DBU, which is followed by treatment in situ of the resulting 1,4-dicarbonyl compound **329** with an arylamine **49** in the presence of *p*-toluenesulfonic acid and molecular sieves.

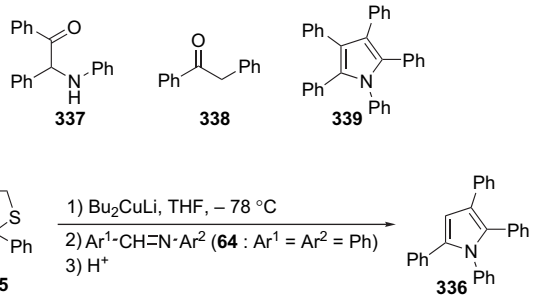
On the other hand, Dhawan and Arndtsen have recently assembled the pyrrole ring of 2,3,5-triaryl-1*H*-pyrrole derivatives **334** by Pd-catalyzed multicomponent coupling of imine **331**, acyl chloride **332** and alkynes **333** (Scheme 39).[190,191] This process has been used to prepare a pyrrole derivative that is a member of a class of multicyclic pyrroles[190] which are of utility as potential therapeutics and retinoic acid regulators.[192]



Scheme 39. Pd-catalyzed synthesis of compounds **334**.

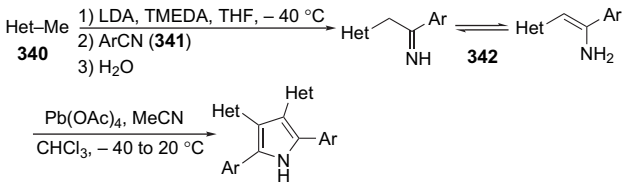
A modification of the Paal–Knorr reaction involving the use of iodine as the catalyst has recently been employed to synthesize 1,2,3,5-tetraphenyl-1*H*-pyrrole (**336**).[193a]

This compound had previously been prepared in 73% yield by a one-pot annulation reaction involving treatment of the propargylic dithioacetal **335** with 0.6 equiv of Bu_2CuLi in THF at −78 °C followed by reaction with imine **64a** (Scheme 40).[193b] Compound **336** had also been synthesized in 70% yield by condensation of aniline (**49a**) with benzoin (**166**) under catalysis by traces of formic acid and treatment of the resulting 2-anilino-2-phenylacetophenone (**337**) with acetophenone (**303**; Ar^2=Ph).[171] On the other hand, reaction of **337** with ketone **338** under acidic catalysis furnished 1,2,3,4,5-pentaphenyl-1*H*-pyrrole (**339**) in 20% yield.[171]



Scheme 40. Synthesis of compound **336** from dithioacetal **335**.

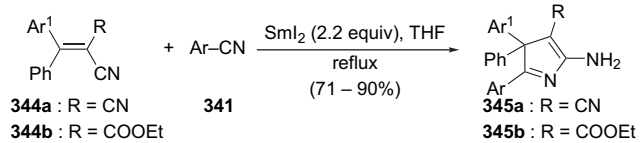
In 1999, symmetrical 2,3,4,5-tetraaryl-1*H*-pyrroles **343**, which include compounds able to prevent Fe^{2+}-induced lipid peroxidation on microsomes, were synthesized in moderate to high yields from the methylheteroarenes **340** and aromatic nitriles **341** according to a two-step reaction sequence in which the second step involved treatment of imine–enamines **342** with $Pb(OAc)_4$ (Scheme 41).[194]



343 : Het = 2-pyrazinyl, 2-pyridyl, 4-pyridyl;
Ar = Ph, 2-$MeOC_6H_4$, 3-$MeOC_6H_4$, 4-$MeOC_6H_4$
2-pyridyl, 4-pyridyl, 2-furyl, 2-thienyl

Scheme 41. Two-step synthesis of compounds **343**.

Finally, several 2,3,3-triaryl-3*H*-pyrroles **345a**,**b** have recently been prepared in good-to-excellent yields by samarium(II) iodide-mediated reductive cyclization of 1,1-diaryl-2,2-dicyanoethylenes **344a**,**b** with aromatic nitriles **341** under neutral and mild conditions (Scheme 42).[195]



344a : R = CN
344b : R = COOEt

345a : R = CN
345b : R = COOEt

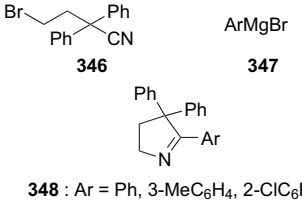
Scheme 42. SmI_2-mediated synthesis of compounds **345a**,**b**.

3. Synthesis of 1-, 2- and 3-pyrrolines with two aryl groups on adjacent positions

Three isomeric groups are possible for the dihydro derivatives of pyrrole: 1-pyrrolines (3,4-dihydro-2*H*-pyrroles), 2-pyrrolines (2,3-dihydro-1*H*-pyrroles) and 3-pyrrolines (2,5-dihydro-1*H*-pyrroles). All of these compounds have been used as intermediates in the synthesis of biologically and/or medically active compounds,[196] but, among these three groups of heterocycles, the 1-pyrrolines are the most interesting. In fact, the latter moieties are present in important biologically active compounds such as hemes,[197] chlorophylls[197] and alkaloids.[198] Moreover, 1-pyrrolines have been used as templates for new drugs.[199] Thus, several methods have been developed for the synthesis of these heterocycles from acyclic, alicyclic or heterocyclic compounds.[200] Nevertheless, the preparation of vicinal diaryl-substituted derivatives of 1-, 2- or 3-pyrrolines has received little attention so far. Here, we summarize the literature data on this subject.

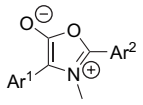
Several years ago, Demoen and Janssen reported that some 2-aryl-3,3-diphenyl-1-pyrrolines **348** can be prepared in satisfactory yields by the reaction of γ-bromonitrile **346** with aryl Grignard reagents **347** in a boiling mixture of Et_2O and xylene.[201]

In 1993, Pal and co-workers synthesized 2,3-diaryl-1-pyrrolines **115** and **352** in high yields by a two-step sequence[202] in which the first step involved alkylation of ketones **349a** and **349b**, respectively, via formation of their zinc enolates prior to a Michael reaction with nitroethylene (**350**). In the second step, the nitroketones **351a** and **351b** were reacted with a catalytic amount of Raney Ni in ethanol at 50 psi H_2, which resulted in the formation of the required 1-pyrrolines.[202]

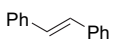


As mentioned in Section 2.2.2, compound **115** has also been prepared from *trans*-2-phenylcyclopropylamine (**113**) via photochemical rearrangement of the corresponding *N*-cyclopropylimine **114**.[112]

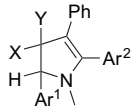
On the other hand, some 2-pyrrolines with two aryl groups on adjacent positions have been synthesized by 1,3-dipolar cycloaddition of münchnones with alkenes.[203,204] Thus, the tetraphenyl-2-pyrroline **355a** was prepared from münchnone **353a** and *trans*-2-stilbene (**354**)[203] and pyrroline **355b** was regioselectively synthesized from münchnone **353b** and alkene **356a**.[204] Compound **355c** was similarly obtained from **353c** and **356b**.[204]



353a : $Ar^1 = Ar^2 = Ph$
353b : $Ar^1 = Ph$; $Ar^2 = 4\text{-}MeOC_6H_4$
353c : $Ar^1 = Ph$; $Ar^2 = 4\text{-}NO_2C_6H_4$



354

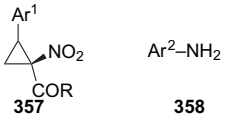


355a : $Ar^1 = Ar^2 = X = Ph$; $Y = H$
355b : $Ar^1 = Ph$; $Ar^2 = 4\text{-}MeOC_6H_4$; $X = CN$; $Y = COOMe$
355c : $Ar^1 = Ph$; $Ar^2 = 4\text{-}NO_2C_6H_4$; $X = Y = CN$

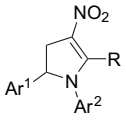
356a : X = CN; Y = COOMe
356b : X = Y = CN

As mentioned in Section 2.2.4, 2-pyrrolines **284a** and **284b** could be obtained by treatment of aniline with the activated cyclopropanes **283a** and **283b**, respectively.[180] A number of 1,5-diaryl-2-pyrrolines **359** were similarly prepared in high yields from the 1-nitrocyclopropyl derivatives **357** and aromatic primary amines **358**.[180]

In Section 2.2.4, it was also reported that some symmetrical 1,3,4-triaryl-3-pyrrolines can be efficiently prepared by McMurry coupling of *N*,*N*-(diarylmethyl)arylamines with $TiCl_4/Zn$.[182]



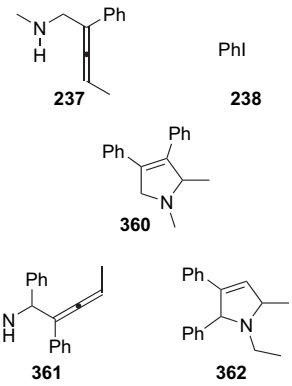
357 **358**



359

Some interesting methods for the synthesis of aryl-substituted 3-pyrrolines are based on the use of aminoallenes. Thus, 3-aryl-3-pyrrolines have been synthesized by a Pd-catalyzed cyclization reaction of α-aminoallenes with aryl iodides in DMF in the presence of K_2CO_3 and Bu_4NCl.[164] This useful reaction has been used to prepare 3-pyrroline **360** from α-aminoallene **237** and iodobenzene (**238**) in 55% yield.[164]

Moreover, a diastereomeric mixture of the 2,3-diphenyl-3-pyrroline **362** has recently been obtained in 88% yield by



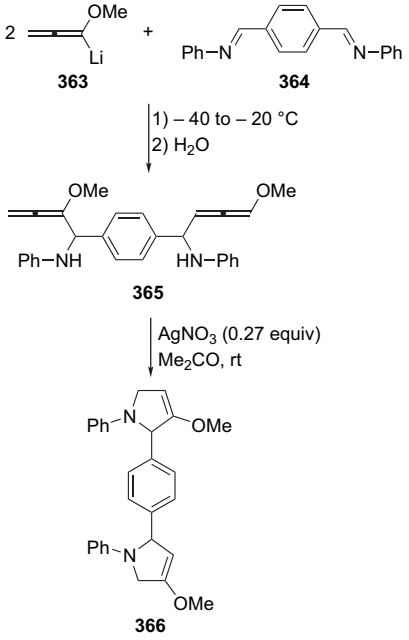
237 **238**

360

361 **362**

an $AgNO_3$-catalyzed reaction of aminoallene **361** in acetone at room temperature.[205] This cyclization reaction could also be performed in dioxane at 100 °C in the presence of 1.5 equiv of Et_3N and 1 mol % $Ru_3(CO)_{12}$ but **362** was obtained only in 56% yield.[205]

Finally, the 3-pyrroline derivative **366** has been synthesized in 94% yield by the reaction of lithiated methoxyallene **363** with diimine **364**, followed by an $AgNO_3$-catalyzed cyclization of the resulting allenyl amine **365** (Scheme 43).[206]



363 **364**

365

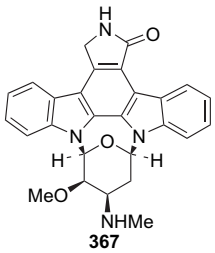
366

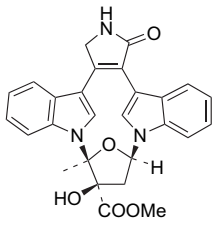
Scheme 43. Synthesis of compound **366**.

4. Synthesis of 3-pyrrolin-2-ones and 2,3-dihydro-1*H*-pyrrole-2,3-diones with two aryl groups on adjacent positions

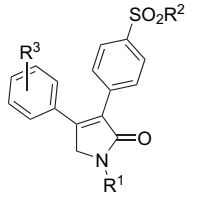
3-Pyrrolin-2-ones (1,5-dihydro-2*H*-pyrrol-2-ones) are important structural units of the structurally related indolocarbazole

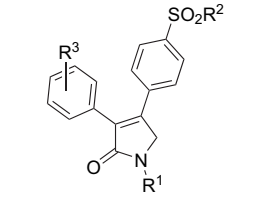
alkaloids (+)-staurosporine (**367**)[207] and (+)-K252a (**368**),[208] which are strong kinase inhibitors widely used as molecular tools.


367

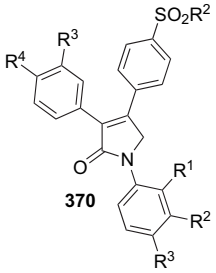

368

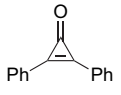
On the other hand, 3,4-diaryl- and 1,3,4-triaryl-3-pyrrolin-2-ones, **369a**,**b** and **370**, have been shown to be a prospective new type of COX-2 selective inhibitors.[209,210] Moreover, the α,β-unsaturated γ-butyrolactam moiety can be utilized as a Michael acceptor for a variety of nucleophiles.[211] Therefore, the synthesis of 3-pyrrolin-2-ones is currently receiving considerable attention[212] and several interesting methods have been reported to prepare 3- and 4-pyrrolin-2-ones with two aryl groups on adjacent positions.[213–225] One of the methods developed for the synthesis of 3-pyrrolin-2-ones is based on a formal [2+3] cycloaddition reaction of diphenylcyclopropenone (**371**)[226] with imines[213] or diimines.[214,215] In fact, some years ago, it was found that the reaction of **371** with acyclic enaminones **372a**,**b** and aminoester **373** in refluxing toluene leads to the formation of the 5-functionalized 3,4-diphenyl-3-pyrrolin-2-ones **374a–c** in good yields.[213]

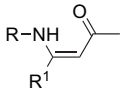

369a : R^1 = Me, Pr, *c*-C_3H_5,
R^2 = Me, NH_2
R^3 = H, 3-Cl, 3-F, 3-Br,
4-F, 4-Cl, 4-Br, 4-Me

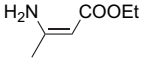

369b : R^1 = Me, Pr, *c*-C_3H_5,
R^2 = Me, NH_2
R^3 = H, 3-Cl, 3-F, 3-Br,
4-F, 4-Cl, 4-Br, 4-Me

The cyclic enaminone **375**, however, proved to be much less reactive towards **371** than compounds **372** and **373** and the 2:1 product **376** was the principal cycloadduct.[213,226]

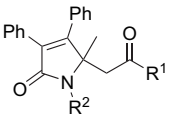

370


371

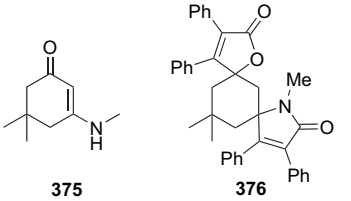

372a : R = H; R^1 = Me
372b : R = R^1 = Me


373

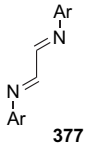
1-Aryl-5-(*N*-aryl)iminomethyl-2,3-diphenyl-2-pyrrolin-4-ones **378** have been reported to be the major products of the reaction of **371** with 1,4-diaryl-1,4-diazabutadienes **377** in refluxing toluene.[214] Nevertheless, it has recently been established that the structure of these compounds corresponds to the tautomers **379** in the *E*-configuration, which are more stable than compounds **378** having extended conjugation and hydrogen bonding.[215]


374a : R^1 = Me; R^2 = H
374b : R^1 = R^2 = Me
374c : R^1 = OEt; R^2 = H

4,5-Diphenyl-3-pyrrolin-2-ones **383a**,**b** have been prepared in good yields by a one-pot procedure that involved the reaction of alkynes **380a**,**b** with Ti(O-*i*-Pr)$_4$, imine **381** and carbon dioxide at atmospheric pressure (Scheme 44).[216] This method, in which an azatitanacyclopentene complex **382a**,**b** is obtained as an intermediate, has also been used to synthesize regioselectively other substituted 3-pyrrolin-2-ones.[216]


375

376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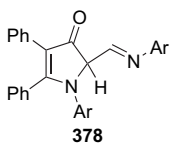
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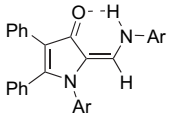
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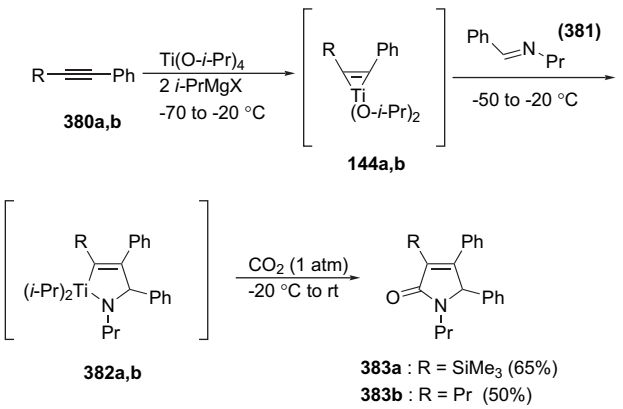
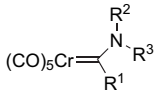


380a,b **144a,b** **382a,b**

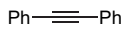
383a : R = $SiMe_3$ (65%)
383b : R = Pr (50%)

Scheme 44. Synthesis of compounds **383a**,**b**.

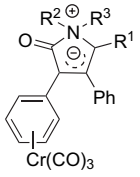
In 1995, Rudler and co-workers demonstrated that the *N*-ylide complexes **385**, obtained upon diphenylacetylene (**380b**) insertion into the carbene complexes **384a**,**b**, are able to react with cyclopentadiene in refluxing benzene to give 3,4-diphenyl-3-pyrrolin-2-ones **386a**,**b** in 65–71% yield.[217]



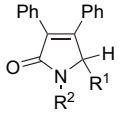
384a : $R^1 = R^2 = R^3 = Me$
384b : $R^1 = Ph$; $R^2 = R^3 = Me$



380b



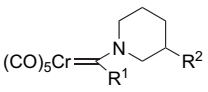
385



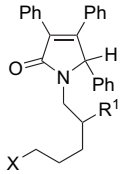
386a : $R^1 = R^2 = Me$
386b : $R^1 = Ph$; $R^2 = Me$

More recently, these authors have reported that aminocarbene complexes **387a–c** are able to react with **380b**, X–H species (X=PhS, PhSe) and, finally, with pyridine to give 3,4,5-triphenyl-3-pyrrolin-2-ones **388a–c** via *N*-ylide complexes of general formula **385**.[218]

A series of 1,5-diaryl-3-arylamino-3-carboxymethyl-3-pyrrolin-2-ones **390** had been previously obtained by the reaction of α-ketoglutaric acid (**389**) with Schiff bases **64**.[219] Compounds **390a–e** were then converted into 1,5-diaryl-3-hydroxy-4-carboxymethyl-3-pyrrolin-2-ones **391a–e** by hydrolysis with hydrochloric acid.[219]



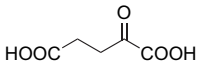
387a : $R^1 = Ph$; $R^2 = H$
387b : $R^1 = Me$; $R^2 = H$
387c : $R^1 = Ph$; $R^2 = Me$



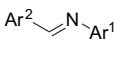
388a : $R^1 = H$; X = PhS
388b : $R^1 = Me$; X = PhS
388c : $R^1 = H$; X = PhSe

3,4-Diheteroaryl-3-pyrrolin-2-one **394**, which was a key intermediate in a stereocontrolled synthesis of the indolocarbazole (+)-K252a (**368**), was synthesized in 92% yield by DBU-catalyzed cyclization of compound **393** in the presence of molecular sieves.[39] This last substance could be obtained in 93% yield by regioselective oxidation of amide **392** with 2 equiv of DDQ in aqueous THF.[39]

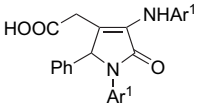
Miller and co-workers[220] prepared the solid phase pyrrolinone **398a** and other 3-carboxy-3-pyrrolin-2-ones by using a protocol in which the polymer-bound malonamides **395** were oxidized to the corresponding ketones **396** by treatment with $CrO_2(O\text{-}t\text{-}Bu)_2$ and these last compounds were cyclized in the presence of LDA or LHMDS to afford the carboxypyrrolinones **397**. Trifluoroacetic acid treatment then released the required compounds **398** in 43–80% overall yield.



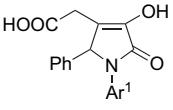
389



64

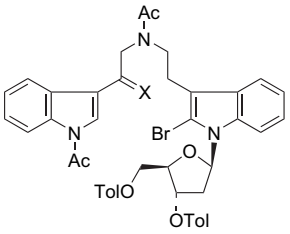


390a-e

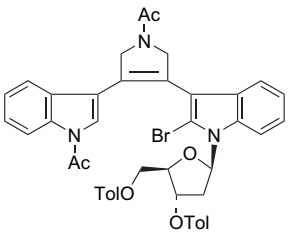


391a-e

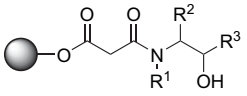
	Ar^1
a	4-IC_6H_4
b	Ph
c	4-$NO_2C_6H_4$
d	4-ClC_6H_4
e	2,4-$(MeO)_2C_6H_3$



392 : X = H_2
393 : X = O



394



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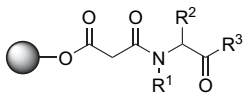
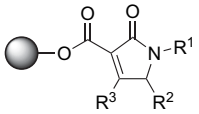
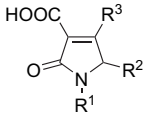


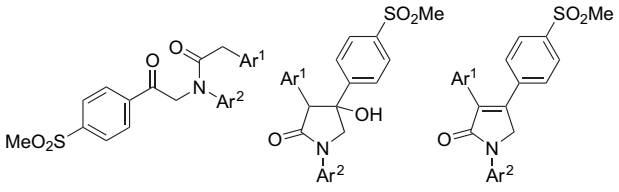
396



397



398
398a : $R^1 = CH_2CH(Et)_2$; $R^2 = R^3 = Ph$

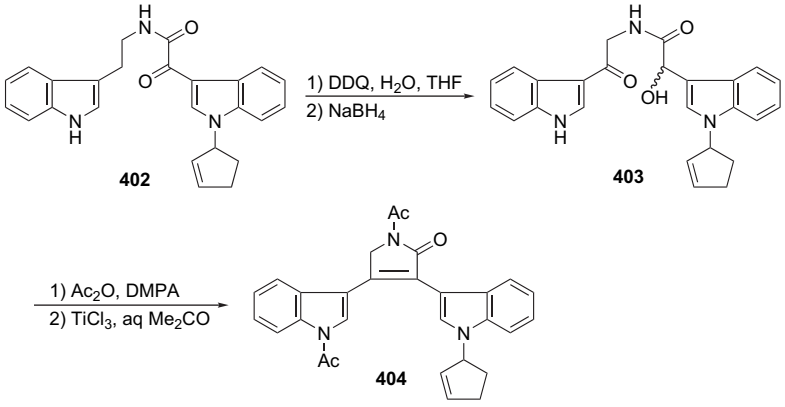


399 **400** **401**

In 2000, several 1,3,4-triaryl-3-pyrrolin-2-ones **401**, which included some novel selective COX-2-inhibitors, were synthesized by a high-yielding aldol-type cyclization of amides **399** with DBU in acetonitrile at 0 °C, followed by dehydration of the resulting lactam alcohols **400** with *p*-toluenesulfonic acid in refluxing benzene.[210]

In 2002, Trost and co-workers elaborated regioselectively the readily available glyoxamide **402** to the corresponding 1-acetyl-3,4-(1-indol-3-yl)-3-pyrrolin-2-one derivative **404** via **403** (Scheme 45)[221] according to a strategy already used in the literature for the synthesis of the staurosporine aglycon.[227]

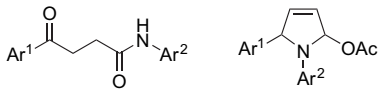
More recently, mixtures of 1,2-diaryl-3- and -4-pyrrolin-2-ones **407a**,**b** and **408a**,**b** have unexpectedly been obtained by the reaction of 3-aroylpropionamides **405a**,**b** with a large excess of refluxing acetyl chloride, followed by alkaline hydrolysis of compounds **406a**,**b**.[222] The pure



402 403 404

Scheme 45. Synthesis of compound **404**.

3-pyrrolin-2-ones **407a**,**b** could be, however, obtained in high yield by recrystallization of the pyrroline mixtures.[222]

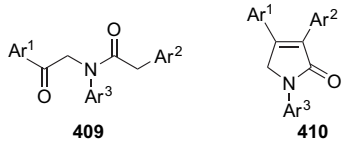


405a,b 406a,b

407a,b 408a,b

	Ar^1	Ar^2
a	Ph	Ph
b	4-MeC_6H_4	Ph

On the other hand, Pal and co-workers have found that cyclization of *N*-aryl-*N*,*N*-di(2-oxo-2-arylethyl)amines **409** by treatment with 1.5 equiv of K_2CO_3 in aqueous ethanol in the presence of atmospheric oxygen at 75 °C for 3 h provides 1,3,4-triaryl-3-pyrrolin-2-ones **410** in good-to-excellent yields.[223]

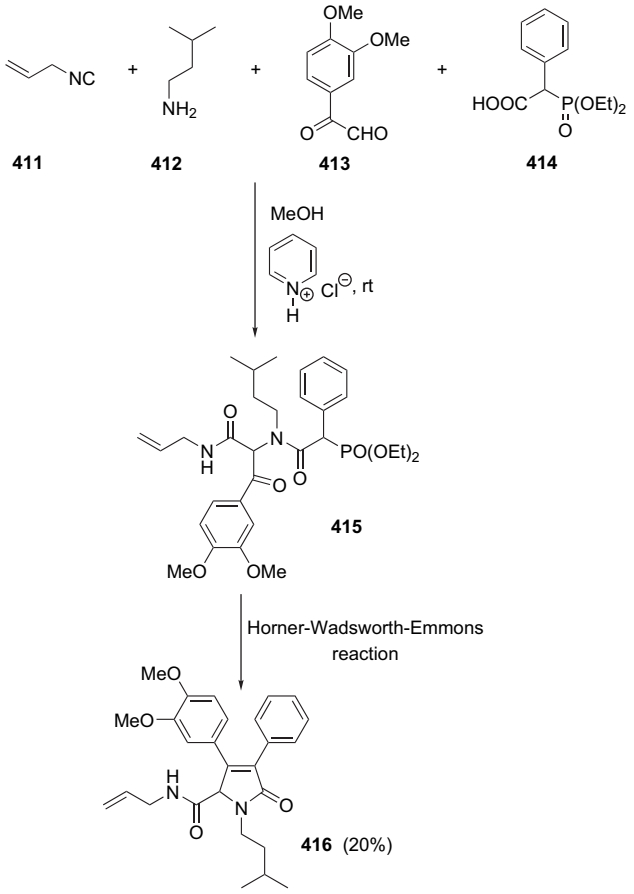


409 410

A convergent assembly of 3,4-diaryl-3-pyrrolin-2-ones **416** has recently been performed by combining a Ugi four-component reaction of isocyanide **411**,[228] amine **412**, α-ketoaldehyde **413** and phosphonic acid diethyl ester **414** to give **415**, with a subsequent Horner–Wadsworth–Emmons ring-closing reaction (Scheme 46).[224] This strategy also allowed the preparation of several other 3-pyrrolin-2-one derivatives in low to high yields.[224]

Recently, the synthesis of 2,3-dihydro-1*H*-pyrrole-2,3-diones with two aryl groups on adjacent positions has also received attention.[229–231]

Thus, a series of 1-aryl-4-cyano-5-phenyl-1*H*-pyrrole-2,3-diones **422** and 1-aryl-4-methoxycarbonyl-5-phenyl-1*H*-

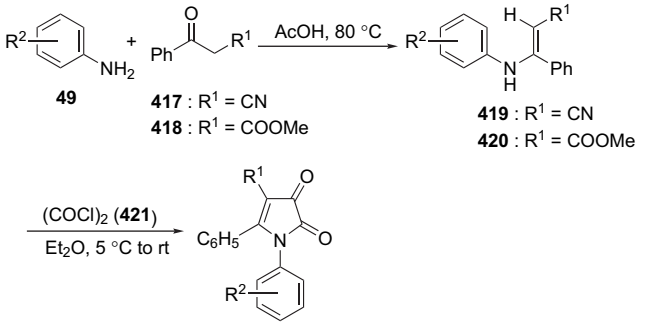


411 412 413 414 415 416 (20%)

Scheme 46. Synthesis of compound **416** via a four-component reaction.

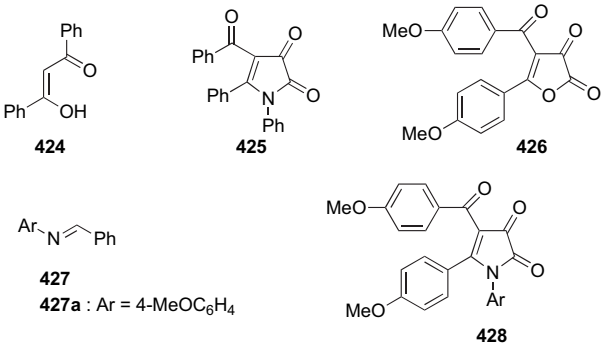
pyrrole-2,3-diones **423** have been synthesized in 75–94% yield by the reaction of oxalyl chloride (**421**) with 3-phenyl-3-arylaminopropenenitriles **419** and ethyl 3-phenyl-3-arylaminoprop-2-enoates **420**, respectively. These last compounds were available from arylamines **49** and benzoylacetonitrile (**417**) and methyl benzoylacetate (**418**), respectively (Scheme 47).[229]

On the other hand, 4-benzoyl-1,5-diphenyl-2,3-dihydro-1*H*-pyrrole-2,3-dione (**425**) has been prepared by reaction of the imine of dibenzoylmethane (**424**) with aniline and oxalyl chloride.[230]



49
417 : R1 = CN
418 : R1 = COOMe
419 : R1 = CN
420 : R1 = COOMe
422 : R1 = CN; R2 = H, 3-F, 4-F, 3,4-F2, 2,3,4-F3
423 : R1 = COOMe; R2 = H, 3-F, 4-F, 3,4-F2, 2,3,4-F3

Scheme 47. Synthesis of compounds **422** and **423**.



424 425 426
427
427a : Ar = 4-MeOC6H4
428

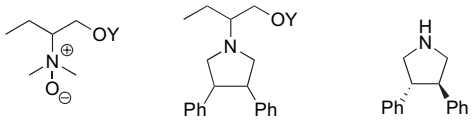
Finally, other red-coloured 4-aroyl-1,5-diaryl-2,3-dihydro-1*H*-pyrrole-2,3-diones **428** have been obtained by treatment of the 4-aroyl-5-aryl-2,3-dihydro-1*H*-furan-2,3-dione **426** with Schiff bases **427** at 60–70 °C.[231]

5. Synthesis of pyrrolidines, 2-pyrrolidinones, 3-hydroxy-3-pyrrolin-2-ones and pyrrolidine-2,4-diones with two aryl groups on adjacent positions

Substituted pyrrolidines and pyrrolidinone derivatives are widespread structural features of natural and designed biologically active molecules.[232] In addition, these heterocycles can be used for pharmaceutical purposes[233] and ligands of transition metal catalysts.[234] Consequently, the efficient preparation of these heterocycles has received significant attention.

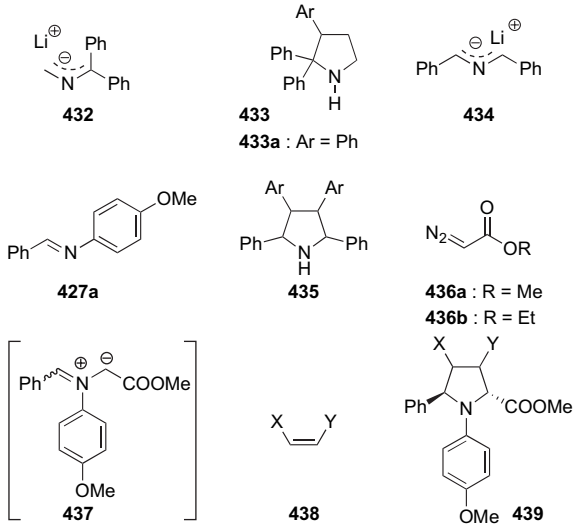
The numerous methods for the synthesis of 2-pyrrolidinones (γ-lactam) derivatives include intramolecular acylation of γ-amino-functionalized carboxylic acids or esters,[235] one-carbon ring expansion of β-lactams,[236] intramolecular C–H insertion reactions[237] and Pd-catalyzed intramolecular allylations.[238]

Several strategies have also been developed for the synthesis of pyrrolidines,[239–250] some of which have been used to prepare vicinal diaryl-substituted pyrrolidine derivatives. Thus, compounds **430a** and **430b** have been synthesized in 34 and 70% yield, respectively, by [3+2] cycloaddition of stilbene (**354**) with the non-stabilized azomethine ylides generated by the reaction of β-aminoalcohol *N*-oxides **429a** and **429b** with LDA at 0 °C.[249] Pyrrolidines **430a** and **430b** could then be converted into 3,4-diphenyl-*trans*-pyrrolidine (**431**) in high yield.[249]



429a : Y = H
429b : Y = *t*-Bu
430a : Y = H
430b : Y = *t*-Bu
431

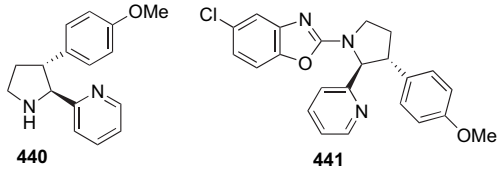
On the other hand, it has been found that the [3+2] cycloaddition reaction of the 2-azaallyllithium **432** with styrene (**282**) provides 2,2,3-triphenylpyrrolidine (**433a**) in 85% yield.[251] Other [3+2] cycloaddition reactions involving the 2-azaallyllithium derivatives **432** and **434** have been usefully employed to prepare other 2,2-diphenyl-3-arylpyrrolidines **433**[240] and some 3,4-diaryl-2,5-diphenylpyrrolidines **435**,[252] respectively.



432
433
433a : Ar = Ph
434
427a
435
436a : R = Me
436b : R = Et
437
438
439

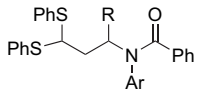
The cycloaddition reaction of dipolarophiles **438** with azomethine ylide **437**, generated by the ruthenium porphyrin-catalyzed reaction of α-diazoester **436a** with imine **427a**, has recently been used for the synthesis of 1-(4-methoxyphenyl)-2-phenylpyrrolidine derivatives **439** in satisfactory yields.[253] It has also been reported that the Cu(I)-catalyzed combination of α-diazoester **436b** and an imine generates a transient azomethine ylide,[254] which is able to undergo diastereoselective cycloaddition with various activated dipolarophiles to afford in a convergent manner highly substituted pyrrolidines which include 1,2-diphenyl derivatives.[254]

An azaallyl cycloaddition strategy has also been used to prepare compound **440**, which is a key intermediate for the synthesis of the LTB_4 inhibitor BIRZ-227 (**441**).[255]

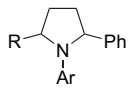


440 **441**

On the other hand, 1,2-diarylpyrrolidines **443a–d** have been obtained by treatment of *N*-[3,3-bis(phenylthio)propyl]anilides **442a–d** with the titanium(II) species $Cp_2Ti[P(OEt)_3]_2$.[256]



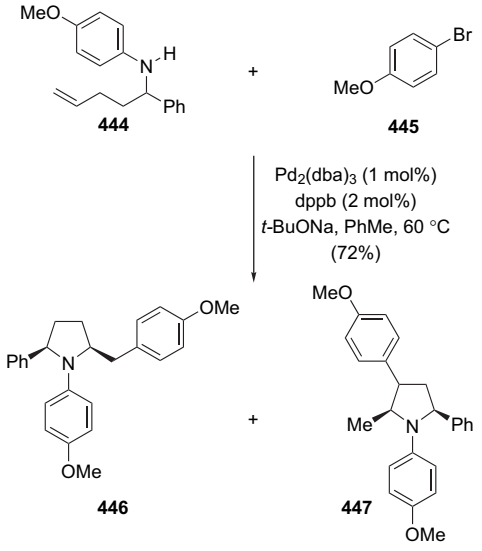
442a-d



443a-d

	R	Ar
a	H	Ph
b	H	2,5-$Me_2C_6H_3$
c	H	4-$MeOC_6H_4$
d	Me	Ph

Recently, a 10:1 mixture of the 1,2-diarylpyrrolidine **446** and the 1,2,4-triarylpyrrolidine **447** have been obtained in 72% yield by a Pd-catalyzed reaction of the *N*-arylamine **444** with the bromo derivative **445** (Scheme 48).[257]

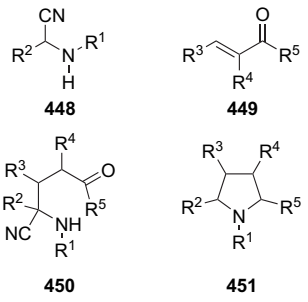


Scheme 48. Pd-catalyzed synthesis of a mixture of compounds **446** and **447**.

Similar Pd-catalyzed tandem *N*-arylation–carboamination reactions of γ-(*N*-arylamino)alkenes with aryl bromides have allowed access to a variety of *N*-arylpyrrolidines with good levels of diastereoselectivity and satisfactory yields.[257,258] Unfortunately, in most cases, the reactions furnished mixtures of regioisomers.

Very recently, polysubstituted pyrrolidines **451** that include some 1,2-diaryl derivatives have been obtained by α-deprotonation of α-aminonitriles **448** and 1,4-addition of the resulting stabilized carbanions to α,β-unsaturated carbonyl compounds **449** and reductive cyclization of the resulting δ-keto-α-aminonitriles **450**.[259] On the other hand, some 1,2-diaryl- and 1,2,5-triarylpyrrolidines **454**, in which the major diastereomer bears a cis relationship between the substituents at the 2- and 5-positions, have been synthesized in high yields by the reaction of aldimines, generated in situ from anilines **49** and aromatic aldehydes **452**, with 1,1-cyclopropanediesters **453** in the presence of a catalytic amount of $Yb(OTf)_3$.[260]

Worthy of mention also is a new catalytic procedure for the synthesis of 1,2-diphenylpyrrolidine (**443a**) via C^3_{sp}–H bond direct arylation of *N*-phenylpyrrolidine (**455**) with iodobenzene in *t*-BuOH at 150 °C for 18 h in the presence of 1.2 equiv of Cs_2CO_3 and 5 mol % of $Ru(H_2)_2(H)_2(PCy_3)_2$.[261] Compound **443a** has been found to be the major product

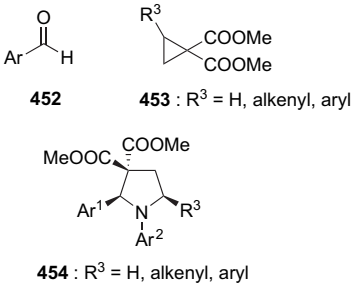


448 **449**

450 **451**

of this reaction which, however, also produces significant amounts of pyrrolidines **456** and **457**.[261]

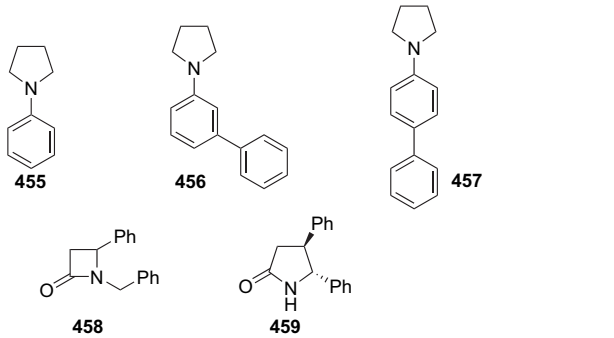
Attention has also been turned in the literature to the development of efficient and convenient methods for the synthesis of 2-pyrrolidinones with two aryl groups on adjacent positions.[233a,262–268]



452 **453** : R^3 = H, alkenyl, aryl

454 : R^3 = H, alkenyl, aryl

Thus, racemic *trans*-4,5-diphenyl-2-pyrrolidinone (**459**) has been obtained in 93% yield by LDA-induced ring enlargement of azetidinone **458**[262] and 1,4-diphenyl-5-aryl-2-pyrrolidinones **462a–c** have been prepared in high yields by deprotonation of the 1,2,4-triazole derivative **460** with 2 equiv of butyllithium followed by reaction with aldimines **64** (Ar^1=Ph) and acidic treatment of the resulting compounds **461a–c**.[263] Recently, racemic **459** has been resolved via the preparation of diastereomers with *N*-phthalyl-L-alanine chloride or D-alanine chloride and the absolute configuration of one of its enantiomers has been determined by X-ray crystallographic analysis.[267]



455 **456** **457**

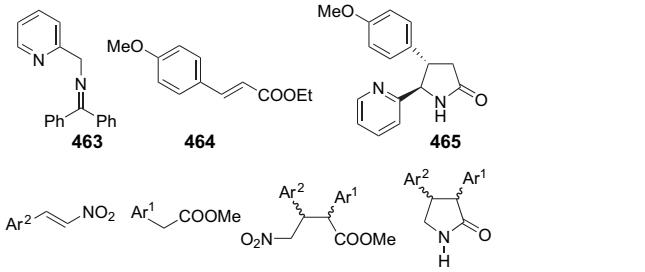
458 **459**

460 **461a-c** **462a-c**

	Ar^2
a	Ph
b	4-ClC_6H_4
c	4-MeC_6H_4

The racemic *trans*-4,5-diaryl-2-pyrrolidinone **465**, used as a key intermediate in the synthesis of the leukotriene-B_4 inhibitor BIRZ-227, has been synthesized on a multigram scale in 52% yield by a one-pot procedure in which the Schiff base **463** was reacted with ethyl 4-methoxycinnamate (**464**) in the presence of 0.5 equiv of aqueous 50% NaOH and 5 mol % $BnEt_3NCl$ and the resulting adduct was hydrolyzed in acidic conditions and then neutralized.[233a,264]

Stereoisomeric mixtures of several 3,4-diaryl-2-pyrrolidinones **469**, which include compound **469a**, have been synthesized by a Michael reaction of the nitroethene derivatives **466** with the esters **467**.[263]

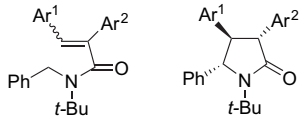


463 **464** **465** **466** **467** **468** **469**

469a : $Ar^1 = Ar^2$ = 3-indolyl

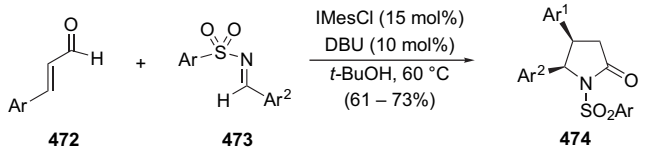
Hydrogenation over Raney Ni of the resulting methyl 4-nitrobutanoates **468** and subsequent lactonization in refluxing toluene in the presence of a small amount of NaH provided the required compounds **469** in low to moderate yields, which were then processed to give staurosporine derivatives.[265] It should be noted that compounds of the basic structure **469a** are known to be biologically active, but often the reported activity is low, probably because mixtures of diastereomers were synthesized and tested.[269] Nevertheless, these mixtures are part of patent claims.[270]

Recently, 3,4-diaryl-5-phenyl-2-pyrrolidinones **471** have been prepared in low to modest yields by the 5-*endo*-*trig*-cyclization reaction of the lithium derivatives obtained by treatment of the substituted acrylamides **470** with LDA in THF at 0 °C.[266]



470 **471**

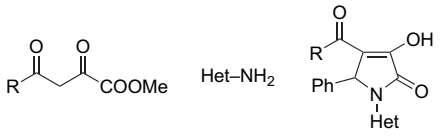
More recently, a variety of *cis*-1-arylsulfonyl-4,5-diaryl-2-pyrrolidinones **474** have been obtained in good yields and modes-to-good diastereomeric purities by annulation of the enals **472** and electrophilic imines **473** in *t*-BuOH at 60 °C in the presence of 15 mol % 1,3-bis(2,4,6-trimethylphenyl)-2-chloroimidazolium chloride (ImesCl) and 10 mol % DBU (Scheme 49).[269]



472 **473** **474**

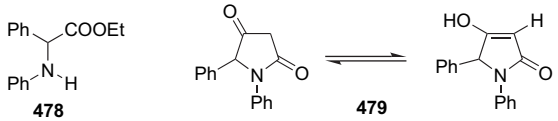
Scheme 49. Synthesis of compounds **474**.

Some data are also available from the literature on the synthesis of 3-hydroxy-3-pyrrolin-2-ones (pyrrolidine-2,3-diones) and pyrrolidin-2,4-diones (4-hydroxy-3-pyrrolin-2-ones) with two aryl groups on adjacent positions. Thus, some 4-acyl-5-phenyl-1-(2-heteroaryl)-3-hydroxy-3-pyrrolin-2-ones **477** have been prepared in high yield by brief heating of mixtures of equimolar amounts of α-ketoesters **475**, benzaldehyde and a heteroarylamine **476** in glacial acetic acid.[271,272] Interestingly, some of these heterocyclic derivatives have been shown to have moderate antimicrobial activity.[272]

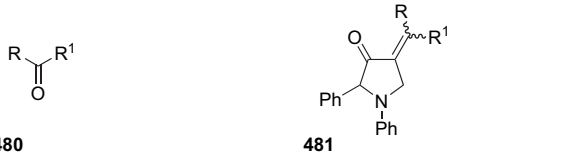


475 **476** **477**

On the other hand, 1,5-diphenylpyrrolidin-2,4-dione (**479**) has been prepared by refluxing ethyl 2-(phenylamino)phenylacetate (**478**) with acetyl chloride and treatment of the resulting *N*-acetyl derivative with Na in toluene at 120–130 °C followed by acidification at 0 °C.[273]



478 **479**



480 **481**

480a : R = 3,4-$Cl_2C_6H_3$; R^1 = H

480b : R = 4-ClC_6H_4 ; R^1 = Me

481a : R = 3,4-$Cl_2C_6H_4$; R^1 = H

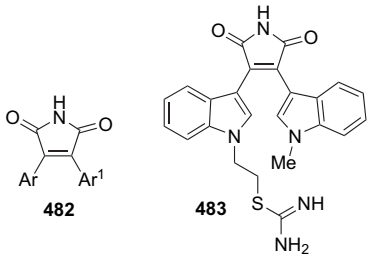
481b : R = 4-ClC_6H_4 ; R^1= Me

It is also worthy of mention that compounds **481a** and **481b**, obtained by condensation of **479** with the carbonyl compounds **480a** and **480b**, respectively, in an acidic medium under reflux, displayed activity against *Staphylococcus aureus* strains.[274]

6. 2,3-Diarylmaleimides (3,4-diaryl-3-pyrroline-2,5-diones) and 2,3-diarylsuccinimides (3,4-diarylpyrrolidine-2,5-diones)

6.1. Biologically active natural and unnatural 2,3-diarylmaleimides

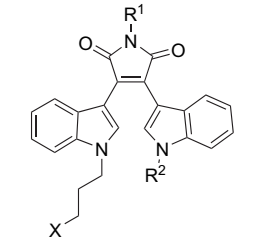
Natural and unnatural 2,3-diarylmaleimides **482** represent a class of compounds, which exhibit diverse biological activities. One of these interesting heterocycles is Ro-31-8220 (**483**), which is a synthetic analogue of naturally occurring (+)-staurosporine (**367**), an alkaloid isolated from the bacterium *Streptomyces staurosporeus*.[207] In fact, **483** is able to induce apoptosis independent of its ability to inhibit protein kinase C (PKC),[28] a family of serine–threonine specific kinases thought to be an essential element in the signal transduction of a variety of hormones, cytokines and growth factors[275] and which is implicated in a wide range of physiological processes including growth differentiation.[276]



482 **483**

The bis-indolylmaleimide GF-109203X (**484a**) like **367** is a potent PKC inhibitor,[29,277] which displays high selectivity as compared to five different protein kinases. Moreover, it is able to inhibit the necrotic cell death induced by oxidative stress in a variety of primary-cultured cells.[29]

Compounds **484b–d** are also PKC inhibitors.[278–280] and **484d**, which is orally adsorbed in rats, may represent an attractive lead in the development of even more potent inhibitors.[279]



484a : $R^1 = R^2 = H$; X = NMe_2
484b : $R^1 = R^2 = H$; X = 
484c : $R^1 = R^2 = H$; X = NH_2
484d : $R^1 = H$; $R^2 = Me$; X = NH_2
484e : $R^1 = R^2 = Me$; X = NH_2
484f : $R^1 = Me$; $R^2 = H$; X = NH_2

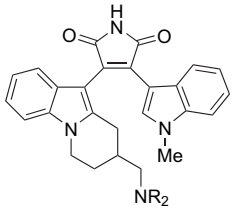
On the other hand, the maleimides **484b** and **484c** are also able to inhibit PDK1, a key kinase from the insulin signalling pathway,[29] and **484e** has been shown to be a potent inhibitor of H_2O_2-induced necrotic death of human leukaemia HL60 cells.[280]

Ro-31-8425 (**485**) and the corresponding *N*,*N*′-dimethyl derivative **486** are two conformationally restricted PKC inhibitors.[281,282] Compound **485** can also inhibit superoxide generation in human neutrophils activated by both receptor and post-receptor stimuli and **486** can antagonize phorbol ester-induced paw edema in mice.[282]

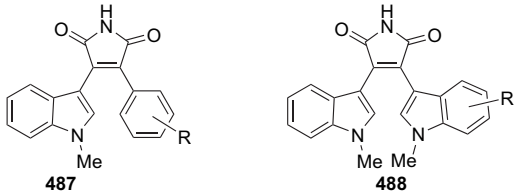
Some 2-phenyl-3-indolylmaleimides **487** have also been shown to be PKC inhibitors, but their activity proved to be lower than that of the 2,3-bis-indolylmaleimides **488**.[283]

The novel indolylindazolylmaleimides **489a** and **489b** have recently been identified as low-nanomolar inhibitors of PKC-β,[30] which is an enzyme induced in response to hyperglycemia in cardiac, aortic, renal and retinal tissues.

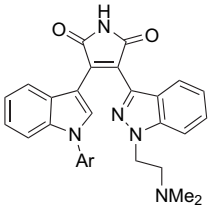
Interestingly, these substances have demonstrated excellent selectivity over other PKC isozymes and glycogen synthase-3β (GSK-3β),[30] a serine–threonine protein kinase involved in signalling from the insulin receptor.[284] On the other hand, **487a** and some 3-aryl-[1,7-aza-annulated-indol-3-yl]maleimides **490** have been reported as potent GSK-3 inhibitors.[285–287]



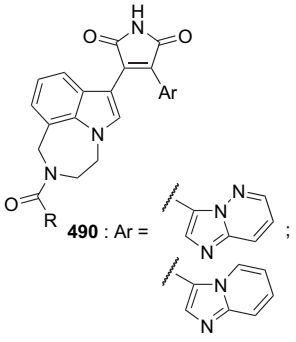
485 : R = H
486 : R = Me



487 **488**
487a : R = 2,4-Cl_2



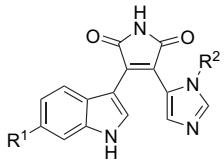
489a : Ar = 2-naphthyl
489b : Ar = 3-quinolinyl



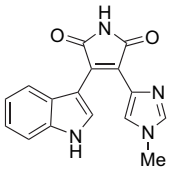
490 : Ar =

Moreover, some compounds **490**, which show a high degree of selectivity against both serine–threonine and tyrosine kinases, have been shown to be highly efficacious oral agents for reduction of blood glucose in the ZDF rat model of non-insulin dependent diabetes mellitus.[287]

The 2,3-diarylmaleimide moiety is also present in a number of naturally occurring compounds, some of which are endowed with relevant biological activities. Thus didemnimides A (**491a**), B (**491b**), C (**491c**) and D (**491d**) are



491a : $R^1 = R^2 = H$
491b : $R^1 = Br$; $R^2 = H$
491c : $R^1 = H$; $R^2 = Br$
491d : $R^1 = Br$; $R^2 = Me$



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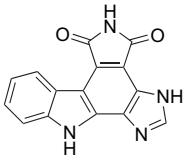

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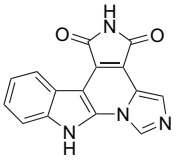
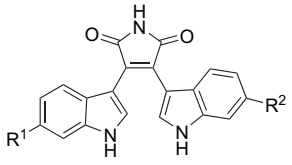


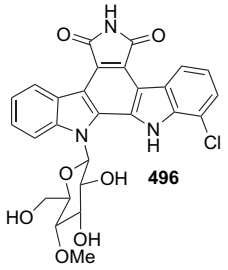
494

members of a class of indole–maleimide–imidazole tricyclic compounds isolated from the Caribbean mangrove ascidian *Didemnum conchyliatum* that are predator deterrents.[288] Compounds **491a** and **491b** have also been found together with didemnimide E (**492**) in the crude extracts of the ascidian *Didemnum granulatum* collected in Brazil.[289] Interestingly, these extracts showed activity in a screen for G2 cell cycle checkpoint inhibitors and this activity was demonstrated to be due to granulatimide (**493**) and isogranulatimide (**494**) present in the extracts.[289]

Arcyriarubins A (**495a**), B (**495b**) and C (**495c**), which represent the simplest members of natural bis-indolylmaleimides,[290] are a family of pigments produced by slime moulds (*Myxomycetes*). These substances are structurally related to the aglycon of (+)-staurosporine (**367**),[207] the potent antitumour agent rebeccamycin (**496**) isolated from *Nocardia aerocoligenes*,[291] SF-2370 (**497**)[292] and other biologically active metabolites from *Streptomycetes*.



495a : $R^1 = R^2 = H$
495b : $R^1 = OH$; $R^2 = H$
495c : $R^1 = R^2 = OH$



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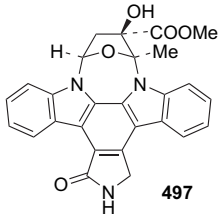
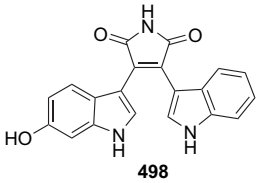


497

Recently, arcyriarubin C (**495c**) has been isolated together with dihydroarcyriarubin C (**498**) and arcyriaflavin C (**499a**) from the fruit bodies of *Arcyria ferruginea*.[293] Moreover, arcyriaflavin C, which has been found to exhibit a cell cycle inhibition effect at G1 and G2/M stage at 10 and 100 ng/mL, respectively, has been isolated from *Tubifera cassaparyi*, together with arcyriaflavin B (**499b**).[293]



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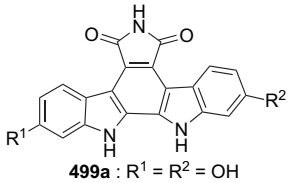
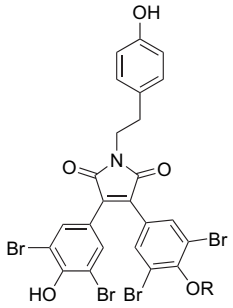


499a : $R^1 = R^2 = OH$
499b : $R^1 = OH$; $R^2 = H$

Arcyriaflavins are also the main pigments of *Arcyria denudata*.[291b,294] Some of their derivatives have shown antimicrobial activity against *Bacillus cereus*,[25] antitumour activity against P388 leukaemia cells,[25] and have been demonstrated to be able to inhibit tyrosine and serine kinases.[25,295,296] On the other hand, some *N*-glucosyl derivatives of arcyriarubin A have demonstrated potent antiproliferative activities.[297]



500a : R = H
500b : R = Me

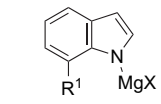
Finally, two 2,3-maleimides, polycitrin A (**500a**) and polycitrin B (**500b**), have been isolated from the ascidian *Polycitor* sp., together with polycitone A (**12**).[17] This last compound was shown to be a potent inhibitor of the HIV-1 RT DNA polymerase activity, but polycitrin A exhibited a significantly lower activity.[27]

6.2. Synthesis of symmetrical and unsymmetrical 2,3-diarylmaleimides and 2,3-diarylsuccinimides

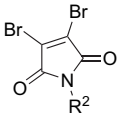
The bis-indolylmaleimides are valuable intermediates in the synthesis of the aglycones of indolocarbazole alkaloids such as staurosporine and rebeccamycin. Thus several methods have been developed for the preparation of these heterocycle derivatives.

A convenient synthesis of symmetrical and unsymmetrical bis-indolylmaleimides involves the reaction of indolyl Grignard reagents with dihalomaleimides. This method was investigated in 1980 by Steglich and co-workers who prepared compound **503a** in 60% yield by reaction of indolylmagnesium iodide (**501a**) with *N*-methyl-2,3-dibromomaleimide (**502a**) in benzene at 25 °C in the presence of a small amount of HMPA.[16] The method was then used by Kaneko,[298] the Weinreb group[299] and Xie and Lown[300] for the synthesis of **503b** from **501b** and **502b** and of **503c** from **501c** and **502c**.

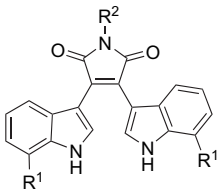
A few years later, Steglich reported that the outcome of the reaction between **501c** and **502a** is strongly dependent on the solvent and that, in toluene, the reaction gives the bis-indolyl



501a : $R^1 = H$; X = I
501b : $R^1 = Cl$; X = I
501c : $R^1 = H$; X = Br



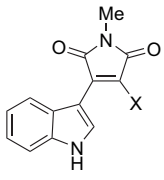
502a : $R^2 = Me$
502b : $R^2 = CH_2OBn$
502c : $R^2 = Bn$



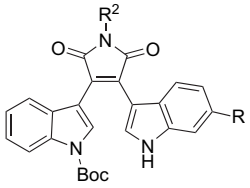
503a : $R^1 = H$; $R^2 = Me$
503b : $R^1 = Cl$; $R^2 = CH_2OBn$
503c : $R^1 = H$; $R^2 = Bn$

compound **503a** in 70% yield, whereas, in THF, the monosubstitution product **504a** is obtained in 74% yield.[301] This last compound, after protection of the indole NH group with a Boc residue, was coupled with **502a** in refluxing THF to give the unsymmetrical substituted bis-indolylmaleimide **505a** in 85% yield.[301] A similar procedure was then followed to prepare **505b**, which was used as a precursor to arcyriarubin B (**495b**).[301]

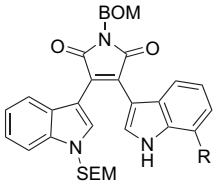
Subsequently, the Danishefsky group employed a similar protocol to prepare compound **506a**, which was used as a key intermediate in a total synthesis of rebeccamycin (**496**),[302] and the unsymmetrical bis-indolylmaleimide **506b**, which was employed as an intermediate in the first total synthesis of naturally occurring (+)-staurosporine (**367**) and its enantiomer.[40]



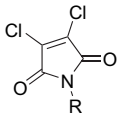
504a : X = Br
504b : X = Cl



505a : R = H
505b : R = THPO



506a : R = Cl
506b : R = H
(SEM = $Me_3SiCH_2CH_2$-O-CH_2)

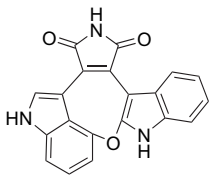


507a : R = Me
507b : R = H
507c : R = Bn

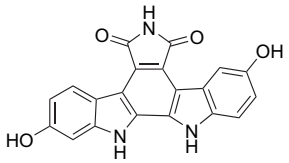
In 1995, Faul and co-workers reported that even 2,3-dichloro-*N*-methylmaleimide (**507a**) can be converted into the bis-indolylmaleimide **503a** by a coupling reaction with 2.2 equiv of the Grignard reagent **501c** in THF and toluene.[303] They also found that the formation of **503a** was reduced and the amount of compound **504b** increased as the ratio of THF to toluene increased and observed that the formation of **503a** became favoured when the solvent was changed from THF to Et_2O.[303] Moreover, they developed a direct method to prepare arcyriarubin A (**495a**) in 72% yield, which involved treatment of 2,3-dichloromaleimide (**507b**) with 5 equiv of **501c** in a 5:1:1 mixture of toluene, Et_2O and THF, respectively, at 90 °C for 24 h.[303]

Bis-indolylmaleimides, prepared as mentioned above or by analogous protocols from 2,3-dibromo-*N*-methylmaleimide (**502a**) or *N*-benzyl-2,3-dichloromaleimide (**507c**) and indolylmagnesium halides or indolyllithium, have been employed in practical syntheses of natural products which include arcyroxin A (**508**),[41] arcyriaflavins A (**499a**), B (**499b**) and D (**509**),[42] the dechlororebeccamycin aglycon[304] and macrocyclic bis-indolylmaleimides in which the indole nitrogens are linked with a tether.[305]

On the other hand, the bis-(7-azaindolyl)maleimide **512a** has recently been prepared according to a strategy that involves a monocoupling reaction of **502a** with 2 equiv of



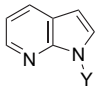
508



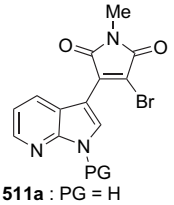
509

the 7-azaindolic Grignard reagent **510b** in toluene and CH_2Cl_2, protection of the NH indolic group of the resulting 3-(7-azaindolyl)-2-bromo-*N*-methylmaleimide (**511a**) and a coupling reaction of the resulting compound **511b** with 2 equiv of the lithium derivative prepared by treatment of 7-azaindole (**510a**) with a molar excess of LHMDS.[305] Compound **512a** was then converted easily into **512b** by reaction with 2 equiv of TBAF in refluxing methanol.[305]

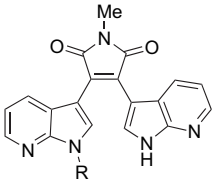
A similar efficient protocol based on the use of 2,3-dibromo-*N*-methylmaleimide (**502a**) has been employed to prepare a series of 3-[1-methyl-2,5-dioxo-4-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-2,5-dihydro-1*H*-pyrrol-3-yl]indole-1-carboxylic acid *tert*-butyl esters **513**.[306] Compounds **512** and **513** have then been used to prepare substances containing an indolocarbazole framework, which could be useful for the preparation of glycosylated derivatives susceptible to target topoisomerase I and/or certain protein kinases.[306]



510a : Y = H
510b : Y = MgBr

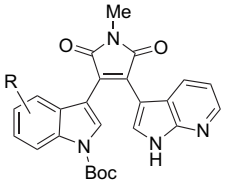


511a : PG = H
511b : PG = $PhSO_2$



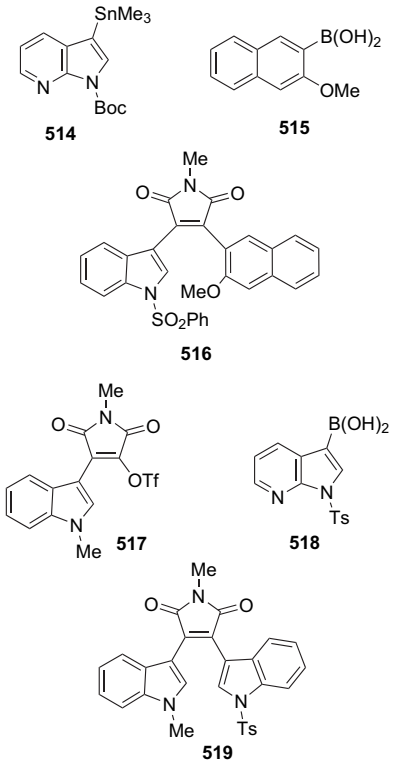
512a : R = $PhSO_2$
512b : R = H

More recently, 1-methyl-3,4-bis(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)pyrrole-2,5-dione (**512b**) has been synthesized in 41% yield by a Stille reaction of **507a** with 3 equiv of 3-trimethylstannylpyrrolo[2,3-*b*]pyridine-1-carboxylic acid *tert*-butyl ester **514** in toluene at 95 °C in the presence of 4.7 equiv of LiCl and 4.7 mol % $PdCl_2(PPh_3)_2$.[307] On the other hand, the unsymmetrical 2,3-disubstituted maleimide **516** has been prepared in 80% yield by a Suzuki reaction of **511b** with 3-methoxynaphthalene-2-boronic acid (**515**) in dioxane and water at 100 °C in the presence of 2 equiv of K_2CO_3, 10 mol % $Pd(OAc)_2$ and 20 mol % PPh_3.[308]

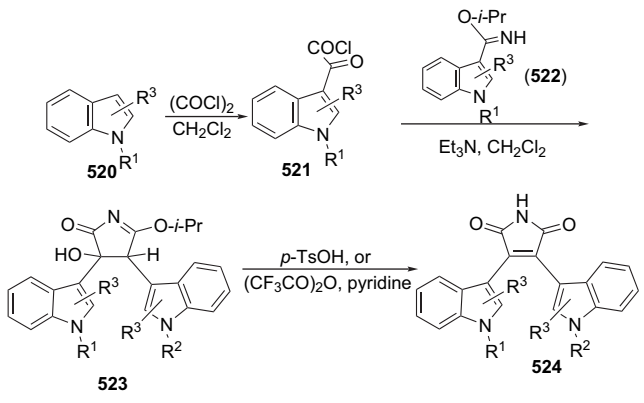


513 : R = H; 9-OBn; 10-OBn; 11-$OCHPh_2$

Very recently, Suzuki-type reactions between 2,3-diiodomaleimides and various organoboron derivatives have been used as key steps in the synthesis of substituted bis(fur-2-yl)-, bis(fur-3-yl)- and bis(thien-2-yl)maleimides with potential antidiabetic properties.[309] A Suzuki reaction had previously been employed to prepare the unsymmetrical 2,3-(3-indolyl)maleimide **519** from the triflate **517** and the boronic acid **518** in 55% yield.[310] This reaction was performed in dioxane at 15–25 °C in the presence of 3 equiv of CsF, 3 equiv of CsBr and a catalytic amount of $Pd_2(dba)_3 \cdot CHCl_3$.



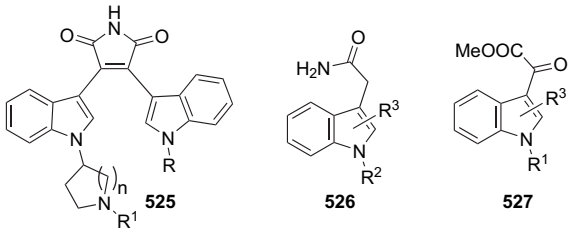
In 1993, Hill and co-workers synthesized symmetrical and unsymmetrical bis-indolylmaleimides **524** by a mild and flexible method that involved the conversion of indoles **520** into indolyl-3-glyoxylyl chlorides **521** and the reaction of these compounds with appropriately substituted indolyl-3-acetimidates **522** in the presence of a molar excess of Et_3N, followed by hydrolysis of the resulting hydroxypyrroline derivatives **523** (Scheme 50).[311]



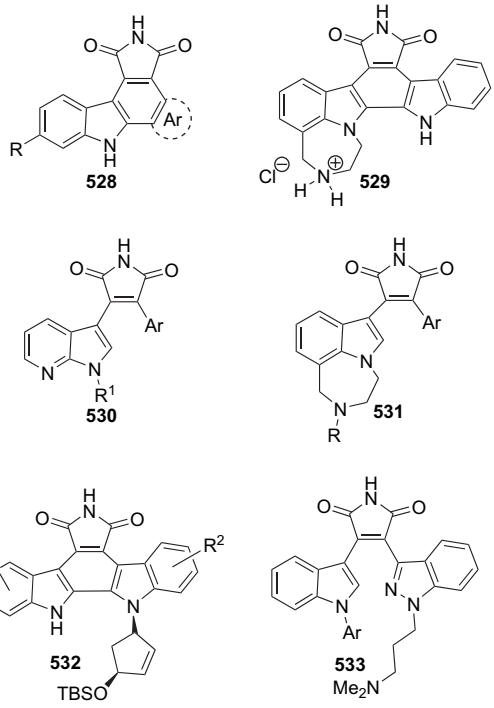
Scheme 50. Synthesis of compounds **524**.

More recently, a similar method has been employed to prepare in low yields a new class of *N*-(azacycloalkyl)bis-indolylmaleimides **525**, which are able to produce selective inhibition of PKCβ.[312]

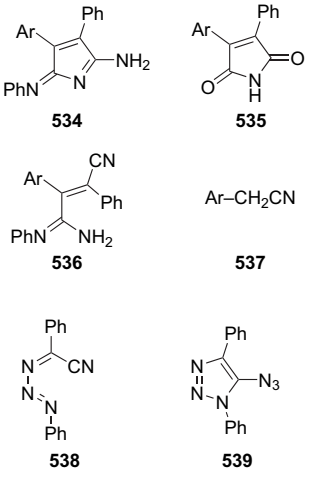
Previously, symmetrical and unsymmetrical bis-indolylmaleimides **524** have alternatively been synthesized by the reaction of readily available indole-3-acetamides **526** with methyl indolyl-3-glyoxylates **527** in THF in the presence of *t*-BuOH.[313]



This protocol, which provides the required compounds in 84–100% yield, has subsequently been applied extensively for the preparation of substances that include natural products such as didemnimides A (**491a**) and B (**491b**),[314] rebeccamycin (**496**),[43] congeners of isogranulatimide,[45] arylpyrrolo[3,4-*c*]carbazoles and indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazoles **528**, which are selective G1 blockers of the cell cycle,[315] *N*-(azacycloalkyl)bis-indolylmaleimides **525**, which are selective inhibitors of PKCβ,[316] indolocarbazole **529**, which was shown to be a potent kinase inhibitor,[317] acyclic 3-(7-azaindolyl)-4-(hetero)arylmaleimides **530**, which include potent and selective inhibitors of GSK-3β,[318] 3-(hetero)aryl-4-[1,7-aza-annulatedindol-3-yl]maleimides **531**, some of which exhibit potent GSK-3 inhibitory activity,[286,287] unsymmetrical indolopyrrolocarbazoles mono-*N*-substituted with a pentacycle, (**532**),[319] and novel indolylindazolylmaleimides **533**, which include potent inhibitors of PKC-β.[30]

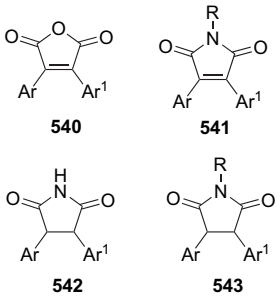


On the other hand, some 2-aryl-3-phenylmaleimides **535** have been prepared by acid-catalyzed hydrolysis of the diarylmaleimidine derivatives **534**, which were easily obtained by isomerization of α-aryl-β-cyano-*N*-phenylcinnamidines **536** by warm alcoholic alkali.[320] The latter compounds could be synthesized in 22–68% yield by a base-catalyzed reaction of arylacetonitriles **537** with 3-(α-cyanobenzylidene)-1-phenyl-1,2,3-triazene (**538**), the product of thermolysis of 5-azido-1,4-diphenyl-1,2,3-triazole (**539**).[320]

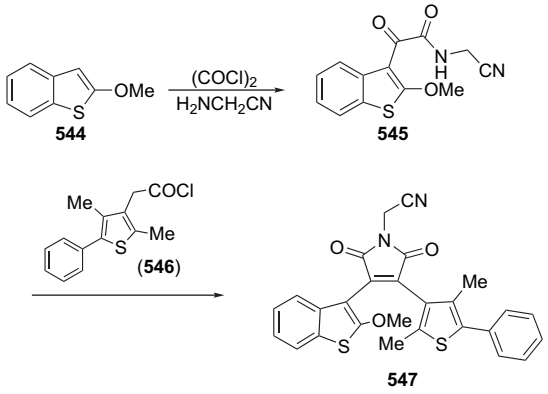


2,3-Diarylmaleimides **482** have occasionally been prepared from the corresponding maleic anhydrides by the standard method of heating at high temperature in the presence of ammonia or an ammonia source[321] or by a procedure also applicable to maleimides containing a sensitive functionality such as an ester or a nitrile group,[322] which involves treatment with a mixture of methanol and hexamethyldisilazane (HMDS) at room temperature.[323] On the other hand, at least in principle, the procedure used to synthesize some *N*-alkyl- and *N*-aryl-succinimides (pyrrolidine-2,5-diones) and -maleimides by a Lewis acid-promoted reaction of HMDS and primary amines with succinic anhydrides and maleic anydrides,[324] respectively, in refluxing benzene[325] might also be employed for preparing *N*-substituted 2,3-diarylsuccinimides **543** and *N*-substituted 2,3-diarylmaleimides **541**.

Established protocols to prepare the latter compounds require heating of maleic anhydrides **540** with primary amines in phenol and Hünig base,[324] in ethanol[141] or DMF[326] or the N-alkylation of the potassium salts of 2,3-diarylmaleimides **482**.[327] Alternatively, maleimides **482** and **541** have been obtained by oxidation of the corresponding 2,3-diarylsuccinimides **542** and **543**, respectively, with 1 equiv of DDQ in CH_2Cl_2 or benzene at room temperature.[44,33]

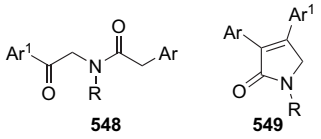


In 1998, the unsymmetrical *N*-cyanomethyl-2,3-diheteroarylmaleimide **547** was synthesized by treatment of 2-methoxythiophene (**544**) with oxalyl chloride and aminoacetonitrile and reaction of the resulting compound **545** with the carboxylic acid chloride **546** (Scheme 51).[328]



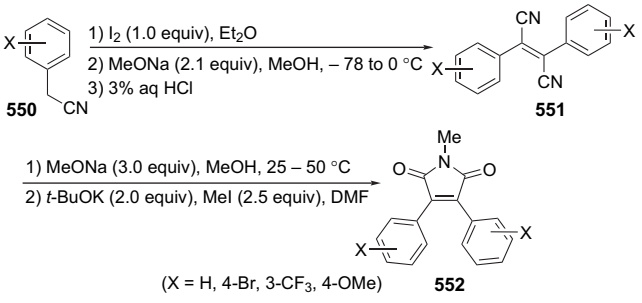
Scheme 51. Synthesis of compound **547**.

More recently, a variety of symmetrical and unsymmetrical *N*-substituted 2,3-diarylmaleimides have been prepared in 59–71% yield by intramolecular ring closure of phenacyl amides **548** with DBU in acetonitrile under an oxygen atmosphere.[224]



Interestingly, this procedure furnished 3,4-diarylpyrrolidin-2-ones **549** in good-to-excellent yields when K_2CO_3 was used in place of DBU.[328]

Finally, in 2004, *N*-methyl-2,3-diarylmaleimides **552** have been conveniently prepared from arylacetonitriles **550** through the diaryl-substituted fumaronitriles **551** by a two-step effective method illustrated in Scheme 52.[329]



Scheme 52. Synthesis of compounds **552**.

7. Conclusions and perspectives

The vicinal diaryl-substituted pyrrole, pyrroline and pyrrolidine derivatives include natural and unnatural compounds with notable biological and pharmacological properties.

These classes of heterocyclic derivatives have stimulated great interest from synthetic and medicinal chemists. We believe that this interest will be secured for some time yet, owing to the continued attention being paid to these and similar heterocycle derivatives in medicinal chemistry and drug development and the progresses in synthetic methodology obtained in recent years. With regard to this last aspect, it is worth mentioning the considerable recent interest, particularly in terms of synthetic and atom efficiency, in the development and application of selective methods to form C–C bonds via C–H activation of (hetero)arenes, in which only one component of the transition metal-catalyzed reaction needs to possess a reactive functional group.[330]

Acknowledgements

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References and notes

1. For some examples of bioactive five-membered heterocycle derivatives with two aryl groups on adjacent positions, see: Bailly, C. *Curr. Med. Chem.—AntiCancer Agents* **2004**, *4*, 364–378.
2. For examples of bioactive 3,4-diaryl-2(5*H*)-furanones, see: (a) Rossi, R.; Bellina, F.; Raugei, E. *Synlett* **2000**, 1749–1752; (b) Bellina, F.; Anselmi, C.; Martina, F.; Rossi, R. *Eur. J. Org. Chem.* **2003**, 2290–2302; (c) Sun, L.; Vasilevich, N. I.; Fuselier, J. A.; Coy, D. H. *Anticancer Res.* **2004**, *24*, 179–186; (d) Kim, Y.; Nam, N.-H.; You, Y.-J.; Ahn, B.-Z. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 719–722; (e) Weber, V.; Coudert, P.; Rubat, E.; Duroux, E.; Vallée-Goyet, D.; Gardette, D.; Bria, M.; Albuisson, E.; Leal, F.; Gramain, J.-C.; Couquelet, J.; Madesclaire, M. *Bioorg. Med. Chem.* **2002**, *10*, 1647–1658.
3. For examples of bioactive imidazole derivatives with two aryl groups on adjacent positions, see: (a) Khanna, I. K.; Weier, R. M.; Xu, X. D.; Koszyk, F. J.; Collins, P. W.; Koboldt, C. A.; Veenhuizen, A. W.; Perkins, W. E.; Casler, J. J.; Masferrer, J. L.; Zhang, Y. Y.; Gregory, S. A.; Seibert, K.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1634–1647; (b) Liverton, N. J.; Butcher, J. W.; Clairborne, C. F.; Claremon, D. A.; Libby, B. E.; Nguyen, K. T.; Pitzenberger, S. M.; Selnick, H. G.; Smith, G. R.; Tebbe, A.; Vacca, J. P.; Varga, S. L.; Agarwal, L.; Dancheck, K.; Forsyth, A. J.; Fletcher, D. S.; Frantz, B.; Hanlon, W. A.; Harper, C. F.; Hofsess, S. J.; Kostura, M.; Lin, J.; Luell, S.; O'Neill, E. A.; Orevillo, C. J.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Visco, D. M.; O'Keefe, S. J. *J. Med. Chem.* **1999**, *42*, 2180–2190; (c) Wang, L.; Woods, K. W.; Li, Q.; Barr, K. J.; McCroskey, R. W.; Hannick, S. M.; Gherke, L.; Credo, R. B.; Hui, Y.-H.; Marsh, K.; Warner, R.; Lee, J. Y.; Zielinski-Mozng, N.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *J. Med. Chem.* **2002**, *45*, 1697–1711; (d) Adams, J. L.; Boehm, J. C.; Gallagher, T. F.; Kassis, S.; Webb, E. F.; Hall, R.; Sorenson, M.; Garigipati, R.; Griswold, D. E.; Lee, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2867–2870; (e) Laufer, S. A.; Wagner, G. K.; Kotschenreuther, D. A.; Albrecht, W. *J. Med. Chem.* **2003**, *46*, 3230–3244; (f) Bellina, F.; Cauteruccio, S.; Mannina, L.; Rossi, R.; Viel, S. *J. Org. Chem.* **2005**, *70*, 3997–4005; (g) Plummer, C. W.; Finke, P. E.; Mills, S. G.; Wang, J.; Tong, X.; Doss, G. A.; Fong, T. M.; Lao, J. Z.; Schaeffer, M.-T.; Chen, J.; Shen, C.-P.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; Van der Ploeg, L. H. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1441–1446; (h) Bellina, F.; Cauteruccio, S.; Mannina, L.; Rossi, R.; Viel, S. *Eur. J. Org. Chem.* **2006**, 693–703.
4. For an example of 1,5-diarylpyrazole derivatives which are cyclooxygenase-2 (COX-2) inhibitors, see: Penning, T.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347–1365.
5. For vicinal diaryl substituted pyrazoles that are estrogen receptor ligands, see: (a) Stauffer, S. R.; Huang, Y.; Coletta, C. J.; Tedesco, R.; Katzellenbogen, J. A. *Bioorg. Med. Chem.* **2001**, *9*, 141–150; (b) Stauffer, S. R.; Huang, Y. R.; Aron, Z. D.; Coletta, C. J.; Katznellenbogen, B. S.; Katznellenbogen, J. A. *Bioorg. Med. Chem.* **2001**, *9*, 151–161; (c) Gusti, R.; Keilitz, R.; Schmidt, K.; von Rauch, M. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *10*, 463–471; (d) Nishiguchi, G. A.; Rodriguez, A. L.; Katznellenbogen, J. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 947–950; (e) Stauffer, S. R.; Coletta, C. J.; Tedesco, R.; Nishiguchi, G.; Carlson, K.; Sun, J.; Katznellenbogen, B. S.; Katznellenbogen, J. A. *J. Med. Chem.* **2000**, *43*, 4934–4947.
6. For vicinal diaryl substituted pyrazoles that are antagonists of the cannabinoid CB_1 receptor, see: (a) Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769–776; (b) Francisco, M. E. Y.; Seltzman, H. H.; Gilliam, A. F.; Mitchell, R. A.; Rider, S. L.; Pertwee, R. G.; Stevenson, L. A.; Thomas, B. F. *J. Med. Chem.* **2002**, *45*, 2708–2719; (c) Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Néliat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Brelière, J. C.; Le Fur, G. *FEBS Lett.* **1994**, *350*, 240–244.
7. For vicinal diaryl substituted thiazoles that are inhibitors of p38 mitogen-activated protein (MAP) kinase, see: (a) Revesz, L.; Blum, E.; Di Padova, F. E.; Buhl, T.; Feifel, R.; Gram, H.; Hiestand, P.; Manning, U.; Rucklin, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3595–3599; (b) Miwatashi, S.; Arikawa, Y.; Naruo, K.-i.; Igaki, K.; Watanabe, Y.; Kimura, H.; Kawamoto, T.; Ohkawa, S. *Chem. Pharm. Bull.* **2005**, *53*, 410–418.
8. For thiophenes with two aryl groups on adjacent positions that are COX-2 inhibitors, see: (a) Gans, K. R.; Gailbraith, W.; Roman, R. J.; Haber, S. B.; Kerr, J. S.; Schmidt, W. K.; Smith, C.; Hewes, W. E.; Ackerman, N. R. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 180–187; (b) Bertenshaw, S. R.; Talley, J. J.; Rogier, D. J.; Graneto, M. J.; Rogers, R. S.; Kramer, S. W.; Penning, T. D.; Koboldt, C. M.; Veenhuizen, A. W.; Zhang, Y.; Perkins, W. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2919–2922.
9. For 1,2,4-triazoles with two aryl groups on adjacent positions that are antifungal compounds, see: (a) Sharma, R. S.; Bahel, S. C. *J. Indian Chem. Soc.* **1982**, *59*, 877–880; (b) Renukadevi, P.; Biradar, J. S. *Indian J. Heterocycl. Chem.* **1999**, *9*, 107–110.

10. For some 2,3-diaryl-2*H*-[1,2,4]-thiadiazoles that are modulators of adenosine A_1 receptors, see: (a) Gao, Z.-G.; Gross, A. S.; Jacobson, K. A. *Life Sci.* **2004**, *74*, 3173–3180; (b) van den Nieuwendijk, A. M. C. H.; Pietra, D.; Heitman, L.; Göblyös, A.; IJezerman, A. P. *J. Med. Chem.* **2004**, *47*, 663–673; (c) Göblyös, A.; de Vries, H.; Brussee, J.; IJezerman, A. P. *J. Med. Chem.* **2005**, *48*, 1145–1151.
11. For examples, see: (a) de Laval, X.; Delarge, J.; Somers, F.; de Tullio, P.; Henrotin, Y.; Pirotte, B.; Dogné, J.-M. *Curr. Med. Chem.* **2000**, *7*, 1041–1062 (for COX-2 inhibitors); (b) Lange, J. H. M.; Kruse, C. G. *Curr. Opin. Drug. Discov. Devel.* **2004**, *7*, 498–506 (for CB_1 cannabinoid receptor antagonists); (c) Nam, N.-H. *Curr. Med. Chem.* **2003**, *10*, 1697–1722 (for combretastatinA-4 analogues as antimitotic antitumor agents); (d) Barth, F.; Rinaldi-Carmona, M. *Curr. Med. Chem.* **1999**, *6*, 745–755 (for CB_1 and CB_2 selective antagonists); (e) Wallace, O. B.; Richardson, T. I.; Dodge, J. A. *Curr. Top. Med. Chem.* **2003**, *3*, 1663–1680 (for estrogen receptor modulators).
12. (a) Katritzky, A. R. *Handbook of Heterocyclic Chemistry*, 1st ed.; Pergamon: New York, NY, 1985; (b) Sundberg, R. J.; Nguyen, P. V. *Prog. Heterocycl. Chem.* **1994**, *6*, 110–128; (c) Gilchrist, T. L. *Contemp. Org. Synth.* **1994**, *1*, 205–217; (d) Sundberg, R. J. *Compr. Heterocycl. Chem.* **1996**, *2*, 119–206; (e) *Second Supplement to the 2nd Edition of Rodd's Chemistry of Carbon Compounds*; Elsevier: Amsterdam, 1997; Vol. IVB; (f) Korostova, S. E.; Mikhaleva, A. I.; Vasil'tsov, A. M.; Trofimov, B. A. *Russ. J. Org. Chem.* **1998**, *34*, 911–948; (g) Gilchrist, T. L. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2491–2515; (h) Ferreira, V. F.; de Souza, M. C. B. V.; Cunha, A. C.; Pereira, L. O. R.; Ferreira, M. L. G. *Org. Prep. Proced. Int.* **2001**, *33*, 411–454; (i) Gilchrist, T. L. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2849–2866; (j) Balme, G. *Angew. Chem., Int. Ed.* **2004**, *43*, 6238–6241; (k) Joshi, U.; Pipelier, M.; Naud, S.; Dubreuil, D. *Curr. Org. Chem.* **2005**, *9*, 261–288.
13. Yoshida, W. Y.; Lee, K. K.; Carrol, A. R.; Scheuer, P. J. *Helv. Chim. Acta* **1992**, *75*, 1721–1725.
14. Kang, H.; Fenical, W. *J. Org. Chem.* **1997**, *62*, 3254–3262.
15. Palermo, J. A.; Brasco, M. F. R.; Seldes, A. M. *Tetrahedron* **1996**, *52*, 2727–2734.
16. Steglich, W.; Steffan, B.; Kopanski, L.; Eckhard, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 459–460.
17. Rudi, A.; Goldberg, I.; Stein, Z.; Frolow, F.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *J. Org. Chem.* **1994**, *59*, 999–1003.
18. Rudi, A.; Evan, T.; Aknin, M.; Kashman, Y. *J. Nat. Prod.* **2000**, *63*, 832–833.
19. Urban, S.; Hickford, S. J. H.; Blunt, J. W.; Munro, M. H. G. *Curr. Org. Chem.* **2000**, *4*, 779–821.
20. Roth, B. D. *Prog. Med. Chem.* **2002**, *40*, 1–22.
21. Chong, P. H.; Seeger, J. D. *Pharmacotherapy* **1997**, *17*, 1157–1177.
22. Deidda, D.; Lampis, G.; Fioravanti, R.; Biava, M.; Porretta, G. C.; Zanetti, S.; Pompei, R. *Antimicrob. Agents Chemother.* **1998**, 3035–3037.
23. Biava, M.; Fioravanti, R.; Porretta, G. C.; Deidda, D.; Maullu, C.; Pompei, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2983–2988.
24. Yee, N. K.; Nummy, L. J.; Byrne, D. P.; Smith, L. L.; Roth, G. P. *J. Org. Chem.* **1998**, *63*, 326–330 and references therein.
25. Pereira, E. R.; Belin, L.; Sancelme, M.; Prudhomme, M.; Ollier, M.; Rapp, M.; Sevère, D.; Riou, J.-F.; Fabbro, D.; Meyer, T. *J. Med. Chem.* **1996**, *39*, 4471–4477.
26. Kashman, Y.; Koren-Goldshlager, G.; Gravalos, M. D. G.; Schleyer, M. *Tetrahedron Lett.* **1999**, *40*, 997–1000.
27. Loya, S.; Rudi, A.; Kashman, Y.; Hizi, A. *Biochem. J.* **1999**, *344*, 85–92.
28. Han, Z.; Pantazis, P.; Lange, T. S.; Wyche, J. H.; Hendrickson, E. A. *Cell Death Differ.* **2000**, *7*, 521–530.
29. Komander, D.; Kular, G. S.; Schüttelkopf, A. W.; Deak, M.; Prakash, K. R.; Bain, J.; Elliott, M.; Garrido-Franco, M.; Kozikowski, A. P.; Alessi, D. R.; van Aalten, D. M. *Structure* **2004**, *12*, 215–226.
30. Zhang, H.-C.; Derian, C. K.; McComsey, D. F.; White, K. B.; Ye, H.; Hecker, L. R.; Li, J.; Addo, M. F.; Croll, D.; Eckardt, A. J.; Smith, C. E.; Li, Q.; Cheung, W.-M.; Conway, B. R.; Emanuel, S.; Demarest, K. T.; Andrade-Gordon, P.; Damiano, B. P.; Maryanoff, B. E. *J. Med. Chem.* **2005**, *48*, 1725–1728.
31. For a review on the design and synthesis of protein kinase C inhibitors, see: Pindur, U.; Kim, Y.-S.; Mehrabani, F. *Curr. Med. Chem.* **1999**, *6*, 29–70.
32. Boger, D. L.; Boyce, C. W.; Labroli, M. A.; Sehon, C. A.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 54–62.
33. Banwell, M.; Flynn, B.; Hockless, D. *Chem. Commun.* **1997**, 2259–2260.
34. Ishibashi, F.; Miyazaki, Y.; Iwao, M. *Tetrahedron* **1997**, *53*, 5951–5962.
35. Peschko, C.; Winklhofer, C.; Steglich, W. *Chem.—Eur. J.* **2000**, *6*, 1147–1152.
36. Boger, D. L.; Soenen, D. R.; Boyce, C. W.; Hedrick, M. P.; Jin, Q. *J. Org. Chem.* **2000**, *65*, 2479–2483.
37. Cironi, P.; Manzanares, I.; Albericio, F.; Álvarez, M. *Org. Lett.* **2003**, *5*, 2952–2962.
38. Mahboobi, S.; Eibler, E.; Koller, M.; Kumar, K. C. S.; Popp, A. *J. Org. Chem.* **1999**, *64*, 4697–4704.
39. Kobayashi, Y.; Fujimoto, T.; Fukuyama, T. *J. Am. Chem. Soc.* **1999**, *121*, 6501–6502.
40. Link, J. T.; Raghavan, S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 552–553.
41. Mayer, G.; Wille, G.; Steglich, W. *Tetrahedron Lett.* **1996**, *37*, 4483–4486.
42. Ohkubo, M.; Nishimura, T.; Jona, H.; Honma, T.; Morishima, H. *Tetrahedron* **1996**, *52*, 8099–8112.
43. Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. *J. Org. Chem.* **1999**, *64*, 2465–2470.
44. Bergman, J.; Koch, E.; Pelcman, B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2609–2614.
45. Piers, E.; Britton, R.; Andersen, R. J. *J. Org. Chem.* **2000**, *65*, 530–535.
46. Faul, M. M.; Sullivan, K. A.; Grutsch, J. L.; Winneroski, L. L.; Shih, C.; Sanchez-Martinez, C.; Cooper, J. T. *Tetrahedron Lett.* **2004**, *45*, 1095–1098.
47. Roy, S.; Eastman, A.; Gribble, G. W. *Synth. Commun.* **2005**, *35*, 595–601.
48. Ramana Rao, V. V.; Wentrup, C. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1232–1235.
49. (a) Urban, S.; Butler, M. S.; Capon, R. J. *Aust. J. Chem.* **1994**, *47*, 1919–1924; (b) Urban, S.; Hobbs, L.; Hooper, J. N. A.; Capon, R. J. *Aust. J. Chem.* **1995**, *48*, 1919–1924.
50. Urban, S.; Hobbs, L.; Hooper, J. N. A.; Capon, R. J. *Aust. J. Chem.* **1995**, *48*, 1491–1494.
51. Andersen, R. J.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492–5495.
52. Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 489–501.

53. Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4570–4574.
54. Urban, S.; Capon, R. J. *Aust. J. Chem.* **1996**, *49*, 711–713.
55. Davies, R. A.; Carroll, A. R.; Pierens, G. K.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 419–424.
56. Krishnaiah, P.; Reddy, V. L. N.; Venkataramana, G.; Ravinder, K.; Srinivasulu, M.; Raju, T. V.; Ravikumar, K.; Chandrasekar, D.; Ramakrishna, S.; Venkateswarlu, Y. *J. Nat. Prod.* **2004**, *67*, 1168–1171.
57. Reddy, S. M.; Srinivasulu, M.; Satyanarayana, N.; Kondapi, A. K.; Venkateswarlu, Y. *Tetrahedron* **2005**, *61*, 9242–9247.
58. Reddy, M. V. R.; Faulkner, D. J.; Venkateswarlu, Y.; Rao, M. R. *Tetrahedron* **1997**, *53*, 3457–3466.
59. Quesada, A. R.; Gravalos, M. D. G.; Puentes, J. L. F. *Br. J. Cancer* **1996**, *74*, 677–682.
60. Facompré, M.; Tardy, C.; Bal-Mahieu, C.; Colson, P.; Perez, C.; Manzanares, I.; Cuevas, C.; Bailly, C. *Cancer Res.* **2003**, *63*, 7392–7399.
61. Ridley, C. P.; Reddy, M. V. Rì; Rocha, G.; Bushman, F. D.; Faulkner, D. J. *Bioorg. Med. Chem.* **2002**, *10*, 3285–3290.
62. Warabi, K.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *J. Org. Chem.* **2003**, *68*, 2765–2770.
63. Kashman, Y.; Koren-Goldshlager, G.; Gravalos, M. D. G. WO 00/20,411. *Chem. Abstr.* **2000**, *132*, 26078.
64. Burnham, B. S.; Gupton, J. T.; Krumpe, K.; Webb, T.; Shuford, J.; Bowers, B.; Warren, A. E.; Barnes, C.; Hall, I. H. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 337–341.
65. Soenen, D. R.; Hwang, I.; Hedrick, M. P.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1777–1781.
66. Fürstner, A.; Krause, H.; Thiel, O. R. *Tetrahedron* **2002**, *58*, 6373–6380.
67. de Laszlo, S. E.; Visco, D.; Agarwai, L.; Chang, L.; Chen, J.; Croft, G.; Forsyth, A.; Flétcher, D.; Frantz, B.; Hacker, C.; Hanlon, W.; Harper, C.; Kostura, M.; Li, B.; Luell, S.; MacCoss, M.; Mantlo, N.; O'Neill, E. A.; Orevillo, C.; Pang, M.; Parsons, J.; Rolando, A.; Sahly, Y.; Sidler, K.; Widmer, W. R.; O'Keefe, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2689–2694.
68. Pargellis, C.; Regan, J. *Curr. Opin. Investig. Drugs* **2003**, *4*, 566–571.
69. Jackson, P. F.; Bullington, J. L. *Curr. Top. Med. Chem.* **2002**, *2*, 1011–1020.
70. Kumar, S.; Boehm, J.; Lee, J. C. *Nat. Rev. Drug Discov.* **2003**, *2*, 717–726.
71. (a) Foster, M. L.; Halley, F.; Houness, J. E. *Drug News Perspect.* **2000**, *13*, 488–497; (b) Johnson, G. V.; Bailey, C. D. *Exp. Neurol.* **2003**, *183*, 263–268; (c) Dalrymple, S. A. *J. Mol. Neurosci.* **2002**, *19*, 295–299; (d) Schultz, R. M. *Prog. Drug Res.* **2003**, *60*, 59–92; (e) Olson, J. M.; Hallahan, A. R. *Trends Mol. Med.* **2004**, *10*, 125–129; (f) Behr, T. M.; Berova, M.; Doe, C. P.; Ju, H.; Augermann, C. E.; Boehm, J.; Willette, R. N. *Curr. Opin. Investig. Drugs* **2003**, *4*, 1059–1064.
72. Gill, A.; Cleasby, A.; Jhoti, H. *ChemBiochem* **2005**, *6*, 506–512.
73. Pelaia, G.; Cuda, G.; Vatrella, A.; Gallelli, L.; Caraglia, M.; Marra, M.; Abbruzzese, A.; Caputi, M.; Maselli, R.; Costanzo, F. S.; Marsico, S. A. *J. Cell Physiol.* **2005**, *202*, 642–653.
74. Roth, B. D.; Blankley, C. J.; Chucholowski, A. W.; Ferguson, E.; Hoefle, M. L.; Ortwine, D. F.; Newton, R. S.; Sekerke, C. S.; Sliskovic, D. R.; Stratton, C. D.; Wilson, M. W. *J. Med. Chem.* **1991**, *34*, 357–366.
75. de Laszlo, S. E.; Hacker, C.; Li, B.; Kim, D.; MacCoss, M.; Mantlo, N.; Pivnichny, J. V.; Colwell, L.; Koch, G. E.; Cascieri, M. A.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 641–646.
76. Goel, A.; Agarwal, N.; Singh, F. V.; Sharon, A.; Tiwari, P.; Dixit, M.; Pratap, R.; Srivastava, A. K.; Maulik, P. R.; Ram, V. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1089–1092.
77. Sloop, K. W.; Michael, M. D. *Drugs Future* **2004**, *29*, 835–841.
78. Kurukulasuriya, R.; Link, J. T.; Mader, D. J.; Pei, Z.; Rhode, J. J.; Richards, S. J.; Souers, A. J.; Szczepankiewicz, B. G. *Curr. Med. Chem.* **2003**, *10*, 99–121.
79. Vanhuyse, M.; Kluza, J.; Tardy, C.; Otero, G.; Cuevas, C.; Bailly, C.; Lansiaux, A. *Cancer Lett.* **2005**, *221*, 165–175.
80. Biava, M.; Porretta, G. C.; Cappelli, A.; Vomero, S.; Manetti, F.; Botta, M.; Sautebin, L.; Rossi, A.; Makovec, F.; Anzini, M. *J. Med. Chem.* **2005**, *48*, 3428–3432.
81. Khanna, I. K.; Weier, R. M.; Yu, Y.; Collins, P. W.; Miyashiro, J. M.; Koboldt, C. M.; Veenhuizen, A. W.; Currie, J. L.; Seibert, K.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1619–1633.
82. Wilkerson, W. W.; Galbraith, W.; Gans-Brangs, K.; Grubb, M.; Hewes, W. E.; Jaffee, B.; Kenney, J. P.; Kerr, J.; Wong, N. *J. Med. Chem.* **1994**, *37*, 988–998.
83. Wilkerson, W. W.; Copeland, R. A.; Covington, M.; Trzaskos, J. M. *J. Med. Chem.* **1995**, *38*, 3895–3901.
84. For reviews on the COX-2 inhibitors, see: (a) De Witt, D. L. *Mol. Pharmacol.* **1999**, *55*, 625–631; (b) Rodrigues, C. R.; Veloso, M. P.; Verli, H.; Fraga, C. A. M.; Miranda, A. L. P.; Barreiro, E. J. *Med. Chem. Rev.* **2004**, *1*, 73–90; (c) Dannhardt, G.; Kiefer, W. *Eur. J. Med. Chem.* **2001**, *36*, 109–126; (d) Michaux, C.; Charlier, C. *Mini Rev. Med. Chem.* **2004**, *4*, 603–615; (e) Garg, R.; Kurup, A.; Mekapati, S. B.; Hansch, C. *Chem. Rev.* **2003**, *103*, 703–731.
85. Zoete, V.; Maglia, F.; Rougée, M.; Bensasson, R. V. *Free Radical Biol. Med.* **2000**, *28*, 1638–1641.
86. Treibs, A.; Derra, R. *Ann.* **1954**, *589*, 176–187.
87. Grimshaw, J.; Hewitt, S. A. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2995–2998.
88. Kruse, C. G.; Bouw, J. P.; van Hes, R.; van de Kuilen, A.; den Hartog, J. A. J. *Heterocycles* **1987**, *26*, 3141–3151.
89. Almerico, A. M.; Montalbano, A.; Diana, P.; Barraja, P.; Lauria, A.; Cirrincione, G.; Dattolo, G. *ARKIVOC* **2001**, 129–142.
90. Delayen, A.; Goossens, R.; Houssin, R.; Hénichart, J.-P. *Heterocycles* **2005**, *65*, 1673–1678.
91. (a) Stetter, H.; Kuhlmann, H. *Org. React.* **1991**, *40*, 407–496; (b) Stetter, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 639–647.
92. Banik, B. K.; Banik, I.; Renteria, M.; Dasgupta, S. K. *Tetrahedron Lett.* **2005**, *46*, 2643–2645.
93. Mohammed, K.; Hilmy, H. *Arch. Pharm. Pharm. Med. Chem.* **2004**, *337*, 15–19.
94. Abdelhamid, A. O.; Negm, A. M.; Abass, I. M. *J. Prakt. Chem.* **1989**, *331*, 31–36.
95. Nasakin, O. E.; Alekseev, V. V.; Terent'ev, P. B.; Bulai, A. K.; Zablotskaya, M. Y. *Khim. Geterotsikl. Soedin.* **1983**, 1062–1066.
96. Korostova, S. E.; Mikhaleva, A. I.; Vasil'tsov, A. M.; Trofimov, B. A. *Russ. J. Org. Chem.* **1998**, *34*, 1691–1714.
97. Magedov, I. V.; Kornienko, A. V.; Zotova, T. O.; Drozd, V. N. *Tetrahedron Lett.* **1995**, *36*, 4619–4622.
98. Kel'in, A. V.; Sromek, A. W.; Gevorgyan, V. *J. Am. Chem. Soc.* **2001**, *123*, 2074–2075.
99. Nishio, T. *Helv. Chim. Acta* **1998**, *81*, 1207–1214.

100. Lagu, B.; Pan, M.; Wachter, M. P. *Tetrahedron Lett.* **2001**, *42*, 6027–6030.
101. Dekura, F.; Honda, T.; Mori, M. *Chem. Lett.* **1997**, 825–826.
102. Wang, Y.; Zhu, S. *Org. Lett.* **2003**, *5*, 745–748.
103. Agarwal, S.; Knölker, H.-J. *Org. Biomol. Chem.* **2004**, *2*, 3060–3062.
104. Barluenga, J.; Tomás, M.; Kouznetsov, V.; Sobrino, A. S.; Rubio, E. *J. Org. Chem.* **1996**, *61*, 2185–2190.
105. (a) Hoberg, H.; Barluenga Mur, J. *Synthesis* **1970**, 142–144; (b) Wittig, G.; Fischer, S.; Tanaka, M. *Justus Liebigs Ann. Chem.* **1973**, 1075–1081.
106. Katritzky, A. R.; Chang, H.-X.; Verin, S. V. *Tetrahedron Lett.* **1995**, *36*, 343–346.
107. Katritzky, A. R.; Wu, H.; Xie, L.; Rachwal, S.; Rachwal, B.; Jiang, J.; Zhang, G.; Lang, H. *Synthesis* **1995**, 1315–1323.
108. (a) Katritzky, A. R.; Zhang, L.; Yao, J.; Denisko, O. V. *J. Org. Chem.* **2000**, *65*, 8074–8076; (b) Katritsky, A. R.; Denisko, O. V. *Pure Appl. Chem.* **2000**, *72*, 1597–1603.
109. Katritzky, A. R.; Yao, J.; Bao, W.; Qi, M.; Steel, P. J. *J. Org. Chem.* **1999**, *64*, 346–350.
110. Trofimov, B. A.; Korostova, S. E.; Belabanova, L. N.; Mikhaleva, A. I. *Zh. Org. Khim.* **1978**, *14*, 2182–2184.
111. Korostova, S. E.; Mikhaleva, A. I.; Vasil'tsov, A. M.; Trofimov, B. A. *Russ. J. Org. Chem.* **1998**, *34*, 911–948.
112. Campos, P. J.; Soldevilla, A.; Sampedro, D.; Rodríguez, M. A. *Org. Lett.* **2001**, *3*, 4087–4089.
113. Katritzky, A. R.; Zhu, L.; Lang, H.; Denisko, O.; Wang, Z. *Tetrahedron* **1995**, *51*, 13271–13276.
114. (a) Katritzky, A. R.; Jiang, J.; Steel, P. J. *J. Org. Chem.* **1994**, *59*, 4551–4555; (b) Katritzky, A. R.; Jiang, J.; Greenhill, J. V. *J. Org. Chem.* **1993**, *58*, 1987–1988.
115. van Leusen, A. M.; Boerma, G. J. M.; Helmholdt, R. B.; Siderius, H.; Strating, J. *Tetrahedron Lett.* **1972**, *13*, 2367–2368.
116. van Leusen, A. M.; Siderius, H.; Hoogenboom, B. E.; van Leusen, D. *Tetrahedron Lett.* **1972**, *13*, 5337–5340.
117. Romashin, Y. N.; Liu, M. T. H.; Bonneau, R. *Chem. Commun.* **1999**, 447–448.
118. Romashin, Y. N.; Liu, M. T. H.; Ma, W.; Moss, R. A. *Tetrahedron Lett.* **1999**, *40*, 7163–7165.
119. Palacios, F.; Herrán, E.; Rubiales, G. *Heterocycles* **2002**, *58*, 89–92.
120. Handy, S. T.; Bregman, H.; Lewis, J.; Zhang, J.; Zhang, X.; Zhang, Y. *Tetrahedron Lett.* **2003**, *44*, 427–430.
121. Handy, S. T.; Zhang, Y.; Bregman, Y. *J. Org. Chem.* **2004**, *69*, 2362–2366.
122. Belanger, P. *Tetrahedron Lett.* **1979**, *20*, 2505–2508.
123. (a) Biftu, T.; Feng, D.; Ponpipom, M.; Giritra, N.; Liang, G.-B.; Qian, X.; Bugianesi, R.; Simeone, J.; Chang, L.; Gurnett, A.; Liberator, P.; Dilski, P.; Leavitt, P. S.; Crumley, T.; Misura, A.; Murphy, T.; Rattray, S.; Samaras, S.; Tamas, T.; Mathew, J.; Brown, C.; Thompson, D.; Schmatz, D.; Fisher, M.; Wyvratt, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3296–3301; (b) Liang, G.-B.; Qian, X.; Biftu, T.; Feng, T.; Fisher, M.; Crumley, T.; Darkin-Rattray, S. J.; Dulski, P. M.; Gurnett, A.; Leavitt, P. S.; Liberator, P. A.; Misura, A. S.; Samaras, S.; Tamas, T.; Schamtz, D.; Wyvratt, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4570–4573.
124. Chan, C.; Bailey, E. J.; Hayman, D. F.; Hutson, J. L.; Inglis, G. A. G.; Jones, P. S.; Keeling, S. E.; Kirk, B. E.; Lamont, R. B.; Lester, M. G.; Pritchard, J. M.; Ross, B. C.; Scicinski, J. J.; Spooner, S. J.; Smith, G.; Steeples, I. P.; Watson, N. S. *J. Med. Chem.* **1993**, *36*, 3646–3657.
125. Procopiou, P. A.; Draper, C. D.; Hutson, J. L.; Inglis, G. A.; Ross, B. C.; Watson, N. S. *J. Med. Chem.* **1993**, *36*, 3658–3662.
126. Gao, Y.; Shirai, M.; Sato, F. *Tetrahedron Lett.* **1996**, *14*, 7787–7790.
127. Dieter, R. K.; Yu, H. *Org. Lett.* **2000**, *2*, 2283–2286.
128. Pandey, P. S.; Rao, T. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 129–131.
129. Roth, B. D.; Blankley, C. J.; Ferguson, E.; Hoefle, M. L.; Ortwine, D. F.; Newton, R. S.; Sekerke, C. S.; Sliskovic, D. R.; Stratton, C. D.; Wilson, M. W. *J. Med. Chem.* **1991**, *34*, 357–366.
130. Crumbie, R. L.; Nimitz, J. S.; Mosher, H. S. *J. Org. Chem.* **1982**, *47*, 4040–4045.
131. Iwasawa, N.; Ochiai, T.; Maeyama, K. *J. Org. Chem.* **1998**, *63*, 3164–3165.
132. (a) Friedman, M. *J. Org. Chem.* **1965**, *30*, 859–863; (b) Gale, P. A.; Camiolo, S.; Tizzard, G. J.; Chapman, C. P.; Light, M. E.; Coles, S. J.; Hursthouse, M. B. *J. Org. Chem.* **2001**, *66*, 7849–7853.
133. Sera, A.; Fukumoto, S.; Yoneda, T.; Yamada, H. *Heterocycles* **1986**, *24*, 697–702.
134. Ono, N.; Miyagawa, H.; Ueta, T.; Ogawa, T.; Tani, H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1595–1601.
135. Barton, D. H. R.; Zard, S. Z. *J. Chem. Soc., Chem. Commun.* **1985**, 1098–1100.
136. Barton, D. H. R.; Kervagoret, J.; Zard, S. Z. *Tetrahedron* **1990**, *46*, 7587–7598.
137. (a) Hashimoto, T.; Takaoka, S.; Yasuda, A.; Akazawa, K.; Tori, M.; Asakawa, Y. *Abstracts of the 34th Symposium on the Chemistry of Natural Products*; Sankawa, U. Ed.: Tokyo 1992, p 470; (b) Hashimoto, T.; Takaoka, S.; Yasuda, A.; Akazawa, K.; Tori, M.; Asakawa, Y. *Tetrahedron Lett.* **1994**, *16*, 2559–2560.
138. Fröde, R.; Hinze, C.; Josten, I.; Schmidt, B.; Steffan, B.; Steglich, W. *Tetrahedron Lett.* **1994**, *35*, 1689–1690.
139. Peschko, C.; Steglich, W. *Tetrahedron Lett.* **2000**, *41*, 9477–9481.
140. Terpin, A.; Polborn, K.; Steglich, W. *Tetrahedron* **1995**, *51*, 9941–9946.
141. Heim, A.; Terpin, A.; Steglich, W. *Angew. Chem., Int. Ed.* **1997**, *36*, 155–156.
142. Ebel, H.; Terpin, A.; Steglich, W. *Tetrahedron Lett.* **1998**, *39*, 9165–9166.
143. Cironi, P.; Manzanares, I.; Albericio, F.; Álvarez, M. *Org. Lett.* **2003**, *5*, 2959–2962.
144. For recent developments in the synthesis of pentacyclic lamellarins, see: (a) Ishibashi, F.; Miyazaki, Y.; Iwao, M. *Tetrahedron* **1997**, *53*, 5951–5962; (b) Banwell, M.; Flynn, B.; Hockless, D. *Chem. Commun.* **1997**, 2259–2260; (c) Ishibashi, F.; Tanabe, S.; Oda, T.; Iwao, M. *J. Nat. Prod.* **2002**, *65*, 500–501; (d) Ploypradith, P.; Jinaglueng, W.; Pavaro, C.; Ruchirawat, S. *Tetrahedron Lett.* **2003**, *44*, 1363–1366; (e) Olsen, C. A.; Parera, N.; Albericio, F.; Álvarez, M. *Tetrahedron Lett.* **2005**, *46*, 2041–2044; (f) Pla, D.; Marchal, A.; Olsen, C. A.; Albericio, F.; Álvarez, M. *J. Org. Chem.* **2005**, *70*, 8231–8234.
145. Smith, N. D.; Huang, D.; Cosford, N. D. P. *Org. Lett.* **2002**, *4*, 3537–3539.
146. (a) Dijkstra, H. P.; ten Have, R.; van Leusen, A. M. *J. Org. Chem.* **1998**, *63*, 5332–5338; (b) van Leusen, D.; van Leusen, A. M. *Org. React.* **2001**, *57*, 417–666.
147. Chang, C. K.; Bag, N. *J. Org. Chem.* **1995**, *60*, 7030–7032.

148. Banwell, M. C.; Bray, A. M.; Edwards, A. J.; Wong, D. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1340–1343.
149. Heinrich, M. R.; Steglich, W.; Banwell, M. G.; Kashman, Y. *Tetrahedron* **2003**, *59*, 9239–9247.
150. Banwell, M. G.; Flynn, B. L.; Hamel, E.; Hockless, C. C. R. *Chem. Commun.* **1997**, 207–208.
151. Bray, B. L.; Mathies, P. M.; Naef, R.; Solas, D. R.; Tidwell, T. T.; Artis, D. R.; Muchowski, J. M. *J. Org. Chem.* **1990**, *55*, 6317–6328.
152. (a) Brown, R. T.; Fox, B. W.; Hadfield, J. A.; McGown, A. T.; Mayalarp, S. P.; Pettit, G. R.; Woods, J. A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 577–582; (b) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666–1672; (c) Cirla, A.; Mann, J. *Nat. Prod. Rep.* **2003**, *20*, 558–564.
153. (a) Banwell, M. G. *Pure Appl. Chem.* **1996**, *68*, 539–542; (b) Shi, Q.; Chen, K.; Brossi, A.; Verdier-Pinard, P.; Hamel, E.; McPhail, A. T.; Lee, K.-H. *Helv. Chim. Acta* **1998**, *81*, 1023–1027 and references therein; (c) Panda, D.; Dayo, J. E.; Jordan, M. A.; Wilson, L. *Biochemistry* **1995**, *34*, 9921–9929.
154. Marfil, M.; Albericio, F.; Álvarez, M. *Tetrahedron* **2004**, *60*, 8659–8668.
155. Fürstner, A.; Weintritt, H.; Hupperts, A. *J. Org. Chem.* **1995**, *60*, 6637–6641.
156. Rolfs, A.; Liebscher, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 712–714.
157. Gupton, J. T.; Krumpe, K. E.; Burnham, B. S.; Dwornik, K. A.; Petrich, S. A.; Du, K. X.; Bruce, M. A.; Vu, P.; Vargas, M.; Keertikar, K.; Hosein, K.; Jones, C. R.; Sikorski, J. A. *Tetrahedron* **1998**, *54*, 5075–5088.
158. Gupton, J. T.; Krumpe, K. E.; Burnham, B. S.; Webb, T. M.; Shuford, J. S.; Sikorski, J. A. *Tetrahedron* **1999**, *55*, 14515–14522.
159. Gupton, J. T.; Clough, S.; Miller, R.; Lukens, J.; Henry, C.; Kanters, R. P. F.; Sikorski, J. *Tetrahedron* **2003**, *59*, 207–215.
160. Kreipl, A. T.; Reid, C.; Steglich, W. *Org. Lett.* **2002**, *4*, 3287–3288.
161. Gupton, J. T.; Miller, R. B.; Krumpe, K. E.; Clough, S. C.; Banner, E. J.; Kanters, R. P. F.; Du, K. X.; Keertikar, K. M.; Lauerman, N. E.; Solano, J. M.; Adams, B. R.; Callahan, D. W.; Little, B. A.; Scharf, A. B.; Sikorski, J. A. *Tetrahedron* **2005**, *61*, 1845–1854.
162. Kim, S.; Son, S.; Kang, H. *Bull. Korean Chem. Soc.* **2001**, *22*, 1403–1406.
163. Bullington, J. L.; Wolff, R. R.; Jackson, P. J. *J. Org. Chem.* **2002**, *67*, 9439–9442.
164. Dieter, R. K.; Yu, H. *Org. Lett.* **2001**, *3*, 3855–3858.
165. Boger, D. L. *Chemtracts: Org. Chem.* **1996**, *9*, 149–189.
166. Boger, D. L. *Chem. Rev.* **1986**, *86*, 781–793.
167. Hamasaki, A.; Zimpleman, J. M.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2005**, *127*, 10767–10770.
168. Liu, J.-H.; Yang, Q.-C.; Mak, T. C. W.; Wong, H. N. C. *J. Org. Chem.* **2000**, *65*, 3587–3595.
169. Liu, J.-H.; Chan, H.-W.; Wong, H. N. C. *J. Org. Chem.* **2000**, *65*, 3274–3283.
170. Mathew, P.; Asokan, C. V. *Tetrahedron Lett.* **2005**, *46*, 475–478.
171. Roth, H. J.; George, H.; Assadi, F.; Rimek, H. J. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 946.
172. James, D. S.; Fanta, P. E. *J. Org. Chem.* **1962**, *27*, 3346–3348.
173. Danks, T. N.; Velo-Rego, D. *Tetrahedron Lett.* **1994**, *35*, 9443–9444.
174. Katritzky, A. R.; Wang, Z.; Li, J.; Levell, J. R. *J. Heterocycl. Chem.* **1997**, *34*, 1379–1381.
175. McEwen, W. E.; Grossi, A. V.; MacDonald, R. J.; Stamegna, A. P. *J. Org. Chem.* **1980**, *45*, 1301–1308.
176. Cooney, J. V.; McEwen, W. E. *J. Org. Chem.* **1981**, *46*, 2570–2573.
177. Ogura, K.; Yanai, H.; Miokawa, M.; Akazome, M. *Tetrahedron Lett.* **1999**, *40*, 8887–8891.
178. Periasamy, M.; Srinivas, G.; Bharathi, P. *J. Org. Chem.* **1999**, *64*, 4204–4205.
179. Rao, H. S. P.; Jothilingam, S.; Scheeren, H. W. *Tetrahedron* **2004**, *60*, 1625–1630.
180. Wurz, R. P.; Charette, A. B. *Org. Lett.* **2005**, *7*, 2313–2316.
181. Aumann, R.; Jasper, B.; Goddard, R.; Krüger, C. *Chem. Ber.* **1994**, *127*, 717–724.
182. Chen, Y.; Zeng, D. X.; Xie, N.; Dang, Y. Z. *J. Org. Chem.* **2005**, *70*, 5001–5005.
183. Schröter, S.; Bach, T. *Synlett* **2005**, 1957–1959.
184. Klappa, J. J.; Rich, A. E.; McNeill, K. *Org. Lett.* **2002**, *4*, 435–437.
185. Farcas, S.; Namy, J.-L. *Tetrahedron* **2001**, *57*, 4881–4888.
186. Katritzky, A. R.; Huang, T.-B.; Voronkov, M. V.; Wang, M.; Kolb, H. *J. Org. Chem.* **2000**, *65*, 8819–8821.
187. Braun, R. U.; Zeitler, K.; Müller, T. J. *Org. Lett.* **2001**, *3*, 3297–3300.
188. Bharadwaj, A. R.; Scheidt, K. A. *Org. Lett.* **2004**, *6*, 2465–2468.
189. Shi, D.; Shi, C.; Wang, X.; Zhuang, Q.; Tu, S.; Hu, F. *Synlett* **2004**, 2239–2241.
190. Dhawan, R.; Arndtsen, B. A. *J. Am. Chem. Soc.* **2004**, *126*, 468–469.
191. For some reviews on the one-pot multicomponent processes, see: (a) Dömling, A. *Curr. Opin. Chem. Biol.* **2002**, *6*, 306–313; (b) Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168–3210; (c) Simon, C.; Constantieux, T.; Rodriguez, J. *Eur. J. Org. Chem.* **2004**, 4957–4980; (d) Ref. 12h.
192. Yoshimura, H.; Nagai, M.; Hibi, S.; Kikuc hi, K.; Abe, S.; Hida, T.; Higashi, S.; Hishinuma, I.; Yamanaka, T. *J. Med. Chem.* **1995**, *38*, 3163–3173.
193. (a) Banik, B. K.; Samajdar, S.; Banik, I. *J. Org. Chem.* **2004**, *69*, 213–216; (b) Lee, C.-F.; Yang, L.-M.; Hwu, T.-Y.; Feng, A.-S.; Tseng, J.-C.; Luh, T.-Y. *J. Am. Chem. Soc.* **2000**, *122*, 4992–4993.
194. Lehuédé, J.; Fauconneau, B.; Barrier, L.; Ourakow, M.; Piriou, A.; Vierfond, J.-M. *Eur. J. Med. Chem.* **1999**, *34*, 991–996.
195. Xu, X.; Zhang, Y. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2836–2839.
196. For some examples, see: (a) Cinquin, C.; Bortolussi, M.; Bloch, R. *Tetrahedron: Asymmetry* **1996**, *7*, 3327–3332; (b) Keely, S. L., Jr.; Tahk, F. C. *J. Am. Chem. Soc.* **1968**, *90*, 5584–5587; (c) Ulbrich, H.; Fiebich, B.; Dannhardt, G. *Eur. J. Med. Chem.* **2002**, *37*, 953–959; (d) Montes de Oca, A. C. B.; Vorreja, C. D. R. *ARKIVOC* **2003**, 390–403; (e) Batey, R. A.; Simonic, P. D.; Lin, D.; Smyj, R. P.; Lough, A. J. *Chem. Commun.* **1999**, 651–652.
197. *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic: San Diego, 2003; Vols. 11–20.
198. For some recent examples, see: (a) Laohakunjit, N.; Biimhorm, A. *Flavour Fragrance J.* **2004**, *19*, 251–259; (b) Pluotno, A.; Varmeli, S. *Tetrahedron* **2005**, *61*, 575–583; (c) Tyroller, S.; Zwickenpflug, W.; Richter, E. *J. Agric. Food Chem.* **2002**, *50*, 4909–4915.

199. Dannhardt, G.; Kiefer, W. *Arch. Pharm. Pharm. Med. Chem.* **2001**, *334*, 183–188.
200. For a recent review, see: Shvekhgeimer, M.-G. A. *Chem. Heterocycl. Compd.* **2003**, *39*, 405–448.
201. Demoen, P. J.; Janssen, P. A. J. *J. Org. Chem.* **1959**, *81*, 6281–6285.
202. Pal, K.; Behnke, M. L.; Tong, L. *Tetrahedron Lett.* **1993**, *34*, 6205–6208.
203. Gotthardt, H.; Huisgen, R.; Schaefer, F. C. *Tetrahedron Lett.* **1964**, *5*, 487–491.
204. Texier, F.; Mazari, M.; Yebdri, O.; Tonnard, F.; Carrié, R. *Tetrahedron* **1990**, *46*, 3515–3526.
205. Dieter, R. K.; Chen, N.; Yu, H.; Nice, L. E.; Gore, V. K. *J. Org. Chem.* **2005**, *70*, 2109–2119.
206. Amombo, M. O.; Hausherr, A.; Reissig, H.-U. *Synlett* **1999**, 1871–1874.
207. (a) Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275–282; (b) Furusaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S. *J. Chem. Soc., Chem. Commun.* **1978**, 800–801; (c) Furusaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3681–3685.
208. (a) Sezaki, M.; Sasaki, T.; Nakazawa, T.; Takeda, U.; Iwata, M.; Watanabe, T.; Koyama, M.; Kai, F.; Shomura, T.; Kojima, M. *J. Antibiot.* **1985**, *38*, 1439–1444; (b) Kase, H.; Iwahashi, K.; Matsuda, Y. *J. Antibiot.* **1986**, *39*, 1059–1065; (c) Nakanishi, S.; Matsuda, Y.; Iwahashi, K.; Kase, H. *J. Antibiot.* **1986**, *39*, 1066–1071.
209. Shen, F.; Bai, A.-P.; Guo, Z.-R.; Cheng, G.-F. *Acta Pharmacol. Sin.* **2002**, 762–768.
210. Bosch, J.; Roca, T.; Catena, J.-L.; Llorens, O.; Pérez, J.-J.; Lagunas, C.; Fernández, A. G.; Miquel, I.; Fernández-Serrat, A.; Farrerons, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1745–1748.
211. For examples, see: (a) Langlois, N.; Radom, M.-O. *Tetrahedron Lett.* **1998**, *39*, 857–860; (b) Langlois, N.; Calvez, O.; Radom, M.-O. *Tetrahedron Lett.* **1997**, *38*, 8037–8040; (c) Luker, T.; Koot, W.-J.; Hiemstra, H.; Speckamp, W. N. *J. Org. Chem.* **1998**, *63*, 220–221.
212. For some examples, see: (a) Mattern, R.-H. *Tetrahedron Lett.* **1996**, *37*, 291–294; (b) Woo, C.-K.; Jones, K. *Tetrahedron Lett.* **1991**, *32*, 6949–6952; (c) Zhang, J.; Blazecka, P. G.; Davidson, J. G. *Org. Lett.* **2003**, *5*, 553–556; (d) Chatani, C.; Kamitani, A.; Murai, S. *J. Org. Chem.* **2002**, *67*, 7014–7018.
213. Kascheres, C.; Kascheres, A.; Pilli, P. S. H. *J. Org. Chem.* **1980**, *45*, 5340–5343.
214. Takahashi, M.; Funaki, T.; Honda, H.; Yokoyama, Y.; Takimoto, H. *Heterocycles* **1982**, *19*, 1921–1924.
215. Gomaa, M. A.-M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 341–344.
216. Gao, Y.; Shirai, M.; Sato, F. *Tetrahedron Lett.* **1997**, *38*, 6849–6852.
217. Bouancheau, C.; Rudler, M.; Chelain, E.; Rudler, H.; Vaissermann, J.; Daran, J.-C. *J. Organomet. Chem.* **1995**, *496*, 127–135.
218. Rudler, H.; Parlier, A.; Ousmer, M.; Vaissermann, J. *Eur. J. Org. Chem.* **1999**, 3315–3321.
219. Gein, V.-L.; Popov, A. V.; Kolla, W. E.; Popova, N. A. *Pharmazie* **1993**, *48*, 107–109.
220. Miller, P. C.; Owen, T. J.; Molyneaux, J. M.; Curtis, J. M.; Jones, C. R. *J. Comb. Chem.* **1999**, *1*, 223–234.
221. Trost, B. M.; Krische, M. J.; Berl, V.; Grenzer, E. M. *Org. Lett.* **2002**, *4*, 2005–2008.
222. Tsolomiti, G.; Tsolomitis, A. *Tetrahedron Lett.* **2004**, *45*, 9353–9355.
223. Pal, M.; Swamy, N. K.; Hameed, P. S.; Padakanti, S.; Yeleswarapu, K. R. *Tetrahedron* **2004**, *60*, 3987–3997.
224. Beck, B.; Picard, A.; Herdtweck, E.; Dömling, A. *Org. Lett.* **2004**, *6*, 39–41.
225. (a) Musicki, B. *J. Org. Chem.* **1991**, *56*, 110–118; (b) Breslow, R.; Eicher, T.; Krebs, A.; Peterson, R. A.; Posner, J. *J. Am. Chem. Soc.* **1965**, *87*, 1320–1325.
226. Kascheres, C. M. *J. Braz. Chem. Soc.* **2003**, *14*, 945–969.
227. Sarsted, B.; Winterfeldt, E. *Heterocycles* **1983**, *20*, 469–476.
228. For a comprehensive review of isocyanide-based multicomponent reactions, see Ref. 191b.
229. Rao, V. V. R.; Wentrup, C. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1232–1235.
230. (a) Wei, H.-Z.; Zhou, C.; Ham, S.; White, J. M.; Birney, D. M. *Org. Lett.* **2004**, *6*, 4289–4292; (b) Kollenz, G.; Igel, H.; Ziegler, E. *Monatsh. Chem.* **1972**, *103*, 450–459.
231. Saripinar, E.; Karatas, S. *J. Heterocycl. Chem.* **2005**, *42*, 787–796.
232. For some recent examples, see: (a) Mitchell, R. E.; Teh, K. L. *Org. Biomol. Chem.* **2005**, *3*, 3540–3543; (b) Okazaki, Y.; Ishihara, A.; Nishida, T.; Iwamura, H. *Tetrahedron* **2004**, *60*, 4765–4771; (c) Enyedy, I. J.; Zaman, W. A.; Sakamuri, S.; Kozikowski, A. P.; Johnson, K. M.; Wang, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1113–1118; (d) O'Hagan, D. *Nat. Prod. Rep.* **2000**, *17*, 435–446; (e) Kato, A.; Adachi, I.; Miyauchi, M.; Ikeda, K.; Komae, T.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Wormald, M. R.; Fleet, G. W. J.; Asano, N. *Carbohydr. Res.* **1999**, *316*, 95–103; (f) Kar, G. K.; Roy, B. C.; Adhikari, S. D.; Ray, J. K.; Brahma, N. K. *Bioorg. Med. Chem.* **1998**, *6*, 2397–2403.
233. For some examples, see: (a) Yee, N. K.; Nummy, L. J.; Byrne, D. P.; Smith, L. L.; Roth, G. P. *J. Org. Chem.* **1998**, *63*, 326–330; (b) Saravanan, P.; Corey, E. J. *J. Org. Chem.* **2003**, *68*, 2760–2764; (c) Crane, S. N.; Corey, E. J. *Org. Lett.* **2001**, *3*, 1395–1397; (d) Xi, N.; Arvedson, S.; Eisenberg, S.; Han, N.; Handley, M.; Huang, L.; Huang, Q.; Kiselyov, A.; Liu, Q.; Lu, Y.; Nunez, G.; Osslund, T.; Powers, D.; Tasker, A. S.; Wang, L.; Xiang, T.; Xu, S.; Zhang, J.; Zhu, J.; Kendall, R.; Dominguez, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2905–2909; (e) Corey, E. J.; Li, W. D. *Chem. Pharm. Bull.* **1997**, *47*, 1–10; (f) Shih, N.-Y.; Lupo, A. T., Jr.; Aslanian, R.; Orlando, S.; Piwinski, J. J.; Gree, M. J.; Ganguly, A. K.; Clark, M. A.; Tozzi, S.; Kreutner, W.; Hey, J. A. *J. Med. Chem.* **1995**, *38*, 1593–1599.
234. Kagan, H. B. *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, NY, 1975; Vol. 5, Chapter 1.
235. Challis, B. C.; Challis, J. A. *Comprehensive Organic Chemistry*; Sutherland, I. O., Ed.; Pergamon: New York, NY, 1979; Vol 2, p 956.
236. For recent examples, see: (a) Park, J.-H.; Ha, J.-R.; Oh, S. J.; Kim, J.-A.; Shin, D.-S.; Won, T.-J.; Lam, Y.-F.; Ahn, C. *Tetrahedron Lett.* **2005**, *46*, 1755–1757; (b) Alcaide, B.; Almendros, P.; Cabrero, G.; Ruiz, M. P. *Org. Lett.* **2005**, *7*, 3981–3984; (c) Alcaide, B.; Almendros, P.; Alonso, J. M. *J. Org. Chem.* **2004**, *69*, 993–996; (d) Banfi, L.; Guanti, G.; Rasparini, M. *Eur. J. Org. Chem.* **2003**, 1319–1336.
237. For examples, see: (a) Yoon, C. H.; Nagle, A.; Gandhi, D.; Jung, K. W. *Org. Lett.* **2003**, *5*, 2259–2262; (b) Wee,

A. G. H.; Duncan, S. C. *Tetrahedron Lett.* **2002**, *43*, 6173–6176; (c) Anada, M.; Hashimoto, S.-I. *Tetrahedron Lett.* **1998**, *39*, 79–82; (d) Wee, A. G. H.; Liu, B.; McLeod, D. D. *J. Org. Chem.* **1998**, *63*, 4218–4227.
238. (a) Craig, D.; Hyland, C. J. T.; Ward, S. E. *Chem. Commun.* **2005**, 3439–3441; (b) Giambastiani, G.; Pacini, B.; Torcelloni, M.; Poli, G. *J. Org. Chem.* **1998**, *63*, 804–807.
239. For 1,3-dipolar cycloaddition reactions of azomethine ylides to C–C double bonds, see: (a) Fokas, D.; Ryan, W. J.; Casabier, D. S.; Coffen, D. L. *Tetrahedron Lett.* **1998**, *39*, 2235–2238; (b) Trávnicek, M.; Potácek, M. *ARKIVOC* **2001**, 156–163; (c) Belyk, K. M.; Beguin, C. D.; Palucki, M.; Grinberg, N.; DaSilva, J.; Askin, D.; Yasuda, N. *Tetrahedron Lett.* **2004**, *45*, 3265–3268; (d) Gao, W.; Zhang, X.; Raghunath, M. *Org. Lett.* **2005**, *7*, 4241–4244; (e) Harwood, L. M.; Vickers, R. J. The Chemistry of Heterocyclic Compounds. In In *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*; Padwa, A., Pearson, W. H., Eds.; Wiley Interscience: New York, NY, 2002; (f) Coldham, I.; Hufton, R. *Chem. Rev.* **2005**, *105*, 2765–2809.
240. For 1,3-dipolar cycloaddition reactions of 2-azaallyllithium compounds to C–C double bonds, see: Kauffmann, T. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 627–639.
241. For the ring-opening of a bisaziridine, see: Fort, S.; McCort, I.; Duréault, A.; Depezay, J.-C. *Synlett* **1997**, 1235–1236.
242. For cyclization reactions by intramolecular addition of radicals to C–C double bonds, see: (a) Bachi, M. D.; Melman, A. *Pure Appl. Chem.* **1998**, *70*, 259–262; (b) Aurrecoechea, J. M.; Fernández, A.; Gorgojo, J. M.; Saornil, C. *Tetrahedron* **1999**, *55*, 7345–7362.
243. For intramolecular 2-propylidene-1,3-bis(silane)-acylnitrilium ion cyclizations and subsequent reduction of the resultant 1-pyrrolines, see: Kercher, T.; Livinghouse, T. *J. Org. Chem.* **1997**, *62*, 805–812.
244. For radical cyclization of unsaturated haloamides promoted by $Mn_2(CO)_{10}$, see: Gilbert, B. C.; Kalz, W.; Lindsay, C. I.; McGrail, P. T.; Parsons, A. F.; Whittaker, D. T. E. *Tetrahedron Lett.* **1999**, *40*, 6095–6098.
245. For Ni-catalyzed cyclization of aminodienal derivatives, see: Sato, Y.; Saito, N.; Mori, M. *Tetrahedron* **1998**, *54*, 1153–1168.
246. For a synthesis from a homochiral α-hydroxyacid, α-amino-acid or aminocarbohydrate, see: (a) Flögel, O.; Amombo, M. G. O.; Reissig, H.-U.; Zahn, G.; Brüdgam, I.; Hartl, H. *Chem.—Eur. J.* **2003**, *9*, 1405–1415; (b) Elliot, R. L.; Kopecka, H.; Lin, N.-H.; He, Y.; Garvey, D. S. *Synthesis* **1995**, 772–774; (c) Kim, Y.-A.; Oh, S.-M.; Han, S.-Y. *Bull. Korean Chem. Soc.* **2001**, *22*, 327–329; (d) Han, S.-Y.; Liddell, P. A.; Joullié, M. M. *Synth. Commun.* **1988**, *18*, 275–283.
247. For the Pd-catalyzed formal [3+2] cycloaddition of a glycine derived 5,5-divinyloxazolidin-2-one to highly activated Michael acceptors, see: Knight, J. G.; Tchabanenko, K.; Stoker, P. A.; Harwood, S. J. *Tetrahedron Lett.* **2005**, *46*, 6261–6264.
248. For the synthesis of pyrrolidines by ring-closing metathesis, see: Felpin, F.-X.; Lebreton, J. *Eur. J. Org. Chem.* **2003**, 3693–3712.
249. For reviews about the synthesis and application of pyrrolidine derivatives, see: (a) Cheng, Y.; Huang, Z.-T.; Wang, M.-X. *Curr. Org. Chem.* **2004**, *8*, 325–351; (b) Notz, W.; Tanaka, F.; Barbas, C. F., III. *Acc. Chem. Res.* **2004**, *37*, 580–581; (c) Pearson, W. H.; Stoy, P. *Synlett* **2003**, 3693–3712; (d) Pearson, W. H. *Pure Appl. Chem.* **2002**, *74*, 1339–1347.
250. Roussi, G.; Zhang, J. *Tetrahedron* **1991**, *47*, 5161–5172.
251. Kaufmann, T.; Berg, H.; Köppelmann, E. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 380–381.
252. Kaufmann, T.; Köppelmann, E. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 290–291.
253. Li, G.-Y.; Chen, J.; Yu, W.-Y.; Hong, W.; Che, C.-M. *Org. Lett.* **2003**, *5*, 2153–2156.
254. Galliford, C. V.; Beenen, M. A.; Nguyen, S. T.; Scheidt, K. A. *Org. Lett.* **2003**, *5*, 3487–3490.
255. Roth, G. P.; Emmanuel, M.; Tong, L. *Tetrahedron: Asymmetry* **1997**, *8*, 185–186.
256. Takeda, T.; Saito, J.; Tsubouchi, A. *Tetrahedron Lett.* **2003**, *44*, 5571–5574.
257. Ney, J. E.; Wolfe, J. P. *Angew. Chem., Int. Ed.* **2004**, *43*, 3605–3608.
258. Yang, Q.; Ney, J. E.; Wolfe, J. P. *Org. Lett.* **2005**, *7*, 2575–2578.
259. Meyer, N.; Werner, F.; Opatz, T. *Synthesis* **2005**, 945–956.
260. Carson, C. A.; Kerr, M. A. *J. Org. Chem.* **2005**, *70*, 8242–8244.
261. Sezen, B.; Sames, D. *J. Am. Chem. Soc.* **2005**, *127*, 5284–5285.
262. Durst, T.; Van Den Elzen, R.; LeBelle, M.-J. *J. Am. Chem. Soc.* **1972**, *94*, 9261–9263.
263. Katritzky, A. R.; Feng, D.; Lang, H. *J. Org. Chem.* **1997**, *62*, 706–714.
264. Yee, N. K. *Tetrahedron Lett.* **1997**, *38*, 5091–5094.
265. Mahoboodi, S.; Eibler, E.; Koller, M.; Kumar, K. C. S.; Popp, A.; Schollmeyer, D. *J. Org. Chem.* **1999**, *64*, 4697–4704.
266. Clayden, J.; Watson, D. W.; Helliwell, M.; Chambers, M. *Chem. Commun.* **2003**, 2582–2583.
267. Escalante, J.; González–Tototzin, M. A. *Tetrahedron: Asymmetry* **2003**, *14*, 981–985.
268. He, M.; Bode, J. W. *Org. Lett.* **2005**, *7*, 3131–3134.
269. (a) Fabre, S.; Proudhomme, M.; Rapp, M. *Bioorg. Med. Chem.* **1993**, *1*, 193–196; (b) Sancelme, M.; Fabre, S.; Proudhomme, M. *J. Antibiot.* **1994**, *47*, 792–798.
270. Slater, M. J.; Cockerill, G. S.; Littler, F. Wellcome Foundation Ltd. PCT WO 93/18. 765. 1993. *Chem. Abstr.* **1994**, *120*, P106760j.
271. Silina, T. A.; Pulina, N. A.; Gein, L. F.; Gein, V. L. *Chem. Heterocycl. Compd.* **1998**, *34*, 844.
272. Gein, V. L.; Platonov, V. S.; Voronina, É. V. *Pharm. Chem. J.* **2004**, *38*, 316–318.
273. Habicht, E. Cilag-Chemie Ltd. Patent CH 362,694 1962.08.15; *Chem. Abstr. 59*, 48278.
274. Saudi, M. N. S.; El Semary, M. M. A.; El Sawaf, G. *Pharmazie* **2002**, *57*, 519–522.
275. (a) Nishizuka, Y. *Nature* **1984**, *308*, 693–698; (b) Farago, A.; Nishizuka, Y. *FEBS Lett.* **1990**, *268*, 350–354.
276. Nishizuka, Y. *Cancer* **1989**, *63*, 1892–1903.
277. Toullec, D.; Pianetti, P.; Coste, H.; Bellevergue, P.; Grand-Perret, T.; Ajakane, M.; Baudet, V.; Boissin, P.; Boursier, E.; Loriolle, F.; Duhamel, L.; Charon, D.; Kirilovsky, J. *J. Biol. Chem.* **1991**, *266*, 15771–15781.
278. Davis, P. D.; Elliott, L. H.; Harris, W.; Hill, C. H.; Hurst, S. A.; Keech, E.; Kumar, M. K. H.; Lawton, G.; Nixon, J. S.; Wilkinson, S. E. *J. Med. Chem.* **1992**, *35*, 994–1001.
279. Asakai, R.; Aoyama, Y.; Fujimoto, T. *Neurosci. Res.* **2002**, *44*, 297–304.

280. Katoh, M.; Dodo, K.; Sodeoka, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3109–3113.
281. Muid, R. E.; Dale, M. M.; Davis, P. D.; Elliott, L. H.; Hill, C. H.; Kumar, H.; Lawton, G.; Twomey, B. M.; Wadsworth, J.; Wilkinson, S. E.; Nixon, J. S. *FEBS Lett.* **1991**, *293*, 169–172.
282. Bit, R. A.; Davis, P. D.; Elliott, L. H.; Harris, W.; Hill, C. H.; Keech, E.; Kumar, H.; Lawton, G.; Maw, A.; Nixon, J. S.; Vesey, D. R.; Wadsworth, J.; Wilkinson, S. E. *J. Med. Chem.* **1993**, *36*, 21–29.
283. Davis, P. C.; Hill, C. H.; Lawton, G.; Nixon, J. S.; Wilkinson, S. E.; Hurst, S. A.; Keech, E.; Turner, S. E. *J. Med. Chem.* **1992**, *35*, 177–184.
284. (a) Eldar-Finkelman, H.; Krebs, E. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9660–9664; (b) Welsh, G. I.; Miller, C. M.; Loughlin, A. J.; Price, N. T.; Proud, C. G. *FEBS Lett.* **1998**, *421*, 125–130.
285. Coghlan, M. P.; Culbert, A. A.; Cross, D. A. E.; Corcoran, S. L.; Yates, J. W.; Pearce, N. J.; Rausch, O. L.; Murphy, G. J.; Carter, P. S.; Cox, L. R.; Mills, D.; Brown, M. J.; Haigh, D.; Ward, R. W.; Smith, D. G.; Murray, K. J.; Reith, A. D.; Holder, J. C. *Chem. Biol.* **2000**, *7*, 793–803.
286. Engler, T. A.; Henry, J. R.; Malhotra, S.; Cunningham, B.; Furness, K.; Brozinick, J.; Burkholder, T. P.; Clay, M. P.; Clayton, J.; Defenbacher, C.; Hawkins, E.; Iversen, P. W.; Li, Y.; Lindstrom, T. D.; Marquart, A. L.; McLean, J.; Mendell, D.; Misener, E.; Briere, D.; O'Toole, J. C.; Porter, W. J.; Queener, S.; Reel, J. K.; Owens, R. A.; Brier, R. A.; Eessalu, T. E.; Wagner, J. R.; Campbell, R. M.; Vaughn, R. *J. Med. Chem.* **2004**, *47*, 3934–3937.
287. Engler, T. A.; Malhotra, S.; Burkholder, T. P.; Henry, J. R.; Mendel, D.; Porter, W. J.; Furness, K.; Diefenbacher, C.; Marquart, A.; Reel, J. K.; Li, Y.; Clayton, J.; Cunningham, B.; McLean, J.; O'Toole, J. C.; Brozinick, J.; Hawkins, E.; Misener, E.; Briere, D.; Brier, R. A.; Wagner, J. R.; Campbell, R. M.; Anderson, B. D.; Vaughn, R.; Bennett, D. B.; Meier, T. I.; Cook, J. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 899–903.
288. Vervoot, H. C.; Richards-Gross, S. E.; Fenical, W.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1997**, *62*, 1486–1490.
289. Berlinck, R. G. S.; Britton, R.; Piers, E.; Lim, L.; Roberge, M.; Moreira da Rocha, R.; Andersen, R. J. *J. Org. Chem.* **1998**, *63*, 9850–9856.
290. (a) Gill, M.; Steglich, W. *Progr. Chem. Org. Nat. Prod.* **1987**, *51*, 216–226; (b) Steglich, W. *Pure Appl. Chem.* **1989**, *61*, 281–289.
291. (a) Nettleton, D. E.; Doyle, T. W.; Krishnan, B.; Matsumoto, G. K.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4011–4014; (b) For recent developments of rebeccamycin analogues as topoisomerase I inhibitors and antitumor agents, see: Prudhomme, M. *Curr. Med. Chem.* **2000**, *7*, 1189–1212.
292. Sezaki, M.; Sasaki, T.; Nakazawa, T.; Takeda, U.; Iwata, M.; Koyama, M.; Kai, F.; Shomura, T.; Kojima, M. *J. Antibiot.* **1985**, *38*, 1437–1439.
293. Nakatami, S.; Naoe, A.; Yamamoto, Y.; Yamauchi, T.; Yamaguchi, N.; Ishibashi, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2879–2881.
294. (a) Steglich, W.; Steffan, B.; Kopanski, L.; Eckhardt, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 459–464; (b) Steglich, W. *Pure Appl. Chem.* **1981**, *53*, 1233–1240.
295. Trinks, U.; Buchdunger, E.; Furet, P.; Kump, W.; Mett, H.; Meyer, T.; Müller, M.; Regenass, U.; Rihs, G.; Lydon, N.; Traxler, P. *J. Med. Chem.* **1994**, *37*, 1015–1027.
296. Sancelme, M.; Fabre, S.; Prudhomme, M. *J. Antibiot.* **1994**, *47*, 792–798.
297. Melnik, S. Y.; Bakhmedova, A. A.; Garaeva, L. D.; Goryunova, O. V.; Miniker, T. D.; Plikhtyak, I. L.; Ivanova, T. P.; Yartseva, I. V. *Bioorg. Khim.* **1991**, *22*, 458–461.
298. Kaneko, T.; Wong, H.; Okamoto, K. T.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4015–4018.
299. Joyce, R. P.; Gainor, J. A.; Weinreb, S. M. *J. Org. Chem.* **1987**, *52*, 1177–1185.
300. Xie, G.; Lown, J. W. *Tetrahedron Lett.* **1994**, *35*, 5555–5558.
301. Brenner, M.; Rexhausen, H.; Steffan, B.; Steglich, W. *Tetrahedron* **1988**, *44*, 2887–2892.
302. Gallant, M.; Link, J. T.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 343–349.
303. Faul, M. M.; Sullivan, K. A.; Winneroski, L. L. *Synthesis* **1995**, 1511–1516.
304. Lakatosh, S. A.; Balzarini, J.; Andrei, G.; Snoeck, R.; De Clercq, E.; Preobrazhenskaya, M. N. *J. Antibiot.* **2002**, *55*, 768–773.
305. Bartlett, S.; Nelson, A. *Org. Biomol. Chem.* **2004**, *2*, 2874–2883.
306. (a) Routier, S.; Coudert, G.; Mérourm, J.-Y.; Caignard, D. H. *Tetrahedron Lett.* **2002**, *43*, 2561–2564; (b) Routier, S.; Ayerbe, N.; Mérour, J.-Y.; Coudert, G.; Bailly, C.; Pierré, A.; Pfeiffer, B.; Caignard, D.-H.; Renard, P. *Tetrahedron* **2002**, *58*, 6621–6630.
307. (a) Kuo, G.-H.; Prouty, C.; De Angelis, A.; Shen, L.; O'Neill, D. J.; Shah, C.; Connolly, P. J.; Murray, W. V.; Conway, B. R.; Cheung, P.; Westover, L.; Xu, J. Z.; Look, R. A.; Demarest, K. T.; Emanuel, S.; Middleton, S. A.; Jolliffe, L.; Beavers, M. P.; Chen, X. *J. Med. Chem.* **2003**, *46*, 4021–4031; (b) Shen, L.; Prouty, C.; Conway, B. R.; Westover, L.; Xu, J. Z.; Look, R. A.; Chen, X.; Beavers, M. P.; Roberts, J.; Murray, W. V.; Demarest, K. T.; Kuo, G.-H. *Bioorg. Med.* **2004**, *12*, 1239–1255.
308. Routier, S.; Coudert, G.; Mérour, J.-Y. *Tetrahedron Lett.* **2001**, *42*, 7025–7028.
309. Dubernet, M.; Coubert, V.; Guillard, J.; Viaud-Massuard, M.-C. *Tetrahedron* **2005**, *61*, 4585–4593.
310. Neel, D. A.; Jirousek, M. R.; McDonald, J. H., III. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 47–50.
311. Bit, R. A.; Crackett, P. H.; Harris, W.; Hill, C. H. *Tetrahedron Lett.* **1993**, *34*, 5623–5626.
312. (a) Faul, M. M.; Gillig, J. R.; Jirousek, M. R.; Ballas, L. M.; Schotten, T.; Kahl, A.; Mohr, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1857–1859; (b) Joy, S. V.; Scates, A. C.; Bearelly, S.; Dar, M.; Taulien, C. A.; Goebel, J. A.; Cooney, M. J. *Ann. Pharmacother.* **2005**, *39*, 1693–1699 and references therein.
313. Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. *J. Org. Chem.* **1998**, *63*, 6053–6058.
314. Hughes, T. V.; Cava, M. P. *Tetrahedron Lett.* **1998**, *39*, 9629–9630.
315. (a) Sanchez-Martinez, C.; Faul, M. M.; Shih, C.; Sullivan, K. A.; Grutsch, J. L.; Cooper, J. T.; Kolis, S. P. *J. Org. Chem.* **2003**, *68*, 8008–8014; (b) Sanchez-Martinez, C.; Shih, C.; Zhu, G.; Li, T.; Brooks, H. B.; Patel, B. K. R.; Schultz, R. M.; DeHahn, T. B.; Spencer, C. D.; Watkins, S. A.; Ogg, C. A.; Considine, E.; Dempsey, J. A.; Zhang, F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3841–3846.
316. Faul, M. M.; Grutsch, J. L.; Kobierski, M. E.; Kopach, M. E.; Krumrich, C. A.; Staszak, M. A.; Udodong, U.; Vicenzi, J. T.; Sullivan, K. A. *Tetrahedron* **2003**, *59*, 7215–7229.

317. Al-awar, R. S.; Ray, J. E.; Hecker, K. A.; Joseph, S.; Huang, J.; Shih, C.; Brooks, H. B.; Spencer, C. D.; Watkins, S. A.; Schultz, R. M.; Considine, E. L.; Faul, M. M.; Sullivan, K. A.; Kolis, S. P.; Carr, M. A.; Zhang, F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3925–3928.
318. Zhang, H.-C.; Ye, H.; Conway, B. R.; Derian, C. K.; Addo, M. F.; Kuo, G.-H.; Hecker, L. R.; Croll, D. R.; Li, J.; Westover, L.; Xu, J. Z.; Look, R.; Demarest, K. H.; Andrade-Gordon, P.; Damiano, B. P.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3245–3250.
319. Fröhner, W.; Monse, B.; Braxmeier, T. M.; Casiraghi, L.; Sehagún, H.; Seneci, P. *Org. Lett.* **2005**, *7*, 4573–4576.
320. Smith, P. A. S.; Friar, J. J.; Resemann, W.; Watson, A. C. *J. Org. Chem.* **1990**, *55*, 3351–3362.
321. Sandler, S. R.; Karo, W. *Organic Functional Group Preparation*; Academic: London, 1972; Vol. 3, Chapter 7.
322. Davis, P. D.; Bit, R. A. *Tetrahedron Lett.* **1990**, *31*, 5201–5204.
323. Roy, S.; Eastman, A.; Grobble, G. W. *Synth. Commun.* **2005**, *35*, 595–601.
324. For the preparation of symmetrical and unsymmetrical 2,3-diarylmaleic anhydrides, see: (a) Davis, P. D.; Bit, R. A.; Hurst, S. A. *Tetrahedron Lett.* **1990**, *31*, 2353–2356; (b) Irie, M.; Uchida, K. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 985–996; (c) Pattabiraman, V. R.; Padakanti, S.; Veeramaneni, V. R.; Pal, M.; Yeleswarapu, K. R. *Synlett* **2002**, 947–951.
325. Reddy, P. Y.; Kondo, S.; Toru, T.; Ueno, Y. *J. Org. Chem.* **1997**, *62*, 2652–2654.
326. Braña, M. F.; Fernández, A.; Garrido, M.; Rodriguez, M. L. L.; Morcillo, M. J.; Sanz, A. M. *Chem. Pharm. Bull.* **1989**, *37*, 2710–2712.
327. Braña, M. F.; Añorbe, L.; Tarrasón, G.; Mitjans, F.; Piulats, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2701–2703.
328. Bergman, J.; Pelcman, B. *Tetrahedron Lett.* **1987**, *28*, 4441–4444.
329. Yeh, H.-C.; Wu, W.-C.; Wen, Y.-S.; Dai, D.-C.; Wang, J.-K.; Chen, C.-T. *J. Org. Chem.* **2004**, *69*, 6455–6462.
330. For examples, see: (a) Miura, M.; Nomura, M. *Topics in Current Chemistry*; Miyaura, N., Ed.; Springer: Berlin, 2002; Vol. 219, pp 211–241; (b) Zificsak, C. A.; Hlasta, D. J. *Tetrahedron* **2004**, *60*, 8991–9016; (c) Kalyani, D.; Deprez, N. R.; Desai, L. V.; Sanford, M. S. *J. Am. Chem. Soc.* **2005**, *127*, 7330–7331; (d) Ref. 3f; (e) Ref. 3h; (f) Tan, K. L.; Parks, S.; Ellman, J. A.; Bergman, R. G. *J. Org. Chem.* **2004**, *69*, 7329–7335; (g) Hughes, C. C.; Trauner, D. *Tetrahedron* **2004**, *60*, 9675–9686; (h) Yokoji, A.; Okazawa, T.; Satoh, T.; Miura, M.; Nomura, M. *Tetrahedron* **2003**, *59*, 5685–5689; (i) Park, C.-H.; Ryabova, V.; Seregin, I. V.; Sromek, A. W.; Gevorgyan, V. *Org. Lett.* **2004**, *6*, 1159–1162 and references therein; (j) Parisien, M.; Valette, D.; Fagnou, K. *J. Org. Chem.* **2005**, *70*, 7578–7584 and references therein; (k) Dajka-Halász, B.; Monsieurs, K.; Éliás, O.; Károlyházy, L.; Tapolcsányi, P.; Maes, B. U. W.; Riedl, Z.; Hajós, G.; Dommisse, R. A.; Lemière, G. F. L.; Komrlj, J.; Mátyus, P. *Tetrahedron* **2004**, *60*, 2283–2291; (l) Bellina, F.; Cauteruccio, S.; Rossi, R. *Eur. J. Org. Chem.* **2006**, 693–701.

Biographical sketch

Renzo Rossi was born in Pisa (Italy) and graduated in Chemistry with first-class honours at the University of Pisa in 1960 defending a thesis performed under the guidance of Professor Piero Pino. In 1969, he became Assistant Professor and in 1971 he earned the *libera docenza* in Organic Chemistry. After holding other intermediate positions at the University of Pisa and the Scuola Normale Superiore of Pisa, in 1980 he became Full Professor of Organic Chemistry at the University of Calabria. In 1982, he joined the University of Pisa again where he has held the Chair of Chemistry of Naturally Occurring Compounds. In 1999, the University of Pisa awarded him the *Ordine del Cherubino*. At the beginning of his career, he was interested in stereochemistry, the study of the chemistry and bioactivity of insect pheromones and the synthesis of insecticidal unsaturated carboxyamides, acetylenic and thiophenic phototoxins, structural analogues of naturally occurring fungicidal compounds of agrochemical interest and natural products useful for controlling insects and fungi which are devasting pests of historical and cultural papery and wooden materials. His current research interests include the total synthesis of naturally occurring compounds of biological and/or pharmacological interest, the study of transition metal-catalyzed carbon–carbon and carbon–heteroatom bond forming reactions and their applications for the synthesis of pharmacologically active compounds, and the design and development of new, efficient and selective methods for the synthesis of vicinal diaryl-substituted heterocycles that include potential antineoplastic derivatives. He is a fellow of the Royal Society of Chemistry and the Società Chimica Italiana.

Fabio Bellina was born in Catania (Italy) in 1964. He studied Chemistry at the University of Pisa and received his Laurea Degree in 1990 under the supervision of Professor R. Rossi. After his national service (1991–1992) in 1992 he joined the University of Pisa as an Organic Chemistry Researcher at the Dipartimento di Chimica e Chimica Industriale, working under the supervision of Professor R. Rossi. In October 2003, he was appointed by the Faculty of Science of the University of Pisa as an Associate Professor of Organic Chemistry. Most of his research has been devoted to the study of transition metal-catalyzed reactions and their application to the selective synthesis of bioactive natural and synthetic heterocyclic compounds, and particularly of substances which are cytotoxic against human tumour cell lines.

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Isotopic labelling of quercetin 3-glucoside

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Abstract—The potentially important dietary antioxidant, quercetin 3-*O*-β-D-glucoside, has been ^{13}C-labelled at C-2 of the flavonoid unit by synthesis in 15% yield over five steps from [^{13}C]carbon dioxide. The route is appropriate for radiochemical synthesis. Formation of the protected 3-glucosylated flavonol appears to result from [1,7]-sigmatropic rearrangement with migration of a benzyl group followed by cyclisation. A free 5-OH results even when a phosphazene superbase is used.

1. Introduction

The flavonol quercetin[1] **1** is a polyphenolic plant secondary metabolite (Fig. 1), and its *O*-glycosides are found in high concentrations in onions, broccoli, apples, red wine and tea.[2] Epidemiological studies have linked the consumption of diets rich in the polyphenolic compounds produced by plants with a range of health benefits. Quercetin in particular reduces the risk of lung cancer.[3] It has been argued that these health benefits arise from the ability of the various polyphenolics to act as biological antioxidants, scavenging reactive oxygen species within the body. Indeed, quercetin is a more effective antioxidant than vitamin E in vitro, reacting 4.5 times more rapidly with oxygen-centred radicals, and quenching more than three oxygen-centred radicals per flavonol molecule.[4,5] However, while a compound's ability to scavenge radicals is a useful predictor of whether it will act as a food antioxidant, preventing rancidity, it is not sufficient evidence for a role as a biological antioxidant, reducing oxidative damage within the body. Vital to this latter role is the compound's bioavailability.[6,7] In a similar vein, a huge range of biological activities have been demonstrated for quercetin in vitro, but most studies have not even considered whether quercetin, consumed as its glycoside derivatives in the quantities found in the diet, can reach cells in sufficient concentration to elicit the effects observed in vitro.[7,8]

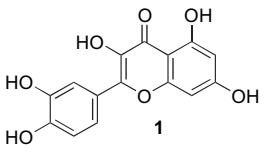

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Figure 1. Quecetin.

In order to understand the absorption, metabolism, distribution and excretion of a compound, it is necessary to identify accurately and quantify the compound and all its metabolites in the various parts of the body. In a recent study,[9,10] we prepared [2-^{14}C]quercetin 4′-glucoside, following our route to [2-^{13}C]quercetin 4′-glucoside,[11] and used it in a feeding study in rats. This was followed by analysis with reversed-phase HPLC with on-line radioactivity detection and ion-trap mass spectrometry capable of performing data dependent MS–MS studies.[12] Radioactivity allowed the location and quantification of quercetin-4′-glucoside-derived material in different tissues (93.6% of ingested radioactivity recovered). The HPLC then separated the metabolites, the radioactivity allowing identification and quantification of those derived from quercetin-4′-glucoside, and MS–MS allowing structural determination so that the HPLC peak assignment was unambiguous. The ion-trap method allowed almost all contributors to HPLC peaks to be identified. In this way we identified 17 different metabolites of the parent flavonol glycoside.[9]

The differential absorption of dietary flavonol glycosides and aglycones is currently attracting a good deal of attention. The parent flavonol, the type of sugar attached, and the position of glycosylation all appear to affect absorption. Indeed, some experiments indicate that lactase phlorizin hydrolase (LPH) hydrolyses quercetin-3-glucoside prior to absorption,[13,14] but that quercetin-4′-glucoside is actively transported by the intestinal sodium-dependent glucose

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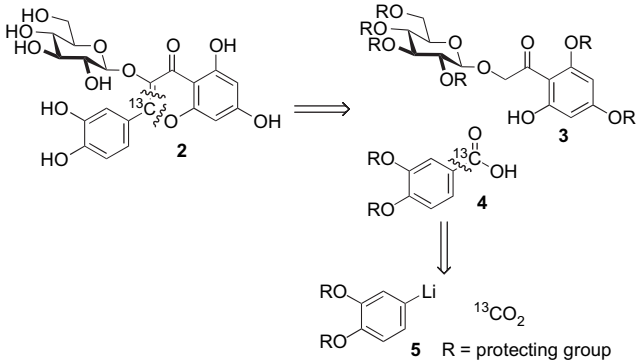
transporter (SGLT1).[15] Other experiments, however, seem to show that these two glucosides interact similarly with SGLT[16] and/or LPH,[17] but that other polyphenols and their glycosides do not. Our synthesis of [2-^{13}C]quercetin-4′-*O*-β-D-glucoside had provided a general route that should be applicable to all flavon-3-ols glycosylated on the B-ring, but did not allow the preparation of 3-*O*-glycosides. The latter are important not only as dietary components themselves, but also as synthetic precursors of dietary anthocyanins[18] (which are invariably glycosylated at the 3-position to enhance stability) and 3-*O*-glucuronides,[19] considered to be important metabolites. Here we report the synthesis of [2-^{13}C]quercetin 3-*O*-β-D-glucoside in a way that should be easy to adapt to ^{14}C-labelling and provides a paradigm for the labelling of other flavonol 3-glycosides:[20,21] the label is introduced from carbon dioxide generated from barium carbonate (barium [^{14}C]carbonate is one of the cheapest sources of carbon-14), and labelling within the flavonoid unit avoids any possibility of loss of radioactivity by exchange under physiological conditions. Labelling using chemical synthesis, rather than biosynthesis,[22] ensures the production of a single compound labelled at a specific site with a controlled amount of the isotope used.

2. Results and discussion

Retrosynthetic analysis of [2-^{13}C]quercetin 3-*O*-β-D-glucoside **2** (Scheme 1) revealed that the key intermediates in the synthesis would be a glycosylated acetophenone **3** with one free phenolic hydroxy group and a labelled carboxylic acid **4**, which would be easily prepared by reaction of an aryllithium **5** with [^{13}C]carbon dioxide. Initially we decided on *O*-benzyl protection, as hydrogenolysis of these groups is known not to affect the flavonoid core or glycosidic links.

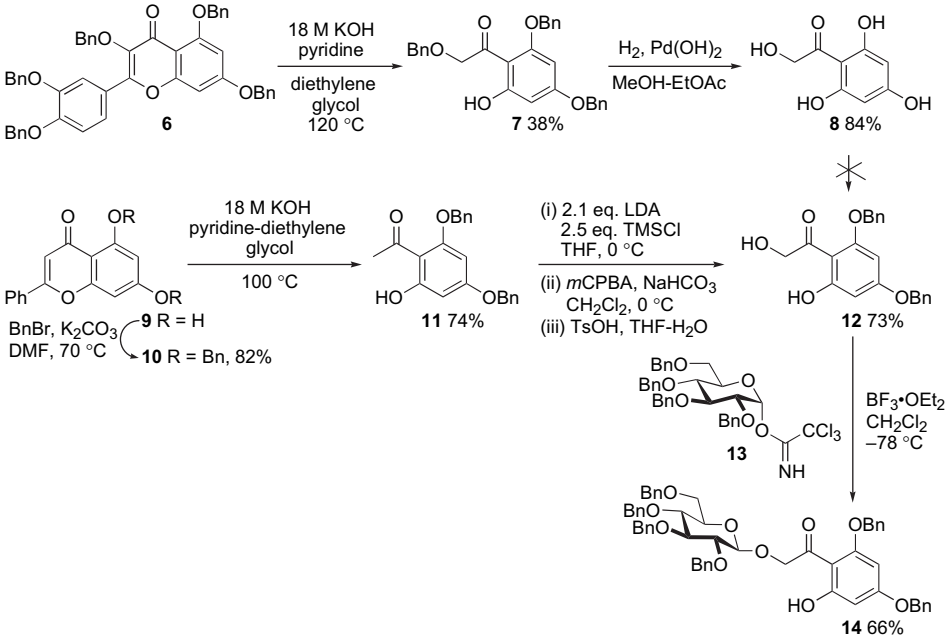
In our first approach to glycosylated acetophenone **14** (Scheme 2), fully benzylated quercetin **6** was fragmented



Scheme 1. Retrosynthetic analysis of [^{13}C]quercetin 3-glucoside.

to give acetophenone **7** as we had previously reported.[11] Hydrogenation removed all protection, yielding tetraol **8**. Unfortunately attempts to selectively benzylate the phenoxides generated from acetophenone **8** under basic conditions led to a range of products including *C*-benzylated adducts. This is not surprising as the aromatic C–H's that appear at δ_H 5.82 ppm in the ^{1}H NMR spectrum were 95% exchanged for deuterium when acetophenone **8** was heated overnight in D4-methanol (*C*–D appears as a 1:1:1 triplet, *J* 96 Hz, at 95.91 ppm in the ^{13}C NMR spectrum).

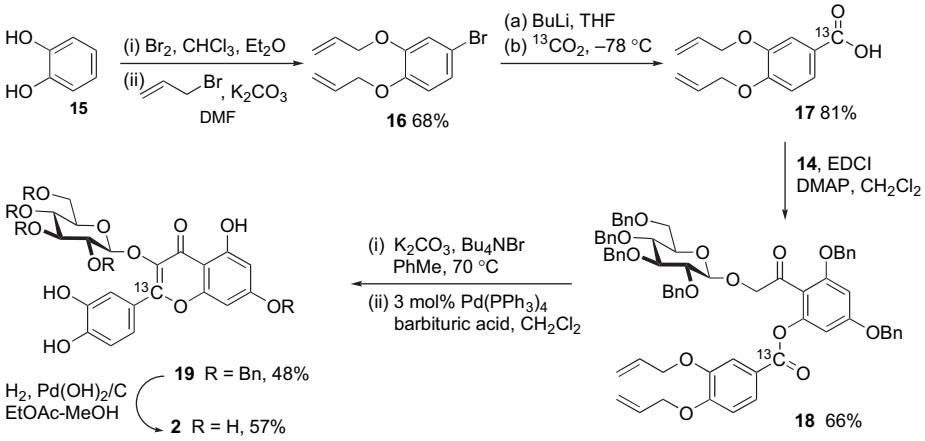
An alternative approach was then investigated. The readily available flavone, chrysin **9**, was benzylated and the resulting flavone **10** fragmented to give acetophenone **11**. Although direct dibenzylation of 2,4,6-trihydroxyacetophenone is possible,[23] we found that reaction under a variety of conditions gave rise to *C*-benzylated and mono-*O*-benzylated by-products that were difficult to remove. The α-hydroxy group was then introduced through Rubottom oxidation[24,25] of the silyl enol ether derived from acetophenone **11**, the phenolic hydroxy group being transiently protected as a TMS ether. The α-hydroxy group of the resulting acetophenone **12** was then glucosylated regioselectively,



Scheme 2. Preparation of acetophenone coupling partner.

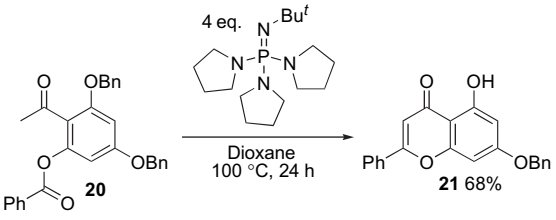
without affecting the phenolic hydroxy, and with good β-selectivity using Schmidt's imidate[26,27] **13**. Pure β-anomer **14** was obtained following a single recrystallisation and the stereochemistry was confirmed by the presence of a doublet of *J* 7.6 Hz at 4.34 ppm in the ^{1}H NMR spectrum.

The carboxylic acid coupling partner to acetophenone **14** was synthesised from catechol **15** (Scheme 3). Bromination and double allylation gave aryl bromide **16**, which was lithiated and reacted with $^{13}CO_2$, freshly generated from barium [^{13}C]carbonate using the procedure and apparatus described by Kratzel and Billek,[28] to give carboxylic acid **17** (this and all later steps were tested unlabelled first). Acetophenone **14** and carboxylic acid **17** were then coupled using a water-soluble coupling agent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, EDCI. Cyclisation of the resulting ester **18** proceeded with loss of the 5-benzyl group, and palladium-catalysed removal of the allyl protecting groups then gave a triol **19** that could be purified. A mixed protecting group strategy was employed because purification had proved too problematic when only benzyl groups were employed, and lithiation–carboxylation of 3,4-dibenzyloxy-1-iodobenzene had proceeded in only 24% yield, probably due to poor solubility of the lithiated species. Global debenzylation of triol **19** completed the synthesis of [2-^{13}C]quercetin 3-*O*-β-D-glucoside **2** in 15% overall yield from barium [^{13}C]carbonate.



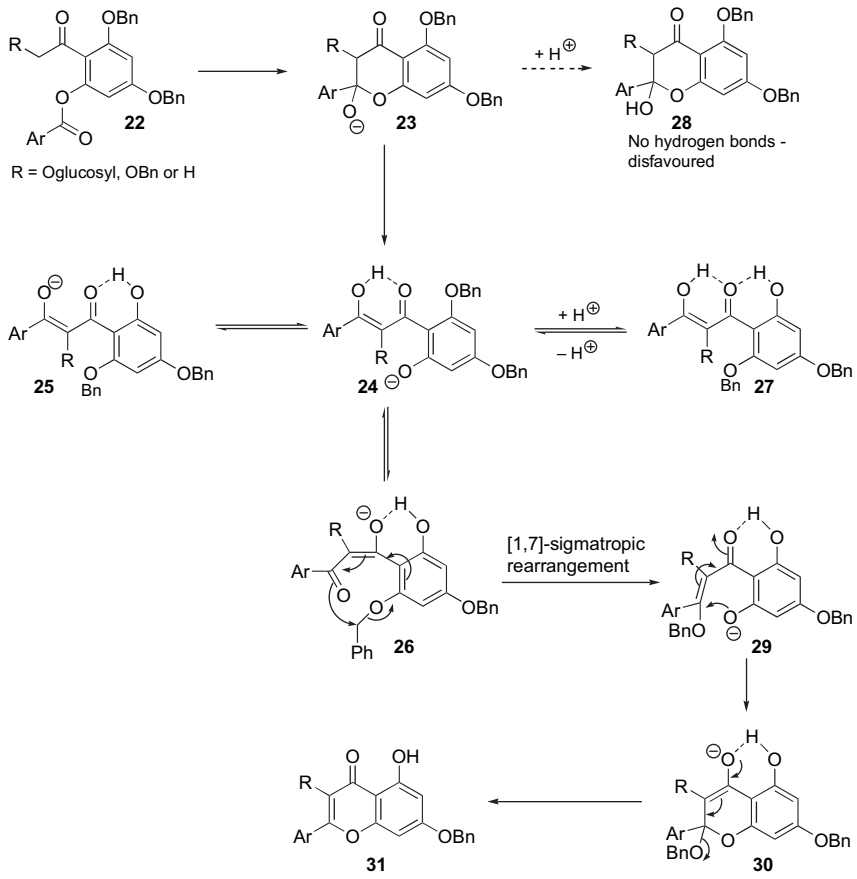
Scheme 3. Synthesis of [^{13}C]quercetin 3-*O*-β-D-glucoside.

The debenzylation to produce a flavonoid with a free 5-OH by cyclisation–dehydration of ester **18** appeared mechanistically interesting and was examined briefly. A similar debenzylation had occurred in our earlier synthesis of labelled quercetin-4′-glucoside.[11] Benzoate **20** derived from acetophenone **11** was used as a model substrate (Scheme 4). One possibility was that nucleophilic attack by bromide had removed the benzyl group, but when the neutral phosphazene P1 superbase[29,30] was used instead of K_2CO_3 and tetrabutylammonium bromide, ester **20** also cyclised to give monobenzylated chrysin **21**. This excludes the involvement of bromide, but raises the possibility that mono-debenzylation occurred after formation of dibenzylated chrysin as a result of nucleophilic attack by hydroxide generated from water produced in the reaction. However, dibenzylated chrysin **10** was isolated unchanged after treatment under the cyclisation conditions with 1 equiv of water added. Thus, debenzylation must have occurred prior to cyclisation.



Scheme 4. Phosphazene superbase-induced rearrangement.

A plausible mechanism for the debenzylation–cyclisation of esters **22** is shown in Scheme 5. Such reactions are known to begin with Baker–Venkataraman rearrangement[31] to give 1,3-diketones **27**, which will exist predominantly in their enol form in non-polar solvents. The hydrogen bonding in keto-enol **27** with Ar=Ph and R=H, which was isolated from fragmentation of dibenzylated chrysin **10**, is evident from its crystal structure (Fig. 2), and its ^{1}H NMR spectrum in $CDCl_3$ shows an enolic proton at 15.64 ppm and a chelated phenolic proton at 13.68 ppm. Cyclic alkoxides **23** are intermediates in the Baker–Venkataraman rearrangement and open to give the tautomeric enolates **24**–**26**, which are in equilibrium with each other and diketones **27** (depending on the strength of the base employed). Protonation of the cyclic alkoxides **23** to give cyclic hemiacetals **28**, which would be intermediates in the formation of fully benzylated flavonoids, is less favourable since no intramolecular hydrogen bonding is possible. We suggest that [1,7]-sigmatropic rearrangement[32] of ketones **26** involving migration of a benzyl group, generates phenoxides **29** (such a rearrangement is symmetry allowed if helical geometry allows antarafacial transfer of the benzyl group). The resulting phenoxides **29** would cyclise easily to give enolates **30** as the α,β-unsaturated ketone is made more reactive by hydrogen bonding. Bois et al.[33] and Rama Rao et al.[34] have previously noted the importance of a free *ortho*-hydroxy group in accelerating cyclisations of this sort. Elimination of benzyloxide would then give flavonoids **31**. It is noteworthy that benzyl esters were produced as side products under most conditions employed to cyclise aryl esters **22** (R≠H) to give flavon-3-ol derivatives **31**, and these are presumably formed by transesterification of



Scheme 5. Proposed mechanism of rearrangement–cyclisation.

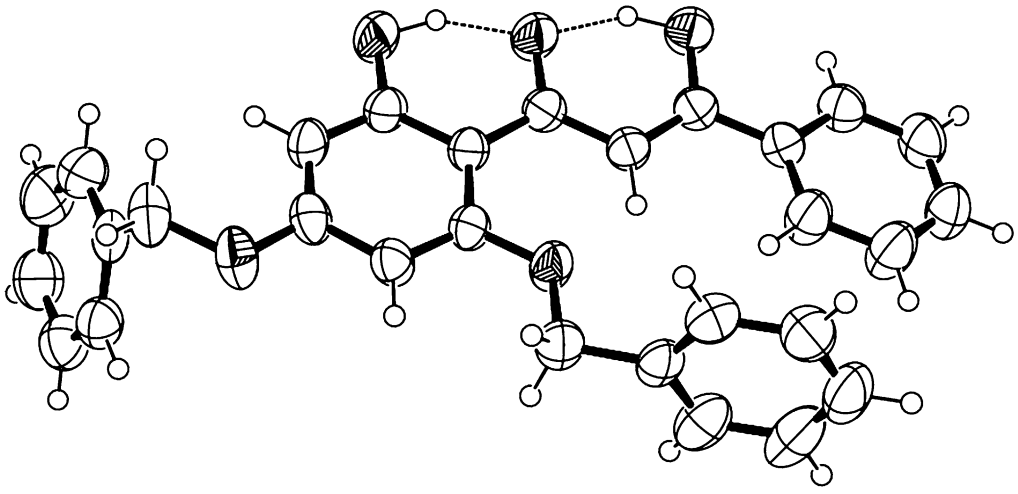


Figure 2. Crystal structure of keto-enol **27** (Ar=Ph, R=H).

the starting aryl esters **22** with benzyloxide produced during the reaction.

3. Conclusion

In summary, we have provided a useful method for the isotopic labelling of the important dietary flavonoid, quercetin 3-*O*-β-D-glucoside, and the route should be easy to adapt for the synthesis of other flavonoid 3-*O*-glycosides. We have also briefly explored the unexpected debenzylation reaction that occurs in the flavonoid-forming step, and propose a [1,7]-sigmatropic rearrangement to account for this.

4. Experimental

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DPX/400 spectrometer operating at 400 and 100 MHz, respectively. All coupling constants are measured in Hertz.

DEPT was used to assign the signals in the ^{13}C NMR spectra as C, CH, CH_2 or CH_3. The entire labelling sequence was first checked with unlabelled material (data not presented), and the ^{13}C NMR spectra of unlabelled material were used to identify coupling in the ^{13}C NMR spectra of labelled material. Mass spectra (MS) were recorded on a Jeol JMS700 (MStation) spectrometer. Infra-red (IR) spectra were obtained on a Perkin–Elmer 983 spectrophotometer. A Golden Gate™ attachment that uses a type IIa diamond as a single reflection element was used in some cases so that the IR spectrum of the compound (solid or liquid) could be directly detected without any sample preparation. Column chromatography was carried out on silica gel, 70–230 mesh, or neutral alumina (Brockmann grade III). Tetrahydrofuran and diethyl ether were dried over sodium and benzophenone, and dichloromethane was dried over calcium hydride. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 297713. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.1. [2-^{13}C]Quercetin 3-*O*-β-D-glucoside 2

Twenty percent palladium hydroxide on carbon (184 mg) was added to a stirring suspension of partly benzyl protected flavonol **19** (597 mg, 652 μmol) in 1:1 EtOAc–MeOH (10 mL) under an atmosphere of hydrogen. The suspension was stirred for 6 h at rt. The suspension was filtered through a plug of Celite eluting with MeOH. The filtrate was concentrated under vacuum and the resulting solid was recrystallised from MeOH–water (1:1) to give the quercetin 3-glucoside **2** as a green solid (174 mg, 57%), mp 224–226 °C. $[\alpha]_D^{22}$ –12.9 (*c* 1.0, MeOH). ν_{max}(Golden Gate)/cm^{-1}: 3170 (OH), 1653 (C═O), 1602 (C═O). δ_H (400 MHz; CD_3OD): 3.10–3.66 (6H, m, H-2″, 3″, 4″, 5″ and 6″), 5.14 (1H, d, *J* 7.4 Hz, H-1″), 6.08 (1H, d, *J* 2.1 Hz, H-6 or H-8), 6.27 (1H, d, *J* 2.1 Hz, H-6 or H-8), 6.76 (1H, d, *J* 8.4 Hz, H-5′), 7.47 (1H, ddd, J 2.1, 3.8 and 8.4 Hz, H-6′), 7.61 (1H, dd, *J* 2.1 and 3.9 Hz, H-2′). δ_C (100 MHz, MeOD-d_4): 62.53 (CH_2), 71.17 (CH), 75.70 (CH), 78.07 (CH), 78.32 (CH), 94.71 (CH, d, *J* 3 Hz), 99.86 (CH), 104.35 (CH), 105.64 (C), 115.97 (CH, d, *J* 5 Hz), 117.57 (CH, d, *J* 2 Hz), 122.94 (CH, d, *J* 68 Hz), 123.36 (C), 135.58 (C, d, *J* 89 Hz), 145.83 (C, d, *J* 6 Hz), 149.81 (C), 158.64 (C), 158.99 (^{13}C-label), 162.95 (C), 165.92 (C), 179.43 (C, d, *J* 6 Hz). *m/z* (FAB): 466 [(M+H)$^+$, 44%], 304 (84), 74 (100). HRMS: 466.1069. $^{12}C_{20}\,^{13}CH_{21}O_{12}$ requires (M+H)$^+$ 466.1063. HPLC Gradient reverse phase HPLC (Phenomonex Max RP C12 250× 4.6 mm×5 μm), solvent A 1% aqueous formic acid–Solvent B acetonitrile, 1.0 mL/min 5–40% B gradient over 60 min, *t*=26.64 min, showed 95% purity by peak area measurement (Absorbance wavelengths 200–600 nm, photodiode array detector). Spectral data in good agreement with literature for unlabelled compound.[19]

4.2. 3,5,7,3′,4′-Pentabenzyloxyflavone 6 and 2-hydroxy-ω,4,6-tribenzyloxyacetophenone 7

The syntheses of these compounds have already been reported.[11]

4.3. 1-(2′,4′,6′-Trihydroxyphenyl)-2-hydroxyethanone 8

Twenty percent palladium hydroxide on carbon (400 mg) was added to a suspension of phenol **7** (4.00 g, 8.8 mmol) in methanol–ethyl acetate (1:1, 20 mL) under an atmosphere of hydrogen. The suspension was then stirred overnight. The resulting solution was filtered through a pad of Celite, which was subsequently washed with methanol (30 mL). The solution concentrated under vacuum and the resulting solid recrystallised from ethanol to yield phenol **8** as an amorphous solid (1.36 g, 84%), mp decomp >225 °C. Lit.: 224 °C.[35] R_f [silica, EtOAc–hexane (7:3)] 0.19. ν_{max}(nujol)/cm^{-1}: 3380 (OH), 3299 (OH), 3153 (OH), 1650 (C═O). δ_H (400 MHz: DMSO-d_6): 4.60 (2H, s, CH_2), 4.73 (1H, broad s, CH_2OH), 5.82 (2H, s, H-3′, H-5′), 10.4 (1H, broad s, OH), 12.1 (2H, broad s, OH). δ_C (100 MHz: DMSO-d_6): 68.27 (CH_2), 94.91 (CH), 102.30 (C), 164.40 (C), 165.35 (C), 203.85 (C). *m/z* (EI) 184 (M$^{+\bullet}$, 18%), 153 (100), 69 (10). HRMS: 184.0372 $C_8H_8O_5$ requires (M$^+$), 184.0371. Microanalysis: C, 52.32; H, 4.62%. $C_8H_8O_5$ requires C, 52.17; H, 4.35%.

4.4. 5,7-Dibenzyloxyflavone 10

Benzyl bromide (46.8 mL, 394 mmol, 4.0 equiv) and K_2CO_3 (54.3 g, 394 mmol, 4.0 equiv) were added to a stirring solution of chrysin (25.00 g, 98.5 mmol) in DMF (150 mL). The resulting suspension was stirred at 70 °C for 4 d under a nitrogen atmosphere. After cooling, the solution was acidified to pH 1 with 1 M HCl and extracted into EtOAc (250 mL). The solution was allowed to stir for 10 min before the resulting precipitate was filtered off and washed with 50 mL of EtOAc to give flavone **10** as an off white solid pure enough for the next step (35.15 g, 82%). A sample was recrystallised from EtOAc–$CHCl_3$ for characterisation purposes, mp 165–167 °C. R_f [silica, EtOAc–hexane (7:3)] 0.52. δ_H (400 MHz: $CHCl_3$): 5.05 (2H, s, OCH_2), 5.16 (2H, s, OCH_2), 6.44 (1H, d, *J* 1.7 Hz, H-6), 6.59 (1H, d, *J* 1.7 Hz, H-8), 6.61 (1H, s, H-3), 7.19–7.81 (15H, m, Ar-H). δ_C (100 MHz: $CHCl_3$): 70.29 (CH_2), 70.48 (CH_2), 94.06 (CH), 98.18 (CH), 108.91 (CH), 109.70 (C), 125.75 (CH), 126.38 (CH), 127.44 (CH), 128.25 (CH), 128.40 (CH), 128.58 (CH), 128.74 (CH), 130.00 (CH), 131.35 (C), 135.35 (C), 136.29 (C), 159.49 (C), 159.56 (C), 160. 41 (C), 162.77 (C), 177.05 (C). *m/z* (EI): 434 (M$^{+\bullet}$, 10%), 91 (100). HRMS: 434.1518. $C_{29}H_{22}O_4$ requires (M$^+$) 434.1515. Microanalysis: C, 80.16; H, 5.13%. $C_{29}H_{22}O_4$ requires C, 80.17; H, 5.10%.

4.5. 2,4-Dibenzyloxy-6-hydroxyacetophenone 11

Diethylene glycol (170 mL) was added slowly to a stirring mixture of flavone 10 (39.6 g, 91.4 mmol) in pyridine (150 mL) and 18 M KOH (150 mL). The solution was heated at 120 °C for 4 h. After cooling, the solution was acidified to pH 1 with 4 M HCl and the precipitate filtered off. The solid was washed with water then extracted into EtOAc (300 mL). The organics were washed with saturated $NaHCO_3$ solution (3×400 mL) then dried over magnesium sulfate and concentrated under vacuum. The resulting solid was recrystallised from MeOH to give acetophenone **11**

as off white prisms (23.4 g, 74%), mp 108–109 °C. Lit.:110–111 °C.[36] R_f [silica, EtOAc–hexane (7:3)] 0.73. ν_{max}(nujol)/cm^{-1}: 1615 (C=O). δ_H (400 MHz: $CDCl_3$): 2.54 (3H, s, CH_3), 5.049 (2H, s, $ArOCH_2$), 5.053 (2H, s, $ArOCH_2$), 6.09 (1H, d, *J* 2.4 Hz, H-3), 6.15 (1H, d, *J* 2.4 Hz, H-5), 7.32–7.40 (10H, m, Ar-H), 14.02 (1H, s, OH). δ_C (100 MHz: $CDCl_3$): 33.26 (CH_3), 70.19 (CH_2), 71.05 (CH_2), 92.29 (CH), 94.69 (CH), 106.28 (C), 127.59 (CH), 127.93 (CH), 128.30 (CH), 128.37 (CH), 128.41 (C), 128.66 (CH), 128.69 (CH), 135.56 (C), 135.81 (C), 161.90 (C), 165.03 (C), 167.51 (C), 203.13 (C). *m/z* (EI): 348 ($M^{+\bullet}$, 15%), 306 (7), 91 (100). HRMS: 348.1361. $C_{22}H_{20}O_4$ requires (M^+), 348.1361. Microanalysis: C, 75.90; H, 5.75%. $C_{22}H_{20}O_4$ requires C 75.86, H 5.75%. ^{1}H NMR agrees with literature.[37,38]

4.6. 1-(2′,4′-Dibenzyloxy-6′-hydroxyphenyl)-2-hydroxyethanone 12

A solution of ketone **11** (10.0 g, 28.7 mmol) in dry THF (30 mL) was added to a solution of LDA (2.1 equiv) in dry THF (100 mL), under N_2, at 0 °C over 10 min. The solution was stirred at 0 °C for 30 min after which point chlorotrimethylsilane (9.0 mL, 71.8 mmol, 2.5 equiv) was added slowly, the solution was then stirred for a further 30 min at 0 °C. After this time, the reaction mixture was quenched with aqueous $NaHCO_3$ (100 mL) and extracted into ether (300 mL). The aqueous layer was re-extracted with ether (200 mL). The combined organic layers were washed with H_2O (2×300 mL) and dried over sodium sulfate and concentrated under vacuum. The whole washing process was performed as quickly as possible to minimise the risk of hydrolysing the silyl enol ether. The crude silyl enol ether was dissolved in CH_2Cl_2 (100 mL) and $NaHCO_3$ (3.61 g, 43.0 mmol, 1.5 equiv) was added. The solution was cooled 0 °C and *m*CPBA (70–75%, 7.45 g, 43.0 mmol, 1.5 equiv) was added slowly. The reaction mixture was allowed to stir at 0 °C for 1.5 h. After this time, the yellow cloudy solution was diluted with CH_2Cl_2 (200 mL) and washed with saturated $NaHCO_3$ (2×300 mL) and H_2O (2×300 mL) and concentrated under vacuum. The product was dissolved in 4:1 THF–H_2O (150 mL) and 2.5 g of *para*-toluenesulfonic acid was added, the solution was then stirred at rt for 30 min. The product was extracted into Et_2O (400 mL), washed with saturated $NaHCO_3$ (2×300 mL) and H_2O (2×300 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was recrystallised from EtOAc–hexanes (3:7) to give ketone **12** as an amorphous solid (7.61 g, 73%), mp 105–107 °C. ν_{max}(nujol)/cm^{-1}: 3451 (OH), 1636 cm^{-1} (C=O). δ_H (400 MHz: $CDCl_3$): 3.71 (1H, t, *J* 4.8 Hz, OH), 4.62 (2H, d, *J* 4.8 Hz, CH_2OH), 5.04 (2H, s, OCH_2), 5.06 (2H, s, OCH_2), 6.10 (1H, d, *J* 2.2 Hz, H-3′), 6.19 (1H, d, *J* 2.2 Hz, H-5′), 7.32–7.42 (10H, m, Ar-H), 13.2 (1H, s, Ar-OH). δ_C (100 MHz: $CDCl_3$): 68.83 (CH_2), 70.40 (CH_2), 71.31 (CH_2), 92.45 (CH), 94.92 (CH), 103.63 (C), 127.60 (CH), 128.14 (CH), 128.42 (CH), 128.72 (CH), 128.75 (CH), 128.88 (CH), 134.89 (C), 135.57 (C), 162.212 (C), 166.02 (C), 167.23 (C), 201.90 (C). *m/z* (CI): 365 [(M+H)$^+$, 100%], 347 (25), 333 (22), 307 (20), 91 (70). HRMS: 365.1389. $C_{22}H_{21}O_5$ requires (M+H)$^+$ 365.1386. Microanalysis: C, 72.38; H, 5.66%. $C_{22}H_{20}O_5$ requires C, 72.51%; H, 5.53%.

4.7. 1-(2′,4′-Dibenzyloxy-6′-hydroxyphenyl)-2-(2″,3″,4″,6″-tetra-*O*-benzyl-β-D-glucopyranosyloxy)ethanone 14

Boron trifluoride diethyl etherate (0.33 mL, 2.6 mmol, 0.3 equiv) was added dropwise to a solution of phenol **12** (3.14 g, 8.6 mmol) and *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)trichloroacetimidate **13** (6.50 g, 9.5 mmol, 1.1 equiv, prepared) in dry CH_2Cl_2 (40 mL) at −78 °C, under an atmosphere of nitrogen. The solution was stirred for 1 h at −78 °C and then quenched with aqueous $NaHCO_3$ (75 mL). The solution was diluted with CH_2Cl_2 (250 mL) and washed with brine (2×300 mL), dried over magnesium sulfate and concentrated under vacuum. The solid was recrystallised from EtOAc–pet. ether to give *glucoside* **14** as an amorphous solid (4.98 g, 66%), mp 118–119 °C. R_f (silica, ether) 0.68. ν_{max}(nujol)/cm^{-1}: 1633 (C=O), 1604 (Ar), 1583 (Ar). δ_H (400 MHz: $CDCl_3$): 3.29–3.72 (6H, m, H-2″, 3″, 4″, 5″, 6″), 4.34 (1H, d, *J* 7.6 Hz, H-1″), 4.48 (1H, d, *J* 12.4 Hz, $ArOCH_AH_B$), 4.52 (1H, d, *J* 10.8 Hz, $ArOCH_CH_D$), 4.56 (1H, d, *J* 12.0 Hz, $ArOCH_AH_B$), 4.74 (1H, d, *J* 18.4 Hz, $ArOCH_EH_F$), 4.75 (1H, d, *J* 12.0 Hz, $ArOCH_GH_H$), 4.77 (1H, d, *J* 11.2 Hz, $ArOCH_IH_J$), 4.81 (1H, d, *J* 10.8 Hz, $ArOCH_CH_D$), 4.96 (1H, d, *J* 10.8 Hz, $ArOCH_IH_J$), 4.97 (1H, d, *J* 18.4 Hz, $ArOCH_EH_F$), 4.99 (2H, s, $ArOCH_2$), 5.12 (2H, s, $ArOCH_2$), 5.14 (1H, d, *J* 10.8 Hz, $ArOCH_GH_H$), 6.08 (1H, d, *J* 2.2 Hz, H-5′), 6.20 (1H, d, *J* 2.1 Hz, H-3′), 7.15–7.40 (30H, m, Ar-H), 13.7 (1H, s, Ar-OH). δ_C (100 MHz: $CDCl_3$): 68.76 (CH_2), 70.33 (CH_2), 71.22 (CH_2), 73.48 (CH_2), 74.23 (CH_2), 74.71 (CH_2), 74.84 (CH), 75.06 (CH_2), 75.66 (CH_2), 77.62 (CH), 81.90 (CH), 84.45 (CH), 92.25 (CH), 95.04 (CH), 103.15 (CH), 104.54 (C), 127.52 (CH), 127.53 (CH), 127.58 (CH), 127.61 (CH), 127.71 (CH), 127.75 (CH), 127.85 (CH), 127.04 (CH), 128.18 (CH), 128.28 (CH), 128.33 (CH), 128.37 (CH), 128.56 (CH), 128.71 (CH), 128.75 (CH), 135.14 (C), 136.26 (C), 138.10 (C), 138.19 (C), 138.48 (C), 138.66 (C), 161.59 (C), 165.33 (C), 167.46 (C), 199.39 (C). *m/z* (FAB): 909.6 [(M+Na)$^+$, 100%], 439.3 (30), 91.5 (82%). HRMS: 909.3611. $C_{56}H_{54}O_{10}Na$ requires (M+Na)$^+$, 909.3614. Microanalysis: C, 75.91; H, 6.12%. $C_{56}H_{54}O_{10}$ requires C, 75.83%; H, 6.14%. $[\alpha]_D^{19}$ −5.0 (*c* 140 mg mL^{-1}, $CHCl_3$).

4.8. 1-Bromo-3,4-diallyloxybenzene 16

Commercially available catechol **15** (20.0 g, 182 mmol, 1 equiv) was dissolved in a mixture of chloroform–diethyl ether (2:1, 150 mL) that was cooled to 0 °C and stirred under argon. Bromine (9.3 mL, 182 mmol, 1 equiv) dissolved in chloroform (200 mL) was added dropwise to the catechol solution over a period of 2 h, the resulting solution was then stirred for another 30 min. The reaction mixture was quenched by adding saturated sodium thiosulfate (400 mL). The organic layer was collected, and the aqueous layer was extracted with EtOAc (300 mL). Both organic fractions were combined, dried ($MgSO_4$) and concentrated to give 3-bromocatechol as an intermediate. The intermediate was dissolved into DMF (300 mL), and the solution was put under argon. K_2CO_3 (55.0 g, 400 mmol, 2.2 equiv) and allyl bromide (35.0 mL, 400 mmol, 2.2 equiv) were added and the resulting suspension was stirred for 20 h at rt. The reaction mixture was then acidified to pH 1 with 1 M HCl. The solution was then extracted with diethyl ether

(300 mL) and the aqueous layer was re-extracted with diethyl ether (150 mL). The combined organic layers were washed with H_2O (2×100 mL), 1 M KOH (200 mL) and brine (100 mL), before the ether layer was dried ($MgSO_4$) and concentrated. Flash chromatography (silica) eluting with cyclohexane–EtOAc (30:1) gave 1-bromo-3,4-diallyloxybenzene **16** as a yellow oil (32.6 g, 68%). R_f [silica, pet. ether–EtOAc (8:1)] 0.83. ν_{max}(nujol)/cm^{-1}: 1586 (Ar), 1495 cm^{-1} (Ar). δ_H (400 MHz; $CDCl_3$): 4.50–4.54 (4H, m, 2×OCH_2), 5.22–5.27 (2H, m, 2×CH_AH$_B$=CH), 5.38–5.42 (2H, m, 2×CH$_A$$H_B$=CH), 5.98–6.05 (2H, m, 2×CH$_A$H$_B$=C*H*), 6.71 (1H, d, *J* 8.4 Hz, H-5), 6.96 (1H, dd, *J* 2.3 and 8.0 Hz, H-6), 6.97 (1H, d, *J* 2.3 Hz, H-2). δ_C (100 MHz, $CDCl_3$): 69.53 (CH_2), 69.65 (CH_2), 112.62 (C), 114.93 (CH), 116.81 (CH), 117.34 (CH_2), 117.49 (CH_2), 123.08 (CH), 132.74 (CH), 133.01 (CH), 147.38 (C), 148.93 (C). *m*/*z* (EI): 270 [M$^{+\bullet}$ (^{81}Br), 22%], 268 [M$^{+\bullet}$ (^{79}Br), 22], 229 [M$^{+\bullet}$(^{81}Br)-$^{\bullet}$C$_3$H$_5$, 22], 227 [M$^{+\bullet}$(^{79}Br)-$^{\bullet}$C$_3$H$_5$, 22], 41 ($^{+}$C$_3$H$_5$, 100). HRMS: 270.0079 and 268.0096. $C_{12}H_{13}O_2{}^{81}Br$ requires M$^+$, 270.0079, $C_{12}H_{13}O_2{}^{79}Br$ requires M$^+$, 268.0099. Microanalysis: C, 53.95; H, 4.99%. $C_{12}H_{13}O_2Br$ requires C, 53.55; H, 4.89%.

4.9. 3,4-Diallyloxy[*carboxy*-^{13}C]benzoic acid 17

Carboxylation of the aryl bromide **16** was carried out using the apparatus described by Kratzel and Billek[28] and a variation of their method. *n*-Butyllithium (4.7 mL, 2.1 M in hexane, 9.9 mmol, 2.0 equiv) was added to a stirred solution of 3,4-diallyloxy-1-bromobenzene **16** (2.69 g, 10.0 mmol, 2 equiv) in dry THF (20 mL) at −78 °C under nitrogen. After 3 min, the reaction mixture was cooled to −198 °C. When the solution was frozen the whole system was put under vacuum. The system was sealed from vacuum and [^{13}C]carbon dioxide was generated by adding an excess of concentrated sulfuric acid (10 mL) dropwise onto powdered [^{13}C] barium carbonate (0.99 g, 5.0 mmol, 1 equiv) in a separate reaction vessel in the same system. The carbon dioxide evolved condensed onto the frozen THF solution of aryllithium. After 20 min the THF solution was allowed to warm up to –78 °C and the reaction mixture was stirred for 40 min. The entire system was filled with nitrogen and reaction mixture was quenched by the addition of HCl (1M, 10 mL). The solution was extracted into EtOAc (2×100 mL) and the combined organic extracts were washed with brine (2×200 mL). The combined organic layers were extracted with 2 M NaOH (2×250 mL). The resulting aqueous layer was acidified with 1 M HCl and then extracted into EtOAc (2×200 mL). The combined extracts were dried over $MgSO_4$ and concentrated to give acid **17** as a powder (956 mg, 81%). R_f [silica, pet. ether–EtOAc (1:1)] 0.56. ν_{max}(Golden Gate)/cm^{-1}: 1638 (C=O), 1581 (Ar). δ_H (400 MHz: $CDCl_3$): 4.66–4.70 (4H, m, 2×OCH_2), 5.30–5.34 (2H, m, 2×CH_AH$_B$=CH), 5.42–5.48 (2H, 2×CH$_A$$H_B$=CH), 6.04–6.15 (2H, m, 2×CH$_A$H$_B$=C*H*), 6.95 (1H, dd, *J* 0.9 and 8.5 Hz, H-5), 7.62 (1H, dd 2.0 and 4.3 Hz, H-2), 7.74 (1H, ddd 2.0, 4.1 and 8.5 Hz, H-6). δ_C (100 MHz: $CDCl_3$): 69.63 (CH_2), 69.84 (CH_2), 112.34 (CH, d, *J* 5.6 Hz), 114.86 (CH, d, *J* 3.2 Hz), 118.08 (CH_2), 118.18 (CH_2), 121.72 (C, d, *J* 74.8 Hz, C-1), 124.63 (CH, d, *J* 2.6 Hz), 132.53 (CH), 132.85 (CH), 147.90 (C, d, *J* 5.3 Hz), 153.22 (C), 171.89 (^{13}C-label). *m*/*z* (EI): 235 (M$^{+\bullet}$, 78%), 194 (M$^{+\bullet}$-$^{\bullet}$C$_3$H$_5$, 32), 41 ($^{+}$C$_3$H$_5$, 100%). HRMS: 235.0926, $^{12}C_{12}{}^{13}CH_{14}O_4$ requires 235.0926.

4.10. 1-[2′-(3″,4″-Dialloxy[*carbonyl*-^{13}C]benzoyloxy)-4′,6′-dibenzyloxyphenol]-2-(2‴,3‴,4‴,6‴-*tetra*-*O*-benzyl-β-D-glucopyranosyloxy)ethanone 18

EDCI (0.76 g, 4.0 mmol, 1.6 equiv) was added to a solution of the labelled benzoic acid **17** (0.64 g, 2.7 mmol, 1.1 equiv), phenol **14** (2.19 g, 2.47 mmol, 1 equiv) and DMAP (0.30 g, 2.5 mmol, 1 equiv) in dry DCM (25 mL) under an atmosphere of nitrogen. The solution was stirred for 24 h at rt, then diluted with DCM (100 mL) and washed with water (2×100 mL) and brine (2×100 mL), dried over $MgSO_4$ and concentrated to give the crude ester as an oil. Flash column chromatography (silica) eluting with hexane–EtOAc (4:1) gave labelled ester **18** as yellow oil (1.81 g, 66%). R_f [silica, pet. ether–EtOAc (4:1)] 0.52. ν_{max}(Golden Gate)/cm^{-1}: 1688 (C=O), 1607 cm^{-1} (Ar). δ_H (400 MHz: $CDCl_3$): 3.18 (1H, dt, *J* 2.4 and 9.6 Hz, H-5‴), 3.40–3.63 (5H, m, H-2‴, 3‴, 4‴, and 6‴), 4.30 (1H, d, *J* 7.4 Hz, H-1‴), 4.44 (1H, d, *J* 12.2 Hz, OCH_2Ph), 4.49 (1H, d, *J* 10.9 Hz, OCH_2Ph), 4.52–4.59 (6H, m, 2×OCH_2CHCH_2, 2×OC*H*HPh), 4.66 (1H, d, *J* 17.0 Hz, OCH_2CO), 4.70 (1H, d, *J* 11.0 Hz, OCH_2Ph), 4.78 (1H, d, *J* 10.8 Hz, OCH_2Ph), 4.81 (1H, d, *J* 17.2 Hz, OCH_2CO), 4.88 (1H, d, *J* 10.9 Hz, OCH_2Ph), 4.93 (1H, d, *J* 11.1 Hz, OCH_2Ph), 4.97 (2H, s, OCH_2Ph), 5.02 (2H, s OCH_2Ph), 5.24–5.30 (2H, m, 2×CH_AH$_B$=CH), 5.38–5.42 (2H, m, 2×CH$_A$$H_B$=CH), 6.00–6.09 (2H, m, 2×CH$_A$H$_B$=C*H*), 6.46 (1H, d, *J* 2.2 Hz, H-5′), 6.53 (1H, d, *J* 2.1 Hz, H-3′), 6.82 (1H, dd, *J* 1.0 and 8.6 Hz, H-5″), 7.13–7.40 (30 H, m, ArH), 7.61 (1H, dd, *J* 2.0 and 4.4 Hz, H-2″), 7.74 (1H, ddd, *J* 2.0, 4.2 and 8.5 Hz, H-6″). δ_C (100 MHz: $CDCl_3$): 68.64 (CH_2), 69.52 (CH_2), 69.72 (CH_2), 70.40 (CH_2), 70.99 (CH_2), 73.37 (CH_2), 74.25 (CH_2), 74.38 (CH_2), 74.74 (CH), 74.86 (CH_2), 75.58 (CH_2), 77.51 (CH), 81.84 (CH), 84.42 (CH), 98.20 (CH), 101.75 (CH), 103.40 (CH), 112.42 (CH, d, *J* 5.7 Hz, C-5″), 114.84 (CH, d, *J* 3.1 Hz, C-2″), 114.91 (C), 117.95 (CH_2), 118.02 (CH_2), 121.39 (C, d, *J* 79.7 Hz, C-1″), 124.71 (CH, d, *J* 2.3 Hz, C-6″), 127.28 (CH), 127.47 (CH), 127.48 (CH), 127.57 (CH), 127.62 (CH), 127.70 (CH), 127.83 (CH), 127.87 (CH), 128.08 (CH), 128.26 (CH), 128.29 (CH), 128.32 (CH), 128.38 (CH), 128.66 (CH), 128.71 (CH), 132.52 (CH), 132.81 (CH), 135.65 (C), 135.85 (C), 138.17 (C), 138.25 (C), 138.66 (C), 138.69 (C), 148.02 (C, d, *J* 6.0 Hz, C-3″), 150.66 (C, d, *J* 7.3 Hz, C-2′), 153.13 (C), 158.41 (C), 161.64 (C), 164.48 (C=O, ^{13}C-label), 197.16 (C=O). *m*/*z* (FAB): 1126.6 [(M+Na)$^+$, 10%], 218 (46), 93 (100). HRMS: 1126.4438, $^{12}C_{68}{}^{13}CH_{66}O_{13}Na$ requires (M+Na)$^+$, 1126.4435.

4.11. 3-(2″,3″,4″,6″-*tetra*-*O*-Benzyl-β-D-glucopyranosyloxy)-7-benzyloxy-3′,4′,5-trihydroxy[2-^{13}C]flavonol 19

The labelled ester **18** (1.58 g, 1.43 mmol, 1 equiv) was stirred in toluene (20 mL) under argon. K_2CO_3 (0.79 g, 5.7 mmol, 4 equiv) was added followed by tetrabutylammonium bromide (0.69 g, 2.2 mmol, 1.5 equiv) and the mixture was heated at 70 °C for 23 h. After the mixture was cooled down, toluene was evaporated in vacuo. The solid was dissolved in DCM (100 mL) and washed with water (2×100 mL) and brine (2×100 mL) and concentrated. The

crude mono-deprotected labelled flavonol was dissolved in DCM (10 mL) and the solution degassed with argon for 20 min. $Pd(PPh_3)_4$ (50 mg, 0.043 mmol, 3 mol%) and barbituric acid (1.34 g, 8.58 mmol, 6 equiv) were added and the solution was stirred for 2 h at rt under argon. After this time, the solvent was removed in vacuo and the resulting solid was dissolved into EtOAc (100 mL). The organic solution was washed with satd $NaHCO_3$ (3×300 mL), dried ($MgSO_4$) and concentrated to give a brown/black oil. Column chromatography (silica, hexane–EtOAc, 2:1) gave flavonol **19** as a pale yellow oil (637 mg, 48%), R_f [SiO_2, pet. ether–EtOAc (4:1)] 0.26. ν_{max}(Golden Gate)/cm^{-1}: 1652 (C═O), 1593 cm^{-1} (C═O). δ_H (400 MHz: $CDCl_3$): 3.42–3.44 (1H, m, H-5″), 3.58–3.80 (5H, m, H-2″, 3″, 4″ and 6″), 4.28 (1H, d, *J* 12.1 Hz, OCH_2Ph), 4.34 (1H, d, *J* 12.0 Hz, OCH_2Ph), 4.50 (1H, d, *J* 10.9 Hz, OCH_2Ph), 4.75 (1H, d, *J* 12.5 Hz, OCH_2Ph), 4.78 (1H, d, *J* 11.1 Hz, OCH_2Ph), 4.79 (1H, d, *J* 10.9 Hz, OCH_2Ph), 4.96 (1H, d, *J* 11.1 Hz, OCH_2Ph), 5.06 (1H, d, *J* 13.1 Hz, OCH_2Ph), 5.08 (2H, s, OCH_2Ph), 5.58 (1H, d, *J* 7.0 Hz, H-1″), 6.41 (1H, d, *J* 2.0 Hz, H-6 or H-8), 6.45 (1H, d, *J* 2.0 Hz, H-6 or H-8), 6.84 (1H, d, *J* 8.5 Hz, H-5′), 7.10–7.42 (25H, m, Ar-H), 7.52 (1H, ddd, *J* 8.5, 3.8, and 2.0 Hz, H-6′), 7.91 (1H, dd, *J* 2.0 and 3.8 Hz, H-2′), 12.51 (1H, s, OH). *m/z* (FAB): 916.7 [$(M+H)^+$, 3%]. 808.6 [$(M+H)^+-HOCH_2Ph$, 1], 393.3 [$(M+H)^+-C_{34}H_{35}O_5$, 4], 364.3 [$(M+H)^+$-$C_{35}H_{36}O_6$, 3], 91.5 ($^+C_7H_7$, 100). HRMS: 938.3224, $^{12}C_{55}{}^{13}CH_{50}O_{12}Na$ requires $(M+Na)^+$, 938.3234.

4.12. 2-Benzoyloxy-4,6-dibenzyloxyacetophenone 20

Benzoyl chloride (4.00 mL, 34.9 mmol, 2.4 equiv) was added to a solution of acetophenone **11** in pyridine (20 mL). The solution was stirred at rt under an atmosphere of nitrogen overnight. The solution was then extracted into EtOAc and washed with 1 M HCl (3×250 mL), dried over magnesium sulfate and concentrated under vacuum. The resulting solid was then recrystallised from Et_2O–hexane (1:1) to give ester **20** as an amorphous solid (4.45 g, 72%), mp 105–106 °C. R_f [silica, EtOAc–hexane (7:3)] 0.67. ν_{max}(nujol)/cm^{-1}: 1730 (C═O of ester), 1693 (C═O). δ_H (400 MHz: $CDCl_3$): 2.47 (3H, s, CH_3), 5.03 (2H, s, $ArOCH_2$), 5.08 (2H, s, $ArOCH_2$), 6.48 (1H, d, *J* 2.2 Hz, H-5), 6.54 (1H, d, *J* 2.2 Hz, H-3), 7.24–7.39 (10H, m, Ar-H), 7.47–7.51 (2H, m, H-3′,5′), 7.60–7.64 (1H, m, H-4′), 8.13–8.15 (2H, m, H-2′,6′). δ_C (100 MHz: $CDCl_3$): 32.03 (CH_3), 70.43 (CH_2), 70.94 (CH_2), 98.52 (CH), 101.38 (CH), 117.90 (C), 127.42 (CH),127.59 (CH), 128.24 (CH), 128.30 (CH), 128.54 (CH), 128.68 (CH), 129.147 (C), 130.26 (CH), 133.63 (CH), 135.79 (C), 135.93 (C), 149.72 (C), 158.17 (C), 158.21 (C), 165.00 (C), 199.21 (C). *m/z* (EI): 452 ($M^{+\bullet}$, 5%), 347 (10), 91 (100). HRMS: 452.1623. $C_{29}H_{24}O_5$ requires M^+, 452.1624. Microanalysis: C, 76.84; H, 5.41%. $C_{29}H_{24}O_5$ requires C 76.98, H 5.35%.

4.13. 7-Benzyloxy-5-hydroxyflavone 21

tert-Butylimino-tri(pyrrolidino)phosphorane[29] (0.57 mL, 1.9 mmol, 4.0 equiv) was added to a solution of ester **20** in dry 1,4-dioxane (2 mL). The reaction mixture was heated at 100 °C under N_2 for 24 h. After cooling, the reaction mixture was extracted into EtOAc (50 mL) and washed with H_2O (3×200 mL). The organic layer was dried over magnesium sulfate and concentrated under vacuum. The crude product was recrystallised from ^{i}PrOH to give flavone **21** as an amorphous solid (92 mg, 61%), mp 177–178 °C. R_f (silica, Et_2O) 0.76. δ_H (400 MHz: $CDCl_3$): 5.05 (2H, s, OCH_2), 6.36 (1H, d, *J* 2.2 Hz, H-6), 6.48 (1H, d, *J* 2.2 Hz, H-8), 6.57 (1H, s, H-3), 7.25–7.49 (8H, m, Ar-H), 7.77–7.80 (2H, m, H-2,6′), 12.65 (1H, broad s, OH). δ_C (100 MHz: $CDCl_3$): 70.39 (CH_2), 93.45 (CH), 98.90 (CH), 105.81 (CH), 126. 23 (CH), 127.45 (CH), 128. 34 (CH), 128.71 (CH), 129.03 (CH), 131.20 (C), 131.81 (CH), 135.68 (C), 157.68 (C), 162.12 (C), 163. 95 (C), 164.59 (C), 182.41 (C), *m/z* (EI): 344 ($M^{+\bullet}$, 70%), 91 (100). HRMS: 344.1045. $C_{22}H_{16}O_4$ requires M^+, 344.1049. Found: C, 76.76; H, 4.67%. $C_{22}H_{16}O_4$ requires C, 76.73; H 4.68%. Literature NMR spectroscopy data which was run in DMSO-d_6 is in close agreement.[39]

4.14. 1-(4′,6′-Dibenzyloxy-2′-hydroxyphenyl)-3-hydroxy-3-phenylpropenone 27

Diethylene glycol (50 mL) was added slowly to a stirring mixture of flavone **10** (12.64 g, 29.0 mmol) in pyridine (50 mL) and 18 M KOH (50 mL). The solution was heated at 100 °C for 2 h. After cooling, the solution was acidified to pH 1 with 1 M HCl and extracted into EtOAc (2× 300mL). The organics were washed with H_2O (3× 400 mL) and 1M HCl (2×400 mL). The EtOAc layer was dried over magnesium sulfate and concentrated under vacuum. The resulting solid was recrystallised from MeOH to give dibenzoylmethane **27** as yellow needles (3.91 g, 39%), mp 131–133 °C.). R_f [silica, EtOAc–hexane (7:3)] 0.73. ν_{max}(nujol)/cm^{-1}: 3170 (OH), 1610 (C═O), 1571 (C═C). δ_H (400 MHz: $CDCl_3$): Keto-enol form: 5.01 (2H, s, OCH_2), 5.07 (2H, s, OCH_2), 6.18 (1H, d, *J* 2.3 Hz, H-3′), 6.21 (1H, d, *J* 2.3 Hz, H-5′), 7.10–7.68 (16 H, m, Ar-H, C═CHCO), 13.68 (1H, s, Ar-OH), 15.64 (1H, s, OH). δ_C (100 MHz: $CDCl_3$): Keto-enol form: 70.18 (CH_2), 71.38 (CH_2), 92.54 (CH), 95.22 (CH), 97.93 (CH), 104.45 (C), 126.48 (CH), 127.64 (CH), 128.31 (CH), 128.36 (CH), 128.68 (CH), 128.72 (CH), 128.92 (CH), 129.15 (CH), 131.51 (CH), 133.90 (C), 135.60 (C), 135.86 (C), 161.07 (C), 164.52 (C), 167.36 (C), 175.76 (C), 193.43 (C). *m/z* (EI): 452.1 ($M^{+\bullet}$, 5%), 434.1 (40), 345.1 (5), 91.1 (100). HRMS: 452.1630. $C_{29}H_{24}O_5$ requires M^+, 452.1624. Microanalysis: C, 76.84; H, 5.39%. $C_{29}H_{24}O_5$ requires C 76.98, H 5.35%.

Acknowledgements

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References and notes

1. Erlund, I. *Nutr. Res.* **2004**, *24*, 851–874.
2. Saltmarch, M.; Crozier, A.; Radcliffe, B. *Plants: Diet and Health*; Goldberg, G., Ed.; Chapman Hall: London, 2003; pp 107–133.

3. Neuhouser, M. L. *Pharm. Biol.* **2004**, *42*, 36–45.
4. McPhail, D. B.; Hartley, R. C.; Gardner, P. T.; Duthie, G. G. *J. Agric. Food Chem.* **2003**, *51*, 1684–1690.
5. Zhou, B.; Miao, Q.; Yang, L.; Liu, Z. L. *Chem.—Eur. J.* **2005**, *11*, 680–691.
6. Walle, T. *Free Radic. Biol. Med.* **2004**, *36*, 829–837.
7. Williamson, G.; Manach, C. *Am. J. Clin. Nutr.* **2005**, *81*, 243S–255S.
8. Williamson, G.; Barron, D.; Shimoi, K.; Terao, J. *Free Radic. Res.* **2005**, *39*, 457–469.
9. Mullen, W.; Graf, B. A.; Caldwell, S. T.; Hartley, R. C.; Duthie, G. G.; Edwards, C. A.; Lean, M. E. J.; Crozier, A. *J. Agric. Food Chem.* **2002**, *50*, 6902–6909.
10. Graf, B. A.; Mullen, W.; Caldwell, S. T.; Hartley, R. C.; Duthie, G. G.; Lean, M. E. J.; Crozier, A.; Edwards, C. A. *Drug Metab. Dispos.* **2005**, *33*, 1036–1043.
11. Caldwell, S. T.; Crozier, A.; Hartley, R. C. *Tetrahedron* **2000**, *56*, 4101–4106.
12. Mullen, W.; Hartley, R. C.; Crozier, A. *J. Chromatogr., A* **2003**, *1007*, 21–29.
13. Sesink, A. L. A.; Arts, I. C. W.; Faassen-Peters, M.; Hollman, P. C. H. *J. Nutr.* **2003**, *133*, 773–776.
14. Nemeth, K.; Plumb, G. W.; Berrin, J. G.; Juge, N.; Jacob, R.; Naim, H. Y.; Williamson, G.; Swallow, D. M.; Kroon, P. A. *Eur. J. Nutr.* **2003**, *42*, 29–42.
15. Day, A. J.; Gee, J. M.; DuPont, M. S.; Johnson, I. T.; Williamson, G. *Biochem. Pharmacol.* **2003**, *65*, 1199–1206.
16. Cermak, R.; Landgraf, S.; Wolffram, S. *Br. J. Nutr.* **2004**, *91*, 849–855.
17. Arts, I. C. W.; Sesink, A. L. A.; Faassen-Peters, M.; Hollman, P. C. H. *Br. J. Nutr.* **2004**, *91*, 841–847.
18. Elhabiri, M.; Figueiredo, P.; Fougerousse, A.; Brouillard, R. *Tetrahedron Lett.* **1995**, *36*, 4611–4614.
19. Bouktaib, M.; Atmani, A.; Rolando, C. *Tetrahedron Lett.* **2002**, *43*, 6263–6266.
20. Du, Y. G.; Wei, G. H.; Linhardt, R. J. *J. Org. Chem.* **2004**, *69*, 2206–2209.
21. Du, Y. G.; Wei, G. H.; Linhardt, R. J. *Tetrahedron Lett.* **2003**, *44*, 6887–6890.
22. Yousef, G. G. Y.; Seigler, D. S.; Grusak, M. A.; Rogers, R. B.; Knight, C. T. G.; Kraft, T. F. B.; Erdman, J. W.; Lila, M. A. *J. Agric. Food Chem.* **2004**, *52*, 1138–1145.
23. Ahuja, M.; Bandopadhyay, M.; Seshadri, T. R. *Indian J. Chem.* **1974**, *12*, 26–28.
24. Rubottom, G. M.; Vazquez, M. A.; Pelegrina, D. R. *Tetrahedron Lett.* **1974**, 4319–4322.
25. Dangles, O.; Elhajji, H. *Helv. Chim. Acta* **1994**, *77*, 1595–1610.
26. El-Desoky, E.-S. I.; Abdel-Rahman, H. A. R.; Schmidt, R. R. *Liebigs Ann. Chem.* **1990**, 877–881.
27. Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, 1249–1256.
28. Kratzel, K.; Billek, G. *Holzforschung* **1953**, *7*, 66–70.
29. Schwesinger, R.; Hasenfratz, C.; Schlemper, H.; Walz, L.; Peters, E. M.; Peters, K.; Vonschnering, H. G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1361–1363.
30. Seebach, D.; Beck, A. K.; Studer, A. *Modern Synthetic Methods 1995*; Leuman, E. C., Ed.; VCH: Weinheim, 1995; pp 1–178.
31. Harborne, J. B.; Mabry, T. J.; Manry, H. *The Flavonoids*; Chapman and Hall: London, 1975.
32. [1, 7] Sigmatropic rearrangement involving migration of a carbon atom does appear to be new. The most common form of [1, 7] sigmatropic rearrangement involves hydride migration (e.g. Akerling, Z. R.; Norton, J. E.; Houk, K. N. *Org. Lett.* **2004**, *6*, 4273–4275), but both boron and sulfur groups have been reported to migrate, e.g.; Dushenko, G. A.; Mikhailov, I. E.; Zschunke, A.; Hakam, N.; Mugge, C.; Minkin, V. I. *Mendeleev Commun.* **1997**, 50–51; Gridnev, I. D.; Tok, O. L.; Gurskii, M. E.; Bubnov, Y. N. *Chem.—Eur. J.* **1996**, *2*, 1483–1488.
33. Bois, F.; Beney, C.; Mariotte, A.-M.; Boumendjel, A. *Synlett* **1999**, 1480–1482.
34. Rama Rao, A. V.; Telang, S. A.; Madhavan Nair, P. *Indian J. Chem.* **1964**, *2*, 431–436.
35. Chavan, J. J.; Robinson, R. *J. Chem. Soc.* **1933**, 368–370.
36. Sethi, M. L.; Taneja, S. C.; Dhar, K. L.; Atal, C. K. *Indian J. Chem., Sect. B* **1981**, *20*, 770–772.
37. Jain, A. C.; Bambah, P. K. *Indian J. Chem., Sect. B* **1987**, *26*, 488–490.
38. Ahuja, M.; Bandopad, M.; Seshadri, T. R. *Indian J. Chem.* **1974**, *12*, 26–28.
39. Comte, G.; Daskiewicz, J. B.; Bayet, C.; Conseil, G.; Viornery-Vanier, A.; Dumontet, C.; Di Pietro, A.; Barron, D. *J. Med. Chem.* **2001**, *44*, 763–768.

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Application of the photocyclization reaction of 1,2-cyclopenta-fused pyridinium perchlorate to formal total syntheses of (−)-cephalotaxine

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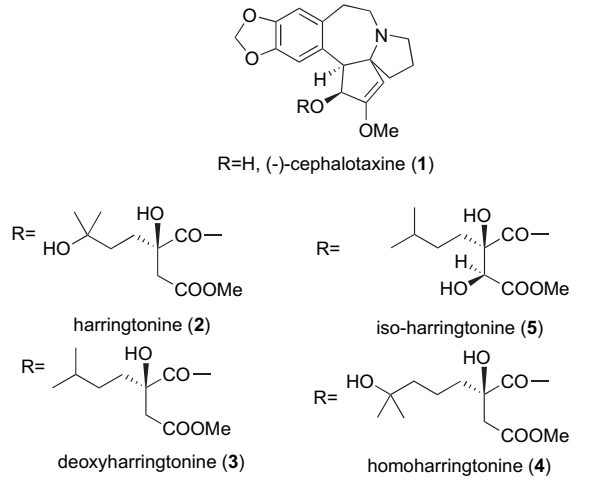
Abstract—Two strategies for the formal total synthesis of (−)-cephalotaxine, based on pyridinium salt photochemistry, are described. The routes begin with photocyclization reaction of 1,2-cyclopenta-fused pyridinium perchlorate. This process efficiently and regioselectively produces a tricyclic aziridine, which undergoes sequential aziridine ring opening and enzymatic desymmetrization to generate enantio-enriched intermediates that contain the spirocyclic D,E-ring system found in cephalotaxine. These substances serve as precursors to late stage key intermediates used by Mori, Tietze, and Yoshida in earlier syntheses of (−)-cephalotaxine.

1. Introduction

(−)-Cephalotaxine is the parent member of the harringtonine alkaloid family. These natural products occur in about eight known species of the genus *Cephalotaxus*, evergreen plum yews that are indigenous to Southeast Asia.[1] (−)-Cephalotaxine was isolated from yew plants by Paudler and his co-workers in 1963[2] and its structure was determined in 1969.[3] This natural product has become an interesting synthetic target,[4a–q] not only because of its unique pentacyclic ring skeleton, containing a 1-azaspiro[4.4]-nonane moiety fused to a benzazepine system, but also as a result of the observed antileukemic and antitumor activities of several of its C-3 2-alkylhydroxysuccinate derivatives, including harringtonine **2**, deoxyharringtonine **3**, homoharringtonine **4**, and isoharringtonine **5** (Scheme 1).[5] The cancer chemotherapeutic effectiveness of homoharringtonine **4** has been evaluated in phase II–III clinical trials,[6] and this substance has been investigated in the treatment of chloroquine-resistant malaria.[7]

Extensive studies have been carried out in our laboratory to both develop and demonstrate the preparative utility of pyridinium salt photochemistry in sequences targeted at a variety of biomedically interesting natural and nonnatural products.[8] Recently, we reported the results of an exploratory study of the photochemistry of a 1,2-cyclopenta-fused pyridinium perchlorate **6** (Scheme 2).[9] Specifically, we observed that irradiation of a basic aqueous solution of this substance promotes a remarkably regioselective photocyclization reaction that results in efficient formation of a single tricyclic-allylic alcohol **8**. The degree of structural, functional, and stereochemical complexity introduced in this 'green' chemical process is remarkable. Moreover, transformation of **8** to the corresponding spirocyclic amido diester **9** by acid promoted, regiocontrolled aziridine ring opening followed by enzymatic desymmetrization was used to produce the enantiomerically pure monoalcohol **10**



Scheme 1.

Keywords: Pyridinium salt photochemistry; (−)-Cephalotaxine formal synthesis.
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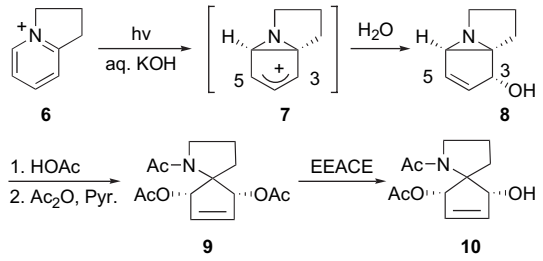
(Scheme 2) that contains a structurally interesting [4.4]-spirocyclic framework.



Scheme 2.

The unique structural and stereochemical features of spirocyclic amide **10** along with the efficiency of its construction should make this substance and its relatives ideal synthons in routes for the preparation of a number of natural product targets. The aim of the effort described below was to demonstrate how the photochemistry of 1,2-cyclopenta-fused pyridinium perchlorate **6** can be readily adapted to routes that rapidly generate two key intermediates in the syntheses of (−)-cephalotaxine.

2. Results and discussion

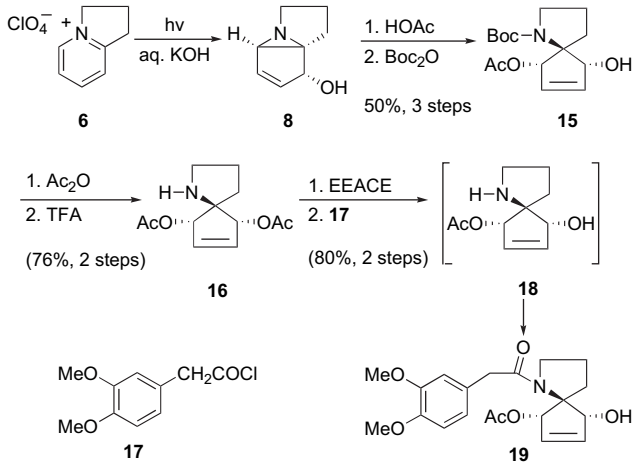
2.1. Retrosynthetic analyses

Two retrosynthetic plans for formal total syntheses of (−)-cephalotaxine, designed on the basis of pyridinium salt photochemistry, are outlined in Scheme 3. A key intermediate in both sequences is pentacyclic alkene **11**, a substance used by Mori and Isono in the first nonracemic total synthesis of this natural product.[4i] We envisaged that in one approach **11** would be derive from the known spirocyclic amido-allylic alcohol **12**, which has already been converted to **11** in the seminal synthesis of (−)-cephalotaxine reported by Mori and Isono.[4i] We assumed that **12** would be easily prepared from an aminomonoalcohol **14**, formed by an enzymatic hydrolytic desymmetrization process[10] on a *meso*-diester derived by application of the pyridinium salt photocyclization/aziridine ring opening sequence (Scheme 2).

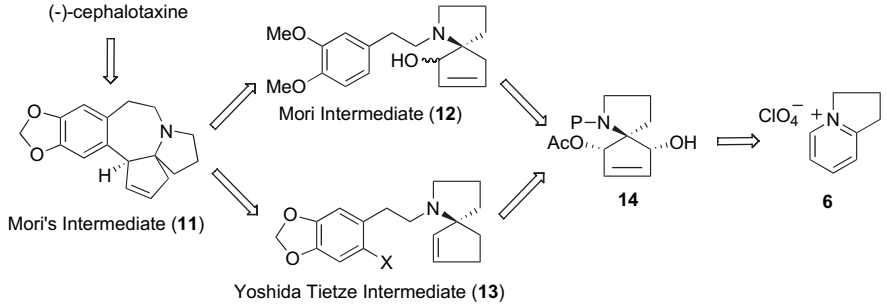
In more recent approaches to the synthesis (−)-cephalotaxine, Tietze[4j] and Yoshida[4l] have utilized Heck coupling reactions of haloarylethyl tethered spirocyclic intermediates **13** to form the late stage intermediate **11** in Mori's synthesis of (−)-cephalotaxine. We visualized that these synthons would arise by appropriate manipulation of the monoalcohol **14**.

2.2. One formal total synthesis of (−)-cephalotaxine

The sequence developed for formal total synthesis of (−)-cephalotaxine that targets construction of the Mori spirocyclic allylic alcohol **12** is initiated by the known photocyclization reaction of the the cyclopenta-fused pyridinium salt **6** (Scheme 4). Irradiation of an aqueous basic solution of this substance gives tricyclic aziridine **8**, which is directly transformed to *N*-Boc-protected spirocyclic monoalcohol **15** by treatment with acetic acid, to promote regioselective aziridine ring opening, followed by protection of the liberated secondary amine through the action of Boc_2O and Et_3N. This three-step sequence serves as an exceptionally convenient method to access the racemic structurally and functionally complex intermediate **15** in a 50% overall yield. Acetylation of **15** with Ac_2O and pyridine followed by deprotection of the Boc group with TFA yields the *meso* aminodiester **16** (81%, two steps). Importantly, this diester serves as an active substrate for EEACE[9] catalyzed hydrolysis (pH=6.9, 0.5 M NaH_2PO_4) that affords a modestly unstable aminomonoalcohol intermediate **18**, which without purification is reacted with (3,4-dimethoxyphenyl)acetyl chloride (**17**) to furnish amidomonoalcohol **19** in a 80% two-step yield. The enantiomeric purity of **18** is determined by its conversion to an *N*-Boc derivative followed by reaction



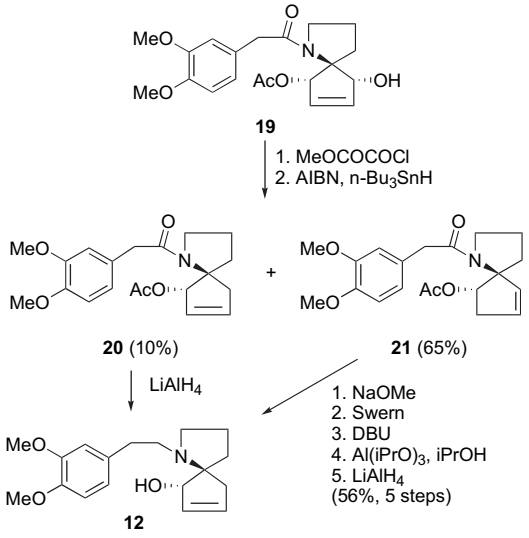
Scheme 4.



Scheme 3.

with both (*R*)- and (*S*)-Mosher acetyl chloride and ^{1}H NMR analysis of the derived esters.[11] The % ee determined in this manner are in the range of 80–90% ee depending on the time/percent conversion of the enzymatic reaction.

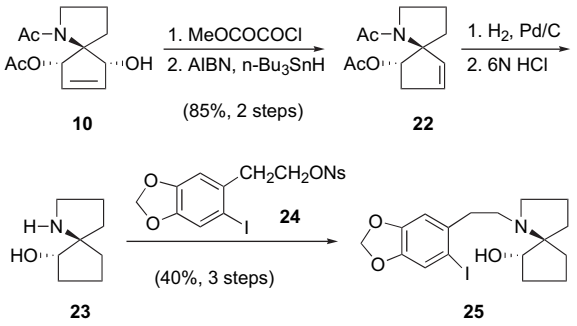
The transformation of spirocyclic monoalcohol **19** to the Mori intermediate **11**, on first thought seemed simple since all that is required is reductive removal of the unblocked hydroxyl group. Among a number of radical reduction approaches explored for this purpose (e.g., *O*-phenoxythiocarbonate reduction), Barton[12] type oxalate reduction was superior. Accordingly, treatment of **19** with methyl(chlorocarbonyl)formate followed by free radical reduction of the intermediate mixed oxalate ester by using AIBN/*n*-Bu_3SnH in refluxing toluene leads to the generation of the desired dehydroxylated product **20** in a 10% yield along with the rearranged homoallylic alcohol **21** as the major product (65%). As expected, treatment of **20** with lithium aluminum hydride gives **12** (95%) (Scheme 5), which has physical and spectroscopic properties that match those reported by Mori and Isono.[4i] In addition, **21** is also transformed to Mori intermediate through a fivestep sequence (55% overall yield) involving alcohol liberation and oxidation, α,β-unsaturated enone formation, and reduction, and amide reduction (Scheme 5).



Scheme 5.

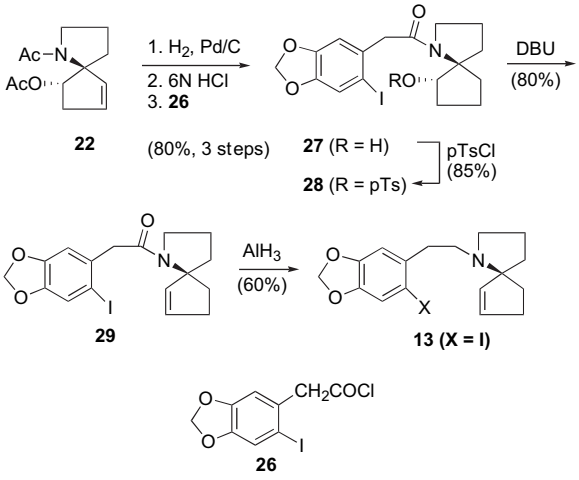
2.3. Another formal total synthesis of (−)-cephalotaxine

The second approach developed for the synthesis of (−)-cephalotaxine links with the iodoarylethyl spirocyclic alkene **13**, which served as a key intermediate in Yoshida and his co-workers' synthesis of this target. The sequence begins with monoalcohol **10**[9] (Scheme 6), a substance we prepared earlier in nonracemic form by using a sequential-photocyclization/aziridine ring opening/enzymatic desymmetrization sequence (Scheme 2). Free radical reduction (AIBN, *n*-Bu_3SnH, toluene, reflux) of the mixed oxalate arising by treatment of **10** with methyl(chlorocarbonyl)formate gives the homoallylic ester **22** (90%). Hydrogenation of **22** with 10% Pd/C in ethanol generates the saturated amide, which upon reaction in refluxing 6 N HCl affords the HCl salt of spirocyclic aminoalcohol **23**. Without purification, this substance is reacted with the iodoarylethyl 4-nitrobenzenesulfonate **24**[4i] to generate the spirocyclic aminoalcohol **25**. A brief exploration for optimal conditions resulted in an optimized 40% yield for the three-step sequence. In order to complete the synthesis of Yoshida spirocyclic alkene **13** all that is needed was dehydration of aminoalcohol **25**. However, we found that this was a difficult task when we observed that several typical time-tested methods ($POCl_3$/Py, Burgess salts, CS_2/MeI, and MeOCOCOCl/Pyr) failed to give satisfactory results.



Scheme 6.

As a result of low yields of the N-alkylation reaction of spirocyclic amine **23** and dehydration reaction of alcohol **25**, the synthetic plan was modified by employing the amidoalcohol **27** as an intermediate (Scheme 7). As anticipated, **23** derived by successive hydrogenation of **22** and amide hydrolysis, reacts with iodoarylacetyl chloride **26** to give spirocyclic amidoalcohol **27** in a dramatically improved 80% three-step yield. Formation of the tosylate derivative **28** by reaction of **27** with *p*-TsCl and pyridine (85%) is followed by smooth DBU[13] catalyzed elimination to afford the unsaturated amide **29** in an 80% yield. Finally, low temperature (−40 °C) aluminum hydride reduction[14] of **29** (60%) gives **13**, the late stage intermediate in Yoshida's formal synthesis of (−)-cephalotaxine along with about 20% of a product (**13**, X=H) missing the iodide group. Importantly, the physical and spectroscopic properties of **13**, prepared by the current methodology, match those reported by Yoshida and his co-workers.[4l]



Scheme 7.

3. Conclusions

The results described above add further to the growing body of observations that suggest that pyridinium salt photochemistry can serve as an important methodology in synthetic organic chemistry. Despite the well recognized limitations (e.g., scale up) of photochemical reactions, the ability to construct structurally, stereochemically, and functionally complex substances by irradiation of pyridinium salts stands as a unique feature of this chemistry that is unmatched by any ground state processes. As with all interesting excited state reactions of organic compounds, this environmentally friendly process will become an important component of the synthetic arsenal, especially if/when the cost of photon production becomes low.

4. Experimental

4.1. General

All reactions were run under a nitrogen atmosphere. Unless otherwise noted, all reagents were obtained from commercial sources and used without further purification. All compounds were isolated as oils and shown to be >90% pure by ^{1}H NMR/or ^{13}C NMR, unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were recorded on $CDCl_3$ solution unless otherwise specified and chemical shifts are reported in parts per million (ppm) relative to residual $CHCl_3$ at 7.24 ppm (for ^{1}H NMR at 250 or 500 MHz) and 77.0 ppm (for ^{13}C NMR at 62.9 MHz). ^{13}C NMR resonance assignments were aided by the use of DEPT technique to determine the number of attached hydrogens.

4.1.1. *N*-Boc-1-acetoxy-6-azaspiro[4.4]non-2-en-4-ol (15). A solution of fused pyridinium salt **6** (2.14 g, 0.01 mol) in water (500 mL) containing KOH (0.60 g, 0.01 mol) was irradiated in a circular reactor with light emitted from 2537 Å lamps for 24 h (70% conversion). Concentration of the photolysate in vacuo provided a residue, which was triturated with chloroform. Concentration of the triturate in vacuo to yield the known[9] bicyclic alcohol **8**, which was used without purification in the next step.

A solution of **8** in CH_2Cl_2 (100 mL) and HOAc (5 mL) was stirred at room temperature for 12 h. Triethylamine (7 mL) and di-*tert*-butyl dicarbonate (3.4 g, 15.6 mmol) were then added and then the mixture was stirred for 12 h at room temperature, diluted sequentially with 5% aq $KHSO_4$ (10 mL) and $CHCl_3$ (200 mL), washed sequentially with 5% aq $KHSO_4$ and satd aq $NaHCO_3$, dried, and concentrated in vacuo to give a residue, which was subjected to column chromatography (silica gel, 30% acetone/hexane) to provide **15** (1.04 g, 50%, three steps). ^{1}H NMR (1:1 mixture of two rotamers) 1.45 (s, 9H), 1.46 (s, 9H), 1.60–1.65 (m, 2H), 1.75–1.83 (m, 3H), 1.95–2.05 (m, 1H), 2.17 (s, 6H), 2.21–2.36 (m, 2H), 3.30–3.54 (m, 5H), 3.71 (br s, 1H), 5.11 (s, 1H), 5.35 (s, 1H), 5.76 (s, 2H), 5.91 (d, J=5.6 Hz, 2H), 6.13 (s, 1H), 6.32 (s, 1H); ^{13}C NMR (1:1 mixture of two rotamers) 20.2 (2), 21.9, 22.3, 26.0, 27.3, 27.6 (2), 46.9, 47.0, 75.5, 76.0, 77.6, 77.7, 77.8, 77.9, 78.9, 79.0, 129.7 (2), 135.3, 135.2, 152.7, 153.0, 169.9 (2); HRMS (ES) *m/z* 320.1452 (M+Na), calcd for $C_{15}H_{23}NO_5Na$ 320.1474.

4.1.2. 1,4-Diacetoxy-6-azaspiro[4.4]non-2-ene (16). A solution of Boc-protected amidomonoalcohol **15** (3.0 g, 0.01 mol) in CH_2Cl_2 (100 mL), DMAP (300 mg), Ac_2O (5 mL), and pyridine (10 mL) was stirred at room temperature for 12 h, poured into satd $NaHCO_3$ solution, and extracted with $CHCl_3$. The chloroform extracts were dried and concentrated in vacuo to provide the crude diacetate, which was subjected to column chromatography (silica gel, 30% acetone/hexane) to give pure diacetate (3.1 g, 90%). ^{1}H NMR (1:0.8 mixture of rotamers) 1.48 (s, 16.2H), 1.65–1.74 (m, 3.6H), 2.00 (t, J=7.0 Hz, 2H), 2.10 (s, 10.8H), 2.14 (t, J=7.0 Hz, 1.6H), 3.31 (t, J=7.0 Hz, 2H), 3.40 (t, J=7.0 Hz, 1.6H), 5.89 (s, 1.6H), 5.90 (s, 2H), 6.14 (s, 1.6H), 6.34 (s, 2H); ^{13}C NMR (1:0.8 mixture of rotamers) 20.9 (4), 22.5, 23.1, 27.7, 28.4 (2), 29.1, 47.2, 47.6, 75.3, 76.2, 78.6 (2), 79.2 (2), 79.6, 80.6, 132.3 (2), 132.4 (2), 153.0, 153.6, 170.0 (4) ; HRMS (ES) *m/z* 362.1535 (M+Na), calcd for $C_{17}H_{25}NO_6Na$ 362.1580.

A solution of the diacetate (1.5 g, 4.4 mmol) in CH_2Cl_2 (10 mL) and TFA (3.5 mL) was stirred overnight at room temperature, diluted with satd aq $NaHCO_3$, and extracted with $CHCl_3$. The extracts were dried and concentrated in vacuo to provide a residue, which was subjected to column chromatography (silica gel, 10% MeOH/ethyl acetate) to yield **16** (0.9 g, 85%). ^{1}H NMR 1.71–1.80 (m, 4H), 2.10 (s, 6H), 2.98 (t, J=6.8 Hz, 2H), 5.37 (s, 2H), 6.07 (s, 2H); ^{13}C NMR 20.8 (2), 24.8, 26.8, 45.9, 74.6, 82.1 (2), 134.0 (2), 170.2 (2); HRMS (ES) *m/z* 240.1233 (M+1), calcd for $C_{12}H_{18}NO_4$ 240.1236.

4.1.3. (1*S*,4*R*,5*S*)-*N*-(3,4-Dimethoxyphenylacetyl)-1-acetoxy-6-azaspiro[4.4]non-2-en-4-ol (19). A solution of 50 mg of sodium azide, 500 units of lypholized electric eel acetyl cholinesterase (EEACE) and amine-diacetate **16** (4.0 g, 16.7 mmol) in 100 mL of 0.58 M sodium dihydrogen phosphate buffer (pH=6.9) at room temperature was gently stirred for 0.5–1 h and extracted with $CHCl_3$. The extracts were dried and concentrated in vacuo to yield the recovered **16** (3.0 g). The aqueous layer was concentrated in vacuo to provide a residue, which was dissolved in methanol and filtered. The filtrate was concentrated in vacuo to yield the monoalcohol **18**, which was used without further purification in the next step. ^{1}H NMR (D_2O) 1.96–2.06 (m, 3H), 2.26 (s, 3H), 2.18–2.21 (m, 1H), 3.32 (t, J=6.5 Hz, 2H), 4.58 (s, 1H), 5.56 (s, 1H), 6.13 (d, J=6.0 Hz, 1H), 6.28 (d, J=5.5 Hz, 1H); ^{13}C NMR (D_2O) 20.1, 21.9, 25.1, 45.0, 76.5, 77.5, 78.9, 130.9, 136.9, 173.1; HRMS (ES) *m/z* 198.1127 (M+1), calcd for $C_{10}H_{16}NO_3$ 198.1130. The % ee of the monoalcohol (80–90%, depending on reaction time) was determined by conversion to the *N*-Boc derivative, followed by reaction with (*R*)- and (*S*)-Mosher acetyl chloride.

To a solution of the crude monoalcohol in CH_3CN (30 mL) at −40 °C were added sequentially Et_3N (2 mL) and a solution of 3,4-dimethoxyphenylacetyl chloride (**17**) (0.7 g, 3.24 mmol) in CH_3CN (10 mL). The mixture was stirred at −40 °C for 70 min, diluted with water, and extracted with $CHCl_3$. The extracts were dried and concentrated in vacuo to yield a residue, which was subjected to column chromatography (silica gel, 33% acetone/hexane) to provide **19** (0.94 g, 80%); $[\alpha]_D^{23}$ +7.4 (*c* 0.41, $CHCl_3$). ^{1}H NMR 1.70–1.81 (m, 1H), 1.85–1.92 (m, 2H), 2.07 (s, 3H), 2.17–2.22

(m, 1H), 3.40–3.44 (m, 1H), 3.47–3.52 (m, 1H), 3.56 and 3.61 (abq, J=15.0 Hz, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 5.48 (d, J=1.0 Hz, 1H), 5.79 (d, J=6.0 Hz, 1H), 5.91 (dd, J=2.0, 6.3 Hz, 1H), 6.42 (d, J=1.5 Hz, 1H), 6.77 (d, J=8.0 Hz, 1H), 6.80 (d, J=8.0 Hz, 1H), 6.82 (s, 1H); ^{13}C NMR 20.9, 23.7, 26.5, 42.7, 48.5, 55.8 (2), 76.2, 78.5, 79.2, 111.1, 112.2, 121.1, 127.1, 130.6, 136.2, 147.7, 148.9, 170.3 (2); HRMS (ES) *m/z* 376.1757 (M+1), calcd for $C_{20}H_{26}NO_6$ 376.1760.

4.1.4. (1*S*,5*S*)-*N*-(3,4-Dimethoxyphenylacetyl)-1-acetoxy-6-azaspiro[4.4]non-2-ene (20) and (4*S*,5*S*)-*N*-(3,4-dimethoxyphenylacetyl)-5-acetoxy-6-azaspiro[4.4]non-1-ene (21). To a solution of monoalcohol **19** (1.1 g, 2.9 mmol) in CH_2Cl_2 (30 mL) containing DMAP (1.1 g, 9.0 mmol) at 0 °C, was added methyl chlorooxoacetate (0.7 mL). The mixture was stirred at room temperature for 4 h, diluted with $CHCl_3$ and water, and separated. The $CHCl_3$ layer was washed with aq NH_4Cl, dried, and concentrated in vacuo to afford the mixed oxalate ester, which was used without further purification in the next step. ^{1}H NMR 1.74–1.86 (m, 2H), 2.05 (t, J=6.8 Hz, 2H), 2.09 (s, 3H), 3.42 (dd, J=2.0, 6.5 Hz, 2H), 3.63 (s, 2H), 3.86 (s, 3H), 3.88 (s, 3H), 3.91 (s, 3H), 5.96 (dd, J=6.0, 12.3 Hz, 2H), 6.45 (s, 1H), 6.58 (s, 1H), 6.78 (d, J=8.0 Hz, 1H), 6.81 (s, 1H), 6.82 (d, J=8.0 Hz, 1H); ^{13}C NMR 20.8, 23.7, 27.4, 42.5, 47.8, 53.5, 55.7 (2), 77.4, 78.0, 81.5, 111.1, 112.1, 121.2, 126.9, 130.8, 133.8, 147.7, 148.9, 156.9, 157.9, 169.9, 170.5; HRMS (ES) *m/z* 462.1765 (M+1), calcd for $C_{23}H_{28}NO_9$ 462.1764.

To a solution of the crude oxalate in toluene (10 mL) were added AIBN (0.3 g, 1.8 mmol) and *n*-Bu_3SnH (3 mL, 11.3 mmol). The mixture was stirred at 100 °C for 2 h, cooled to room temperature, diluted with $CHCl_3$, washed with satd aq $NaHCO_3$, dried, and concentrated in vacuum to afford a residue, which was subjected to column chromatography (silica gel, 30% acetone/hexane) to afford **20** (0.11 g, 10%), **21** (0.68 g, 65%), and recovered starting material **19** (0.3 g).

Compound **20**: $[\alpha]_D^{23}$ +90.2 (*c* 0.1, $CHCl_3$); ^{1}H NMR 1.62 (dt, J=6.0, 13.0 Hz, 1H), 1.78 (dt, J=7.0, 13.0 Hz, 2H), 2.20 (s, 3H), 2.22 (ddd, J=7.5, 5.5, 4.5 Hz, 2H), 3.27 (dd, J=1.5, 16.3 Hz, 1H), 3.42 (abq, J=7.5 Hz, 1H), 3.51 (abq, J=6.0 Hz, 1H), 3.59 (abq, J=15.0 Hz, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 5.66 (dd, J=1.5, 6.0 Hz, 1H), 5.95 (dd, J=2.0, 6.5 Hz, 1H), 6.49 (s, 1H), 6.77 (d, J=9.5 Hz, 1H), 6.80 (s, 1H), 6.82 (d, J=9.5 Hz, 1H); ^{13}C NMR 21.1, 23.7, 35.2, 42.8, 44.0, 48.3, 55.8, 72.7, 81.9, 111.1, 111.9, 121.1, 127.3, 129.2, 133.2, 147.7, 148.9, 169.6, 170.5; HRMS (ES) *m/z* 360.1823 (M+1), calcd for $C_{20}H_{26}NO_5$ 360.1811.

Compound **21**: $[\alpha]_D^{23}$ +94.9 (*c* 0.28, $CHCl_3$); ^{1}H NMR 1.61–1.65 (m, 1H), 1.81 (abq, J=7.5 Hz, 2H), 2.06 (s, 3H), 2.21–2.27 (m, 2H), 3.10 (dd, J=8.0, 17.0 Hz, 1H), 3.44–3.47 (m, 1H), 3.50–3.57 (m, 1H), 3.57 (abq, J=15.0 Hz, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 5.52 (d, J=4.0 Hz, 1H), 5.75–5.77 (m, 1H), 5.88–5.90 (m, 1H), 6.76 (d, J=8.5 Hz, 1H), 6.80 (s, 1H), 6.81 (d, J=8.5 Hz, 1H); ^{13}C NMR 21.1, 23.7, 32.8, 38.1, 42.9, 48.1, 55.8, 76.7, 77.6, 111.1, 111.9, 121.1, 127.3, 128.1, 134.1, 147.7, 148.9, 169.6, 170.4; HRMS (ES) *m/z* (M+1), 360.1810 calcd for $C_{20}H_{26}NO_5$ 360.1811.

4.1.5. (1*S*,5*S*)-*N*-[2-(3,4-Dimethoxyphenylethyl)]-6-azaspiro[4.4]non-2-en-1-ol (12) from 20. To a solution of **20** (0.10 g, 0.3 mmol) in THF (10 mL) was added $LiAlH_4$ (1 mL, 1 M solution, 1 mmol). The mixture was stirred at reflux for 3 h, cooled, sequentially diluted by addition of a solution of 0.5 mL water in 10 mL THF followed by 0.6 mL 10% aq NaOH, and filtered. The filtrate was concentrated in vacuo to afford a residue, which was subjected to column chromatography (silica gel, 10% $CHCl_3$/MeOH) to afford **12** (0.09 g, 95%), $[\alpha]_D^{23}$ +44.4 (*c* 0.03, $CHCl_3$) [lit.[4i] 86.4 (*c* 1.02, $CHCl_3$)] whose spectroscopic properties matched with those previously reported.[4i] ^{1}H NMR 1.56–1.61 (m, 1H), 1.80–1.86 (m, 2H), 2.02 (d, J=17.0 Hz, 1H), 2.23 (ddd, J=8.0, 12.0, 12.0 Hz, 1H), 2.39 (d, J=17.0 Hz, 1H), 2.40–2.45 (m, 1H), 2.64–2.74 (m, 4H), 3.11 (dd, J=3.5, 9.0 Hz, 1H), 3.83 (s, 3H), 3.84 (s, 3H), 4.63 (s, 1H), 5.70 (dd, J=2.0, 6.0 Hz, 1H), 5.82 (d, J=5.5 Hz, 1H), 6.70 (s, 1H), 6.71 (d, J=8.0 Hz, 1H), 6.75 (d, J=8.0 Hz, 1H); ^{13}C NMR 20.7, 32.4, 35.4, 36.5, 51.4, 51.8, 55.8, 55.8, 75.3, 78.0, 111.1, 112.0, 120.4, 132.2, 132.9, 133.0, 147.3, 148.7; HRMS (ES) *m/z* 304.1906 (M+1), calcd for $C_{18}H_{26}NO_3$ 304.1913.

4.1.6. Spirocyclic cyclopentenol 12 from 21. A solution of **21** (0.36 g, 1.0 mmol) and NaOMe (0.10 g, 19 mmol) in MeOH (10 mL) was stirred at room temperature for 2 h, diluted with water and extracted with $CHCl_3$, The extracts were washed with satd aq NaCl, dried and concentrated in vacuo to yield the crude homoallylic alcohol (0.32 g). $[\alpha]_D^{23}$ +99.4 (*c* 0.264, $CHCl_3$); ^{1}H NMR 1.68–1.72 (m, 1H), 1.78–1.81 (m, 1H), 1.91–2.17 (m, 2H), 2.53 (dd, J=2.0, 19.3 Hz, 1H), 2.73 (dd, J=5.5, 20.5 Hz, 1H), 3.53 (abq, J=7.5 Hz, 1H), 3.64 (abq, J=18.0 Hz, 2H), 3.86 (s, 6H), 3.96–3.99 (m, 1H), 4.4 (d, J=11.5 Hz, 1H), 5.60 (d, J=6.0 Hz, 1H), 5.82 (d, J=5.5 Hz, 1H), 6.78 (d, J=8.5 Hz, 1H), 6.81 (s, 1H), 6.81 (d, J=8.0 Hz, 1H); ^{13}C NMR 23.0, 36.6, 41.8, 42.4, 49.0, 55.7 (2), 77.8, 79.3, 111.1, 111.8, 121.0, 126.7, 128.0, 133.2, 147.8, 148.9, 173.1; HRMS (ES) *m/z* 318.1702 (M+1), calcd for $C_{18}H_{24}NO_4$ 318.1705.

To a solution of oxalyl chloride (1 mL, 2 M solution, 2 mmol) and DMSO (0.31 g, 4 mmol) in CH_2Cl_2 (15 mL) at −78 °C was added a solution of the crude homoallylic alcohol (0.32 g, 1 mmol) in CH_2Cl_2 (20 mL) slowly. The mixture was stirred at −78 °C for 2 h, and diluted with Et_3N (5 mL). After stirring for 1 h, the mixture was diluted with satd aq NH_4Cl, and extracted with $CHCl_3$. The extracts were washed with satd aq NaCl, dried, and concentrated in vacuo to afford the crude unsaturated enone, which was used without purification in the next step. ^{1}H NMR 1.75–1.78 (m, 1H), 1.95–2.05 (m, 3H), 2.74 and 3.28 (abq, J=23.0 Hz, 2H), 3.51–3.52 (m, 2H), 3.53 (abq, J=7.0 Hz, 2H), 3.81 (s, 3H), 3.85 (s, 3H), 5.85 (dt, J=2.0, 7.0 Hz, 1H), 6.23 (dt, J=2.0, 7.0 Hz, 1H), 6.68 (d, J=7.5 Hz, 1H), 6.69 (s, 1H), 6.76 (d, J=7.5 Hz, 1H); ^{13}C NMR 24.6, 35.7, 41.0, 41.4, 48.1, 55.8 (2), 71.8, 111.2, 111.6, 120.9, 126.6, 129.8, 132.5, 147.8, 149.0, 168.9, 213.6; HRMS (ES) *m/z* 316.1553 (M+1), calcd for $C_{18}H_{22}NO_4$ 316.1549.

A solution of the crude nonconjugated enone and DBU (2.5 mL) in CH_3CN (10 mL) was stirred at room temperature for 12 h, diluted with satd aq $NaHCO_3$, and extracted with $CHCl_3$. The extracts were dried and concentrated

in vacuo to yield a residue, which was subjected to column chromatography (silica gel, 1:1 acetone/hexane) to give the crude conjugated enone as a colorless oil (0.19 g, 60%, three steps). $[\alpha]_D^{23}$ −27.4 (*c* 0.13, $CHCl_3$); ^1H NMR 1.74–1.76 (m, 1H), 1.97–2.00 (m, 2H), 2.08–2.17 (m, 1H), 2.54 and 3.13 (abq, *J*=23.0 Hz, 2H), 3.50–3.53 (m, 1H), 3.55 (abq, *J*=9.0 Hz, 2H), 3.59–3.65 (m, 1H), 3.85 (s, 3H), 3.90 (s, 3H), 6.27 (dt, *J*=2.0, 6.0 Hz, 1H), 6.75 (d, *J*=8.0 Hz, 1H), 6.80 (d, *J*=8.0 Hz, 1H), 6.82 (s, 1H), 7.72 (m, 1H); ^{13}C NMR 24.5, 38.0, 41.4, 42.1, 48.4, 55.7, 55.8, 68.4, 111.1, 111.7, 120.9, 126.7, 132.3, 147.7, 148.9, 159.2, 168.8, 206.9; HRMS (ES) *m/z* 316.1551 (M+1), calcd for $C_{18}H_{22}NO_4$ 316.1549.

To a solution of the conjugated enone (0.15 g, 0.48 mmol) in ^{i}PrOH (5 mL) was added Al(^{i}PrO)$_3$ (4.7 g, 18.3 mmol). The solvent was removed by distillation at 80 °C and the residue was stirred at 130 °C for 2 h, cooled, and poured into 100 mL of dilute HCl at 0 °C. The mixture was stirred for 30 min and extracted with $CHCl_3$. The extracts were dried and concentrated in vacuo to yield the crude allylic alcohol, which was used without purification in the next step. ^1H NMR 1.49–1.58 (m, 1H), 1.70–1.83 (m, 1H), 1.93 (dt, *J*=6.0, 12.5 Hz, 1H), 2.04 (d, *J*=6.5 Hz, 1H), 2.12 (dd, *J*=2.0, 16.0 Hz, 1H), 2.46–2.51 (m, 1H), 3.23 (dd, *J*=2.0, 16.0 Hz, 1H), 3.50 (t, *J*=6.8 Hz, 2H), 3.57 (abq, *J*=18.0 Hz, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 5.56 (br s, 1H), 5.67 (d, *J*=6.0 Hz, 1H), 5.85 (d, *J*=5.5 Hz, 1H), 6.76 (d, *J*=8.0 Hz, 1H), 6.80 (d, *J*=8.0 Hz, 1H), 6.82 (s, 1H); ^{13}C NMR 23.7, 34.1, 43.0, 43.1, 48.9, 55.8 (2), 74.4, 78.9, 111.1, 111.9, 121.0, 127.3, 131.6, 133.0, 147.7, 148.9, 169.5; HRMS (ES) *m/z* 318.1707 (M+1), calcd for $C_{18}H_{24}NO_4$ 318.1705.

To a solution of the crude allylic alcohol (0.1 g, 0.28 mmol) in anhydrous THF (5 mL) was added $LiAlH_4$ (1 mL, 1 mmol). The mixture was stirred at reflux for 1 h, cooled, diluted with ether, and then slowly diluted with a solution of 0.5 mL water in 10 mL THF followed by 0.6 mL 10% aq NaOH, and filtered. The filtrate was concentrated in vacuo to afford a residue, which was subjected to column chromatography (silica gel, 10% $CHCl_3$/MeOH) to afford **12** (0.09 g, 92%).

4.1.7. (4*S*,5*S*)-*N*-Acetyl-4-acetoxy-6-azaspiro[4.4]non-1-ene (22). To a solution of the known[9] alcohol **10** (2.4 g, 10 mmol, 80% ee) in CH_2Cl_2 (50 mL) containing DMAP (2.5 g, 20 mmol) was added methyl chlorooxoacetate (2 mL) at 0 °C. The reaction mixture was stirred at 25 °C for 2 h, diluted with chloroform and water, and separated. The combined organic layers were washed with satd aq NH_4Cl, dried, and concentrated in vacuo giving the oxalate ester, which was used without further purification.

A solution of the crude oxalate, AIBN (0.6 g, 3.6 mmol), and *n*-Bu_3SnH (12 mL, 45 mmol) in 20 mL toluene was stirred at 100 °C for 1 h, cooled, diluted with chloroform, and washed with satd aq $NaHCO_3$. The organic layer was dried and concentrated in vacuo to afford the residue, which was subjected to silica gel column chromatography (30% acetone/hexane) to afford the unsaturated amido-ester **22** [1.34 g, 85% based on recovered 0.64 g (25%) starting material]. $[\alpha]_D^{22}$ +111.6 (*c* 0.7, $CHCl_3$); ^1H NMR (4:1 mixture of two rotamers) major rotamer 1.66 (dt, *J*=8.5, 13.0 Hz, 1H), 1.88 (dd, *J*=7.5, 8.0 Hz, 1H), 2.04 (s, 3H), 2.06 (s, 3H), 2.21–2.30 (m, 3H), 3.05–3.09 (m, 1H), 3.47–3.53 (m, 2H), 5.55 (dt, *J*=6.0, 2.0 Hz, 1H), 5.75 (dt, *J*=6.0, 2.5 Hz, 1H), 5.83 (dd, *J*=5.5, 8.0 Hz, 1H); ^{13}C NMR (major rotamer) 20.9, 23.4, 23.9, 32.7, 37.7, 48.7, 76.0, 77.6, 127.5, 134.2, 168.9, 170.2; HRMS (ES) *m/z* 246.1106 (M+Na), calcd for $C_{12}H_{17}NO_3Na$ 246.1105.

4.1.8. 2-Iodo-4,5-methylenedioxyphenylacetyl chloride (26). To a solution of CrO_3 (6 g, 0.06 mol) and concd H_2SO_4 (4 mL) in 20 mL water was added 2-(3,4-methylenedioxy-6-iodophenyl)ethanol[4c] (6.0 g, 0.021 mol) in 80 mL acetone and the mixture was stirred at room temperature for 5 h. Then 10 mL ^{i}PrOH was added and the solution was stirred for an additional 1 h and filtered through Celite. The filtrate was concentrated in vacuo, diluted with 50 mL 3 N NaOH, washed with chloroform, and acidified to pH 1–2 by the addition of concd HCl. The formed solid was filtered and dried to give 3.9 g (60%) of 2-(3,4-methylenedioxy-6-iodophenyl)acetic acid. ^1H NMR (CD_3COCD_3) 3.75 (s, 2H), 6.04 (s, 2H), 6.97 (s, 1H), 7.29 (s, 1H); ^{13}C NMR 46.0, 89.4, 102.9, 111.7, 118.9, 132.8, 148.6, 149.5, 171.8.

A solution of (3,4-methylenedioxy-6-iodophenyl)acetic acid (1.37 g, 0.0045 mol) in 10 mL $SOCl_2$ containing two drops of DMF was stirred at room temperature for 4 h and concentrated in vacuo to give the crude 2-(3,4-methylenedioxy-6-iodophenyl)acetyl chloride (**26**) (1.46, 100%).

4.1.9. (1*S*,5*R*)-*N*-(2-Iodo-4,5-methylenedioxyphenylacetyl)-6-azaspiro[4.4]nonan-1-ol (27). A solution of **22** (2.23 g, 10 mmol) in EtOH (30 mL) containing 10% Pd/C (2.0 g) and H_2 (1 atm) was stirred under an atmosphere of hydrogen at 25 °C for 12 h, and filtered through a Celite pad. The filtrate was concentrated in vacuo to yield a crude amido-ester (2.24 g, ca. 100%, >95% purity), which was used without purification. $[\alpha]_D^{22}$ +48.4 (*c* 0.79, $CHCl_3$). ^1H NMR 1.45–1.55 (m, 2H), 1.62–1.66 (m, 2H), 1.76–1.94 (m, 3H), 2.03 (s, 6H), 2.22–2.31 (m, 2H), 2.59–2.64 (m, 1H), 3.43 (dd, *J*=16.0, 7.0 Hz, 2H), 5.88 (t, *J*=7.5 Hz, 1H); ^{13}C NMR 20.1, 21.1, 23.2, 24.5, 30.0, 34.4, 34.9, 49.2, 71.8, 76.5, 168.9, 170.3; HRMS (ES) *m/z* 248.1255 (M+Na), calcd for $C_{12}H_{19}NO_3Na$ 248.1263.

A solution of amido-ester (2.24 g, 10 mmol) in 6 N HCl (20 mL) was stirred at 100 °C for 3 h, cooled, and concentrated in vacuo to afford the spirocyclic aminoalcohol **23** as its HCl salt (1.69 g, 95%, >95% purity), which was used without purification. ^1H NMR (D_2O) 1.53–1.69 (m, 2H), 1.75–1.95 (m, 4H), 1.97–2.10 (m, 4H), 3.32 (m, 2H), 4.04 (2d, *J*=5.0 Hz, 1H); ^{13}C NMR (D_2O) 22.3, 26.0, 34.5, 36.0, 36.5, 48.0, 78.0, 78.3.

To a solution of aminoalcohol **23** (0.89 g, 5 mmol) and Et_3N (10 mL) in CH_3CN (30 mL) at −40 °C was slowly added a solution of 2-(3,4-methylenedioxy-6-iodophenyl)acetyl chloride (**26**) (1.46 g, 4.5 mmol) in CH_3CN (10 mL). The mixture was stirred at −40 °C for 70 min, diluted with water, and extracted with chloroform. The combined extracts were dried and concentrated in vacuo to give a residue, which was subjected to silica gel column chromatography (33%

acetone/hexane) to provide arylacetamide **27** (1.54 g, 80% based on **26**). $[\alpha]_D^{22}$ +14.2 (*c* 0.65, $CHCl_3$). ^1H NMR 1.45–1.51 (m, 2H), 1.77–1.80 (m, 1H), 1.89–1.94 (m, 6H), 2.69–2.76 (m, 1H), 3.63–3.64 (m, 1H), 3.70 (abq, *J*=16.5 Hz, 2H), 3.77 (dt, *J*=10.0, 5.0 Hz, 1H), 4.44 (dd, *J*=3.5, 10.5 Hz, 1H), 5.94 (d, *J*=1.5 Hz, 2H), 6.80 (s, 1H), 7.23 (s, 1H); ^{13}C NMR 21.2, 23.1, 34.5, 35.3, 41.2, 47.5, 49.4, 74.2, 81.7, 89.0, 101.5, 110.3, 118.3, 131.5, 147.3, 148.4, 171.4; HRMS (ES) 430.0507 (M+1), calcd for $C_{17}H_{21}NO_4I$ 430.0515.

4.1.10. (1*S*,5*R*)-*N*-(2-Iodo-4,5-methylenedioxyphenylacetyl)-6-azaspiro[4.4]nonan-1-yl-*p*-toluenesulfonate (28). To a solution of amidoalcohol **27** (0.75 g, 1.75 mmol) and pyridine (1 mL) in CH_2Cl_2 (10 mL) at 0 °C was added *p*-toluenesulfonyl chloride (0.67 g, 3.5 mmol). The mixture was stirred at 0 °C for 4 h and at 25 °C for 12 h, diluted with 15% aq NaOH, and extracted with chloroform. The extracts were washed with satd aq NaCl, dried, and concentrated in vacuo to yield a residue, which was subjected to silica gel column chromatography (30% acetone/hexane) to give the tosylate **28** (0.71 g, 85% based on recovered 0.11 g starting material). $[\alpha]_D^{22}$ +16.4 (*c* 0.37, $CHCl_3$). ^1H NMR 1.29–1.39 (m, 1H), 1.46–1.50 (m, 1H), 1.63–1.65 (m, 1H), 1.86–2.06 (m, 4H), 2.12–2.21 (m, 2H), 2.43 (s, 3H), 2.87–2.90 (m, 1H), 3.59–3.65 (m, 2H), 3.68 (abq, *J*=16.5 Hz, 2H), 4.52 (t, *J*=7.0 Hz, 1H), 5.93 (d, *J*=8.0 Hz, 2H), 6.83 (s, 1H), 7.22 (s, 1H), 7.30 (d, *J*=8.0 Hz, 2H), 7.76 (d, *J*=8.5 Hz, 2H); ^{13}C NMR 21.5, 21.7, 22.7, 32.5, 34.4, 41.6, 47.8, 49.2, 71.2, 88.0, 89.0, 101.5, 110.7, 118.1, 127.5 (2), 129.6 (2), 132.3, 134.5, 144.3, 147.2, 148.4, 168.7; HRMS (ES) 584.0592 (M+1), calcd for $C_{24}H_{27}NO_6I$ 584.0604.

4.1.11. (5*S*)-*N*-(2-Iodo-4,5-methylenedioxyphenylacetyl)-6-azaspiro[4.4]non-1-ene (29). A solution of tosylate **28** (0.59 g, 1 mmol) and DBU (5 mL) in DMF (10 mL) was stirred at 120 °C for 12 h, cooled, diluted with EtOAc, washed with satd aq NaCl, and concentrated in vacuo, giving a residue, which was subjected to silica gel column chromatography (20% acetone/hexane) to give amidoalkene **29** (0.25 g, 80% based on recovered 0.15 g starting material). $[\alpha]_D^{22}$ −57.6 (*c* 0.18, $CHCl_3$). ^1H NMR (2.5:1 mixture of two rotamers) major rotamer 1.75–1.79 (m, 1H), 1.83–1.95 (m, 4H), 2.25–2.32 (m, 1H), 2.39–2.45 (m, 1H), 2.60–2.69 (m, 1H), 3.51–3.65 (m, 4H), 5.54 (t, *J*=3.0 Hz, 1H), 5.81 (t, *J*=3.0 Hz, 1H), 5.92 (d, *J*=5.5 Hz, 2H), 6.77 (s, 1H), 7.21 (s, 1H); ^{13}C NMR (major rotamer) 23.6, 31.7, 34.5, 39.7, 47.6, 48.4, 76.0, 88.9, 101.5, 110.6, 118.2, 131.5, 132.3, 133.7, 147.2, 148.4, 167.8; HRMS (ES) 412.0404 (M+1), calcd for $C_{17}H_{19}NO_3I$ 412.0410.

4.1.12. (5*S*)-*N*-[2-(2-Iodo-4,5-methylenedioxyphenylethyl)]-6-azaspiro[4.4]non-1-ene (13). To a solution of amidoalkene **29** (0.041 g, 0.1 mmol) in THF (3 mL) at −40 °C was added a solution of AlH_3 in THF (0.2 mL, 0.67 M, 0.134 mmol). The solution was stirred at −40 °C for 15 min, diluted with satd aq Na_2SO_4, and filtered. The filtrate was dried and concentrated in vacuo, giving a residue, which was subjected to silica gel column chromatography (EtOAc then 10:1 EtOAc/MeOH containing 1% Et_3N) to yield aminoalkene **13** (24 mg, 60%) whose spectroscopic properties (except for its optical rotation, which was not reported in Ref. 41) matched with those reported earlier.[41] $[\alpha]_D^{22}$ −32.0 (*c* 0.08, $CHCl_3$). ^1H NMR 1.49–1.66 (m, 1H), 1.75–1.96 (m, 5H), 2.30 (m, 2H), 2.38–2.49 (m, 2H), 2.78–2.84 (m, 3H), 2.97–3.01 (m, 1H), 5.56–5.57 (m, *J*=1 Hz, 1H), 5.80–5.81 (m, 1H), 5.93 (s, 2H), 6.74 (s, 1H), 7.20 (s, 1H); ^{13}C NMR 21.4, 29.7, 31.5, 38.2, 40.9, 50.0, 51.3, 77.7, 87.8, 101.4, 109.6, 118.5, 132.1, 134.7, 136.8, 146.7, 148.4; HRMS (ES) 398.0610 (M+1), calcd for $C_{17}H_{21}NO_2I$ 398.0617.

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Supplementary data

Within this section are (1) the general experimental section, and (2) ^1H and ^{13}C NMR spectra of **12**, **13**, **15**, **16**, **18**–**23**, **27**–**29**, (*S*)-Mosher ester derivative of *N*-Boc-**13**, and (*R*)-Mosher ester derivative of *N*-Boc-**13**. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tet.2006.05.045.

References and notes

1. For a review see: Jalil Miah, M. A.; Hudlicky, T.; Reed, J. W. *The Alkaloids*; Brossi, A., Ed.; Academic: New York, NY, 1998; Vol. 51, p 199.
2. Paudler, W. m.; Kerley, G. I.; Mckay, J. *J. Org. Chem.* **1963**, *28*, 2194.
3. Powell, R. G.; Weisleder, D.; Smith, C. R., Jr.; Wolff, I. A. *Tetrahedron Lett.* **1969**, 4081.
4. (a) Auerbach, J.; Weinreb, S. M. *J. Am. Chem. Soc.* **1972**, *94*, 7172; (b) Weinreb, S. M.; Auerbach, J. *J. Am. Chem. Soc.* **1975**, *97*, 2503; (c) Semmelhack, M. F.; Chong, B. P.; Stauffer, R. D.; Rogerson, T. D.; Chong, A.; Jones, L. D. *J. Am. Chem. Soc.* **1975**, *97*, 2507; (d) Yasuda, S.; Yamada, T.; Hanaoka, M. *Tetrahedron Lett.* **1986**, *27*, 2023; (e) Burkholder, T. P.; Fuchs, P. L. *J. Am. Chem. Soc.* **1990**, *112*, 9601; (f) Ishibashi, H.; Okano, M.; Tamaki, H.; Maruyama, K.; Yakura, T.; Ikeda, M. *J. Chem. Soc., Chem. Commun.* **1990**, 1436; (g) Kuehne, M. E.; Bornmann, W. E.; Parsons, W. H.; Spitzer, T. D.; Blount, J. F.; Zubieta, J. *J. Org. Chem.* **1988**, *53*, 3439; (h) Lin, X. D.; Kavash, R. W.; Mariano, P. S. *J. Org. Chem.* **1996**, *61*, 7335; (i) Isono, N.; Mori, M. *J. Org. Chem.* **1995**, *60*, 115; (j) Tietze, L. F.; Schirok, H. *J. Am. Chem. Soc.* **1999**, *121*, 10264; (k) Nagasaka, T.; Sato, H.; Saeki, S. *Tetrahedron: Asymmetry* **1997**, *8*, 191; (l) Suga, S.; Watanabe, M.; Yoshida, J. *J. Am. Chem. Soc.* **2002**, *124*, 14824; (m) Koseki, Y.; Sato, H.; Watanabe, Y.; Nagasaka, T. *Org. Lett.* **2002**, *4*, 885; (n) Li, W. D.; Wang, Y. Q. *Org. Lett.* **2003**, *5*, 2931; (o) Li, W. D.; Ma, B. C. *J. Org. Chem.* **2005**, *70*, 3277; (p) Ma, B. C.; Wang, Y. Q.; Li, W. D. *J. Org. Chem.* **2005**, *70*, 4528; (q) Planas, L.; Perard-Viret, J.; Royer, J. *J. Org. Chem.* **2004**, *69*, 3087.
5. Milkolajczak, K. L.; Smith, C. R., Jr. *J. Med. Chem.* **1977**, *20*, 328.
6. Sinha, S.; Jain, S. *Prog. Drug Res.* **1994**, *42*, 53.

7. Whaun, J. M.; Brown, N. D. *Ann. Trop. Med. Parasitol.* **1990**, *84*, 229.
8. For recent examples, see: Song, L.; Duesler, E. N.; Mariano, P. S. *J. Org. Chem.* **2004**, *69*, 7284; Zhao, Z.; Song, L.; Duesler, E. N.; Mariano, P. S. *Tetrahedron* **2005**, *61*, 8888; Feng, X.; Duesler, E. N.; Mariano, P. S. *J. Org. Chem.* **2005**, *70*, 5618.
9. Zhao, Z. M.; Duesler, E.; Wang, C. H.; Guo, H.; Mariano, P. S. *J. Org. Chem.* **2005**, *70*, 8508.
10. Deardorff, D. R.; Windham, C. Q.; Craney, C. L. *Org. Synth.* **1995**, *73*, 25.
11. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.
12. Barton, D. H. R.; Motherwell, W. B.; Stange, A. *Synthesis* **1981**, 743.
13. Mihelcic, H.; Moeller, K. *J. Am. Chem. Soc.* **2004**, *126*, 9106.
14. Brown, H. C.; Yoon, N. Y. *J. Am. Chem. Soc.* **1966**, *88*, 1464.

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The role of terminal tyrosine residues in the formation of tripeptide nanotubes: a crystallographic insight

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Abstract—Terminally protected acyclic tripeptides containing tyrosine residues at both termini self-assemble into nanotubes in crystals through various non-covalent interactions including intermolecular hydrogen bonds. The nanotube has an average internal diameter of 5 Å (0.5 nm) and the tubular ensemble is developed through the hydrogen-bonded phenolic-OH side chains of tyrosine (Tyr) residues [*Org. Lett.* **2004**, *6*, 4463]. We have synthesized and studied several tripeptides **3**–**6** to probe the role of tyrosine residues in nanotube structure formation. These peptides either have only one Tyr residue at N- or C-termini or they have one or two terminally located phenylalanine (Phe) residues. These tripeptides failed to form any kind of nanotubular structure in the solid state. Single crystal X-ray diffraction studies of these peptides **3**–**6** clearly demonstrate that substitution of any one of the terminal Tyr residues in the Boc-Tyr-X-Tyr-OMe (X=Val or Ile) sequence disrupts the formation of the nanotubular structure indicating that the presence of two terminally located Tyr residues is vital for nanotube formation.

1. Introduction

Organic nanotubular architectures[1] have applications in the field of materials science, nanotechnology, and artificial ion channel[2] systems. Peptide nanotubular systems find useful applications in biology and medical sciences. They can be used as glucose transporter[3] or as transmembrane ion channels[2] or even as potential antibiotics against drug resistant bacteria.[4] Ghadiri and co-workers have compellingly demonstrated that 24- and 30-membered ring forming *cyclo* α-peptides with an even number of alternating D and L amino acid residues stack in an antiparallel β-sheet-like arrangement to form a hydrogen-bonded nanotubular structure.[5] Interestingly, related cyclic peptides consisting exclusively of β-amino acid residues,[6] or with an alternating arrangement of α- and β-amino acids,[7] or of vinylogous δ-amino acids[8] can also form nanotubular structures. Many successful attempts have previously been made to create nanotubular structures using various self-assembling organic compounds including cyclic oligoureas,[9] cyclodextrin based polyionic amino acids,[10] 7-deaza-2-deoxy xanthosine dihydrate,[11] and others.[12] While the self-association of cyclic peptides or peptide derivatives into hollow nanotubes has been studied in detail, the formation of acyclic peptide based nanotubes has been paid relatively less attention, there being only a few examples.[13] A recent study demonstrates the formation of nanotubes using self-assembly of a dipeptide D-Phe–D-Phe and insertion of platinum nanoparticles inside the tubes.[13c] Other studies of acyclic peptide based nanotube formation include the self-assembly of a truncated variants of Alzheimer's Aβ-peptide residue 16–22 (CH_3CO-KLVFFAE-NH_2) into a nanotubular structure in aqueous

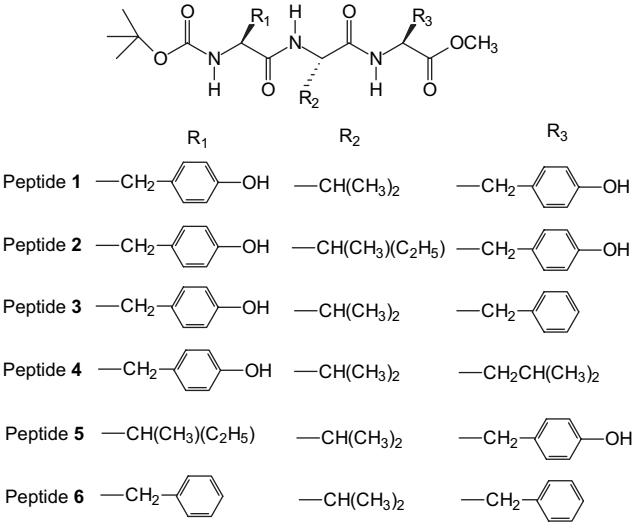

Figure 1. Schematic presentation of tripeptides **1**, **2**, **3**, **4**, **5**, and **6**.

Keywords: Acyclic peptides; Tyrosine; Nanotube; Self-assembly.
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solution[13d] and the self-association of surfactant-like peptides with variable glycine tails into nanotubes of diameter 30–50 nm at neutral pH.[13e]

In our previous communication, we demonstrated that peptides **1** and **2**, each containing two terminal Tyr residues are able to form nanotubes in crystals. In this paper, we are addressing the question whether any change of the terminal Tyr residue can retain (or not) the nanotubular structure in crystals. Keeping this in mind, several tripeptides **3**–**6** (Boc-Tyr(1)-Val(2)-Phe(3)-OMe **3**, Boc-Tyr(1)-Val(2)-Leu(3)-OMe **4**, Boc-Ile(1)-Val(2)-Tyr(3)-OMe **5**, and Boc-Phe(1)-Val(2)-Phe(3)-OMe **6**) (Fig. 1) with only one terminally positioned tyrosine or no Tyr residue, have been synthesized, purified, characterized, and their self-assembling behavior in crystals have been studied in detail to probe whether any of these peptides are able to form nanotubular structures in the solid state.

2. Results and discussion

Single crystals suitable for X-ray diffraction of tripeptides **1**–**6** were obtained from methanol–water solution by slow evaporation. Tripeptides **1** and **2** contain tyrosine residues at both termini and adopt β-sheet-like conformation in the crystal state. The individual peptide subunits are stacked one over another, maintaining proper registry, and form an open ended tube having an average internal diameter of 5.0 Å (0.5 nm) including van der Waals' contacts along the crystallographic *b* axis. The top view of the supramolecular cylindrical ensembles of peptide **2** in ball and stick models (Fig. 2a) and space-filling models (Fig. 2b) show that the interior of the peptide supramolecular channel is hydrophilic (due to the presence of hydrogen-bonded CONH moieties and phenolic-OH groups of Tyr residues), while the exterior is hydrophobic as it is occupied by the valine and isoleucine side chains and the N-terminally protecting Boc groups.[13f]

Tripeptide **3** contains a tyrosine residue at the N-terminus whereas there is a phenylalanine residue at the C-terminus. For peptide **3** there are two molecules in the asymmetric unit and they are held together by van der Waals' forces. The ORTEP diagram of this peptide is given in Figure 3a. Backbone torsion angles of each conformer (A and B) of peptide **3** are mostly in the extended region of the Ramachandran diagram[14] (Table 1). Each conformer then self-assembles by three intermolecular hydrogen bonds to form a columnar structure (for conformer A, N3A–H3A···O2A 2.13 Å, 2.92 Å, 149°, N9A–H9A···O8A 1.99 Å, 2.79 Å, 150°, and N6A–H6A···O5A 2.01 Å, 2.87 Å, 165° and for conformer B, N3B–H3B···O2B 2.17 Å, 2.93 Å, 146°, N9B–H9B···O8B 2.05 Å, 2.92 Å, 175°, and N6B–H6B···O5B 2.06 Å, 2.93 Å, 170°) (Table 2) along the crystallographic *b* direction (Fig. 4a). This columnar structure on further aggregation using van der Waals' interactions along the crystallographic *a* axis formed a complex quaternary β-sheet structure (Fig. 4b).

Tripeptide **4** contains a tyrosine residue at the N-terminus whereas tripeptide **5** possesses a tyrosine residue at the C-terminus with a centrally located Val residue in each case. The molecular conformation of tripeptide **4** in the crystal state is illustrated in Figure 3b. Most of the torsion angles (ϕ_1 −137.0(3), ψ_1 118.4(3), ϕ_2 −120.0(3), ψ_2 111.4(3), and ϕ_3 −122.8(3)) of the constituent amino acids residues of the tripeptide **4** fall within the parallel β-sheet region of the Ramachandran plot[14] (Table 1). Hence, the tripeptide **4** adopts an extended backbone conformation, which self-assembles through three intermolecular hydrogen bonds (N3–H3···O2 2.27, 3.01 Å, 144°, N9–H9···O8 2.13 Å, 2.99 Å, 173°, and N6–H6···O5 2.13 Å, 2.99 Å, 172°) (Table 2) along the crystallographic *c* axis to form a columnar structure (Fig. 5a). These columnar structures of tripeptide **4** are further self-assembled into higher order supramolecular β-sheet structures through intermolecular hydrogen bonds (O48–H48···O16 1.97 Å, 2.78 Å, 168°) along the

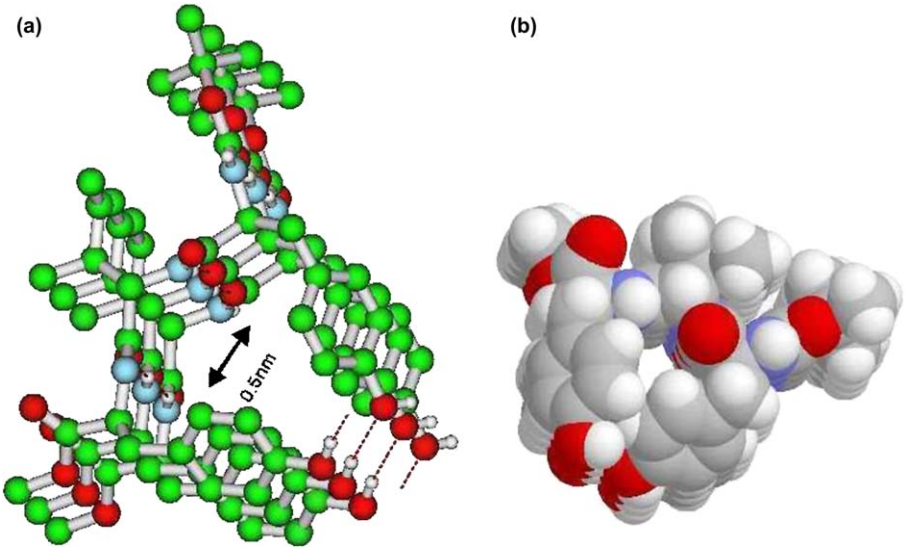

Figure 2. (a) Top view of the development of intermolecularly hydrogen-bonded nanotubular structure along the crystallographic *b* axis exhibiting internal tubular diameter of about 5.0 Å in ball and stick model. The tubular structure is composed of the acyclic peptide **2** subunit with extended backbone conformation (which adopts a β-strand like structure). (b) Space-filling model of the nanotubular ensemble obtained from a higher order self-assembly of the peptide **2**.

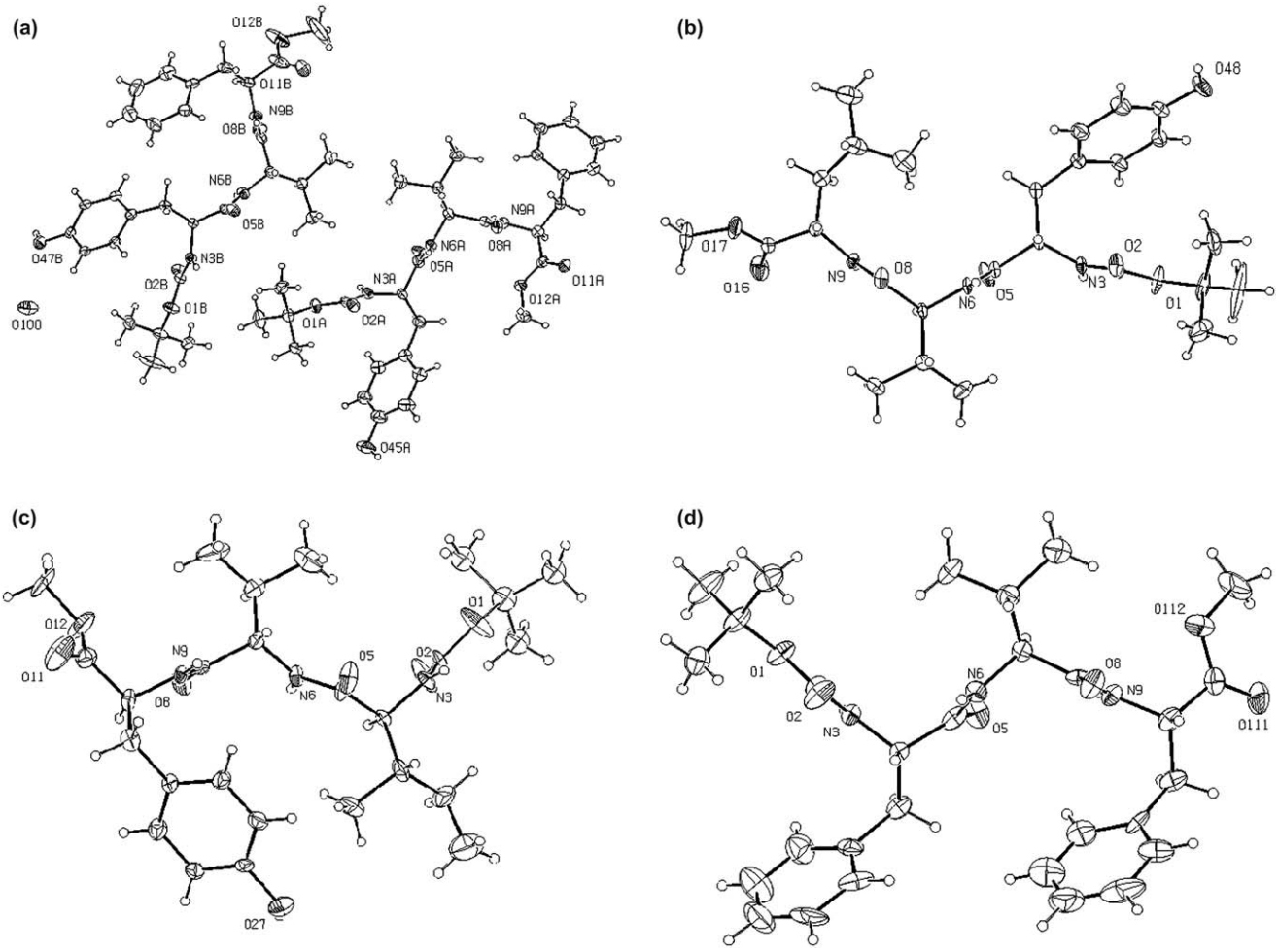

Figure 3. ORTEP diagrams with atomic numbering scheme for the (a) peptide **3**, (b) peptide **4**, (c) peptide **5**, and (d) peptide **6**. Thermal ellipsoids are shown at 30% probability level. Only nitrogen and oxygen atoms are labeled due to clarity.

crystallographic *a* direction and via van der Waals' interactions along the crystallographic *b* axis (Fig. 5b).

From the backbone torsion angles (ϕ_1 −116.6(8), ψ_1 110.6(7), ϕ_2 −124.5(7), ψ_2 119.8(7), and ϕ_3 −102.9(8)), it is clear that tripeptide **5** also adopts an extended backbone in its molecular structure. The molecular conformation of tripeptide **5** in the crystal state is illustrated in Figure 3c. Each subunit of tripeptide **5** self-assembles to form a columnar structure using three intermolecular hydrogen bonds (N3–H3···O2 2.08 Å, 2.94 Å, 173°, N9–H9···O8 2.05 Å, 2.91 Å, 173°, and N6–H6···O5 2.17 Å, 3.02 Å, 168°) (Table 2) along the crystallographic *b* axis (Fig. 6a). These columnar structures of tripeptide **5** are further self-assembled into higher order supramolecular structures using intermolecular hydrogen bonds (O27–H27···O27 2.08 Å, 2.85 Å, 157° with symmetry element $1-x$, $-1/2+y$, $-z$) involving the phenolic-OH groups of the Tyr residue, along the crystallographic *a* direction (Fig. 6b). Although tripeptides **4** and **5** contain one tyrosine residue at the C and N terminus, respectively, they are unable to form any nanotubular structure like peptides **1** and **2**.

The molecular conformation of the tripeptide **6** (Fig. 3d) was also established by a single crystal X-ray diffraction studies. Most of the ϕ and ψ values (ϕ_1 −103.5(6), ψ_1 97.8(6), ϕ_2 −102.1(7), ψ_2 97.8(7), and ϕ_3 −117.4(7)) with the exception of ψ_3 at 33.4(8) of the constituent amino acid residues of tripeptide **6** fall within the parallel β-sheet region of the Ramachandran plot and the peptide adopts an extended backbone structure. The individual peptide subunits self-assembles through intermolecular hydrogen bonds (N3–H3···O2 2.12 Å, 2.95 Å, 162°, N9–H9···O8 2.16 Å, 2.87 Å, 140°, and N6–H6···O5 2.12 Å, 2.97 Å, 167°) (Table 2) maintaining the proper registry to form a supramolecular columnar structure along the crystallographic *b* axis (Fig. 7a). Figure 7b clearly shows that peptide **6** also fails to form a nanotubular structure, instead it forms a complex sheet-like structure using van der Waals' interaction along the crystallographic *a* axis. Crystal data for peptides **3**, **4**, **5**, and **6** are listed in Table 3.

3. Conclusion

This study clearly demonstrates that there is a definite role of both terminally located Tyr residues for the formation of nanotubular structures. Any change in the terminally located Tyr residue by Phe or any other residues completely disrupts the crystal packing arrangement, which is

Table 1. Selected torsion angles (°) of peptides **3**, **4**, **5**, and **6**

Peptide **3**			
Molecule A			
O1A–C2A–N3A–C4A	−167.4(3) (ω_0)	N6A–C7A–C8A–N9A	104.4(4) (ψ_2)
C2A–N3A–C4A–C5A	−128.6(4) (ϕ_1)	C7A–C8A–N9A–C10A	−177.8(3) (ω_2)
N3A–C4A–C5A–N6A	115.0(4) (ψ_1)	C8A–N9A–C10A–C11A	48.6(4) (ϕ_3)
C4A–C5A–N6A–C7A	177.0(3) (ω_1)	N9A–C10A–C11A–O12A	45.2(4) (ψ_3)
C5A–N6A–C7A–C8A	−104.0(4) (ϕ_2)		
Molecule B			
O1B–C2B–N3B–C4B	−164.3(3) (ω_0)	N6B–C7B–C8B–N9B	108.0(4) (ψ_2)
C2B–N3B–C4B–C5B	−133.2(4) (ϕ_1)	C7B–C8B–N9B–C10B	178.3(4) (ω_2)
N3B–C4B–C5B–N6B	122.8(4) (ψ_1)	C8B–N9B–C10B–C11B	−87.8(5) (ϕ_3)
C4B–C5B–N6B–C7B	167.6(3) (ω_1)	N9B–C10B–C11B–O12B	146.1(5) (ψ_3)
C5B–N6B–C7B–C8B	−115.7(4) (ϕ_2)		
Peptide **4**			
O1–C2–N3–C4	17.5(5) (ω_0)	N6–C7–C8–N9	111.4(3) (ψ_2)
C2–N3–C4–C5	−137.0(3) (ϕ_1)	C7–C8–N9–C10	−174.4(3) (ω_2)
N3–C4–C5–N6	118.4(3) (ψ_1)	C8–N9–C10–C15	−122.8(3) (ϕ_3)
C4–C5–N6–C7	175.7(3) (ω_1)	N9–C10–C15–O16	−26.9(5) (ψ_3)
C5–N6–C7–C8	−120.0(3) (ϕ_2)		
Peptide **5**			
O1–C2–N3–C4	174.1(7) (ω_0)	N6–C7–C8–N9	119.8(7) (ψ_2)
C2–N3–C4–C5	−116.6(8) (ϕ_1)	C7–C8–N9–C10	179.7(7) (ω_2)
N3–C4–C5–N6	110.6(7) (ψ_1)	C8–N9–C10–C11	−102.9(8) (ϕ_3)
C4–C5–N6–C7	−168.1(7) (ω_1)	N9–C10–C11–O12	61.8(9) (ψ_3)
C5–N6–C7–C8	−124.5(7) (ϕ_2)		
Peptide **6**			
O1–C2–N3–C4	173.6(5) (ω_0)	N6–C7–C8–N9	97.8(7) (ψ_2)
C2–N3–C4–C5	−103.5(6) (ϕ_1)	C7–C8–N9–C10	175.8(6) (ω_2)
N3–C4–C5–N6	97.8(6) (ψ_1)	C8–N9–C10–C11	−117.4(7) (ϕ_3)
C4–C5–N6–C7	−169.0(5) (ω_1)	N9–C10–C11–O12	33.4(8) (ψ_3)
C5–N6–C7–C8	−102.1(7) (ϕ_2)		

necessary for nanotubular architecture formation. So, the presence of both the Tyr residues is essential for nanotubular structure formation as the phenolic-OH groups from these two terminally located Tyr residues are responsible for the construction of polar nanochannel-like structures. This study not only sheds some light on the future design and construction of acyclic peptide based nanotubular structure but also implies the active involvement of important functional residues in the formation and stability of the nanotubular structure.

Table 2. Intermolecular hydrogen bonding parameters of peptides **3**, **4**, **5**, and **6**

Peptides	D–H···A	H···A/Å	D···A/Å	D–H···A/°	Symmetry
Peptide **3**					
Molecule A	N3A–H3A···O2A	2.13	2.92	149	$x, -1+y, z$
	N6A–H6A···O5A	2.01	2.87	165	$x, 1+y, z$
	N9A–H9A···O8A	1.99	2.79	150	$x, -1+y, z$
Molecule B	N3B–H3B···O2B	2.17	2.93	146	$x, 1+y, z$
	N6B–H6B···O5B	2.06	2.93	170	$x, -1+y, z$
	N9B–H9B···O8B	2.05	2.92	175	$x, 1+y, z$
	O47B–H47C···O11A	1.91	2.71	160	$2-x, y, 2-z$
Peptide **4**					
	N3–H3···O2	2.27	3.01	144	$-1+x, y, z$
	N6–H6···O5	2.13	2.99	172	$1+x, y, z$
	N9–H9···O8	2.13	2.99	173	$-1+x, y, z$
	O48–H48···O16	1.97	2.78	168	$1+x, y, -1+z$
Peptide **5**					
	N3–H3···O2	2.08	2.94	173	$x, 1+y, z$
	N6–H6···O5	2.17	3.02	168	$x, -1+y, z$
	N9–H9···O8	2.05	2.91	173	$x, 1+y, z$
	O27–H27···O27	2.08	2.85	157	$1-x, -1/2+y, -z$
Peptide **6**					
	N3–H3···O2	2.12	2.95	162	$x, -1+y, z$
	N6–H6···O5	2.12	2.97	167	$x, 1+y, z$
	N9–H9···O8	2.16	2.87	140	$x, -1+y, z$

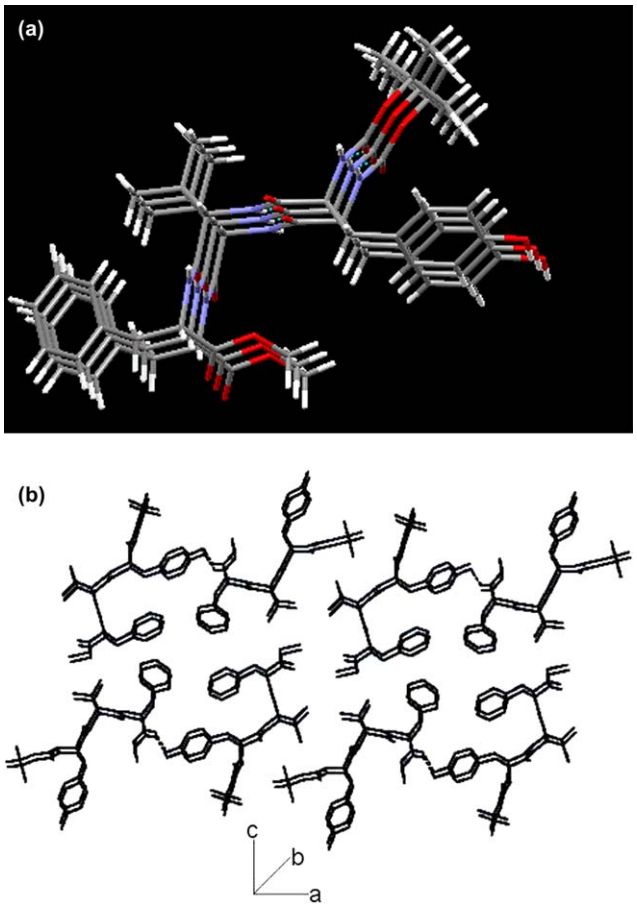

Figure 4. (a) Columnar packing of peptide **3** along crystallographic *b* direction and (b) packing diagram of the peptide **3** showing the formation of intermolecular hydrogen-bonded sheet-like structure along crystallographic *a* axis.

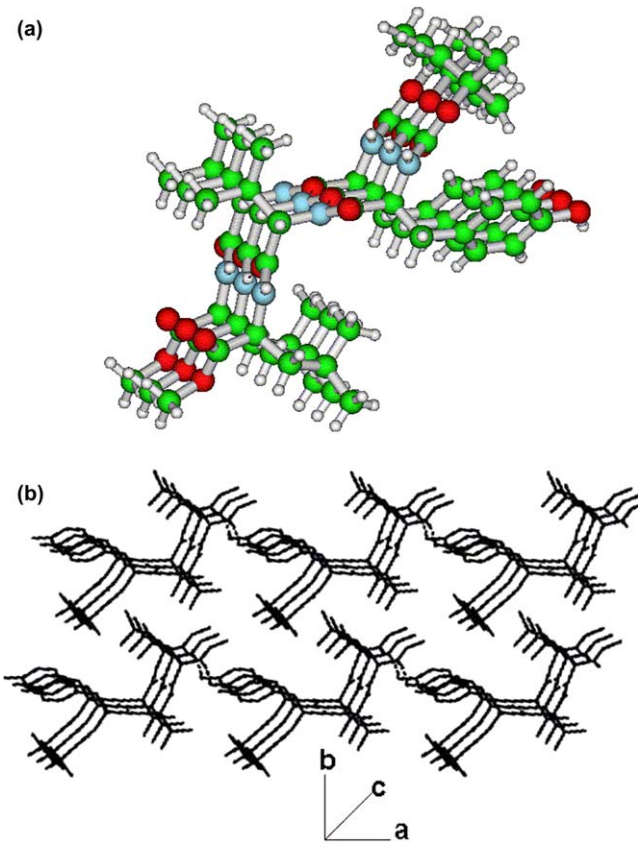

Figure 5. (a) Crystallographic view of a single molecule of peptide **4** along *c* axis. Nitrogen atoms are blue, oxygen atoms are red, carbon atoms are green, and hydrogen atoms are gray. Hydrogen bonds are shown as dotted lines. (b) Packing diagram of the peptide **4** showing the formation of intermolecular hydrogen-bonded complex sheet-like structure along crystallographic *a* axis.

4. Experimental

4.1. General

The tripeptides **1**, **2**, **3**, **4**, **5**, and **6** employed in this report have been synthesized by the conventional solution phase methodology.[15] The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by di-cyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The final compounds were fully characterized by IR spectroscopy, ^{1}H NMR spectroscopy, and mass spectrometry.

4.2. Synthesis of peptide

4.2.1. Boc-Tyr(1)-OH 7. See Ref. 13f.

4.2.2. Boc-Tyr(1)-Val(2)-OMe 8. See Ref. 13f.

4.2.3. Boc-Tyr(1)-Val(2)-OH 9. See Ref. 13f.

4.2.4. Boc-Tyr(1)-Val(2)-Tyr(3)-OMe 1. See Ref. 13f.

4.2.5. Boc-Tyr(1)-Ile(2)-OMe 10. See Ref. 13f.

4.2.6. Boc-Tyr(1)-Ile(2)-OH 11. See Ref. 13f.

4.2.7. Boc-Tyr(1)-Ile(2)-Tyr(3)-OMe 2. See Ref. 13f.

4.2.8. Boc-Tyr(1)-Val(2)-Phe(3)-OMe 3. Boc-Tyr(1)-Val(2)-OH **9** (1.9 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (2.15 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL, and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.675 g, 5 mmol). The reaction mixture was stirred for three days. The reaction mixture was then taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuo to yield peptide **3** as a white solid. Purification was done by silica gel column (100–200 mesh) using 3:1 ethyl acetate/toluene as eluent.

Yield=2.16 g (4 mmol, 80%); R_f=0.66 (25% toluene/ethyl acetate); mp 70–72 °C; IR (KBr): 3324, 3293, 1714, 1691, 1647, 1521 cm^{-1}; $[\alpha]_D^{20}$ −20.3 (*c* 0.5, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (ring Hs of Tyr(1), 2H, d, *J*=6.6 Hz); 7.02 (ring Hs of Tyr(1), 2H, d, *J*=8.3 Hz); 6.74–6.71 (ring Hs of Phe(3), 5H, m); 6.45 (Phe(3) NH, 1H, d, *J*=9 Hz); 6.29 (Val(2) NH, 1H, d, *J*=6 Hz); 4.98 (Tyr(1) NH, 1H, d, *J*=6 Hz); 4.82 (C$^{\alpha}$H of Phe(3), 1H, m);

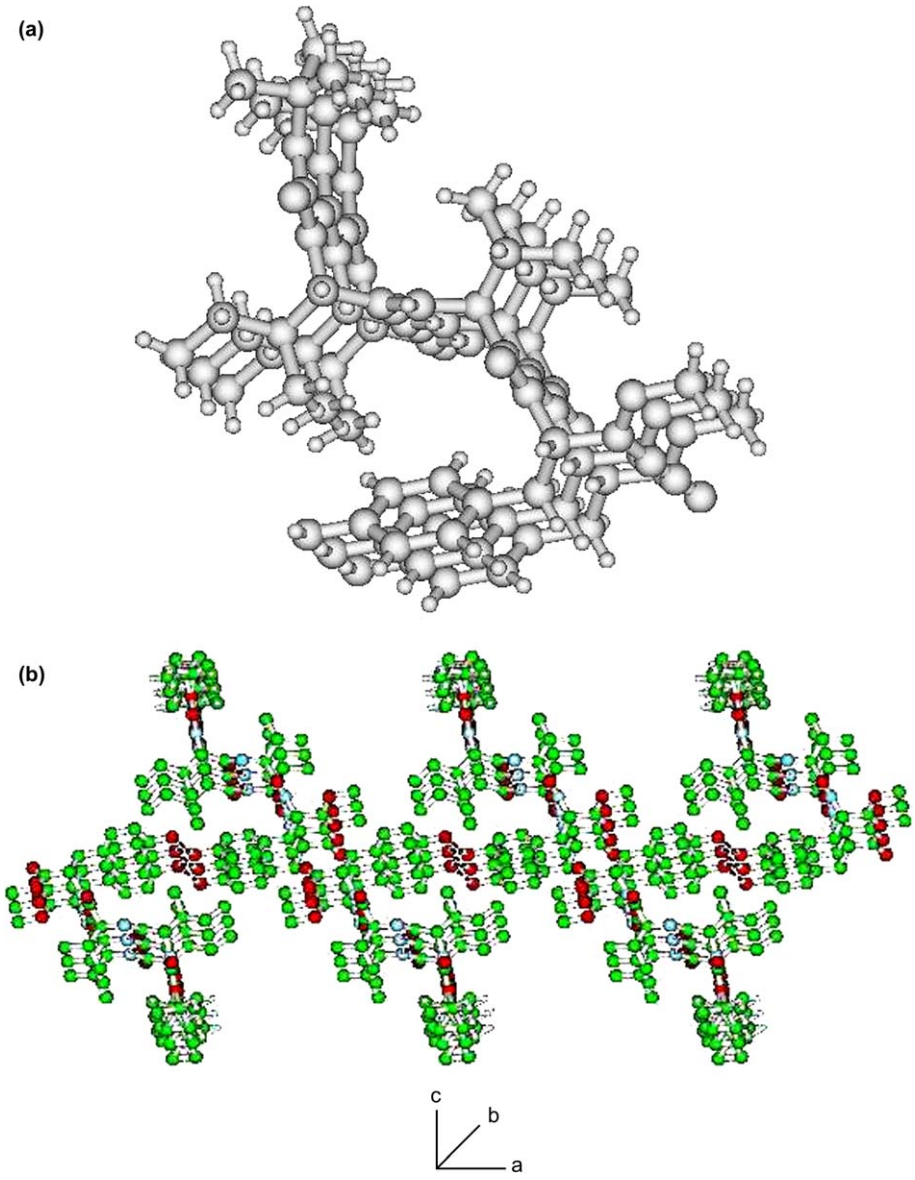

Figure 6. (a) Packing diagram of peptide **5** along crystallographic *b* direction and (b) the crystallographic view of peptide **5** along the axis parallel to the crystallographic *a* axis exhibits that the peptide has failed to form any nanotubular structure, instead it forms quaternary sheet-like structure. Nitrogen atoms are blue, oxygen atoms are red, carbon atoms are green, and hydrogen atoms are gray. Hydrogen bonds are shown as dotted lines.

4.27 ($C^{\alpha}H$ of Val(2), 1H, m); 4.16 ($C^{\alpha}H$ of Tyr(1), 1H, m); 3.70 (–OCH_3, 3H, s); 3.11 ($C^{\beta}Hs$ of Tyr(1), 2H, m); 2.97 ($C^{\beta}Hs$ of Phe(3), 2H, m); 2.05 ($C^{\beta}H$ of Val(2), 1H, m); 1.41 (Boc–CH_3s, 9H, s); 0.88–0.77 ($C^{\gamma}Hs$ of Val(2), 6H, m); (found: C, 64.2; H, 7.1; N, 7.65. $C_{29}H_{39}N_3O_7$ (541) requires C, 64.32; H, 7.21; N, 7.76%); ESI-MS *m*/*z* (%): 542.3 (100) $(M+H)^+$, 543.3 (35) $(M+2H)^+$, M_{calcd}=541.

4.2.9. Boc-Tyr(1)-Val(2)-Leu(3)-OMe 4. Boc-Tyr(1)-Val(2)-OH **9** (1.9 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Leu-OMe was isolated from the corresponding methyl ester hydrochloride (1.81 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL, and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.675 g, 5 mmol). The reaction mixture was stirred for three days. The reaction mixture was then taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuo to yield peptide **4** as a white solid. Purification was done by silica gel column (100–200 mesh) using 3:1 ethyl acetate/toluene as eluent.

Yield=2.03 g (4 mmol, 80%); R_f=0.58 (25% toluene/ethyl acetate); mp 76–78 °C; IR (KBr): 3322, 1691, 1648, 1517 cm^{-1}; $[\alpha]_D^{20}$ −24.3 (*c* 0.5, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 7.02 (ring Hs of Tyr(1), 2H, d, *J*=8.4 Hz), 6.75 (ring Hs of Tyr(1), 2H, d, *J*=8.1 Hz); 6.6 (Leu(3) NH, 1H, d, *J*=8.7 Hz); 6.45 (Val(2) NH, 1H, d, *J*=7.5 Hz); 5.0 (Tyr(1) NH, 1H, d, *J*=6 Hz); 4.56 ($C^{\alpha}H$ of Leu(3), 1H, m); 4.29 ($C^{\alpha}H$ of Val(2), 1H, m); 4.22 ($C^{\alpha}H$ of Tyr(1), 1H, m); 3.72 (–OCH_3, 3H, s); 3.00 ($C^{\beta}Hs$ of

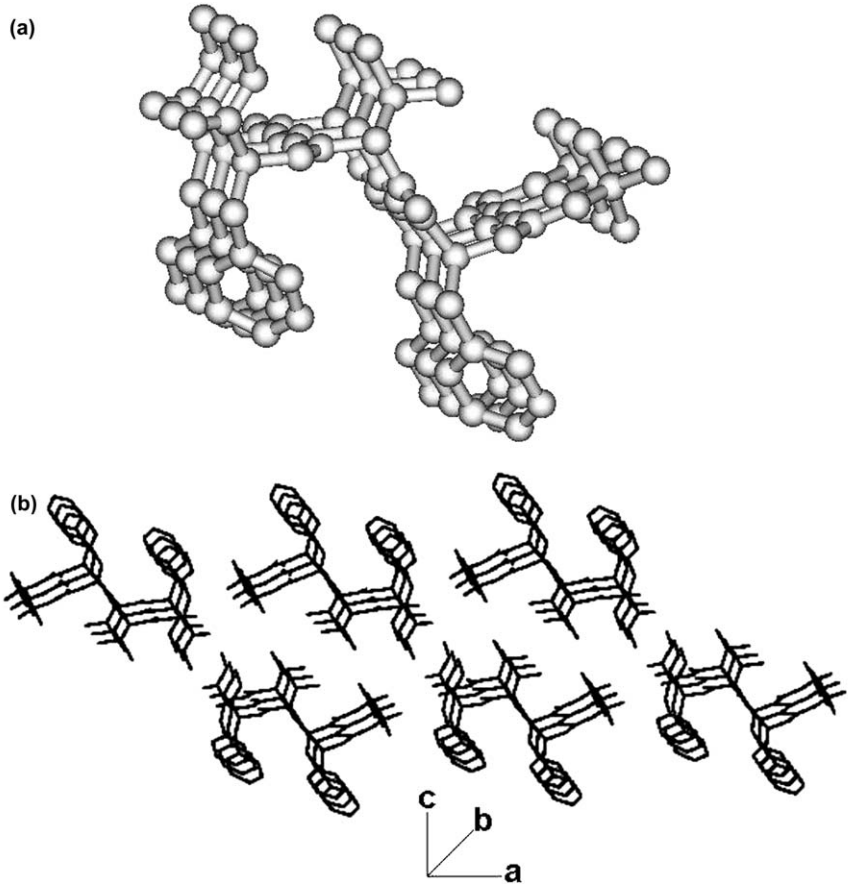

Figure 7. (a) Packing diagram of the peptide **6** showing the formation of intermolecular hydrogen-bonded structure along crystallographic *b* axis and (b) higher order packing of peptide **6**, showing the formation of complex sheet-like structure along *a* axis.

Tyr(1), 2H, m); 2.17–2.03 ($C^{\beta}H$ of Val(2), 1H, m); 1.67–1.58 ($C^{\beta}Hs$ and $C^{\gamma}H$ of Leu(3), 3H, m); 1.41 (Boc–CH_3s, 9H, s); 0.94–0.86 ($C^{\gamma}Hs$ of Val(2) and $C^{\delta}Hs$ of Leu(3), 12H, m); (found: C, 61.2; H, 7.8; N, 8.2. $C_{26}H_{41}N_3O_7$ (507) requires C, 61.54; H, 8.08; N, 8.28%); ESI-MS *m*/*z* (%): 508.4 (100) $(M+H)^+$, M_{calcd}=507.

4.2.10. Boc-Ile(1)-OH 12.[16] A solution of isoleucine (1.31 g, 10 mmol) in a mixture of dioxane (20 mL), water (10 mL), and 1 M NaOH (10 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butylpyrocarbonate (2.2 g, 11 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated

Table 3. Crystal and data collection parameters of peptides **3**, **4**, **5**, and **6**

	Peptide **3**	Peptide **4**	Peptide **5**	Peptide **6**
Empirical formula	$C_{29}H_{38}N_3O_7 \cdot 0.5H_2O$	$C_{26}H_{41}N_3O_7$	$C_{26}H_{41}N_3O_7$	$C_{29}H_{39}N_3O_6$
Mol. wt.	1097.25	507.62	507.56	525.63
Data collection	X-Calibur CCD	Image Plate	Image Plate	Image Plate
Radiation, temperature	Cu Kα, 100	Mo Kα, 293	Mo Kα, 293	Mo Kα, 293
Crystallizing solvent	Methanol–water	Methanol–water	Methanol–water	Methanol–water
Crystal system	Monoclinic	Triclinic	Monoclinic	Monoclinic
Space group	*P*2	*P*1	$P2_1$	$P2_1$
a (Å)	22.2063(12)	5.021(7)	16.289(18)	15.327(17)
b (Å)	4.9902(2)	10.435(12)	4.979(7)	5.096(7)
c (Å)	29.0502(12)	14.009(15)	18.931(19)	20.73(2)
α (°)	(90)	80.26(1)	(90)	(90)
β (°)	108.288(4)	87.91(1)	103.57(1)	109.30(1)
γ (°)	(90)	80.23(1)	(90)	(90)
U (Å^3)	3056.6(3)	713(3)	1492(3)	1528(3)
Z	4	1	2	2
Density (calcd, mg/mm^3)	1.192	1.184	1.129	1.142
Unique data	9049	4586	4571	4833
Observed reflections ($I>2\sigma(I)$)	5466	3875	3588	2562
R	0.0465	0.0557	0.1148	0.1018
*wR*2	0.1050	0.1587	0.1931	0.1923
Max, residual e/Å^3	0.264, −0.264	0.193, −0.181	0.245, −0.269	0.283, −0.296

in vacuo to about 15–20 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 30 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4, and evaporated in vacuo to obtain **12** as a solid material.

Yield=2.2 g (9.5 mmol, 95%). Elemental Analysis Calcd for $C_{11}H_{21}NO_4$ (231): C, 57.14; H, 9.09; N, 6.06. Found: C, 56.9; H, 8.9; N, 5.9%. Mp 65–67 °C, lit. mp 66–69 °C.

4.2.11. Boc-Ile(1)-Val(2)-OMe 13. Boc-Ile-OH **12** (1.84 g, 8 mmol) was dissolved in dichloromethane (DCM) (10 mL) in an ice-water bath. H-Val-OMe was isolated from the corresponding methyl ester hydrochloride (2.68 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL, and it was added to the reaction mixture, followed immediately by di-cyclohexylcarbodiimide (DCC) (1.64 g, 8 mmol). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, residue was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **13** as a solid compound.

Yield=2.4 g (7 mmol, 87%); R_f=0.65 (ethyl acetate); mp 78–82 °C; $[\alpha]_D^{20}$ −38.8 (*c* 0.69, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (Val(2) NH, 1H, d, *J*=7.5 Hz); 5.03 (Ile(1) NH, 1H, d, *J*=6.6 Hz); 4.54 (C^{α}H of Val(2), 1H, m); 3.94 (C^{α}H of Ile(1), 1H, m); 3.73 (–OCH_3, 3H, s); 2.15 (C^{β}H of Val(2), 1H, m); 1.86 (C^{β}H of Ile(1), 1H, m); 1.67–1.62 (C^{γ}Hs of Ile(1), 2H, m); 1.42 (Boc–CH_3s, 9H, s); 0.94–0.89 (C^{γ}Hs of Val(2), C^{γ}Hs and C^{δ}Hs of Ile(1), 12H, m); (found: C, 59.0; H, 8.21; N, 8.03. $C_{17}H_{32}N_2O_5$ (344) requires C, 59.30; H, 9.30; N, 8.14%).

4.2.12. Boc-Ile(1)-Val(2)-OH 14. Boc-Ile(1)-Val(2)-OMe **13** (2.23 g, 6.5 mmol), MeOH (20 mL), and 2 M NaOH (10 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h methanol was removed under vacuo, the residue was taken in 50 mL of water and washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **14** as a waxy solid.

Yield=1.8 g (5.5 mmol, 84%); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 12.46 (–COOH, 1H, b); 7.70 (Val(2) NH, 1H, d, *J*=6 Hz); 6.69 (Ile(1) NH, 1H, d, *J*=9 Hz); 4.08 (C^{α}H of Val(2), 1H, m); 3.79 (C^{α}H of Ile(1), 1H, m); 2.44 (C^{β}H of Val(2), 1H, m); 1.97 (C^{β}H of Ile(1), 1H, m); 1.60 (C^{γ}Hs of Ile(1), 2H, m); 1.3 (Boc–CH_3s, 9H, s); 1.03–0.96 and 0.82–0.70 (C^{γ}Hs and C^{δ}Hs of Ile(1) and C^{γ}Hs of Val(2), 12H, m); (found: C, 58.01; H, 9.13; N, 8.34. $C_{16}H_{30}N_2O_5$ (330) requires C, 58.18; H, 9.09; N, 8.48%).

4.2.13. Boc-Ile(1)-Val(2)-Tyr(3)-OMe 5. Boc-Ile(1)-Val(2)-OH **14** (1.65 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Tyr-OMe was isolated from the corresponding methyl ester hydrochloride (2.31 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL, and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.675 g, 5 mmol). The reaction mixture was stirred for three days. The reaction mixture was then taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuo to yield peptide **5** as a white solid. Purification was done by silica gel column (100–200 mesh) using 3:1 ethyl acetate/toluene as eluent.

Yield=2.23 g (4.4 mmol, 89%); R_f=0.64 (25% toluene/ethyl acetate); mp 110–112 °C; IR (KBr): 3488, 3313, 1739, 1692, 1645, 1522 cm^{-1}; $[\alpha]_D^{20}$ −35 (*c* 0.5, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (ring Hs of Tyr(3), 2H, d, *J*=9 Hz), 6.74 (ring Hs of Tyr(3), 2H, d, *J*=8.4 Hz); 6.60 (Tyr(3) NH, 1H, d, *J*=8.7 Hz); 6.45 (Val(2) NH, 1H, d, *J*=8.1 Hz); 5.01 (Ile(1) NH, 1H, d, *J*=9 Hz); 4.82 (C^{α}H of Tyr(3), 1H, m); 4.23 (C^{α}H of Val(2), 1H, m); 3.94 (C^{α}H of Ile(1), 1H, m); 3.72 (–OCH_3, 3H, s); 3.10–2.94 (C^{β}Hs of Tyr(3), 2H, m); 2.12–2.05 (C^{β}H of Val(2), 1H, m); 1.95–1.90 (C^{β}H of Ile(1), 1H, m); 1.44 (Boc–CH_3s, 9H, s); 1.28 (C^{γ}Hs of Ile(1), 2H, m); 0.91–0.87 (C^{γ}Hs of Val(2) and C^{γ}Hs and C^{δ}Hs of Ile(1), 12H, m); (found: C, 61.34; H, 8.10; N, 8.06. $C_{26}H_{41}N_3O_7$ (507) requires C, 61.53; H, 8.09; N, 8.28%); ESI-MS *m/z* (%): 508.4 (100) $(M+H)^+$, 509.4 (30) $(M+2H)^+$, M_{calcd}=507.

4.2.14. Boc-Phe(1)-OH 15. See Ref. 17.

4.2.15. Boc-Phe(1)-Val(2)-OMe 16. Boc-Phe-OH (2.65 g, 10 mmol) was dissolved in dichloromethane (DCM) (10 mL) in an ice-water bath. H-Val-OMe was isolated from the corresponding methyl ester hydrochloride (3.34 g, 20 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL, and it was added to the reaction mixture, followed immediately by di-cyclohexylcarbodiimide (DCC) (2.06 g, 10 mmol). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, residue was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **16** as a white solid.

Yield=3.5 g (9.2 mmol, 92%); R_f=0.76 (ethyl acetate); mp 58–60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.19 (ring Hs of Phe(1), 5H, m); 6.36 (Val(2) NH, 1H, d, *J*=8.4 Hz); 5.01 (Phe(1) NH, 1H, d, *J*=8.1 Hz); 4.45 (C^{α}H of Val(2), 1H, m); 4.35 (C^{α}H of Phe(1), 1H, m); 3.69 (–OCH_3, 3H, s); 3.07 (C^{β}Hs of Phe(1), 2H, d, *J*=6 Hz); 2.13–2.04 (C^{β}H of Val(2), 1H, m); 1.42 (Boc–CH_3s, 9H, s); 0.88–0.83 (C^{γ}Hs of Val(2), 6H, m); (found: C, 63.2; H, 7.6; N, 7.03. $C_{20}H_{30}N_2O_5$ (378) requires C, 63.49; H, 7.94; N, 7.4%).

4.2.16. Boc-Phe(1)-Val(2)-OH 17. Boc-Phe(1)-Val(2)-OMe **16** (2.3 g, 6 mmol), MeOH (20 mL), and 2 M NaOH (10 mL) were added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuo, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **17** as a white solid sample.

Yield=1.89 g (5.2 mmol, 86.5%); mp 56–58 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 12.57 (–COOH, 1H, b); 7.86 (Val(2) NH, 1H, d, J=8.6 Hz); 7.27–7.19 (ring Hs of Phe(1), 5H, m); 6.95 (Phe(1) NH, 1H, d, J=8.7 Hz); 4.22 (C^{α}H of Val(2), 1H, m); 4.01 (C^{α}H of Phe(1), 1H, m); 2.99–2.93 (C^{β}Hs of Phe(1), 2H, m); 2.77–2.69 (C^{β}H of Val(2), 1H, m); 1.29 (Boc–CH_3s, 9H, s); 1.15–0.81 (C^{γ}Hs of Val, 6H, m); (found: C, 62.45; H, 7.54; N, 7.52. $C_{19}H_{28}N_2O_5$ (364) requires C, 62.63; H, 7.69; N, 7.69%).

4.2.17. Boc-Phe(1)-Val(2)-Phe(3)-OMe 6. Boc-Phe(1)-Val(2)-OH **17** (1.82 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (2.15 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL, and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.675 g, 5 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine (2×50 mL), 1 M sodium carbonate (3×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuo to yield peptide **6** as a white solid. Purification was done by silica gel column (100–200 mesh) using 3:1 ethyl acetate/toluene as eluent.

Yield=2.3 g (4.4 mmol, 87%); R_f=0.70 (25% toluene/ethyl acetate); mp 76–78 °C; IR (KBr): 3329, 3295, 1741, 1691, 1649, 1530 cm^{-1}, $[\alpha]_D^{20}$ −29.4 (c 0.5, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.08 (ring Hs of Phe(1) and Phe(3), 10H, m); 6.47 (Phe(3) NH, 1H, d, J=8.4 Hz); 6.23 (Val(2) NH, 1H, d, J=7.5 Hz); 4.93 (Phe(1) NH, 1H, d, J=8.1 Hz); 4.80 (C^{α}H of Phe(3), 1H, m); 4.35 (C^{α}H of Val(2), 1H, m); 4.16 (C^{α}H of Phe(1), 1H, m); 3.7 (–OCH_3, 3H, s); 3.15–3.02 (C^{β}Hs of Phe(1) and Phe(3), 4H, m); 2.10–2.00 (C^{β}H of Val(2), 1H, m); 1.40 (Boc–CH_3s, 9H, s); 0.81 (C^{γ}Hs of Val(2), 6H, m); (found: C, 61.92; H, 7.31; N, 7.92. $C_{29}H_{39}N_3O_6$ (525) requires C, 66.28; H, 7.42; N, 8.00%); ESI-MS m/z (%): 526.3 (100) $(M+H)^+$, 1073.6 (50) $(2M+H)^+$, M_{calcd}=525.

4.3. Single crystal X-ray diffraction study

Single crystals suitable for X-ray diffraction studies for tripeptides **3**–**6** were grown from methanol–water solution by slow evaporation. Diffraction data were measured for tripeptide **3** with Cu Kα radiation at 100 K using the Oxford Instruments X-Calibur CCD system and for **4**, **5**, and **6** with Mo Kα radiation at 293 K using the MAR research Image Plate System. A crystal of peptide **3** was positioned at 50 mm from the CCD and 330 frames were measured with a counting time of 10 s. Data analysis was carried out with the Crysalis program.[18] For peptides **4**, **5**, and **6**, the crystals were positioned at 70 mm from the Image Plate and 100 frames were measured at 2° intervals with a counting time of 2–5 min for various peptides. Data analyses were carried out with the XDS program.[19] The structures were solved using direct methods with the Shelx97[20] program. All non-hydrogen atoms of all peptides were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structures were refined on F^2 using Shelx97. Crystallographic data for the peptides **3**–**6** have been deposited at the Cambridge Crystallographic Data Centre (CCDC 264909–264911 and 298754).

4.4. ^{1}H NMR experiments

All NMR studies were carried out on a Brüker DPX 300 MHz spectrometer at 300 K. Peptide concentrations were in the range of 1–10 mmol in $CDCl_3$ and $(CD_3)_2SO$.

4.5. Mass spectrometry

Mass spectra were recorded on a Hewlett Packard Series 1100MSD mass spectrometer by positive mode electrospray ionization.

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References and notes

1. (a) Harada, A.; Li, J.; Kamachi, M. *Nature* **1993**, *346*, 516–518; (b) Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. *Science* **1997**, *277*, 1793–1796; (c) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988–1011; (d) Fenniri, H.; Deng, B.-L.; Ribbe, A. E.; Hallenga, K.; Jacob, J.; Thiyagarajan, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6487–6492.
2. (a) Lear, J. D.; Wasserman, Z. R.; Degrado, W. F. *Science* **1988**, *240*, 1177–1181; (b) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Kazanovich, N. *Nature* **1993**, *366*, 324–327; (c) Sakai, N.; Brennan, K. C.; Weiss, L. A.; Matile, S. *J. Am. Chem. Soc.* **1997**, *119*, 8726–8727; (d) Sakai, N.; Majumdar, N.; Matile, S. *J. Am. Chem. Soc.* **1999**, *121*, 4294–4295.
3. Granja, J. R.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 10785–10786.
4. Sanchez-Ouesada, J.; Isler, M. P.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2002**, *124*, 10004–10005.
5. (a) Fernandez-Lopez, S.; Kim, H.-S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature*

2001, *412*, 452–455; (b) Hartgerink, J. D.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 43–50; (c) Amorin, M.; Castedo, L.; Granja, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 2844–2845; (d) Rosenthal-Aizman, K.; Svensson, G.; Unden, A. *J. Am. Chem. Soc.* **2004**, *126*, 3372–3373; (e) Horne, W. S.; Ashkenasy, N.; Ghadiri, M. R. *Chem.—Eur. J.* **2005**, *11*, 1137–1144.

6. (a) Gademann, K.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 957–962; (b) Seebach, D.; Matthews, J. L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, L. B. *Helv. Chim. Acta* **1997**, *80*, 173–182; (c) Clark, T. D.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651–656.
7. Karle, I. L.; Handa, B. K.; Hassell, C. H. *Acta Crystallogr.* **1975**, *B31*, 555–560.
8. Gauthier, D.; Baillargeon, P.; Drouin, M.; Dory, Y. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 4635–4638.
9. Semetey, V.; Didierjean, C.; Briand, J.-P.; Aubry, A.; Guichard, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1895–1898.
10. Kraus, T.; Buděšínský, M.; Císařová, I.; Závada, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1715–1717.
11. Seela, F.; Wiglenda, T.; Rosemeyer, H.; Eickmeier, H.; Reuter, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 603–604.
12. (a) Fenniri, H.; Mathivanan, P.; Vidale, K. L.; Sherman, D. M.; Hallenga, K.; Wood, K. V.; Stowell, J. G. *J. Am. Chem. Soc.* **2001**, *123*, 3854–3855; (b) Fenniri, H.; Deng, B.-L.; Ribbe, A. E. *J. Am. Chem. Soc.* **2002**, *124*, 11064–11072; (c) Moralez, G. J.; Raez, J.; Yamazaki, T.; Motkuri, K. R.; Kovalenko, A.; Fenniri, H. *J. Am. Chem. Soc.* **2005**, *127*, 8307–8309.
13. (a) Görbitz, C. H. *Chem.—Eur. J.* **2001**, *7*, 5153–5159; (b) Reches, M.; Gazit, E. *Science* **2003**, *300*, 625–627; (c) Song, Y.; Challa, S. R.; Medforth, C. J.; Qiu, Y.; Watt, R. K.; Peňa, D.; Miller, J. E.; Swol, F. v.; Shelnutt, J. A. *Chem. Commun.* **2004**, 1044–1045; (d) Lu, K.; Jacob, J.; Thiyagarajan, P.; Conticello, V. P.; Lynn, D. G. *J. Am. Chem. Soc.* **2003**, *125*, 6391–6393; (e) Vauthey, S.; Santoso, S.; Gong, H.; Watson, N.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5355–5360; (f) Ray, S.; Haldar, D.; Drew, M. G. B.; Banerjee, A. *Org. Lett.* **2004**, *6*, 4463–4465.
14. Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 284–438.
15. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: New York, NY, 1984; pp 1–282.
16. Keller, O.; Keller, W. E.; van Look, G.; Wersin, G. *Organic Syntheses*; Wiley & Sons: New York, NY, 1990; Collect. Vol. VII, p 70.
17. Das, A. K.; Banerjee, A.; Drew, M. G. B.; Ray, S.; Haldar, D.; Banerjee, A. *Tetrahedron* **2005**, *61*, 5027–5036.
18. Crysalis Version 1; Oxford Instruments: 2005.
19. Kabsch, W. *J. Appl. Crystallogr.* **1988**, *21*, 916–924.
20. Sheldrick, G. M. *Shelx97, Program for Crystal Structure Refinement*; University of Gottingen: 1997.

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Synthesis and biological evaluation of anthranilamide-based non-peptide mimetics of ω-conotoxin GVIA

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Abstract—Non-peptide mimetics based on an anthranilamide 'scaffold' possessing fragments that mimic Lys2, Tyr13 and Arg17 in ω-conotoxin GVIA have been prepared. Compounds were assayed for binding to the voltage-gated calcium channels $Ca_v2.2$ ('N-type') and $Ca_v2.1$ ('P/Q-type') in rat brain. The primary synthetic target, 2-(6-amino-hexanoylamino)-5-(3-guanidino-propoxy)-*N*-[4-(4-hydroxyphenoxy)-phenyl]-benzamide (**2a**), exhibited low μM binding to $Ca_v2.2$ and was more than 30-fold selective for $Ca_v2.2$ over $Ca_v2.1$.

1. Introduction

ω-Conotoxins are pharmacologically active toxins derived from the venom of cone snails.[1] ω-Conotoxin GVIA (GVIA) is produced by *Conus geographus*, while ω-conotoxin MVIIA (MVIIA) is produced by *Conus magus*. Both of these polypeptides potently block the neuronal voltage-gated N-type calcium channel ($Ca_v2.2$), which in humans is a target for the relief of neuropathic pain. Indeed, ω-conotoxin MVIIA (Prialt®, Ziconotide®, SNX-111) was approved late in 2004 for the treatment of severe chronic pain and shows efficacy in cases where morphine-based analgesics are less effective.[2]

Such therapeutic promise has driven the search for other inhibitors of $Ca_v2.2$. Another ω-conopeptide, ω-conotoxin CVID (AM336), has recently[3] been taken into phase II clinical trials by AMRAD and reportedly has a better therapeutic index than Elan's MVIIA. However, being polypeptides, these agents suffer drawbacks in that they are relatively expensive to manufacture and possess poor pharmacokinetic properties. Indeed, both MVIIA and CVID of necessity are administered intrathecally.

Pain management is one of the largest pharmaceutical markets in the world and is expected[4] to increase at a compounded annual growth rate (CAGR) of 10% to reach $29.8 billion (US) in 2008. It is therefore not surprising that intensive screening events have focused on the discovery of small molecule $Ca_v2.2$ inhibitors that might be orally active. This has culminated in NeuroMed's NMED-160, which is an orally available blocker of $Ca_v2.2$ channels that is now in Phase II clinical trials for a variety of pain conditions.[5]

Our interest is in the rational design of small molecule mimetics of peptide and protein binding epitopes. GVIA and MVIIA are structurally defined and their binding epitopes have been extensively mapped, making them attractive targets for mimetic design.[1,6]

We recently reported[7] the synthesis and biological activity of the benzothiazole derivative **1a** (Fig. 1), which was designed to mimic the side chains of K2, Y13 and R17 in GVIA and bound $Ca_v2.2$ channels with a Ki of 1.8 μM. This simple 3-residue mimetic was also selective for $Ca_v2.2$ over $Ca_v2.1$ ('P/Q-type calcium channels') and this was seen as therapeutically desirable in order to minimise off-target side effects.[8] The key to designing these mimetics is the application of interactive de novo design since no commercially available software could adequately address the challenge posed by the large, discontinuous and disparate ω-conotoxin binding epitope.

Keywords: ω-Conotoxin; Mimetic; $Ca_v2.2$ ('N-type') calcium channel blocker.
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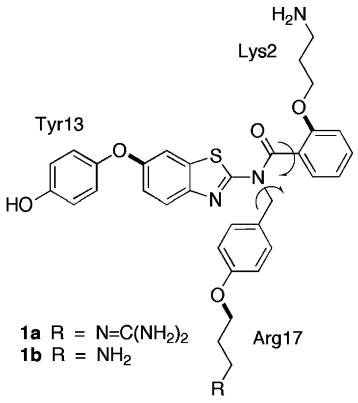


Figure 1. The structure of the type-III mimetic of ω-conotoxin GVIA based on an *N*′-benzylated benzamidobenzothiazole core (**1a**), along with the structure of an analogue (**1b**), both recently reported by us.[7] Annotations indicate the GVIA residue mimicked, and emboldened bonds are those that mimic the corresponding α–β bond vectors in GVIA.

Since the side chain termini targeted for the mimicry of GVIA are not spatially well defined in solution, our approach is to concentrate on the design of scaffolds that mimic the targeted α–β bond vectors as these are conformationally better resolved. In order to investigate whether other scaffolds could be developed besides those based on a benzothiazole template, we have designed the anthranilamide derivative **2a** (Fig. 2). A solid-state structure of the anthranilamide scaffold confirmed that it could serve as a suitable K2–Y13–R17 mimetic of GVIA. We have previously reported this crystal structure along with preliminary synthetic and functional activity details for this mimetic.[9] Herein, we characterise the full synthesis of **2a** and its analogue **2b**, using a different and more efficient synthetic route to that which we have previously outlined, and report binding data for these compounds to $Ca_v2.2$ and $Ca_v2.1$.

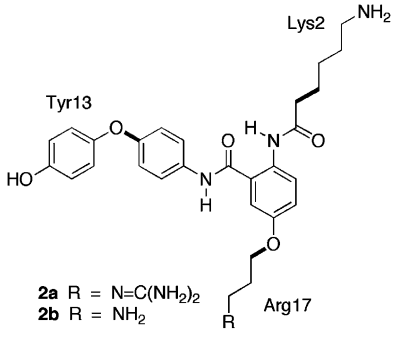


Figure 2. The structure of the type-III mimetic of ω-conotoxin GVIA based on an *N*′-aryl-*N*″-acyl anthranilamide core (**2a**), along with the structure of an analogue (**2b**), reported herein. Annotations indicate the GVIA residue mimicked, and emboldened bonds are those that mimic the corresponding α–β bond vectors in GVIA.

2. Results

2.1. Mimetic synthesis

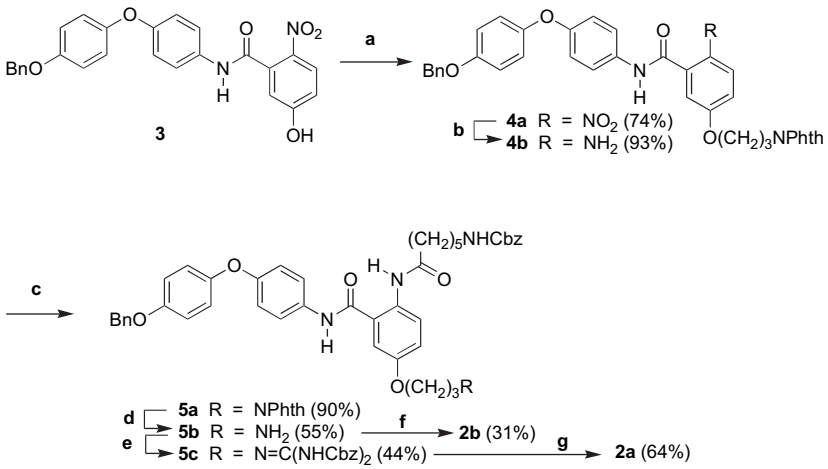
Mimetics were synthesised from key intermediate **3**, as shown in Scheme 1. Firstly, the phenolic group was alkylated with *N*-(3-bromopropyl)phthalimide and potassium carbonate in DMF at 65 °C for 4 h to give **4a** in 74% yield. The nitro group of **4a** was then reduced, after dissolution in hot 60% ethanolic THF, by portionwise addition of sodium dithionite and water at 80 °C, to give aniline **4b** in 93% yield. This aniline was then acylated with Cbz-protected 6-aminohexanoic acid using a typical HOBT/HBTU coupling protocol, to give **5a** in 90% yield. The phthalimide group was cleaved using sodium borohydride in aqueous isopropanol[10] followed by treatment with acetic acid at 60 °C for 48 h to give the free amine **5b** in 55% yield. In turn, this amine was converted into a protected guanidyl group by treatment with *N*,*N*′-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine and triethylamine in methanolic DCM, to give **5c** in 44% yield. Finally, this was fully deprotected by catalytic hydrogenation in trifluoroethanol to afford **2a** as a colourless solid in 64% yield.

Analogue **2b** was obtained from a sample of precursor **5b** that was deprotected by catalytic hydrogenation in methanolic ethanol, to give **2b** in 31% yield.

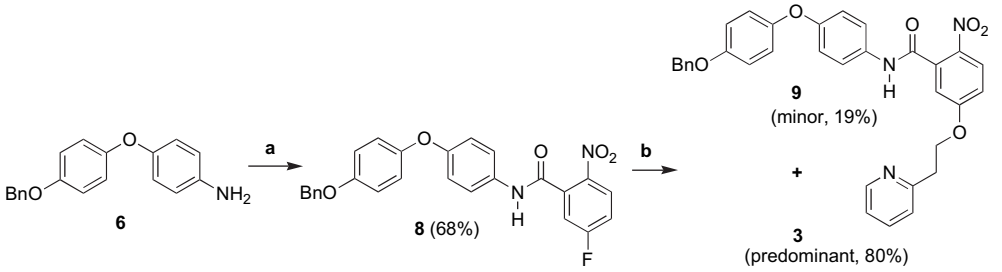
Intermediate **3** was not our initial choice of mimetic precursor. Instead, the aryl fluoride **8** in Scheme 2 was targeted on the basis that previous preliminary studies showed that the labile fluorine atom could be displaced by *N*-Boc-protected 3-aminopropanol as its alkoxide in DME. The aryl fluoride **8** was readily made by coupling our previously reported[7] aniline **6** with 5-fluoro-2-nitrobenzoic acid (**7**). In an initial side project on R17 guanidine isosteres, we investigated the use of pyridin-2-ylethanol and sodium hydride in DME to see if we could displace the labile fluoride to give a compound such as **9**. However, only occasionally did **9** result, and even then only in small yields, and the predominant product instead was repeatedly **3**. With large amounts of **3** at our disposal, we realised that alkylation of the phenolic group in **3** using readily available 3-aminopropylbromide, N-protected as the phthalimide, should provide a facile route to our other target mimetics **2a** and **2b**. As described above for Scheme 1, this indeed proved to be the case.

Although labile aryl fluoride atoms are reported to readily furnish phenols through alkaline hydrolysis,[11,12] model reactions led us to suggest that a mechanism involving base-catalysed beta-elimination of initially formed intended product **9** is involved in the production of **3**, facilitated by the acidic nature of the pyrid-2yl-methylene proton and the excellent leaving group ability of the *para*-nitrophenoxide anion, as shown in Scheme 3. The model reactions involved addition of 2,4-dinitrofluorobenzene to pyridin-2-ylethanol/NaH in DME, whereupon the only pyridine-containing species produced was 2-vinylpyridine. On the other hand, addition of the alkoxide to the model aryl fluoride, conditions under which excess strong base would not exist, gave the model product as intended. Our proposed mechanism is also supported by the published use of *S*-pyrid-2-ylethyl groups as surrogates for thiols, which are unmasked through a base-catalysed beta-elimination mechanism analogous to that postulated here.[13]

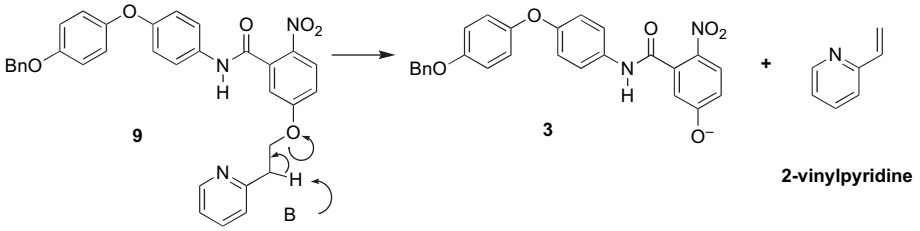
Other unexpected behaviour was encountered for this scaffold when we synthesised mimetics with a greater degree of orthogonal protection than that exhibited in **5c**. Thus,



Scheme 1. Synthesis of mimetic **2a** and its analogue **2b** from key precursor **3**. Reagents and conditions: (a) *N*′-(3-bromopropyl)phthalimide, K_2CO_3, DMF, 65 °C; (b) $Na_2S_2O_4$, EtOH, H_2O, reflux; (c) $CbzN(CH_2)_5CO_2H$, HBTU, HOBt, Et_3N, DMF, rt; (d) (i) $NaBH_4$, *i*-PrOH, H_2O, DCM, rt; (ii) CH_3CO_2H, reflux; (e) *N*,*N*′-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine, DCM, rt; (f) H_2, Pd/C, MeOH/EtOH, rt; (g) H_2, Pd/C, trifluoroethanol, rt.



Scheme 2. Synthesis of key precursor **3** as the predominant by-product instead of the intended target **9**. Reagents and conditions: (a) 5-fluoro-2-nitrobenzoic acid (**7**), HBTU, Et_3N, DMF, rt; (b) 2-(2-hydroxyethyl)pyridine, NaH, DME, rt.

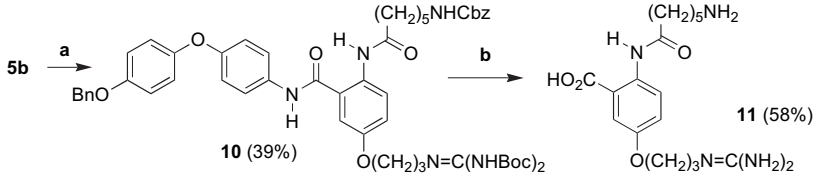


Scheme 3. Proposed base-induced (B) beta-elimination in **9** to account for the observed production of **3** under anhydrous conditions (see Scheme 2) where the intended product was **9**.

5b was readily converted to **10** by reaction with *N*,*N*′-bis(*tert*-butyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine in DCM as shown in Scheme 4. However, when we attempted simultaneous bis-BOC and benzyl ether deprotection, using the TFA/thioanisole method of Kiso et al.,[14] only the cleaved compound **11** was isolated after work up and purification.

2.2. Mimetic binding affinity for $Ca_v2.2$ and $Ca_v2.1$

Mimetics **2a** and **2b** were assayed for binding to $Ca_v2.2$ using ^{125}I-GVIA as a ligand. The K2–Y13–R17 mimetic **2a** bound with a Ki of 3.5 μM while the K2–Y13–R17K mimetic **2b** bound with a Ki of 13 μM. These same mimetics were



Scheme 4. Synthesis of orthogonally protected mimetic **10** and its subsequent decomposition to **11** during attempted deprotection. Reagents and conditions: (a) *N*,*N*′-bis(*tert*-butyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine, DCM, rt; (b) TFA/thioanisole, DCM, rt.

assayed for binding to $Ca_v2.1$ using ^{125}I-MVIIC as a ligand. The K2–Y13–R17 mimetic **2a** bound with a Ki of 111 μM while the K2–Y13–R17K mimetic **2b** bound with a Ki of 176 μM (Table 1).

Table 1. Binding potencies (μM) for mimetics **2a** and **2b** to $Ca_v2.2$ (N-type) and $Ca_v2.1$ (P/Q-type) calcium channels (95% confidence intervals shown in parentheses)

Compound	Ki ($Ca_v2.2$)	Ki ($Ca_v2.1$)
2a	3.5 (2.5–4.8)	111 (70–180)
2b	13.1 (9.5–18.0)	176 (140–220)

3. Discussion

The relatively potent, low micromolar binding affinity for the K2–Y13–R17 mimetic **2a** to $Ca_v2.2$ (N-type calcium) channels is impressive and is made more remarkable by the selectivity of this compound for these channels over $Ca_v2.1$ (P/Q-type calcium) channels. This compound appears to be highly optimisable since the K2 and R17 mimetic side chains contain significant flexibility and are suited to conformational constraint. It is not proven that **2a** mimics the three targeted residues in GVIA exactly in binding to $Ca_v2.2$ as designed, but this is a reasonable assumption since molecular modelling has shown[9] that this scaffold is a good structural mimic of K2, Y13 and R17 in GVIA. This is more clearly shown in Figure 3, where the solid-state conformation of the scaffold[9] is superimposed on the NMR-derived solution conformation of GVIA, which is represented by its peptide backbone conformation as a yellow tube. All residues in GVIA have been removed apart from the side chains of the targeted residues. This makes it quite clear how the anthranilamide scaffold can mimic the α–β bond vectors of the targeted residues, K2, Y13 and R17, in GVIA and how, if suitably functionalised, it would be a structural K2–Y13–R17 GVIA mimetic.

We have previously reported[7] another K2–Y13–R17 mimetic using a benzothiazole-based scaffold (**1a**) and for this system, undertook more extensive testing of truncated analogues to show that each of the three mimetic side chains contributed to the low micromolar affinity for $Ca_v2.2$. For the current anthranilamide system, we have only investigated the variant **2b**, which contains a lysine-like side chain instead of an arginine-like side chain as the R17 side chain mimetic. However, the drop in potency that we see parallels the drop in potency that accompanies the analogous change in the benzothiazole-based mimetic system (**1a** to **1b**), and we propose that the mimetic side chains in **2a** all interact favourably with the ion channel as they do for **1a** and moreover, that both compounds are true GVIA mimetics.

Interactive de novo design can be the most efficient way to develop type-III mimetics of binding epitopes.[1,6,7,9,15,16] Here, synthetic and conformational knowledge is applied by the medicinal chemist interactively with a modelling package to design a suitable mimetic scaffold that is synthetically tractable, conformationally appropriate, not too

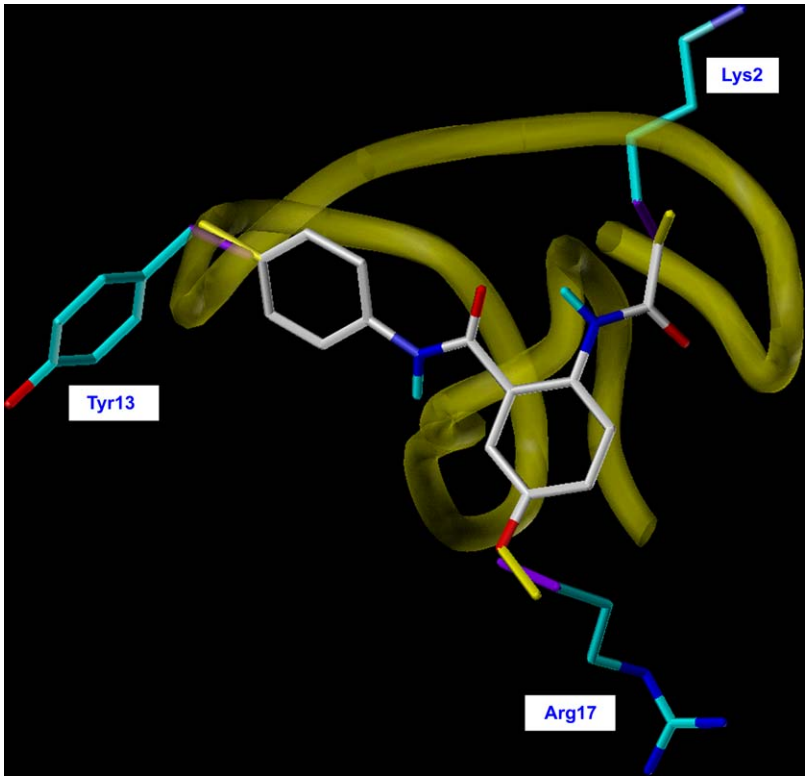


Figure 3. Three-dimensional picture of the anthranilamide scaffold 'core' superimposed on the NMR solution structure of ω-conotoxin GVIA. The peptide backbone is shown as a mustard yellow translucent tube. Only residues Lys2, Tyr13 and Arg17 of GVIA are shown, these in cyan with their respective C_α–C_β bonds being coloured purple. The scaffold is coloured by atom type with only the NH hydrogens being displayed for the sake of clarity. The mimetic C_α–C_β bonds are coloured yellow. It can be seen that the purple GVIA bonds match closely with their yellow counterparts in the mimetic. The conformation of the scaffold used is that determined by X-ray crystallographic analysis, as previously described.[9]

drug-unlike and highly analogable. Occasional structural relaxation with reference to known crystallographic substructure conformation is used in the process. Although this procedure is inherently suited to automation and computational approaches have been developed,[17] in our hands we find our approach to be the most successful and efficient. Binding epitopes targeted by interactive de novo design may vary from continuous and contiguous, to discontinuous and discontiguous[1,6,7,9,15,16] and so although we have targeted mimetics of pharmacologically active toxins, our approach may be suitable for other polypeptide–protein or protein–protein interactions. We are currently applying this technique with considerable success to the design of mimetics of the alpha-helical binding domain of pro-apoptotic BH3-only proteins in their complex with anti-apoptotic Bcl-2 homologues.[18] In the current work, we target a pharmacophore that is both discontinuous and discontiguous. In a sense, the disparate nature of the pharmacophore allows for more choice in the scaffold construction, and we have now shown how two, quite distinct scaffolds can act as GVIA mimetics in targeting the same three residues. This gives some additional versatility to the process where one scaffold may be interchanged for another with better ADMET properties.

One important factor in the success of our approach is the choice of neither more nor less than three residues to target. Less than three is unlikely to register activity, more than three is likely to render initial design and synthesis overly complex. In this regard, we found it quite surprising that mimetic **2a** is as potent as it is. Rudimentary thermodynamic considerations suggest that the side chains in this type of mimetic might contribute significantly more to the binding process than they do in GVIA itself.[1,7,15]

4. Conclusion

Non-peptide mimetics of ω-conotoxin GVIA have been prepared based on an anthranilamide scaffold that projects side chain mimetics of lysine, tyrosine and arginine in a similar respective manner to the projection of K2, Y13 and R17 in GVIA. In so doing, mimetic **2a** exhibits a Ki to $Ca_v2.2$ of 3.5 μM and is over 30-fold more selective for this channel than $Ca_v2.1$ channels. We have selected **2a** for further optimisation and assessment of functional antagonism of the $Ca_v2.2$ channel.

5. Experimental

All commercially obtained chemicals and reagents were used as received. Melting points were recorded on a Reichert hot stage melting point apparatus. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 300 MHz spectrometer using the solvents specified; exchangeable NH and OH protons are only assigned where specified. ^{19}F-decoupled ^1H and ^{13}C NMR spectra were run on Bruker DPX 300 MHz spectrometer FTIR were run on Perkin–Elmer 1600 Series FTIR. ATR IR spectra were run on a Bruker IFS 55 FTIR Specac single reflection ATR system fitted with a single bounce diamond top plate. HRMS of compounds were recorded on a Bruker BioApex 47e Fourier Transform mass spectrometer. Low Resolution MS was recorded on a Micromass Platform II mass spectrometer. The compounds analysed were dissolved in organic solvent and ionised using an electrospray ionisation source. MS data are recorded as positive electrospray ions unless indicated by (ESI-) where negative ions are reported. Elemental analyses were conducted by CMAS Chemical and Micro Analytical Services Pty. Ltd. (P.O. Box 248, Belmont, Victoria 3216, Australia).

5.1. 2-(6-Aminohexanoylamino)-5-(3-guanidinopropoxy)-*N*-[4-(4-hydroxyphenoxy)-phenyl]-benzamide (2a)

The fully protected compound (**5c**) (17.5 mg, 0.017 mmol) was dissolved in trifluoroethanol (6 mL) and 10% Pd/C was added. The reaction mixture was stirred under H_2 at atmospheric pressure for 4 h. The catalyst was then removed by filtration and washed with MeOH (2×3 mL) and the combined filtrates and washings concentrated to dryness under reduced pressure. The solid was then triturated with DCM (2×3 mL), dissolved in water and centrifuged. Freeze-drying of the supernatant afforded **2a** as a white solid (5.8 mg, 64%). Mp 134–136 °C; ^1H NMR (300 MHz, methanol-d_4) δ ppm: 1.44 (m, 4H), 1.70 (p, J=7.2 Hz, 2H), 2.10 (p, J=7.2 Hz, 2H), 2.40 (t, J=6.0 Hz, 2H), 2.60 (t, J=6.9 Hz, 2H), 3.44 (t, J=6.8 Hz, 2H), 4.16 (t, J=5.9 Hz, 2H), 6.76–6.94 (m, 6H), 7.14 (dd, J=3.0, 9.0 Hz, 1H), 7.32 (d, J=2.7 Hz, 1H), 7.58 (dd, J=2.1, 9.0 Hz, 2H), 7.89 (d, J=8.7 Hz, 1H); ^{13}C NMR-APT (75 MHz, methanol-d_4; 1 unassigned ArC) δ ppm: 27.5 (CH_2), 28.3 (CH_2), 30.5 (CH_2), 34.3 (CH_2), 38.9 (CH_2), 40.4 (CH_2), 43.2 (CH_2), 67.4 (CH_2), 116.0 (CH), 118.6 (CH), 119.1 (CH), 119.3 (CH), 122.7 (CH), 124.7 (CH), 127.2 (CH), 130.4 (C), 132.1 (C), 134.7 (C), 150.5 (C), 157.7 (C), 158.3 (C), 159.6 (C), 169.5 (C), 175.5 (C); ATR (neat) cm^{-1}: 3278 (O–H), 2932, 2847 (aliphatic C–H), 1652, 1600 (C═O), 1497 (C═N); HRMS calcd for $C_{29}H_{36}N_6O_5$ (M+H$^+$) 549.28254, found 549.2828.

5.2. 2-(6-Aminohexanoylamino)-5-(3-aminopropoxy)-*N*-[4-(4-hydroxyphenoxy)-phenyl]-benzamide (2b)

The protected compound (**5b**) (26 mg, 0.036 mmol) was dissolved in absolute EtOH (5 mL) and MeOH (2 mL) and stirred under H_2 with 10% Pd/C for 3 h. The mixture was filtered and the filtrate evaporated to dryness. The residue was triturated with hexanes (4×8 mL) and ether (3×8 mL) and then dissolved in water and centrifuged. The supernatant was removed and freeze-dried to give **2b** as a green solid (4.8 mg, 31%). Mp 146–148 °C; ^1H NMR-COSY (300 MHz, methanol-d_4) δ ppm: 1.46 (p, J=7.5 Hz, 2H), 1.71 (m, J=8.2, 7.2 Hz, 4H), 2.20 (p, J=6.6 Hz, 2H), 2.43 (t, J=7.4 Hz, 2H), 2.91 (t, J=7.4 Hz, 2H), 3.19 (t, J=7.2 Hz, 2H), 4.22 (t, J=5.9 Hz, 2H), 6.81–6.96 (m, 6H), 7.14–7.18 (dd, J=3.0, 9.0 Hz, 1H), 7.37 (d, J=2.7 Hz, 1H), 7.52 (d, J=9.0 Hz, 2H), 7.93 (d, J=9.0 Hz, 1H); ^{13}C NMR (75 MHz, methanol-d_4) δ ppm: 26.9 (CH_2), 27.7 (CH_2), 29.1 (CH_2), 29.4 (CH_2), 38.6 (CH_2), 39.5 (CH_2), 41.4 (CH_2), 67.7 (CH_2), 112.5 (CH), 116.0 (CH), 116.1 (2CH), 117.9 (2CH), 118.2 (CH), 119.3 (2CH), 119.5 (2CH), 130.0 (C), 132.4 (C), 134.9 (C), 151.5 (C), 155.8 (C), 157.4 (C), 158.1 (C), 169.6 (C), 175.0 (C); ATR (neat) cm^{-1}: 3237 (O–H), 2927, 2849 (aliphatic C–H), 1647, 1596 (weak), 1496 (C═O); MS (ESI) (M+H$^+$) 507.3; HRMS calcd for $C_{28}H_{34}N_4O_5$ (M+H$^+$) 507.26075, found 507.2606.

5.3. *N*-[4-(4-Benzyloxyphenoxy)-phenyl]-5-hydroxy-2-nitrobenzamide (3)

2-(2-Hydroxyethyl)pyridine (0.1 mL, 0.89 mmol) was added to a slurry of NaH (60% in mineral oil, 21.6 mg, 0.54 mmol) in anhydrous DME (0.25 mL) under N_2. After 20 min, a solution of **8** (36 mg, 0.079 mmol) dissolved in anhydrous DMF (3 mL) was added dropwise over 25 min at room temperature. The reaction mixture changed from an orange-yellow colour to a brown colour overnight (24 h). DME was removed under reduced pressure and the resulting residue dissolved in EtOAc (30 mL). The EtOAc solution was washed with 2 M HCl (2×40 mL). Washings with 2 M NaOH (2×40 mL) were carried out until the aqueous solution became colourless. The organic layer was then washed with saturated brine (2×30 mL), dried ($MgSO_4$) and filtered. The organic solvent was removed under reduced pressure. Purification by radial chromatography using 100% EtOAc afforded **3** (28.7 mg, 80%) as a yellow solid after removal of solvent (a small amount of **9** (8.6 mg, 19%) was also furnished as a brown amorphous solid). ^{1}H NMR (300 MHz, acetone-d_6) δ ppm: 5.08 (s, 2H), 6.88–7.05 (m, 8H), 7.35–7.47 (m, 5), 7.62 (d, J=9.3 Hz, 2H), 8.09 (d, J=9.3 Hz, 1H), 10.50 (s, 1H, N*H*); ^{13}C NMR (75 MHz, acetone-d_6; 1 ArCH remains unassigned) δ ppm: 70.30 (CH2), 115.52 (CH), 116.13 (2CH), 118.40 (2CH), 120.31 (2CH), 121.50 (2CH), 127.41 (CH), 127.76 (2CH), 127.94 (CH), 128.59 (2CH), 134.48 (C), 136.49 (C), 137.68 (C), 138.21 (C), 151.05 (C), 154.60 (C), 155.20 (C), 163.08 (C), 164.43 (C); ATR (neat) cm^{-1}: 3217 s, 3047 m, 2875 w, 1580 m, 1494 s, 1444 m, 1386 w, 1323 s, 1263 m, 1209 s, 1102 w, 1063 m, 878 m, 819 m, 742 m, 695 m cm^{-1}. MS (ESI) *m/z* 457.3 [M+H]$^+$. HRMS: Found 456.1303 (requires 456.1321 for $C_{26}H_{20}N_2O_6$). Microanalysis: Found (%) C 68.51 H 4.38 N 6.19 (requires (%) C 68.42 H 4.42 N 6.14 for $C_{26}H_{20}N_2O_6$).

5.4. *N*-[4-(4-Benzyloxyphenoxy)-phenyl]-5-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propoxy]-2-nitrobenzamide (4a)

Phenol (**3**) (0.223 g, 0.489 μmol) and *N*-(3-bromopropyl)-phthalimide (0.131 g, 0.489 μmol) were dissolved in DMF (10 mL) with K_2CO_3 (0.27 g, 0.19 mmol) and the reaction mixture heated under nitrogen at 65 °C for 4 h. After allowing to cool to room temperature, the mixture was poured into iced 2 M HCl (60 mL) with stirring. The beige flocculant was filtered off and the residue washed with water. This precipitate was then dissolved in 40:60 EtOAc/THF, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude residue was re-crystallised from EtOAc to give **4a** as a beige coloured powder (0.232 g, 74%). Mp 186–188 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ ppm: 2.28 (p, J=6.2 Hz, 2H), 3.94 (t, J=6.5 Hz, 2H), 4.35 (t, J=5.9 Hz, 2H), 5.16 (s, 2H), 6.99–7.15 (m, 9H), 7.34–7.57(m, 5H), 7.75–7.91 (m, 5H+NH), 8.12–8.16 (m, 1H); ^{13}C NMR-APT (75 MHz, acetone-d_6) δ ppm: 27.9 (CH_2), 35.2 (CH_2), 66.7 (CH_2), 70.5 (CH_2), 114.1 (CH), 115.1 (CH), 115.8 (2CH), 116.2 (2CH), 120.1 (2CH), 121.9 (2CH), 123.2 (2CH), 126.9 (CH), 127.8 (CH), 127.3 (2CH), 128.4 (2CH), 131.7 (C), 132.6 (C), 134.1 (2CH), 135.4 (C), 136.7 (C), 138.6 (2C), 150.5 (C), 154.7 (C), 154.8 (C), 162.6 (2C), 164.5 (C), 168.4 (C); ATR (neat) cm^{-1}: 3274 (aromatic C–H), 2940, 2875 (aliphatic C–H), 1705 (C═O) 1510, 1208 (NO_2); HRMS calcd for $C_{37}H_{29}N_3O_8$ (M+Na$^+$) 666.1852, found 666.1846.

5.5. 2-Amino-*N*-[4-(4-benzyloxyphenoxy)-phenyl]-5-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propoxy]-benzamide (4b)

Nitrobenzene (**4a**) (0.288 g, 0.448 mmol) was dissolved in 95% EtOH (20 mL) and THF (15 mL) and heated to 80 °C. Upon dissolution of the solid, $Na_2S_2O_4$ (0.312 g, 1.8 mmol) was added, followed by H_2O (4 mL). The temperature was maintained at 80 °C for a further 2 h. The solvent was then removed under reduced pressure. EtOAc (40 mL) and 2 M HCl (40 mL) were added to the residue and the aqueous layer extracted with EtOAc (2×30 mL). The combined organic extracts were then washed with saturated $NaHCO_3$ (2×40 mL), saturated brine (2×30 mL) and dried ($MgSO_4$), filtered and evaporated to dryness to give **4b** as a light yellow solid (0.255 g, 93%). Mp 140–143 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.03 (p, J=6.4 Hz, 2H), 3.87 (t, J=6.9 Hz, 2H), 3.94 (t, J=5.7 Hz, 2H), 4.98 (s, 2H), 6.57–6.92, 7.08–7.09, 7.26–7.38, 7.52–7.55 (m, 16H), 7.60–7.76 (m, 4H); ^{13}C NMR-APT (75 MHz, $CDCl_3$; 1 ArCH and 11 ArC unassigned) δ ppm: 28.1 (CH_2), 35.3 (CH_2), 67.7 (CH_2), 70.5 (CH_2), 115.4 (CH), 115.8 (2CH), 118.4 (2CH), 119.0 (CH), 120.2 (2CH), 122.3 (2CH), 123.2 (2CH), 127.4 (2CH), 127.9 (CH), 128.5 (2CH), 131.9 (C), 132.9 (C), 133.9 (2CH); ATR (neat) cm^{-1}: 3461, 3372 (N–H), 3271 (aromatic C–H), 2951, 2862 (aliphatic C–H), 1811 (C═O), 1639 (phthalimide C═O); HRMS calcd for $C_{37}H_{31}N_3O_6$ (M+H$^+$) 614.2291, found 614.2291.

5.6. (5-{2-[4-(4-Benzyloxyphenoxy)-phenyl-carbomoyl]-4-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propoxy]-phenylcarbomyl}-pentyl)carbamic acid benzyl ester (5a)

Aniline (**4b**) (0.180 g, 0.29 mmol) and 6-benzyloxy-carbonyl aminohexanoic acid (0.156 g, 0.58 mmol) were dissolved in DMF (10 mL). Triethylamine (0.16 mL, 0.11 mol) was added, followed by HOBt (0.080 g, 0.5 mmol) and HBTU (0.223 g, 0.5 mmol). The reaction mixture was stirred at room temperature overnight then poured into 2 M HCl (50 mL). This aqueous solution was extracted with DCM (3×30 mL). The combined DCM extracts were washed with 2 M HCl (30 mL), then with saturated $NaHCO_3$ (2×30 mL) followed by saturated brine (2×30 mL). After drying ($MgSO_4$) and filtering, the DCM was removed under reduced pressure to give the crude product as a white precipitate. This precipitate was re-crystallised from EtOAc to afford **5a** as a white solid (0.228 g, 90%). Mp 157–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.39 (p, J=6.6 Hz, 2H), 1.52 (p, J=7.5 Hz, 2H), 1.71 (p, J=7.5 Hz, 2H), 2.11 (p, J=6.2 Hz, 2H), 2.35 (t, J=7.4 Hz, 2H), 3.17 (q, J=6.4 Hz, 2H), 3.92 (t, J=6.8 Hz, 2H), 4.05 (t, J=5.6 Hz, 2H), 4.84 (s, 2H, 2NH), 5.06 (s, 2H), 5.07 (s, 2H), 6.90–7.04 (m, 8H), 7.15–7.16 (m, 2H), 7.28–7.49 (m, 9H), 7.56–7.59 (m, 2 H), 7.67–7.81 (m, 4H); ^{13}C NMR-APT (75 MHz, $CDCl_3$) δ ppm: 25.1 (CH_2), 26.3 (CH_2), 28.1 (CH_2), 29.7 (CH_2), 35.3 (CH_2), 37.7 (CH_2), 40.9 (CH_2), 66.6 (CH_2), 70.5 (CH_2), 77.0 (CH_2), 114.9 (CH), 115.9 (2CH), 118.2 (2CH), 119.1 (CH), 120.5 (2CH), 122.4 (2C), 122.5 (2CH), 123.2 (2CH), 123.4 (CH), 127.3 (4CH), 127.4 (CH), 127.9 (CH), 128.4 (2CH), 128.5 (2CH), 131.9

(C), 132.0 (2C), 133.1 (C), 134.0 (2CH), 136.5 (C), 136.8 (C), 150.3 (C), 153.6 (C), 155.0 (C), 155.4 (C), 166.8 (C), 168.4 (2C), 171.4 (C); ATR (neat) cm^{-1}: 3270, 3058 (amide N–H), 2936, 2869 (aliphatic C–H), 1711, 1502 (C═O); HRMS calcd for $C_{51}H_{48}N_4O_9$ ($M+Na^+$) 883.3319, found 883.3306.

5.7. (5-{4-(3-Aminopropoxy)-[4-(4-benzyloxyphenoxy)-phenylcarbonoyl]-phenylcarbamoyl}-pentyl)-carbamic acid benzyl ester (5b)

The phthalimide protected precursor (**5a**) (85 mg, 99 μmol) was dissolved in a solution of 6:1 *i*-PrOH/H_2O (10 mL), and DCM was added until a homogeneous mixture was obtained. To this was added $NaBH_4$ (0.019 g, 0.5 mmol) was added and the reaction mixture stirred at room temperature for 18 h. Acetic acid was added to adjust the pH to 2 and the reaction heated at 60 °C for 48 h. The solvent was then evaporated from the reaction mixture. Saturated $NaHCO_3$ (20 mL) was added and the resulting mixture was extracted with EtOAc (3×20 mL). The combined EtOAc extracts were washed with water (20 mL), saturated brine (2×20 mL), dried ($MgSO_4$) and filtered. After the removal of solvent from the filtrate, the residue was purified by radial chromatography using a 2:1 $CHCl_3$/MeOH eluent, or by first flushing through an 80:20 EtOAc/hexanes eluent, then eluting the product **5b** (40 mg, 55%) with MeOH. ^1H NMR (300 MHz, methanol-d_4) δ ppm: 1.39 (p, J=6.6 Hz, 2H), 1.50 (p, J=6.6 Hz, 2H), 1.69 (p, J=6.6 Hz, 2H), 2.19 (p, J=6.6 Hz, 2H), 2.39 (t, J=7.2 Hz, 2H), 3.07 (t, J=6.9 Hz, 2H), 3.18 (t, J=7.2 Hz, 2H), 4.21 (t, J=5.9 Hz, 2H), 5.06 (s, 2H), 5.09 (s, 2H), 6.94–7.05 (m, 6H), 7.13–7.6 (m, 15H); ATR (neat) cm^{-1}: 3463, 3367 (N–H), 3272, 3049 (aromatic C–H), 2946, 2877 (aliphatic C–H), 1710 (C═O); HRMS calcd for $C_{43}H_{46}N_4O_7$ ($M+H^+$) 731.3445, found 731.3444.

5.8. (5-{4-(3-[*N*,*N*′-Bis(benzyloxycarbonyl)-guanidino-propoxy])-[4-(4-benzyloxyphenoxy)-phenylcarbonoyl]-phenylcarbamoyl}-pentyl)-carbamic acid benzyl ester (5c)

Crude amine (**5b**) (0.106 g, 0.12 mmol) was dissolved in MeOH (4 mL) with DCM (2 mL) added to aid dissolution. Triethylamine was then added (0.05 mL, 0.51 mmol), followed by *N*,*N*′-bis(benzyloxycarbonyl)-1*H*-pyrazole-1-caboxamidine (38.3 mg, 0.10 mmol). The reaction mixture was stirred at room temperature for 8 h during which a white precipitate formed. The precipitate was filtered off and the filtrate concentrated to afford more precipitate. The combined precipitate was dissolved in DCM (15 mL) and washed with 2 M HCl (3×10 mL) followed by water (20 mL) and saturated brine (2×10 mL). The product was purified by radial chromatography using a 1:4 EtOAc/DCM solvent system to give **5c** as a white amorphous solid (56 mg, 44%) which solidified only after an extended period under vacuum (25 °C, 0.1 mmHg). Mp 114–116 °C; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 1.36 (p, J=8.4 Hz, 2H), 1.51 (p, J=7.5 Hz, 2H), 1.70 (p, J=7.5 Hz, 2H), 2.05 (p, J=6.3 Hz, 2H), 2.35 (p, J=7.5 Hz, 2H), 3.16 (q, J=6.4 Hz, 2H), 3.66 (q, J=6.0 Hz, 2H), 4.06 (t, J=5.6 Hz, 2H), 5.05 (s, 2H), 5.06 (s, 2H), 5.06 (s, 2H), 5.09 (s, 2H), 6.90–6.93 (m, 6H), 7.20–7.45 (m, 25H); ^{13}C NMR-APT (75 MHz, $CDCl_3$) δ ppm: 25.0 (CH_2), 26.2 (CH_2), 28.4 (CH_2), 29.6 (CH_2), 37.9 (CH_2), 39.4 (CH_2), 40.8 (CH_2), 66.5 (CH_2), 67.0 (CH_2), 67.3 (CH_2), 38.2 (CH_2), 70.5 (CH_2), 114.1 (CH), 115.9 (2CH), 117.2 (CH), 118.2 (2CH), 120.5 (2CH), 122.8 (2CH), 123.3 (CH), 127.5 (4CH), 127.9 (2CH), 127.9 (2CH), 128.0 (2CH), 128.1 (2CH), 128.3 (2CH), 128.4 (2CH), 128.5 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 132.0 (C), 132.7 (C), 134.3 (C), 136.5 (C), 136.7 (C), 136.9 (2C), 150.3 (C), 153.7 (C), 153.9 (C), 155.1 (C), 155.6 (C), 155.8 (C), 156.4 (C), 163.4 (C), 167.1 (C), 171.6 (C); ATR (neat) cm^{-1}: 3228 (N–H), 3078, 3036 (aromatic C–H), 3743, 3871 (aliphatic C–H), 1729, 1679 (C═O), 1500 (C═N); HRMS calcd for $C_{60}H_{60}N_6O_{11}$ ($M+Na^+$) 1063.4218, found 1063.4220.

5.9. 5-Fluoro-2-nitrobenzoic acid (7)

2-Fluorobenzoic acid (1.807 g, 12.9 mmol) was dissolved in concentrated H_2SO_4 (100 mL) after which P_2O_5 (~8 g) was added. The mixture was then cooled to 0 °C and concentrated HNO_3 (15 mL) was added. After stirring for 3 h at 0 °C, the reaction mixture was poured into iced water (500 mL) and filtered. The precipitate was dissolved in DCM (30 mL) and the filtrate was extracted with DCM (3×30 mL). The combined DCM extracts were washed with water (2×30 mL) and saturated brine (2×40 mL). After drying ($MgSO_4$) and filtering, DCM was removed under reduced pressure to give **7** as a white solid (2.280 g, 95%). Mp 136–138 °C (lit.[19] 138–139 °C); ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.37 (m, 1H), 7.53 (dd, J=2.7, 7.8 Hz, 1H), 8.00 (dd, J=4.5, 9.0 Hz, 1H); ^1H (^{19}F-decoupled) NMR (300 MHz, $CDCl_3$) δ ppm: 7.37 (dd, J=1.8, 8.9 Hz, 1H), 7.52 (d, J=2.7 Hz, 1H), 8.00 (d, J=9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 117.5 (d, J=25.4 Hz), 119.2 (d, J=23.1 Hz), 126.7 (d, J=9.4 Hz), 129.0 (d, J=8.6 Hz), 144.3 (s), 164 (d, J=256.7 Hz), 168.4 (s).

5.10. *N*-[4-(4-Benzyloxyphenoxy)-phenyl]-5-fluoro-2-nitrobenzamide (8)

4-(4-Benzyloxyphenoxy)-phenylamine (**6**) (0.889 g, 3 mmol) and 5-fluoro-2-nitrobenzoic acid (**7**) (0.558 g, 3 mmol) were dissolved in DMF (15 mL). Triethylamine (1.2 mL, 12 mmol) was added followed by HBTU (1.14 g, 3 mmol). The reaction mixture was stirred for 5 h at room temperature then poured into 2 M HCl (40 mL). The acidic mixture was then extracted with EtOAc (3×30 mL). The combined organic extracts were washed with water (2×25 mL), saturated brine (2×20 mL), dried ($MgSO_4$) and filtered. The EtOAc solution was then concentrated to one-third volume and cooled in ice to afford the coupled product as a beige coloured precipitate which was filtered off (0.940 g, 68%). Mp 177–179 °C; ^1H (^{19}F-decoupled) NMR (300 MHz, $CDCl_3$) δ ppm: 5.07 (s, 2H), 6.98 (s, 4H), 7.00 (s, 1H), 7.28–7.53 (m, 10H), 8.22 (d, J=9.0 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 70.52 (CH_2), 116.8 (2CH), 117.5 (d, J=25.7 Hz, CH), 118.5 (d, J=23.3 Hz, CH), 119.0 (2CH), 120.8 (2CH), 122.2 (2CH), 128.4 (CH), 128.5 (2CH), 128.6 (CH), 129.2 (2CH), 134.5 (C), 136.4 (C), 137.9 (C), 143.5 (d, J=3.1 Hz, C), 151.0 (C), 154.6 (C), 155.2 (C), 163.2 (C), 164.9 (d, J=253.6 Hz, C); ATR (neat) cm^{-1}: 3250 m, 3070 m, 2940 w, 2870 w, 1650 s, 1550 s, 1490 s, 1340 s, 1210 s, 1010 m, 820 m, 740 w; MS (ESI): *m/z* 459.2 $[M+H]^+$. HRMS: Found 481.1177 (requires 481.1176 for $[C_{26}H_{19}FN_2O_5]Na^+$). Microanalysis: Found

(%) C 68.07 H 4.12 N 6.08 (requires (%) C 68.12 H 4.18 N 6.11 for $C_{26}H_{19}FN_2O_5$).

5.11. *N*-[4-(4-Benzyloxyphenoxy)-phenyl]-2-nitro-5-(2-pyridin-2-yl-ethoxy)-benzamide (9)

This was obtained only in low yields from the reaction of 2-(2-hydroxyethyl)pyridine with **8**, as described earlier for the synthesis of **3**. Mp 99–102 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.31 (t, J=6. 5 Hz, 2H), 4.52 (t, J=6. 5 Hz, 2H), 5.05 (s, 2H), 6.95–7.05 (m, 9H), 7.19–7.52 (m, 8H), 7.67 (dt, J= 1.8, 7.8 Hz, 1H), 8.14 (d, J=8.7 Hz, 1H), 8.54 (dd, J=0.9, 5.7 Hz, 1H); ^{13}C NMR-APT (75 MHz, $CDCl_3$) δ ppm: 37.4 (CH_2), 68.0 (CH_2), 70.5 (CH_2), 114.2 (CH), 115.3 (CH), 115.8 (CH), 118.2 (CH), 120.2 (CH), 121.9 (CH), 122.2 (CH), 123.8 (CH), 127.0 (CH), 127.4 (CH), 127.9 (CH), 128.4 (CH), 132.1 (C), 135.1 (C), 136.6 (CH), 136.8 (C), 138.3 (C), 149.1 (CH), 150.4 (C), 154.8 (C), 155.1 (C), 157.1 (C), 162.8 (C), 164.4 (C); ATR (neat) cm^{-1}: 3283, 3065 (aromatic C–H), 2997, 2941 (aliphatic C–H), 1620 (C═O), 1582 (C═N); MS (ESI) (M+H$^+$) 562.5; HRMS calcd for $C_{33}H_{27}N_3O_6$ (M+Na$^+$) 548.17975, found 584.1793.

5.12. (5-{4-(3-[*N*,*N*′-Bis(*tert*-butoxycarbonyl)-guanidino-propoxy])-[4-(4-benzyloxyphenoxy)-phenylcarbonoyl]-phenylcarbamoyl}-pentyl)-carbamic acid benzyl ester (10)

The protected anthranilamide derivative (**5b**) (55 mg, 0.064 mmol) was stirred with 1-*H*-pyrazole-1-(*N*,*N*′-bis-(*tert*-butoxycarbonyl)]-caboxamidine (20 mg, 0.064 mmol) in DCM (5 mL) overnight. The DCM was then removed under reduced pressure. Excess guanidylating agent was removed by triturating with hexanes (3×3 mL). After purification of the residue by radial chromatography using a 50:50 EtOAc/hexanes solvent system, **10** was obtained as a white amorphous solid (24 mg, 39%). Mp 56–59 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.43, 1.45 (s, 18H), 1.34 (p, J=7.2 Hz, 2H), 1.49 (p, J=7.2 Hz, 2H), 1.65 (p, J=7.2 Hz, 2H), 2.02 (p, J=7.2 Hz, 2H), 2.31 (t, J=7.5 Hz, 2H), 3.14 (t, J=6.4 Hz, 2H), 3.58 (t, J=6.0 Hz, 2H), 4.03 (t, J= 5.4 Hz, 2H), 5.29 (s, 2H), 5.31 (s, 2H), 6.94–6.97 (m, 6H), 7.10–7.54 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 25.1 (CH_2), 26.3 (CH_2), 28.1 ($3CH_3$), 28.3 ($3CH_3$), 28.7 (CH_2), 29.7 (CH_2), 37.9 (CH_2), 39.1 (CH_2), 40.9 (CH_2), 66.5 (CH_2), 67.0 (CH_2), 70.5 (CH_2), 79.4 (C), 83.1 (C), 113.7 (CH), 115.8 (2CH), 117.3 (CH), 118.2 (2CH), 120.4 (2CH), 122.6 (2CH), 122.9 (CH), 123.2 (CH), 127.4 (2CH), 127.7 (2CH), 128.4 (2CH), 128.5 (3CH), 132.1 (C), 132.3 (C), 136.5 (C), 136.8 (C), 150.3 (C), 152.9 (C), 153.9 (2C), 154.9 (C), 155.3 (C), 156.0 (C), 156.2 (C), 163.3 (C), 167.0 (C), 171.4 (C); ATR (neat) cm^{-1}: 3314 (N–H), 2925, 2856 (aliphatic C–H), 1718, 1607 (C═O), 1494 (C═N); HRMS calcd for $C_{54}H_{64}N_6O_{11}$ (M+H$^+$) 973.4711, found 973.4713.

5.13. 2-(6-Aminohexanoylamino)-5-(3-guanidino-propoxy)-benzoic acid (11)

To the bis-Boc protected compound (**10**) (25.6 mg, 26 μmol) was added thioanisole (0.15 mL, 1.3 mmol) and TFA (1 mL). The clear, colourless solution turned red upon addition of TFA. The resulting mixture was stirred at room temperature for 8 h. The solvent was then removed under reduced pressure and the residue triturated with hexanes (6×5 mL) followed by ether (4×5 mL) to remove any residual thioanisole. The crude compound was dissolved in DCM and purified by flushing through a silica plug with a 50:50 EtOAc/hexanes eluant. The most polar band was eluted with MeOH to give **11** (5.6 mg, 58%) as a brown solid. Mp 99–102 °C; ^{1}H NMR (300 MHz, methanol-d_4) δ ppm: 1.51 (p, J=6.3 Hz, 2H), 1.78 (m, 4H), 2.09 (q, J=6.3 Hz, 2H), 2.47 (t, J=7.4 Hz, 2H), 2.97 (t, J=7.4 Hz, 2H), 3.44 (t, J=6.8 Hz, 2H), 4.11 (t, J=5.7 Hz, 2H), 7.09 (d, J=9.0 Hz, 1H), 7.64 (s, 1H), 8.43 (d, J=9.0 Hz, 1H); ^{13}C NMR (75 MHz, methanol-d_4; 2 ArC remain unassigned) δ ppm: 26.9 (CH_2), 27.69 (CH_2), 29.09 (CH_2), 30.59 (CH_2), 39.59 (CH_2), 40.59 (CH_2), 41.39 (CH_2), 67.29 (CH_2), 118.3 (CH), 119.7 (C), 120.3 (CH), 123.6 (CH), 131.8 (C), 156.2 (C), 159.7 (C); ATR (neat) cm^{-1}: 3440 (O–H), 3166, 3063 (aromatic C–H), 2952, 2923, 2868 (aliphatic C–H), 1660, 1589 (C═O), 1449 (C═N); HRMS calcd for $C_{17}H_{27}N_5O_4$ (M+H$^+$) 366.21423, found 366.2153.

6. Biological methods

Peptide synthesis, radiolabelling of the peptides with ^{125}I and rat brain preparation were conducted following previously described procedures.[20–22] Radioligand binding assays were run in triplicate in 96-well plates at room temperature. Each well of the 96-well plate (Polystyrene, Round bottom, Nunc™, Denmark) contained compound **2a** or **2b** (first dilution 0.6 μM of compound total of seven dilutions, 1:10), 5–10 fmol of radiolabelled peptide (^{125}I-GVIA) and 8 μg of crude rat membrane (added last). All dilutions were made in assay buffer (20 mM HEPES, 75 mM NaCl, 0.2 mM EDTA, 0.2 mM EGTA, 2 μM Leupeptin, 2 μL apoprotinin (to 30 mL assay buffer), 0.1% BSA, pH 7.4) and the final volume in each well was 150 μL. The plate was left on a shaker for 1 h at room temperature before being filtered. Incubation was terminated by washing the plate with wash buffer (20 mM HEPES, 125 mM NaCl, pH 7.4) and filtered under vacuum (Tomtec). The glass fibre filter used (90×120 mm, double thickness, Wallac, Finland) was soaked in 0.6% polyethyleneimine immediately prior to filtering to reduce non-specific binding. The filter was put in a Sample Bag (Wallac, Finland) containing 8 mL BetaPlate Scint (Wallac, Finland) and the radioactivity bound to the filter was counted using a 1450 MicroBeta Wallac Jet (Wallac, Finland). The data were analysed using GraphPad Prism 2.0 (GraphPad Software, Inc, San Diego, USA).

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References and notes

1. Norton, R. S.; Baell, J. B.; Angus, J. A. *Calcium Channel Pharmacology*; McDonough, S. I., Ed.; Kluwer/Academic/Plenum: New York, NY, 2003; pp 143–181.

2. Staats, P. S.; Yearwood, T.; Charapata, S. G.; Presley, R. W.; Wallace, M. S.; Byas-Smith, M.; Fisher, R.; Bryce, D. A.; Mangieri, E. A.; Luther, R. R.; Mayo, M.; McGuire, D.; Ellis, D. *J. Am. Med. Assoc.* **2004**, *291*, 63–70.
3. Smith, M. T.; Cabot, P. J.; Ross, F. B.; Robertson, A. D.; Lewis, R. J. *Pain* **2002**, *96*, 119–127.
4. Taken from a Frontline Strategic Consulting Report. See: (a) http://66.102.7.104/search?q=cache:ooJ21LCiKkgJ:www.frontlinesmc.com/SMR/PainManagement/painPR.pdf+Pain+and+compounded+annual+growth+rate+(CAGR)+of+%2510+to+reach+%2429.8+in+2008&hl=en; (b) http://www.newsrx.com/newsletters/Pharma-Business-Week/2003-04-28/0428200333327PB.html; accessed 5th May 2006.
5. McGivern, J. G. *Drug Discov. Today* **2006**, *11*, 245–253.
6. Baell, J. B.; Duggan, P. J.; Lok, Y. P. *Aust. J. Chem.* **2004**, *57*, 179–185.
7. Baell, J. B.; Duggan, P. G.; Forsyth, S. A.; Lewis, R. J.; Lok, Y. P.; Schroeder, C. I. *Bioorg. Med. Chem.* **2004**, *12*, 4025–4037.
8. Cohen, C. J.; Kraus, R. L. *Calcium Channel Pharmacology*; McDonough, S. I., Ed.; Kluwer/Academic/Plenum: New York, NY, 2003; pp 73–93.
9. Baell, J. B.; Forsyth, S. A.; Gable, R. W.; Norton, R. S.; Mulder, R. J. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 1119–1136.
10. Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093–2096.
11. Farmer, J. D.; Rudnicki, S. M.; Suggs, J. W. *Tetrahedron Lett.* **1988**, *29*, 5105–5108.
12. Anderson, J. S.; Brown, K. C. *Synth. Commun.* **1983**, *13*, 233–236.
13. Itoh, T.; Mase, T. *Org. Lett.* **2004**, *6*, 4587–4590.
14. Kiso, Y.; Ukawa, K.; Nakamura, S.; Ito, K.; Akita, T. *Chem. Pharm. Bull.* **1980**, *28*, 673–676.
15. Baell, J. B.; Harvey, A. J.; Norton, R. S. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 245–262.
16. Harvey, A. J.; Gable, R. W.; Baell, J. B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3193–3196.
17. Lauri, G.; Bartlett, P. A. *J. Comput.-Aided Mol. Des.* **1994**, *8*, 51–66.
18. Baell, J. B.; Lessene, G. PCT/AU2005/000968, 2005.
19. Kissener, W.; Kuprat, J.; Emde, H; Paetz, K. C. U.S. Patent 5,756,831, 1998.
20. Kristipati, R.; Nadasdi, L.; Tarczy-Hornoch, K.; Lau, K.; Miljanich, G. P.; Ramachandran, J.; Bell, J. R. *Mol. Cell Neurosci.* **1994**, *5*, 219–228.
21. Lewis, R. J.; Nielsen, K. J.; Craik, D. J.; Loughnan, M. L.; Adams, D. A.; Sharpe, I. A.; Luchian, T.; Adams, D. J.; Bond, T.; Thomas, L.; Jones, A.; Matheson, J. L.; Drinkwater, R.; Andrews, P. R.; Alewood, P. F. *J. Biol. Chem.* **2000**, *275*, 35335–35344.
22. Nadasdi, L.; Yamashiro, D.; Chung, D.; Tarczy-Hornoch, K.; Adriaenssens, P.; Ramachandran, J. *Biochemistry* **1995**, *34*, 8076–8081.

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Ceric Ammonium Nitrate (CAN) catalyzes the one-pot synthesis of polyhydroquinoline via the Hantzsch reaction

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Abstract—A facile and efficient one-pot synthesis of high yields of polyhydroquinoline derivatives at ambient temperature using Ceric Ammonium Nitrate (CAN) as catalyst via the Hantzsch reaction was reported. The process is simple and environmentally benign and the catalyst is commercially available and inexpensive.

1. Introduction

In recent years, much attention has been focused on the synthesis of 1,4-dihydropyridyl compounds, due to their significant biological activity.[1] Cardiovascular agents such as nifedipine, nicardipine, amlodipine, and other related derivatives are dihydropyridyl compounds, which are effective for the treatment of hypertension.[2] 4-Aryl-1,4-dihydropyridines are analogues of NADH coenzymes, which have been explored for their calcium channel activity and the heterocyclic rings are found in a variety of bioactive compounds such as bronchodilator, antiatherosclerotic, antitumour, vasodilator, antidiabetic, geroprotective, and heptaprotective agents.[3] Extensive studies indicate that these compounds exhibit different medical functions, acting as neuroprotectants, platelet antiaggregators, cerebral antiischemic agents, and chemosensitizers.[4] For these reasons, polyhydroquinoline compounds not only have attracted the attention of chemists to synthesize but also represent an interesting research challenge. Numerous methods have been reported for the synthesis of polyhydroquinoline derivatives, because of the biological importance associated with these compounds. The classical method involves the three-component coupling of an aldehyde with ethyl acetoacetate, and ammonia in acetic acid or in refluxing alcohol.[5,6] However, these methods suffer from several drawbacks such as a long reaction time, an excess of organic solvent, lower product yields, and harsh refluxing conditions. Thus, chemists have developed several alternate and more efficient methods for the synthesis of polyhydroquinoline derivatives, which include the use of microwaves,[7] ionic liquids,[8] refluxing at high temperature,[9] TMSCl–NaI,[10] metal triflates,[11] and I_2.[12] However, the use of high temperatures, expensive metal precursors, catalyst that are harmful to the environment, and long reaction times limits the use of these methods. Thus, the development of a simple and efficient method for the preparation of polyhydroquinoline derivatives is an active area of research and there is scope for further improvement involving milder reaction conditions and higher product yields.

The use of Ceric Ammonium Nitrate (CAN) has recently received considerable attention as an inexpensive, nontoxic, commercially available catalyst for various organic transformations to afford the corresponding products in excellent yields. Due to the numerous advantages associated with this eco-friendly compound, CAN has been explored as a powerful catalyst for different reactions, such as oxidation, nitration, 1,3-dipolar cycloaddition, thiocyanation, protection, esterification, 1,4-addition, and the Biginelli reaction.[13] Because polyhydroquinoline derivatives are important biologically active compounds, which have potential medical applications, improvement and the development of a preparation of this type of compound using CAN are worthy of study.

2. Results and discussion

We had the opportunity to further explore the catalytic activity of CAN in the synthesis of 1,4-dihydropyridines. Herein, we wish to report on a novel synthesis of 1,4-DHP promoted by a catalytic amount of CAN under ambient conditions to give excellent yields. In an initial endeavor, 1 equiv each of benzaldehyde **1a**, 1,3-cyclohexanedione **2**, ethyl acetoacetate **3a**, and ammonium acetate **4** were stirred at ambient temperature in ethanol. After 4 h, only 56% of the expected

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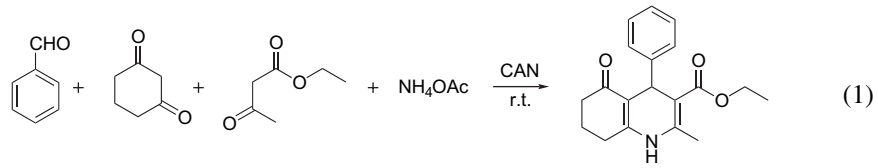
product **5aa** was obtained when after workup and recrystallization of the crude product from ethanol (Eq. 1 and entry 1 of Table 1). To improve the yield and optimize the reaction conditions, the same reaction was carried out in the presence of a catalytic amount of 2 mol % of CAN under similar conditions. Surprisingly, a significant improvement was observed and the yield of **5aa** was dramatically increased to 93% after stirring; the mixture was stirred for only 2 h (entry 2). With this optimistic result in hand, we further investigated the best reaction conditions by using different amounts of CAN. An increase in the quantity of CAN from 2 mol % to 5 mol % not only decreased the reaction time from 2 h to 1.5 h, but also increased the product yield slightly from 93% to 98% (entry 3). Although the use of 10 mol % of CAN permitted the reaction time to be decreased to 1 h, the yield unexpectedly decreased to 65% (entry 4). A possible explanation for the low product yield is that the starting material or the product may have been destroyed during the reaction when excess amount (10 mol %) of CAN was used in the exothermic reaction and that 5 mol % CAN was sufficient to catalyze the reaction effectively.

Based on above observations, we conducted the same reactions using aromatic and heteroaromatic aldehydes **1b**–**1k**, **2**, **3a**, and **4** in the presence of 5 mol % of CAN under similar conditions. As expected, satisfactory results were observed and the results are summarized as Eq. 2 and Table 2. Both aromatic (entries 1–9) and heteroaromatic aldehydes (entries 10 and 11) gave the corresponding products in good yields (88–98%). Concerning aromatic aldehyde, it appears that the presence of different or the same substituted groups does not have a significant effect on the final products.

We next tried to observe the effect of substituents in 1,3-cyclohexanedione **2** using 5,5-dimethyl-1,3-cyclohexanedione **6**. Both aromatic and heteroaromatic aldehydes **1** reacted well with **6**, **3a**, and **4** in the presence of 5 mol % of CAN to afford **7** in good to high yields under similar conditions (Table 3). Compared to the results shown in Table 2, the decrease in reaction time can be explained by the assumption that **6** is slightly more reactive than **2** in most cases due to the presence of the methyl groups in the ring.

Finally, we also examined whether other β-keto compounds such as 2,4-pentanedione **3b**, methyl acetoacetate **3c**, and 2-methoxyethyl acetoacetate **3d** also react with these reagents to produce similar results. Aromatic and heteroaromatic aldehydes **1** such as benzaldehyde **1a** and 2-thiophenecarboxaldehyde **1k** were reacted with **2**, **3b**–**3d**, and **4** in the presence of 5 mol % of CAN to afford the expected product

Table 1. Optimizing the reaction conditions[a] **(1)**



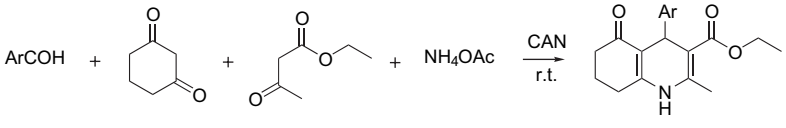
(1)

1a **2** **3a** **4** **5aa**

Entry	CAN (equiv)	Time (h)	Yield (%)[b]
1	0	4	56
2	0.02	2	93
3	0.05	1.5	98
4	0.1	1	65

[a] Benzaldehyde/1,3-cyclohexanedione/ethyl acetoacetate/ammonium acetate = 1:1:1:1.
[b] Isolated yields.

Table 2. CAN catalyzed the synthesis of polyhydroquinoline derivatives through Hantzsch reaction **(2)**



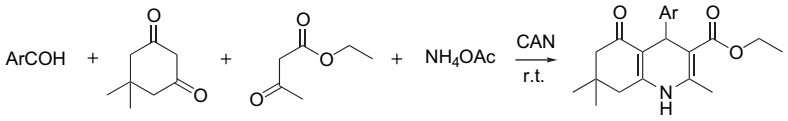
(2)

1 **2** **3a** **4** **5**

Entry	1	Ar	Time	Product	Yield (%)[a]	Mp (°C)
1	**1a**	Ph	1.5 h	**5aa**	98	240–241
2	**1b**	*p*-MeC_6H_4	1.5 h	**5ba**	98	241–242
3	**1c**	*p*-$OMeC_6H_4$	2.5 h	**5ca**	88	193–195
4	**1d**	*p*-FC_6H_4	2 h	**5da**	98	243–244
5	**1e**	*p*-ClC_6H_4	4 h	**5ea**	90	234–235
6	**1f**	*p*-HOC_6H_4	2.5 h	**5fa**	93	220–222
7	**1g**	*o*-$NO_2C_6H_4$	50 min	**5ga**	93	190–191
8	**1h**	*m*-$NO_2C_6H_4$	50 min	**5ha**	98	198–200
9	**1i**	*p*-$NO_2C_6H_4$	25 min	**5ia**	98	204–205
10	**1j**	2-Furyl	35 min	**5ja**	90	210–212
11	**1k**	2-Thienyl	25 min	**5ka**	90	233–234

[a] Isolated yields.

Table 3. CAN catalyzed the synthesis of polyhydroquinoline derivatives through Hantzsch reaction with 5,5-dimethyl-1,3-cyclohexanedione **(3)**



(3)

1 **6** **3a** **4** **7**

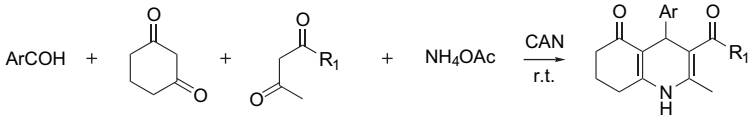
Entry	1	Ar	Time	Product	Yield (%)[a]	Mp (°C)
1	**1a**	Ph	1 h	**7aa**	92	209–210
2	**1c**	*p*-$MeOC_6H_4$	1.75 h	**7ca**	98	243–245
3	**1e**	*p*-ClC_6H_4	35 min	**7ea**	88	230–232
4	**1f**	*p*-HOC_6H_4	2 h	**7fa**	93	237–238
5	**1k**	2-Thienyl	1 h	**7ka**	85	224–226

[a] Isolated yields.

8 in medium to high yields (Table 4). It was surprising to find that the use of **3b** only led to medium yields (60–65%) of products (entries 1 and 4) compared to substrates **3c** and **3d** (entries 2, 3, 5, and 6). A possible explanation of the differences is that **3b** (approximate pK_a value of 9) is much more reactive than **3c** and **3d** (approximate pK_a value of 11) so that the other side reactions might have occurred or side reaction products also could have been formed when **3b** was used.

It is important to understand the role of CAN in the reaction. One possibility is that both Ce(IV) and NH_4^+ in CAN can be used as Lewis acids to catalyze the reaction. To prove this assumption, different Lewis acids such as CeF_4, NH_4Cl, and $CeCl_3\cdot 7H_2O$ were used in the same reactions (Table 5). Surprisingly, CeF_4 and NH_4Cl catalyzed the reaction more efficiently than $CeCl_3\cdot 7H_2O$. The reaction completed more rapidly and the yields were also higher when CeF_4 and NH_4Cl were used (entries 1 and 2). These results indicate that both cerium (IV) ions and ammonium ions, which are present in CAN actually can also catalyze the reaction. Although $CeCl_3\cdot 7H_2O$ can also induce the reaction, the results were not as good as the two other reagents.

Table 4. CAN catalyzed the synthesis of polyhydroquinoline derivatives through Hantzsch reaction with different β-keto compounds **(4)**



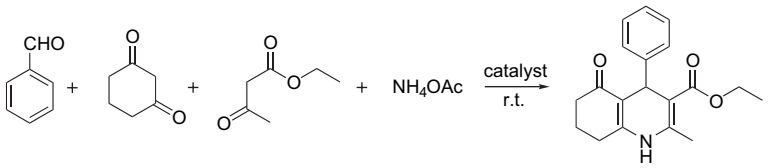
(4)

1 **2** **3b-d** **4** **8**

Entry	1	3	R_1	Time (h)	Product	Yield (%)[a]	Mp (°C)
1	**1a**	**3b**	CH_3	1	**8ab**	65	229–230
2	**1a**	**3c**	OMe	1	**8ac**	85	222–224
3	**1a**	**3d**	OCH_2CH_2OMe	1	**8ad**	87	150–152
4	**1k**	**3b**	CH_3	5	**8kb**	60	218–219
5	**1k**	**3c**	OMe	2	**8kc**	90	216–217
6	**1k**	**3d**	OCH_2CH_2OMe	7	**8kd**	64	207–209

[a] Isolated yields.

Table 5. To address the role of CAN in this reaction[a] **(5)**



(5)

1a **2** **3a** **4** **5aa**

Entry	Catalyst (equiv)	Time (h)	Yield (%)[b]
1	CeF_4 (0.05)	1.75	90
2	NH_4Cl (0.1)	2	85
3	$CeCl_3\cdot 7H_2O$ (0.05)	4	60

[a] Benzaldehyde/1,3-cyclohexanedione/ethyl acetoacetate/ammonium acetate = 1:1:1:1.
[b] Isolated yields.

3. Conclusion

In conclusion, we successfully developed a facile and efficient method for preparing a variety of 4-substituted-1,4-dihydropyridines from the reactions of different aromatic or heteroaromatic aldehydes, β-keto compounds, including 1,3-cyclohexanedione, 5,5-dimethyl-1,3-cyclohexanedione, or 2,4-pentadione, and alkyl acetoacetate, and ammonium acetate in the presence of a catalytic amount of CAN at room temperature. The catalytic activity of CAN is remarkable and the use of the environmentally benign, commercially available CAN as catalyst in the synthesis of 4-substituted-1,4-dihydropyridines in good yields is also significant. The advantages such as shorter reaction times, milder conditions, simplicity of the reaction, good product yields, and the easy procedures involved in the reaction make the inexpensive and commercially available CAN a powerful catalyst for the synthesis of different organic compound.

4. Experimental

4.1. Material and general

All reactions were performed at room temperature. All chemicals were purchased from Aldrich Chemical Co. and the solvent were used directly without further purification. Analytical thin-layer chromatography was performed with E. Merck silica gel 60F glass plates. MS or HRMS were measured by JEOL JMS-D300 or JEOL JMS-HX110 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with Bruker Aavance EX 400.

4.1.1. Typical experimental procedure for the synthesis of Hantzsch polyhydroquinoline derivatives 5, 7, and 8. A typical experimental procedure for the preparation of **5** is described as follows: a 10 mL round-bottomed flask charged with aldehyde **1** (1.0 mmol), 1,3-cyclohexanedione **2** or 5,5-dimethyl-1,3-cyclohexanedione **6** (1.0 mmol), 2,4-pentadione or acetoacetate derivatives **3** (1.0 mmol), ammonium acetate **4** (1.0 mmol), and Ceric Ammonium Nitrate (CAN) (0.05 mmol) followed by 0.5 mL of ethanol. The mixture was then stirred at room temperature until the reaction was completed (monitored by TLC). The reaction mixture was treated with brine solution, extracted with ethyl acetate (2×20 mL). After evaporation of the solvent, the crude yellow product was recrystallized from ethanol to give a yellow or brown solid.

4.1.1.1. 2-Methyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5aa). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (t, 3H, J=6.8 Hz), 1.80–2.10 (m, 2H), 2.30–2.44 (m, 7H), 4.05 (q, 2H, J=6.8 Hz), 5.09 (s, 1H), 6.07 (s, 1H), 7.10 (t, 1H, J=7.6 Hz), 7.20 (t, 2H, J=7.6 Hz), 7.30 (d, 2H, J=7.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.16, 19.34, 21.01, 27.46, 36.38, 37.00, 59.78, 106.06, 113.46, 125.99, 127.90, 127.98, 143.30, 147.12, 149.58, 167.41, 195.52. MS *m/z* (relative intensity) 311 (M^+, 27), 282 (8), 235 (15), 234 (100), 206 (27). HRMS calcd for $C_{19}H_{21}NO_3$ (M^+) 311.1521; found 311.1526.

4.1.1.2. 2-Methyl-5-oxo-4-(4-methylphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ba). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.21 (t, 3H, J=7.2 Hz), 1.80–2.10 (m, 2H), 2.20–2.51 (m, 10H), 4.07 (q, 2H, J=7.2 Hz), 5.05 (s, 1H), 7.00 (d, 2H, J=7.6 Hz), 7.18 (d, 2H, J=7.6 Hz), 7.36 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.15, 18.92, 20.93, 26.93, 35.88, 37.03, 59.66, 105.71, 112.68, 127.69, 128.51, 129.56, 135.28, 143.87, 144.42, 151.42, 167.63, 196.29. MS *m/z* (relative intensity) 325 (M^+, 33), 296 (10), 235 (15), 234 (100), 206 (22). HRMS calcd for $C_{20}H_{23}NO_3$ (M^+) 325.1678; found 325.1685.

4.1.1.3. 2-Methyl-5-oxo-4-(4-methoxyphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ca). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (t, 3H, J=6.8 Hz), 1.80–2.10 (m, 2H), 2.30–2.60 (m, 7H), 3.74 (s, 3H), 4.06 (q, 2H, J=6.8 Hz), 5.04 (s, 1H), 5.95 (s, 1H), 7.10 (d, 2H, J=8.4 Hz), 7.20 (d, 2H, J=8.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.20, 19.36, 21.05, 27.49, 35.52, 37.02, 55.12, 59.77, 106.33, 113.29, 113.72, 128.94, 139.67, 142.92, 149.20, 157.80, 167.47, 195.71. MS *m/z* (relative intensity) 341 (M^+, 52), 312 (20), 268 (16), 235 (15), 234 (100), 206 (33). HRMS calcd for $C_{20}H_{23}NO_4$ (M^+) 341.1627; found 341.1623.

4.1.1.4. 2-Methyl-5-oxo-4-(4-fluorophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5da). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.17 (t, 3H, J=7.2 Hz), 1.80–2.10 (m, 2H), 2.20–2.70 (m, 7H), 4.06 (q, 2H, J=7.2 Hz), 5.07 (s, 1H), 6.03 (s, 1H), 6.85–6.89 (m, 2H), 7.23–7.27 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.60, 18.72, 21.26, 26.58, 35.55, 37.15, 59.54, 103.89, 111.52, 114.78, 114.99, 129.54, 129.62, 144.49, 145.57, 151.90, 167.27, 195.16. MS *m/z* (relative intensity) 329 (M^+, 36), 235 (15), 234 (100), 206 (28). HRMS calcd for $C_{19}H_{20}NFO_3$ (M^+) 329.1427; found 329.1425.

4.1.1.5. 2-Methyl-5-oxo-4-(4-chlorophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ea). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (t, 3H, J=7.2 Hz), 1.80–2.10 (m, 2H), 2.30–2.50 (m, 7H), 4.05 (q, 2H, J=7.2 Hz), 5.05 (s, 1H), 6.34 (s, 1H), 7.18 (d, 2H, J=8.8 Hz), 7.24 (d, 2H, J=8.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.17, 19.33, 20.96, 27.37, 36.07, 36.87, 59.93, 105.77, 112.96, 128.01, 129.40, 131.61, 143.53, 145.63, 150.29, 167.22, 195.92. MS *m/z* (relative intensity) 345 (M^+, 24), 316 (12), 235 (16), 234 (100), 206 (36). HRMS calcd for $C_{19}H_{20}ClNO_3$ (M^+) 345.1132; found 345.1130.

4.1.1.6. 2-Methyl-5-oxo-4-(4-hydroxyphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5fa). ^{1}H NMR (Acetone-d_6, 400 MHz) δ 1.17 (t, 3H, J=7.2 Hz), 1.70–2.00 (m, 2H), 2.19–2.23 (m, 1H), 2.34 (s, 3H), 2.50–2.54 (m, 2H), 4.02 (q, 2H, J=7.2 Hz), 5.05 (s, 1H), 6.63 (d, 2H, J=8.4 Hz), 7.08 (d, 2H, J=8.4 Hz). ^{13}C NMR (Acetone-d_6, 100 MHz) δ 15.07, 19.12, 22.41, 27.75, 36.57, 38.23, 60.24, 106.30, 113.84, 115.66, 130.14, 140.66, 145.27, 151.47, 156.63, 168.50, 195.72. MS *m/z* (relative intensity) 327 (M^+, 37), 298 (15), 254 (12), 235 (15), 234 (100), 206 (35). HRMS calcd for $C_{19}H_{21}NO_4$ (M^+) 327.1471; found 327.1465.

4.1.1.7. 2-Methyl-5-oxo-4-(2-nitrophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ga). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.10 (t, 3H, J=7.2 Hz), 1.70–2.00 (m, 2H), 2.20–2.50 (m, 7H), 4.02–4.09 (m, 2H), 5.87 (s, 1H), 7.22–7.24 (m, 1H), 7.39 (s, 1H), 7.40–7.60 (m, 2H), 7.69 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.82, 18.66, 20.70, 26.68, 32.23, 36.63, 59.67, 104.55, 111.86, 123.49, 126.41, 131.01, 132.49, 141.75, 145.20, 148.12, 152.27, 167.22, 196.11. MS *m*/*z* (relative intensity) 356 (M^+, 26), 327 (12), 235 (16), 234 (100), 206 (36). HRMS calcd for $C_{19}H_{20}N_2O_5$ (M^+) 356.1372; found 356.1380.

4.1.1.8. 2-Methyl-5-oxo-4-(3-nitrophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ha). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.19 (t, 3H, J=7.2 Hz), 1.80–2.10 (m, 2H), 2.30–2.50 (m, 7H), 4.07 (q, 2H, J=7.2 Hz), 5.18 (s, 1H), 6.88 (s, 1H), 7.35–7.40 (m, 1H), 7.72 (d, 2H, J=8.0 Hz), 7.98 (d, 2H, J=8.0 Hz), 8.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.19, 19.30, 21.02, 27.24, 33.01, 36.88, 60.07, 105.77, 112.29, 121.26, 122.89, 128.65, 134.83, 144.63, 148.29, 149.42, 151.03, 167.02, 196.03. MS *m*/*z* (relative intensity) 356 (M^+, 12), 339 (15), 235 (15), 234 (100), 206 (31). HRMS calcd for $C_{19}H_{20}N_2O_5$ (M^+) 356.1372; found 356.1378.

4.1.1.9. 2-Methyl-5-oxo-4-(4-nitrophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ia). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (t, 3H, J=6.8 Hz), 1.80–2.10 (m, 2H), 2.30–2.60 (m, 7H), 4.03–4.09 (m, 2H), 5.18 (s, 1H), 7.30 (s, 1H), 7.48 (d, 2H, J=7.6 Hz), 8.10 (d, 2H, J=7.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.08, 19.19, 20.94, 27.12, 36.88, 37.13, 60.10, 104.63, 111.89, 123.32, 128.99, 134.83, 144.91, 146.41, 151.62, 154.78, 167.07, 196.23. MS *m*/*z* (relative intensity) 356 (M^+, 12), 339 (40), 235 (17), 234 (100), 206 (50). HRMS calcd for $C_{19}H_{20}N_2O_5$ (M^+) 356.1372; found 356.1365.

4.1.1.10. 2-Methyl-5-oxo-4-(furan-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ja). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, 3H, J=7.2 Hz), 1.90–2.10 (m, 2H), 2.30–2.60 (m, 7H), 4.12–4.19 (m, 2H), 5.27 (s, 1H), 5.95–5.99 (m, 1H), 6.02–6.10 (m, 1H), 6.20–6.26 (m, 1H), 7.21 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.27, 19.37, 21.08, 27.56, 30.21, 36.95, 59.88, 103.02, 104.74, 110.10, 140.81, 144.20, 150.56, 157.94, 167.23, 195.56. MS *m*/*z* (relative intensity) 301 (M^+, 50), 272 (22), 256 (19), 245 (29), 228 (100), 200 (19). HRMS calcd for $C_{17}H_{19}NO_4$ (M^+) 301.1314; found 301.1313.

4.1.1.11. 2-Methyl-5-oxo-4-(thiophene-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (5ka). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.19 (t, 3H, J=7.2 Hz), 1.80–2.10 (m, 2H), 2.30–2.50 (m, 7H), 4.07 (q, 2H, J=7.2 Hz), 5.45 (s, 1H), 6.18 (s, 1H), 6.80–6.90 (m, 2H), 7.13–7.20 (d, 1H, J=7.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.27, 19.35, 21.08, 27.42, 31.11, 36.98, 59.96, 105.59, 112.81, 123.15, 123.33, 126.46, 143.73, 149.85, 151.18, 167.15, 195.57. MS *m*/*z* (relative intensity) 317 (M^+, 100), 288 (67), 244 (68), 234 (55), 206 (45), 161 (24). HRMS calcd for $C_{17}H_{19}SNO_3$ (M^+) 317.1086; found 317.1084.

4.1.1.12. 2,7,7-Trimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (7aa). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.94 (s, 3H), 1.07(s, 3H), 1.20 (t, 3H, J=7.2 Hz), 2.10–2.40 (m, 7H), 4.06 (q, 2H, J=7.2 Hz), 5.06 (s, 1H), 6.22 (s, 1H), 7.10 (t, 1H, J=7.6 Hz), 7.20 (t, 2H, J=7.6 Hz), 7.31 (d, 2H, J=7.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.18, 19.22, 27.19, 29.33, 32.50, 36.25, 41.27, 50.66, 59.87, 106.16, 112.56, 125.97, 127.88, 127.99, 143.35, 146.52, 148.58, 167.61, 195.97. MS *m*/*z* (relative intensity) 339 (M^+, 12), 263 (16), 262 (100), 234 (28). HRMS calcd for $C_{21}H_{25}NO_3$ (M^+) 339.1834; found 339.1840.

4.1.1.13. 2,7,7-Trimethyl-5-oxo-4-(4-methoxyphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (7ca). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.94 (s, 3H), 1.07 (s, 3H), 1.21 (t, 3H, J=7.2 Hz), 2.13–2.36 (m, 7H), 3.74 (s, 3H), 4.06 (q, 2H, J=7.2 Hz), 5.00 (s, 1H), 6.01 (s, 1H), 6.74 (d, 2H, J=8.4 Hz), 7.22 (d, 2H, J=8.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.21, 19.40, 27.17, 29.40, 32.69, 35.67, 41.12, 50.72, 55.10, 59.77, 106.36, 112.42, 113.23, 128.95, 139.56, 139.56, 143.02, 147.72, 157.75, 167.49, 195.52. MS *m*/*z* (relative intensity) 369 (M^+, 33), 340 (13), 263 (15), 262 (100), 234 (23). HRMS calcd for $C_{22}H_{27}NO_4$ (M^+) 369.1940; found 369.1931.

4.1.1.14. 2,7,7-Trimethyl-5-oxo-4-(4-chlorophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (7ea). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92 (s, 3H), 1.06 (s, 3H), 1.17 (t, 3H, J=7.2 Hz), 2.10–2.50 (m, 7H), 4.02–4.10 (m, 2H), 5.04 (s, 1H), 6.13 (s, 1H), 7.15–7.20 (m, 2H), 7.25–7.30 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.17, 19.30, 27.04, 29.39, 32.64, 36.20, 40.93, 50.65, 59.87, 105.65, 111.71, 127.95, 129.39, 131.56, 143.71, 145.58, 167.21, 195.56. MS *m*/*z* (relative intensity) 373 (M^+, 14), 263 (16), 262 (100), 234 (26). HRMS calcd for $C_{21}H_{24}ClNO_3$ (M^+) 373.1445; found 373.1442.

4.1.1.15. 2,7,7-Trimethyl-5-oxo-4-(4-hydroxyphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (7fa). ^{1}H NMR (Acetone-d_6, 400 MHz) δ 0.91 (s, 3H), 1.04 (s, 3H), 1.21 (t, 3H, J=7.2 Hz), 2.13–2.36 (m, 7H), 3.74 (s, 3H), 4.06 (q, 2H, J=7.2 Hz), 5.00 (s, 1H), 6.01 (s, 1H), 6.74 (d, 2H, J=8.4 Hz), 7.22 (d, 2H, J=8.4 Hz). ^{13}C NMR (Acetone-d_6, 100 MHz) δ 15.01, 19.09, 19.16, 27.49, 33.40, 36.70, 41.14, 51.77, 54.98, 60.20, 106.29, 112.65, 115.57, 130.13, 131.38, 140.43, 145.31, 149.71, 156.62, 168.41, 195.36. MS *m*/*z* (relative intensity) 356 (M^+, 28), 326 (12), 282 (11), 263 (14), 262 (100), 234 (24). HRMS calcd for $C_{21}H_{25}NO_4$ (M^+) 355.1784; found 355.1776.

4.1.1.16. 2,7,7-Trimethyl-5-oxo-4-(thiophene-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid ethyl ester (7ka). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (s, 3H), 1.13 (s, 3H), 1.28(t, 3H, J=7.2 Hz), 2.20–2.55 (m, 7H), 4.17 (q, 2H, J=7.2 Hz), 5.42 (s, 1H), 6.20 (s, 1H), 6.81–6.90 (m, 2H), 7.00–7.05 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.28, 19.40, 27.25, 29.50, 31.22, 32.69, 41.04, 50.67, 59.96, 105.55, 111.68, 123.05, 123.42, 126.40, 143.87, 148.31, 150.98, 167.17, 195.36. MS *m*/*z* (relative intensity) 345 (M^+, 100), 316 (65), 272 (68), 262

(69), 234 (34). HRMS calcd for $C_{19}H_{23}NSO_3$ (M^+) 345.1399; found 345.1397.

4.1.1.17. 2-Methyl-3-acetyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline (8ab).[14] ^1H NMR ($CDCl_3$, 400 MHz) δ 1.80–2.00 (m, 2H), 2.14 (s, 3H), 2.32–2.43 (m, 7H), 5.13 (s, 1H), 6.22 (s, 1H), 7.14 (t, 1H, J=7.6 Hz), 7.22–7.32 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.14, 20.81, 27.42, 29.50, 36.98, 37.01, 113.10, 113.98, 126.37, 127.89, 128.38, 143.04, 145.91, 149.21, 195.78, 199.60.

4.1.1.18. 2-Methyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid methyl ester (8ac). ^1H NMR (DMSO-d_6, 400 MHz) δ 1.60–1.75 (m, 1H), 1.85–2.00 (m, 1H), 2.20–2.60 (m, 7H), 3.56 (s, 3H), 4.95 (s, 1H), 7.00–7.30 (m, 5H), 9.19 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 18.25, 20.78, 26.12, 35.38, 36.71, 50.66, 103.16, 111.12, 125.68, 127.21, 127.91, 145.24, 147.63, 151.41, 167.39, 194.69. MS *m/z* (relative intensity) 297 (M^+, 16), 221 (15), 220 (100). HRMS calcd for $C_{18}H_{19}NO_3$ (M^+) 297.1365; found 297.1366.

4.1.1.19. 2-Methyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (2-methoxyethyl) ester (8ad). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.80–2.00 (m, 2H), 2.20–2.50 (m, 7H), 3.31 (s, 3H), 3.52 (t, 2H, J=4.8 Hz), 4.14–4.18 (m, 2H), 5.11 (s, 1H), 6.66 (s, 1H), 7.08–7.32 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.24, 20.99, 27.22, 36.39, 37.01, 58.77, 62.75, 70.44, 105.53, 113.16, 125.97, 127.88, 127.95, 144.03, 147.12, 150.20, 167.35, 195.89. MS *m/z* (relative intensity) 341 (M^+, 12), 265 (12), 264 (100). HRMS calcd for $C_{20}H_{23}NO_4$ (M^+) 341.1627; found 341.1620.

4.1.1.20. 2-Methyl-3-acetyl-5-oxo-4-(thiophene-2-yl)-1,4,5,6,7,8-hexahydroquinoline (8kb).[14] ^1H NMR ($CDCl_3$, 400 MHz) δ 1.92–2.01 (m, 2H), 2.22 (s, 3H), 2.34–2.48 (m, 7H), 5.39 (s, 1H), 6.80–6.88 (m, 2H), 7.01–7.03 (m, 1H), 7.23 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.00, 20.92, 27.05, 29.27, 32.06, 36.87, 112.61, 113.31, 123.68, 123.87, 126.67, 144.18, 150.13, 150.62, 195.85, 199.10.

4.1.1.21. 2-Methyl-5-oxo-4-(thiophene-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid methyl ester (8kc). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.94–2.01 (m, 2H), 2.30–2.55 (m, 7H), 3.72 (s, 3H), 5.42 (s, 1H), 6.79–6.86 (m, 2H), 6.94 (br, 1H), 7.02–7.06 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.14, 21.05, 27.16, 36.97, 51.11, 104.97, 112.42, 123.12, 123.19, 126.53, 144.58, 151.15, 167.68, 196.00. MS *m/z* (relative intensity) 303 (M^+, 90), 288 (25), 244 (57), 220 (100). HRMS calcd for $C_{16}H_{17}NSO_3$ (M^+) 303.0929; found 303.0927.

4.1.1.22. 2-Methyl-5-oxo-4-(thiophene-2-yl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (2-methoxyethyl) ester (8kd). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.93–2.01 (m, 2H), 2.32–2.46 (m, 7H), 3.33 (s, 3H), 3.58 (t, 2H, J=4.8 Hz), 4.22–4.27 (m, 2H), 5.51 (s, 1H), 6.80–6.83 (m, 2H), 7.01–7.07 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.18, 21.05, 27.09, 31.12, 36.99, 58.80, 62.89, 70.47, 104.91, 112.40, 123.09, 123.33, 126.50, 144.69, 150.79, 151.27, 167.14, 195.89. MS *m/z* (relative intensity) 347 (M^+, 100), 288 (65), 264 (48), 244 (70). HRMS calcd for $C_{18}H_{21}NSO_4$ (M^+) 347.1191; found 347.1191.

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References and notes

1. (a) Mauzeral, D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2261; (b) Baraldi, P. G.; Chiarini, A.; Budriesi, R.; Roberti, M.; Casolar, A.; Manfredini, S.; Simoni, D.; Zanirato, V.; Varani, K.; Borea, P. A. *Drug Des. Deliv.* **1989**, *5*, 13; (c) Baraldi, P. G.; Budriesi, R.; Cacciari, B.; Chiarini, A.; Garuti, L.; Giovanninetti, G.; Leoni, A.; Roberti, M. *Collect. Czech. Chem. Commun.* **1992**, 169; (d) Di Stilo, A.; Visentin, S.; Clara, C.; Gasco, A. M.; Ermondi, G.; Gasco, A. *J. Med. Chem.* **1998**, *41*, 5393; (e) Kawase, M.; Shah, A.; Gaveriya, H.; Motohashi, N.; Sakagami, H.; Varga, A.; Molnar, J. *J. Bioorg. Chem.* **2002**, *10*, 1051; (f) Suarez, M.; Verdecia, Y.; Illescas, B.; Martinez-Alvarez, R.; Avarez, A.; Ochoa, E.; Seoane, C.; Kayali, N.; Martin, N. *Tetrahedron* **2003**, *59*, 9179; (g) Shan, R.; Velazquez, C.; Knaus, E. E. *J. Med. Chem.* **2004**, *47*, 254; (h) Sawada, Y.; Kayakiri, H.; Abe, Y.; Mizutani, T.; Inamura, N.; Asano, M.; Hatori, C.; Arsmori, I.; Oku, T.; Tanaka, H. *J. Med. Chem.* **2004**, *47*, 2853.
2. (a) Buhler, F. R.; Kiowski, W. *J. Hypertens.* **1987**, *5*, S3; (b) Reid, J. L.; Meredith, P. A.; Pasanisi, F. *J. Cardiovasc. Pharmacol.* **1985**, *7*, S18.
3. (a) Godfraid, T.; Miller, R.; Wibo, M. *Pharmacol. Rev.* **1986**, *38*, 321; (b) Sausins, A.; Duburs, G. *Heterocycles* **1988**, *27*, 269; (c) Mager, P. P.; Coburn, R. A.; Solo, A. J.; Triggle, D. J.; Rothe, H. *Drug Des. Discov.* **1992**, *8*, 273; (d) Mannhold, R.; Jablonka, B.; Voigdt, W.; Schoenafinger, K.; Schravan, K. *Eur. J. Med. Chem.* **1992**, *27*, 229.
4. (a) Klusa, V. *Drugs Future* **1995**, *20*, 135; (b) Bretzel, R. G.; Bollen, C. C.; Maeser, E.; Federlin, K. F. *Am. J. Kidney Dis.* **1993**, *21*, 53; (c) Bretzel, R. G.; Bollen, C. C.; Maeser, E.; Federlin, K. F. *Drugs Future* **1992**, *17*, 465; (d) Boer, R.; Gekeler, V. *Drugs Future* **1995**, *20*, 499.
5. Love, B.; Sander, K. M. *J. Org. Chem.* **1965**, *30*, 1914.
6. (a) Hantzsch, A. *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 942; (b) Hantzsch, A. *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 1747; (c) Wiley, R. H.; England, D. C.; Behr, L. C. In *Organic Reactions*; Wiley: Toronto, 1951; Vol. 6, 367; (d) Dondoni, A.; Massi, A.; Minghini, E.; Bertolasi, V. *Tetrahedron* **2004**, *60*, 2311.
7. (a) Khadikar, B. M.; Gaikar, V. G.; Chitnavis, A. A. *Tetrahedron Lett.* **1995**, *36*, 8083; (b) Ohberg, L.; Westman, J. *Synlett* **2001**, 1296; (c) Agarwal, A.; Chauhan, P. M. S. *Tetrahedron Lett.* **2005**, *46*, 1345.
8. (a) Ji, S.-J.; Jiang, Z.-Q.; Lu, J.; Loh, T.-P. *Synlett* **2004**, 831; (b) Sridhar, R.; Perumal, P. T. *Tetrahedron* **2005**, *61*, 2465.
9. (a) Phillips, A. P. *J. Am. Chem. Soc.* **1949**, *71*, 4003; (b) Anderson, G., Jr.; Berkelhammer, G. *J. Am. Chem. Soc.* **1958**, *80*, 992; (c) Singh, H.; Chimni, D. S. S.; Kumar, S. *Tetrahedron* **1995**, *51*, 12775; (d) Gordeev, M. F.; Patel, D. V.; Gordon, E. M. *J. Org. Chem.* **1996**, *61*, 924; (e) Breitenbucher, J. G.; Figliozzi, G. *Tetrahedron Lett.* **2000**, *41*, 4311; (f) Liang, J.-C.; Yeh, J.-L.; Wang, C.-S.; Liou,

S.-F.; Tasi, C.-H.; Chen, I.-J. *Bioorg. Med. Chem.* **2002**, *10*, 719; (g) Miri, R.; Niknahad, H.; Vesal, Gh.; Shafiee, A. *Farmaco* **2002**, *57*, 123; (h) Dondoni, A.; Massi, A.; Minghini, E.; Sabbatini, S.; Bertoasi, V. *J. Org. Chem.* **2003**, *68*, 6172; (i) Dondoni, A.; Massi, A.; Minghini, E.; Bertoasi, V. *Tetrahedron* **2004**, *60*, 2311; (j) Tewari, N.; Dwivedi, N.; Tripathi, R. P. *Tetrahedron Lett.* **2004**, *45*, 9011; (k) Zolfigol, M. A.; Safaiee, M. *Synlett* **2004**, 827; (l) Moseley, J. D. *Tetrahedron Lett.* **2005**, *46*, 3179.

10. Sabitha, G.; Reddy, G. S. K. K.; Reddy, Ch. S.; Yadav, J. S. *Tetrahedron Lett.* **2003**, *44*, 4129.
11. Wang, L.-M.; Sheng, J.; Zhang, L.; Han, J.-W.; Fan, Z.; Tian, H.; Qian, C.-T. *Tetrahedron* **2005**, *61*, 1539.
12. Ko, S.; Sastry, M. N. V.; Lin, C.; Yao, C.-F. *Tetrahedron Lett.* **2005**, *46*, 5771.
13. For example: (a) Nair, V.; Nair, L. G. *Tetrahedron Lett.* **1998**, *39*, 4585; (b) Yadav, J. S.; Reddy, B. V. S.; Reddy, K. B.; Raj, K. S.; Prasad, A. R. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1939; (c) Nair, V.; Panicker, S. B.; Augustine, A.; George, T. G.; Thomas, S.; Vairamani, M. *Tetrahedron* **2001**, *57*, 7417; (d) Asghedom, H.; LaLonde, R. T.; Ramdayal, F. *Tetrahedron Lett.* **2002**, *43*, 3989; (e) Ji, S.-J.; Wang, S.-Y. *Synlett* **2003**, 2074; (f) Itoh, K.-I.; Horiuchi, C. A. *Tetrahedron* **2004**, *60*, 1671; (g) Pan, W.-B.; Chang, F.-R.; Wei, L.-M.; Wu, M.-J.; Wu, Y.-C. *Tetrahedron Lett.* **2003**, *44*, 331; (h) Nair, V.; Balagopal, L.; Rajan, R.; Mathew, J. *Acc. Chem. Res.* **2004**, *37*, 21; (i) Chuang, C.-P.; Wu, Y.-L. *Tetrahedron* **2004**, *60*, 1841; (j) Comin, M. J.; Elhalem, E.; Rodriguez, J. B. *Tetrahedron* **2004**, *60*, 11851; (k) More, S. V.; Sastry, M. N. V.; Yao, C.-F. *Green Chem.* **2006**, *8*, 91; (l) Ko, S.; Lin, C.; Tu, Z.; Wang, Y.-F.; Wang, C.-C.; Yao, C.-F. *Tetrahedron Lett.* **2006**, *47*, 487.
14. Safak, C.; Erdemli, I.; Sunal, R. *Arzneimittelforschung* **1993**, *43*, 1052.

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Kinetic resolution of poly(ethylene glycol)-supported carbonates by enzymatic hydrolysis

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Abstract—The enzyme-mediated enantioselective hydrolysis of poly(ethylene glycol) (PEG)-supported carbonates is disclosed. The water-soluble carbonates were prepared by immobilization of a racemic secondary alcohol (4-benzyloxy-2-butanol) onto low-molecular weight (av MW 550 and 750) monomethoxy PEG through a carbonate linker. For the screening of the hydrolytic enzymes, the substrate was enantioselectively hydrolyzed by commercially available lipase from porcine pancreas (PPL; Type II, Sigma) to afford the optically active compounds. In this system, the separation of the remaining (*S*)-substrate and the resulting (*R*)-alcohol was achieved by an extraction process without a laborious column chromatography. The (*S*)-carbonate was easily hydrolyzed with K_2CO_3 to afford the corresponding (*S*)-alcohol. Other MPEG-supported substrates were also hydrolyzed to afford the corresponding optically active alcohols.

1. Introduction

Optically active secondary alcohols are versatile intermediates in organic syntheses. The use of enzymes in the preparation of such compounds is especially attractive due to its benign effect on the environment. In particular, the kinetic resolution of racemic alcohols and esters using hydrolytic enzymes is one of the practical methods for the preparation of the optically active compounds, and a significant number of examples have been reported.[1] In the reaction process, the enantiomers, the remaining substrate and the resulting product, could be separated mainly by column chromatography. However, the tedious and wasteful separation step is the bottleneck to an easy operation and a sustainable product. Although, in order to resolve this irritating problem, several studies of an easy separation have been published,[2–9] facile and efficient procedures are still desired.

On the other hand, organic synthesis based on polymer supports has made rapid progress, especially in the field of combinatorial chemistry. Because insoluble polymers (polystyrene, silica gel, and so on) are usually used, it is called 'solid-phase' chemistry. While the methodology provides us an easy separation of the products, the heterogeneous reaction causes a low reactivity and a difficult analysis of the polymer-supported intermediate. Although enzymatic transformation on a polymer support is also of contemporary interest and can be potentially useful for the easy isolation of the products, there have been relatively few reports on polymer-supported reactions by enzymes so far.[6–12] Recently, poly(ethylene glycol) (PEG) has been recognized as an inexpensive and convenient soluble polymer.[13,14] The synthetic approach using a soluble polymer is termed as 'liquid-phase' chemistry and couples the advantages of homogeneous solution chemistry with those of solid-phase chemistry. We have noted that a PEG-supported strategy could be suitable for enzymatic transformation because the broad solubility of PEG facilitates the analysis of the PEG-supported substrates and could significantly enhance the reactivity under homogeneous conditions. Actually, PEG-supported esters and amides have been studied as prodrugs, which are hydrolyzed in vitro or in vivo to gradually release native drugs.[15] In this report, we disclose the first example of the kinetic resolution of PEG-supported substrates with a carbonate linker by a hydrolytic enzyme to afford the corresponding optically active compounds, and the method enables us to achieve the easy separation of the remaining substrates and the resulting alcohols by an extraction process without laborious column chromatography.[16]

In general, previously reported PEG use during enzymatic synthesis has been restricted as the reagent for the modification of enzymes[17] and the additive for improving the enzyme

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activities.[18] To the best of our knowledge, there has been only one report on the PEG-supported substrate used for enzymatic transformation.[19] However, the substrate only worked as a nucleophile to an acyl–enzyme intermediate, and the example did not make the most of the advantage of PEG. We now present a new aspect of PEG use.

2. Results and discussion

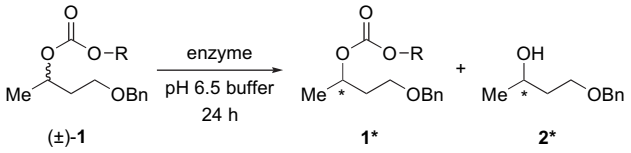
2.1. Screening test of enzymes

We used low-molecular weight monomethoxy PEG (MPEG, av MW 750 and 550) as the matrix.[20] It has the desired solubility profile and the higher loading capacity ($MPEG_{750}$, 1.3 mmol/g; $MPEG_{550}$, 1.8 mmol/g), while that of $MPEG_{5000}$ (av MW 5000), which has been used in many previous reports, is only 0.2 mmol/g. In addition, the terminal methyl group becomes a reference for the determination of the loading ratio in the reaction steps.

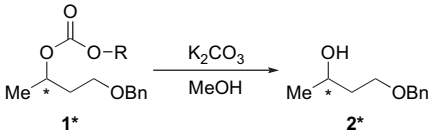
For the screening test of enzymes, we selected the carbonate (±)-**1a** ($MPEG_{750}$), which was afforded by the coupling of racemic 4-benzyloxy-2-butanol ((±)-**2**) with $MPEG_{750}$–OH through a carbonate linker. The carbonate is a typical linker for organic synthesis on a polymer support and can be easily constructed. In general, carbonate is recognized as a poor substrate for hydrolase. However, we have already succeeded in the development of the enzymatic hydrolysis of cyclic carbonates,[21] and realize that carbonate is merely a kind of ester, which can be hydrolyzed by enzymes.

The substrate (±)-**1a** was readily synthesized as shown in Scheme 1. The reaction of (±)-**2** with *N*,*N*′-carbonyldiimidazole in CH_2Cl_2 proceeded to afford the corresponding (±)-**3**. The compound (±)-**3** was immobilized on MPEG–OH with DMAP in DMF at 120 °C to give nearly the pure $MPEG_{750}$-supported (±)-**1a** in 67% yield. In the same way, the $MPEG_{550}$-supported (±)-**1b** and other substrates were also prepared. The yields of substrates were determined by the weights with the assumption that the MW was 750 or 550 for MPEG–OH.

In the first screening test, 12 hydrolytic enzymes were used. The selection of the enzyme was carried out on the basis of hydrolytic activity without paying attention to the enantioselectivity. The assay was performed by checking the production of **2** using TLC, and we selected three enzymes. In the second screening, the enantiomeric excesses (ees) of the products were determined after purification (Scheme 2). The ee of **2** was determined by HPLC analysis (CHIRALCEL OD-H, Daicel Chemical Industries, Ltd), and a similar analysis of **2** derived from **1a** with K_2CO_3 was also performed (Scheme 3). These results are shown in Table 1. Although pig liver esterase (PLE; Amano Enzyme, Inc.) smoothly catalyzed the hydrolysis of **1a** to afford (*R*)-**2**, the enantioselectivity was quite low (*E* value=3).[22] Interestingly, the esterase SNSM-87 (Nagase & Co., Ltd) preferentially hydrolyzed the opposite enantiomer with moderate enantioselectivity to give the alcohol (*S*)-**2**, but the conversion was very low (conv.=0.08, *E* value=11). Finally, lipase from porcine pancreas (PPL; Type II, Sigma) was found to be the best enzyme. Under the given reaction conditions, the reaction of (±)-**1a** with PPL proceeded with a higher enantioselectivity (conv.=0.29, *E* value=23) to afford the optically active (*S*)-**1a** (50%, 36% ee) and (*R*)-**2** (28%, 89% ee; $[\alpha]_D^{26}$ −12.2 (*c* 0.24, MeOH)). The absolute configurations of the products were determined by comparing the optical rotation of **2** with that of an authentic sample ($[\alpha]_D^{27}$ +19.0 (*c* 0.95, MeOH)) derived from ethyl (*S*)-3-hydroxybutanoate (Scheme 4). Changing the MPEG part to a lower molecular weight $MPEG_{550}$ did not negatively affect the reactivity. The reaction of the $MPEG_{550}$-supported **1b** also proceeded with a high enantioselectivity (conv.=0.35, *E* value=28). In the reaction of (±)-**1b** at 10 °C, the *E* value was up to 32 and (*R*)-**2** with 93% ee was obtained, although the conversion apparently decreased. On the other hand, the methyl carbonate (±)-**1c** (R=Me) was also hydrolyzed, but the enantioselectivity was very low (*E* value=1.4). The substrate (±)-**1c** is not supported on MPEG and basically insoluble in water. These facts indicate that the hydrophilic MPEG matrix could change the physical property of the alcohol **2** and that the substrate would favorably fit into the enzyme active site.



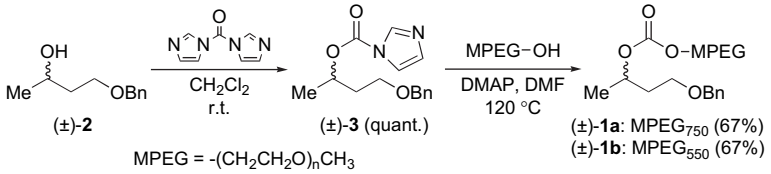
Scheme 2.



Scheme 3.

2.2. Separation of the products

During the reaction process, we succeeded in the establishment of a more facile separation of the remaining substrate (*S*)-**1a**,**b** and the resulting alcohol (*R*)-**2** due to the suitable water-solubility of the MPEG-supported substrates. Scheme 5 illustrates this extraction procedure. First, the extraction

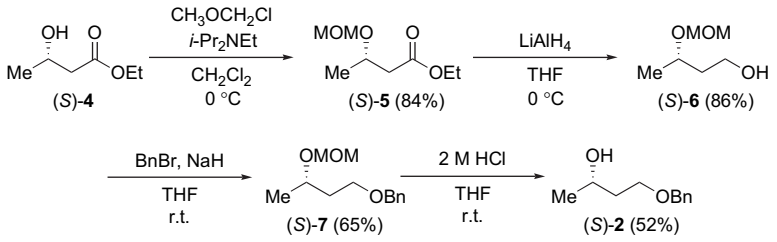


Scheme 1.

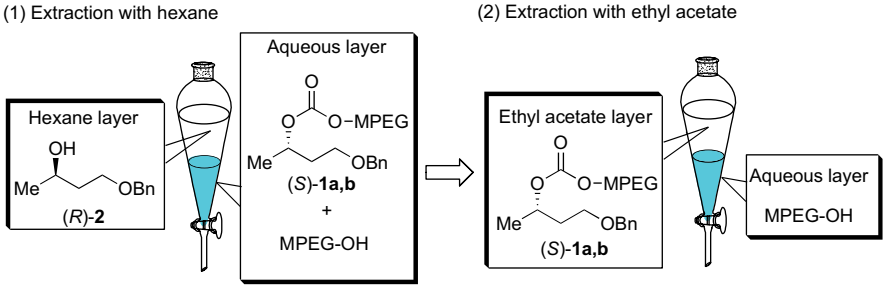
Table 1. Enantioselective hydrolysis of carbonates (±)-**1**[a]

	Substrate	R	Temp (°C)	Carbonate **1**		Alcohol **2**		Conv.[e]	E[f]
				Yield [b](%)	ee (%)[c]	Yield (%)	ee (%)[d]		
PLE	**1a**	$MPEG_{750}$	30	4	96(*S*)	76	7(*R*)	0.93	3
Esterase SNSM-87	**1a**	$MPEG_{750}$	30	56	7(*R*)	11	82(*S*)	0.08	11
PPL	**1a**	$MPEG_{750}$	30	50	36(*S*)	28	89(*R*)	0.29	23
PPL	**1b**	$MPEG_{550}$	30	54	47(*S*)	30	89(*R*)	0.35	28
PPL	**1b**	$MPEG_{550}$	10	76	10(*S*)	21	93(*R*)	0.10	32
PPL	**1c**	Me	30	65	5(*S*)	28	12(*R*)	0.27	1.4

[a] The reaction was performed using 5 mM of the substrate with an enzyme in 0.1 M phosphate buffer (pH 6.5) for 24 h.
[b] Determined by its weight on the basis of the weight of the racemic substrate.
[c] Determined by HPLC analysis after the hydrolysis of the carbonate.
[d] Determined by HPLC analysis.
[e] Calculated by ee(carbonate)/[ee(carbonate)+ee(alcohol)].
[f] Calculated by ln[(1−conv.)(1−ee(carbonate))]/ln[(1−conv.)(1+ee(carbonate))].



Scheme 4.

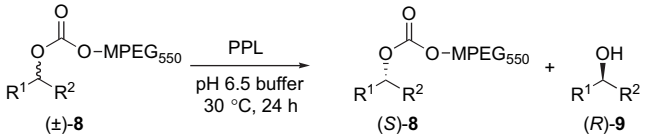


Scheme 5.

process is performed with hexane after the enzymatic reaction. In this step, only the alcohol (*R*)-**2** is selectively extracted into the hexane layer. Second, the substrate (*S*)-**1a**,**b** is successfully extracted from the aqueous layer with AcOEt. MPEG–OH, which is removed from (*R*)-**1a**,**b**, still remains in the aqueous layer. In order to purify these compounds, only a pad of silica gel is needed.

2.3. Application of the enzymatic reaction

We next examined the enzymatic reactions of several substrates supported on $MPEG_{550}$ under the same conditions (Scheme 6). These results are summarized in Table 2. As expected, the hydrolysis of 1-phenylethanol derivative (±)-**8a** (R^1=Me, R^2=Ph) enantioselectively proceeded, and the conversion was greater than those of **1** (conv.=0.55, E value=29). In this case, the corresponding optically active compounds, (*S*)-**8a** (36%, 95% ee) and (*R*)-**9a** (31%, 77% ee), were obtained. While the reaction of (±)-**8c** (R^1=vinyl, R^2=CH_2CH_2OBn) showed a good enantioselectivity (E value=16), (±)-**8b** (R^1=Me, R^2=CH_2CH_2Ph) was



Scheme 6.

Table 2. Enantioselective hydrolysis of carbonates (±)-**8** with PPL[a]

Substrate	R^1	R^2	Carbonate (*S*)-**8**		Alcohol (*R*)-**9**		Conv.	E
			Yield (%)[b]	ee (%)	Yield (%)	ee (%)		
8a	Me	Ph	36	95[c]	31	77[d]	0.55	29
8b	Me	CH_2CH_2Ph	37	23[c]	47	27[d]	0.46	2
8c	Vinyl	CH_2CH_2OBn	47	56[e]	41	80[f]	0.41	16

[a] The reaction was performed using 5 mM of (±)-**8** with PPL in 0.1 M phosphate buffer (pH 6.5) for 24 h.
[b] Determined by its weight on the basis of the weight of the racemic substrate.
[c] Determined by HPLC analysis after the hydrolysis of the carbonate.
[d] Determined by HPLC analysis.
[e] Determined by ^{1}H NMR analysis of the corresponding MTPA ester after the hydrolysis of the carbonate.
[f] Determined by ^{1}H NMR analysis of the corresponding MTPA ester.

hydrolyzed with a very low enantioselectivity. Because the bulkiness of the substituent of **8c** is not much different from those of **1** and **8b**, the interaction between the enzyme and the oxygen atom in the benzyloxy group should be important for the enantioselectivity. In all cases, the substrates **8** and the alcohols **9** were successfully separated by the two step-extraction procedure as expected.

2.4. What is the real active enzyme?

Although commercially available PPL (Type II, Sigma) works well in this enzymatic reaction, the crude enzyme contains a number of hydrolases besides the true PPL. The existence of several active enzymes might affect the reactivity and enantioselectivity. In addition, the active-site model proposed by Jones for the PPL-catalyzed hydrolysis of a primary ester does not predict the reaction mode in this case.[23] In order to research the accurate result of the reaction for MPEG-supported substrates, we then investigated the reaction using the commercially available purified PPL (lipase Type VI-S, Sigma) and two kinds of major contaminant hydrolases, α-chymotrypsin (Type II, Sigma) and cholesterol esterase (Sigma). We selected (±)-**8a** as the substrate because it was the most reactive amongst all the examined substrates (Table 3). It is noteworthy that all enzymes show no or very low enantioselectivities. These results suggest that the activity could be due, not to the true PPL, but to another unknown enzyme. Further detailed investigations are now in progress.

Table 3. Enantioselective hydrolysis of carbonates **8a**[a]

Enzyme	Carbonate (*S*)-**8a**	Alcohol (*R*)-**9a**	Conv.	*E*
	ee (%)	ee (%)		
Purified PPL[b]	2.1	30	0.07	2
α-Chymotrypsin[c]	1.2	21	0.06	1.5
Cholesterol esterase[d]	1.2	0.4	0.77	~1

[a] The reaction was performed using 5 mM of (±)-**8a** with the enzyme in 0.1 M phosphate buffer (pH 6.5, 4 mL) for 24 h at 30 °C.
[b] Using 0.3 mg (81,000 U/mg).
[c] Using 5 mg (30,000 U/mg).
[d] Using 1 mg (54 U/mg).

3. Conclusions

In summary, we have demonstrated the first example for the hydrolase-mediated kinetic resolution of low-molecular weight MPEG-supported carbonates. We succeeded in the highly enantioselective hydrolysis of several substrates to give the substituted methyl (**2** and **9a**) and vinyl (**9c**) carbinols, which were optically active. In our method, the separation of the resulting alcohols from the remaining substrates was achieved by an extraction process without time- and solvent-consuming column chromatography. We anticipate that the use of a soluble polymer as the matrix of the substrates will provide an operationally simple and eco-friendly protocol.

4. Experimental

4.1. General

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were measured on JEOL JNM AL-300, with tetramethylsilane (TMS) as the internal standard. ^{1}H (500 MHz) NMR spectra were measured on JEOL α-500. IR spectra were recorded with a Shimadzu IR Prestige-21 spectrometer. Mass spectra were obtained with a JEOL EI/FAB mate BU25 instrument (EI method). The optical rotations were measured with a Jasco DIP-1000 polarimeter. HPLC data were obtained on Shimadzu LC-10AD$_{VP}$, SPD-10A$_{VP}$, and sic 480II data station (System Instruments Inc.). Kieselgel 60 F_{254} Art.5715 (E. Merck) was used for analytical thin-layer chromatography (TLC). Preparative TLC was performed on a Kieselgel 60 F_{254} Art.5744 (E. Merck). Flash column chromatography was performed with Silica Gel 60N (63–210 mm, Kanto Chemical Co. Inc.). MPEG$_{550}$–OH and MPEG$_{750}$–OH were purchased from Aldrich and the containing water was removed as the toluene azeotrope prior to use. Racemic secondary alcohols were prepared from the suitable starting material in the usual way. All other chemicals and enzymes were also obtained from commercial sources.

4.2. Preparation of carbonates as the substrate

4.2.1. MPEG$_{750}$-supported substrate coupled with 4-benzyloxy-2-butanol ((±)-1a). Under an argon atmosphere, to a solution of *N*,*N*′-carbonyldiimidazole (1.98 g, 12.2 mmol) in CH_2Cl_2 (10 mL) was added a solution of 4-benzyloxy-2-butanol ((±)-**2**, 2.00 g, 11.1 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred overnight at room temperature. After the mixture was diluted with CH_2Cl_2, the solution was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, 4-(benzyloxy)butan-2-yl 1*H*-imidazole-1-carboxylate ((±)-**3**) was obtained as a colorless oil (3.17 g, quant). This was used in the following reaction without further purification; IR (neat) 2862, 2359, 1759, 1472, 1393, 1319, 1290, 1242, 1180, 1096, 1003, 743 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, J=6.0 Hz, 3H), 1.94–2.11 (m, 2H), 3.51–3.59 (m, 2H), 4.44 (d, J=12.0 Hz, 1H), 4.50 (d, J=12.0 Hz, 1H), 5.33 (dqd, J_1=5.0 Hz, J_2=6.0 Hz, J_3=8.0 Hz, 1H), 7.04 (t, J=0.8 Hz, 1H), 7.19–7.34 (m, 5H), 7.36 (t, J=1.3 Hz, 1H), 8.07 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 35.8, 65.8, 73.1, 73.9, 117.0, 127.6, 127.7, 128.3, 130.4, 137.0, 137.9, 148.2; MS *m*/*z* (EI, rel intensities) 274 (M^+, 6.1%), 168 (100), 162 (93), 107 (42), 91 (100); HRMS *m*/*z* (EI) 274.1315 (calcd for $C_{15}H_{18}O_3N_2$: 274.1318, M^+).

Under an argon atmosphere, to a solution of *N*,*N*-dimethylaminopyridine (DMAP) (445 mg, 3.65 mmol) in DMF (10 mL) were added a solution of MPEG$_{750}$–OH (2.74 g, 3.65 mmol) in DMF (20 mL) and (±)-**3** (1.00 g, 3.65 mmol) in DMF (10 mL) at 0 °C. After the mixture was stirred overnight at 120 °C, it was washed with 2 M HCl in order to remove DMAP, the resulting imidazole, and the remaining MPEG–OH. After evaporation under reduced pressure, the residue was purified by column chromatography on silica gel (AcOEt → AcOEt/MeOH=3/1) to give the MPEG$_{750}$-supported carbonate (±)-**1a** as a colorless oil (3.50 g, 67%). The yield was determined by the weight with the assumption that the molecular weight was 750 for MPEG–OH; IR (neat) 2870, 2359, 1742, 1454, 1350, 1263, 1105, 949, 847 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, J=6.5 Hz, 3H), 1.77–2.03 (m, 2H), 3.38 (s, 3H, CH_3O–PEG), 3.50–3.57 (m, 2H, PEG), 3.55–3.70 (m, ca. 62H,

PEG), 3.70 (t, J=4.5 Hz, 2H, PEG), 4.17–4.33 (m, 2H), 4.49 (s, 2H), 4.89–5.01 (m, 1H), 7.26–7.37 (m, 5H).

Other substrates were synthesized by the same procedure.

4.2.2. MPEG$_{550}$-supported substrate coupled with 4-benzyloxy-2-butanol ((±)-1b). Yield 61% from 4-benzyloxy-2-butanol (*dl*-**2**); IR (neat) 2870, 2359, 1742, 1454, 1350, 1263, 1107, 949, 851 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, J=6.5 Hz, 3H), 1.77–2.04 (m, 2H), 3.38 (s, 3H, CH_3O–PEG), 3.51–3.57 (m, 2H, PEG), 3.57–3.67 (m, ca. 44H, PEG), 3.70 (t, J=4.5 Hz, 2H, PEG), 4.17–4.33 (m, 2H), 4.49 (s, 2H), 4.89–5.01 (m, 1H), 7.26–7.37 (m, 5H).

4.2.3. MPEG$_{550}$-supported substrate coupled with 1-phenylethanol ((±)-8a). Yield 53% from 1-phenylethanol ((±)-**9a**); IR (neat) 2872, 2359, 1744, 1454, 1348, 1261, 1103, 949, 849 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (d, J=6.5 Hz, 3H), 1.77–2.03 (m, 2H), 3.38 (s, 3H, CH_3O–PEG), 3.53–3.57 (m, 2H, PEG), 3.60–3.72 (m, ca. 44H, PEG), 3.70 (t, J=5.0 Hz, 2H, PEG), 5.72 (q, J=6.5 Hz, 1H), 7.25–7.40 (m, 5H).

4.2.4. MPEG$_{550}$-supported substrate coupled with 4-phenyl-2-butanol ((±)-8b). Yield 52% from 4-phenyl-2-butanol (*dl*-**9b**); IR (neat) 2870, 2359, 1740, 1454, 1350, 1267, 1107, 949, 847 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, J=6.0 Hz, 3H), 1.77–2.03 (m, 2H), 3.38 (s, 3H, CH_3O–PEG), 3.50–3.57 (m, 2H, PEG), 3.55–3.70 (m, ca. 46H, PEG), 3.70 (t, J=4.5 Hz, 2H, PEG), 4.73–4.84 (m, 1H), 7.14–7.31 (m, 5H).

4.2.5. MPEG$_{550}$-supported substrate coupled with 5-benzyloxy-1-hepten-3-ol ((±)-8c). Yield 60% from 5-benzyloxy-3-ol (*dl*-**9c**); IR (neat) 3113, 2870, 2359, 1744, 1454, 1350, 1261, 1105, 947, 851 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.38 (s, 3H, CH_3O–PEG), 3.50–3.60 (m, 2H, PEG), 3.60–3.72 (m, ca. 46H, PEG), 3.70 (t, J=5.0 Hz, 2H, PEG), 4.22–4.33 (m, 2H), 4.49 (s, 2H), 5.21 (td, J_1=1.5 Hz, J_2=10.5 Hz, 1H), 5.31 (td, J_1=1.5 Hz, J_2=17.0 Hz, 1H), 5.81 (ddd, J_1=7.0 Hz, J_2=10.5 Hz, J_3=17.0 Hz, 1H), 7.25–7.37 (m, 5H).

4.2.6. 4-(Benzyloxy)butan-2-yl methyl carbonate ((±)-1c). Under an argon atmosphere, to a solution of (±)-**2** (400 mg, 2.23 mmol) in CH_2Cl_2 (20 mL) were added pyridine (1.08 mL, 13.3 mmol) and methyl chlorocarbonate (0.34 mL, 4.44 mmol), and the mixture was stirred overnight at room temperature. After the addition of methyl chlorocarbonate (0.68 mL, 8.88 mmol), the mixture was stirred overnight at room temperature again. The reaction was stopped with 0.1 M phosphate buffer (pH 6.5) and the products were extracted with CH_2Cl_2 (×3). The combined organic layer was washed with 2 M HCl (×2), brine, satd $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=5/1) to give (±)-**1c** as a colorless oil (454 mg, 86%); IR (neat) 2955, 2859, 2359, 1746, 1443, 1271, 1096, 941 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, J=7.0 Hz, 3H), 3.50–3.75 (m, 8H), 4.33 (q, J=7.0 Hz, 1H), 4.47 (d, J=11.5 Hz, 1H), 4.59 (d, J=11.5 Hz, 1H), 7.26–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.8, 42.5, 45.6, 66.7, 67.0, 71.1, 75.3, 127.8, 127.9, 128.5, 137.4, 170.5; MS m/z (EI, rel intensities) 238 (M^+, 5.5%), 162 (100), 147 (6.8), 131 (30), 105 (100), 91 (100), 77 (100); HRMS m/z (EI) 238.1205 (calcd for $C_{13}H_{18}O_4$: 238.1205, M^+).

4.3. First screening of enzymes

In the screening test, we used the following enzymes: Lipase from porcine pancreas (PPL; Type II, Sigma), Lipase AK, Lipase PS, Lipase D, Lipase AP, Lipase AY, Newlase F, PLE (Amano Enzyme, Inc.), Lipase OF (Meito Sangyo Co., Ltd), Esterase SNSM-87 (Nagase & Co., Ltd), Trypsin, α-Chymotrypsin (E. Merck). The substrate (±)-**1a** (200 mg) and 50 mg of enzyme were incubated in 40 mL of 0.1 M phosphate buffer (pH 6.5) for 24 h at 30 °C. The products were extracted with AcOEt (×3) and detected by TLC (hexane/AcOEt=3/1).

4.4. Typical procedure of enantioselective hydrolysis of MPEG-supported substrates

To a 200-mL Erlenmeyer flask containing 200 mg (ca. 0.208 mmol; sub. concn, 0.5 mM) of (±)-**1a** was added 40 mL of 0.1 M phosphate buffer (pH 6.5). To the mixture was added 50 mg of Lipase from porcine pancreas (PPL; Type II, Sigma) (994 U/mg, using olive oil at pH 7.7), and the solution was incubated for 24 h at 30 °C. First, only the resulting alcohol **2** was extracted with hexane (×3), and the hexane layer was dried over Na_2SO_4. After evaporation, the residue was passed through a pad of silica gel with hexane/AcOEt (3/1) to give the alcohol (*R*)-**2** (10.1 mg, 28%, 89% ee). Second, the remaining carbonate **1a** was re-extracted with AcOEt from the water layer, and the organic layer was dried over Na_2SO_4. After evaporation, the residue was passed through a pad of silica gel (AcOEt/MeOH=3/1) to give the carbonate (*S*)-**1a** (99.7 mg, 50%, 36% ee). The yields of **1a** and **2** were determined by their weights on the basis of the weight of the substrate (±)-**1a**. The carbonate (*S*)-**1a** was easily hydrolyzed with K_2CO_3 in MeOH to afford the corresponding alcohol (*S*)-**2**.

4.5. Chemical hydrolysis of (*S*)-1a

To a solution of (*S*)-**1a** (40.3 mg, 0.053 mmol) in MeOH (6 mL) was added K_2CO_3 (36.6 mg, 0.265 mmol), and the mixture was stirred for 1 h at room temperature. After the reaction was stopped with water, MeOH was evaporated in vacuo. The products were extracted with AcOEt (×3), and the combined organic layer was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=4/1) to give (*S*)-**2** as a colorless oil (8.1 mg, 85%).

4.6. Several data of alcohols

4.6.1. 4-Benzyloxy-2-butanol (2). Compound (*R*)-**2**, $[\alpha]_D^{26}$ −12.2 (*c* 0.24, MeOH) (89% ee); IR (neat) 3416, 2965, 2864, 1494, 1454, 1368, 1206, 1099, 1028, 737, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, J=6.0 Hz, 3H), 1.66–1.85 (m, 2H), 2.82 (br s, 1H), 3.60–3.75 (m, 2H), 3.95–4.07 (m, 1H) 4.53 (s, 2H), 7.25–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 38.1, 67.4, 69.0, 73.2,

127.6, 127.7, 128.4, 137.9; MS *m/z* (EI, rel intensities) 180 (M^+, 14%), 161 (57), 107 (100), 91 (100), 89 (42); HRMS *m/z* (EI) 180.1146 (calcd for $C_{11}H_{16}O_2$: 180.1150, M^+). The spectral data were in full agreement with those reported.[24] HPLC conditions: column, CHIRALCEL OD-H (Daicel Chemical Industries, Ltd); eluent, hexane/2-propanol=90/10; flow rate, 0.5 mL/min; 254 nm; temperature, 25 °C; retention time, 13 (*S*) and 14 (*R*) min.

Enantioselective hydrolysis of the other cases was carried out by the same procedure. In the case of (±)-**1c**, the remaining **1c** and the resulting **2** were separated by flash column chromatography (hexane/AcOEt=5/1→hexane/AcOEt=3/1).

4.6.2. 1-Phenylethanol (9a). Compound (*R*)-**9a**, $[\alpha]_D^{21}$ +24.8 (*c* 0.85, MeOH) (77% ee), lit.[25] $[\alpha]_D^{20}$ +45 (*c* 5.15, MeOH) for the (*R*)-enantiomer. The spectral data were in full agreement with that of commercial source. The ee of (*R*)-**9a** was determined by GLC analysis. GLC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm×50 m; injection, 160 °C; detection, 160 °C; oven, 140 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 8.9 (*R*) and 9.2 (*S*) min.

4.6.3. 4-Phenyl-2-butanol (9b). Compound (*R*)-**9b**, $[\alpha]_D^{27}$ −3.4 (*c* 0.69, $CHCl_3$) (27% ee), lit.[26] $[\alpha]_D^{27}$ +17.45 (*c* 2.04, $CHCl_3$) for the (*S*)-enantiomer; IR (neat) 3358, 2965, 2926, 2361, 1713, 1603, 1495, 1454, 1373, 1128, 1055, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (d, *J*=4.0 Hz, 3H, CH_3), 1.72–1.82 (m, 2H), 2.60–2.82 (m, 2H), 3.83 (tq, J_1=J_2=6.0 Hz, 1H), 7.14–7.32 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5, 32.1, 40.8, 67.5, 125.8, 128.4, 142.0; MS *m/z* (EI, rel intensities) 150 (M^+, 25%), 132 (100), 117 (100), 105 (47), 91 (100); HRMS *m/z* (EI) 150.1042 (calcd for $C_{10}H_{14}O$: 150.1045, M^+). HPLC conditions: column, CHIRALCEL OD-H (Daicel Chemical Industries, Ltd); eluent, hexane/2-propanol=90/10; flow rate, 0.5 mL/min; 254 nm; temperature, 25 °C; retention time, 15 (*R*) and 20 (*S*) min.

4.6.4. 5-Benzyloxy-1-hepten-3-ol (9c). Compound (*R*)-**9c**, $[\alpha]_D^{28}$ +5.5 (*c* 0.40, MeOH) (80% ee); IR (neat) 3417, 2862, 2359, 1454, 1366, 1277, 1099, 1028, 993, 922, 737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67–1.97 (m, 2H), 2.90 (br s, 1H), 3.59–3.76 (m, 2H), 4.30–4.40 (m, 1H), 4.52 (s, 2H), 5.10 (td, J_1=1.5 Hz, J_2=10.5 Hz, 1H), 5.26 (td, J_1=1.5 Hz, J_2=17.0 Hz, 1H), 5.87 (ddd, J_1=5.5 Hz, J_2=10.5 Hz, J_3=17.0 Hz, 1H), 7.25–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.2, 68.1, 71.6, 73.2, 114.3, 127.6, 128.4, 137.8, 140.5; MS *m/z* (EI, rel intensities) 192 (M^+, 9.3%), 107 (93), 91 (100), 68 (100); HRMS *m/z* (EI) 192.1177 (calcd for $C_{12}H_{16}O_2$: 192.1150, M^+). The ee of **9c** was determined by ^{1}H NMR analysis of the corresponding (+)-methoxytrifluoromethylphenylacetate (MTPA) ester, which was converted from **9c**.

^{1}H NMR of the MTPA ester (500 MHz, $CDCl_3$) δ 4.40 (d, *J*=6.5 Hz, 1H, OC*H*HPh) and 4.42 (d, *J*=6.5 Hz, 1H, OCH*H*Ph)(*S*), 4.48 (s, 2H, OC*HH*Ph)(*R*). The absolute configuration was determined by comparing the NMR signal pattern of the MTPA ester with that of the authentic sample.

4.7. Preparation of authentic (*S*)-2

Under an argon atmosphere, to a solution of ethyl (*S*)-(+)-3-hydroxybutanoate (**4**, 1.00 g, 7.57 mmol) in CH_2Cl_2 (20 mL) were added diisopropylethylamine (5.27 mL, 30.3 mmol) and a solution of chloromethylmethylether (1.82 g, 22.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction was stopped with 0.1 M phosphate buffer (pH 6.5) and the products were extracted with AcOEt (×3). The organic layer was washed with brine (×2) and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by column chromatography (hexane/AcOEt=5/1) to give ethyl (*S*)-3-(methoxymethoxy)butanoate as a colorless oil (**5**, 1.12 g, 84%); IR (neat) 2978, 1738, 1449, 1377, 1300, 1186, 1150, 1103, 1036, 918 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (d, *J*=6.0 Hz, 3H), 1.26 (t, *J*=7.0 Hz, 3H), 2.41 (dd, J_1=5.5 Hz, J_2=15.0 Hz, 1H), 2.60 (dd, J_1=7.5 Hz, J_2=15.0 Hz, 1H), 3.36 (s, 3H), 4.08–4.22 (m, 1H), 4.15 (q, *J*=7.0 Hz, 2H), 4.66 (d, *J*=7.0 Hz, 1H), 4.67 (d, *J*=7.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 20.5, 42.4, 55.3, 60.3, 70.3, 95.3, 171.2.

Under an argon atmosphere, to a suspension of $LiAlH_4$ (200 mg, 5.26 mmol) in THF (5 mL) was added a solution of (*S*)-**5** (901 mg, 5.12 mmol) in THF (10 mL) at 0 °C. After the mixture was stirred for 1 h at room temperature, the reaction was quenched with water (200 μL), 15% NaOH aqueous solution (200 μL), and water (400 μL). After filtration thorough a Celite pad and evaporation, the residue was purified by column chromatography (hexane/AcOEt=1/1→AcOEt) to give (*S*)-3-(methoxymethoxy)-1-butanol as a colorless oil (**6**, 588 mg, 86%); IR (neat) 3428, 2963, 1449, 1411, 1377, 1261, 1103, 1036, 797 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, *J*=6.0 Hz, 3H), 1.75 (dt, J_1=J_2=6.0 Hz, 2H), 2.69 (br s, 1H), 3.39 (s, 3H), 3.68–3.85 (m, 2H), 3.93 (tq, J_1=J_2=6.0 Hz, 1H), 4.63 (d, *J*=7.0 Hz, 1H), 4.72 (d, *J*=7.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.2, 39.1, 55.4, 59.9, 72.0, 94.9.

Under an argon atmosphere, to a suspension of NaH (60% in oil, 337 mg, 8.43 mmol) in THF (5 mL) were added a solution of (*S*)-**6** (501 mg, 3.74 mmol) in THF (10 mL) and benzyl bromide (0.44 mL, 3.74 mmol) at 0 °C. The mixture was stirred for 4 h at room temperature and the reaction was quenched with 0.1 M phosphate buffer (pH 6.5). The products were extracted with AcOEt (×3), and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=10/1→5/1) to give (*S*)-1-benzyloxy-3-(methoxymethoxy)butane as a colorless oil (**7**, 541 mg, 65%); IR (neat) 2930, 2882, 1452, 1375, 1207, 1103, 1040, 918, 737, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, *J*=6.0 Hz, 3H), 1.70–1.89 (m 2H), 3.35 (s, 3H), 3.50–3.64 (m, 2H), 3.87 (tq, J_1=J_2=6.5 Hz, 1H), 4.50 (s, 3H), 4.60 (d, *J*=7.0 Hz, 1H), 4.67 (d, *J*=7.0 Hz, 1H), 7.22–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 37.2, 55.3, 66.9, 70.6, 73.0, 95.1, 127.5, 127.7, 128.3, 138.4.

To a solution of (*S*)-**7** (400 mg, 1.79 mmol) in THF (10 mL) was added 2 M HCl (4 mL). After the mixture was stirred overnight at room temperature, the reaction mixture was diluted with water. The products were extracted with AcOEt

(×3), and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=10/1→4/1) to give the remaining (*S*)-**7** (193 mg, 48%) and (*S*)-**2** as colorless oils (167 mg, 52%); (*S*)-**2**, $[\alpha]_D^{27}$ +19.0 (*c* 0.95, MeOH). The spectral data were in full agreement with those of the (*R*)-**2** obtained by the enzymatic reaction.

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References and notes

1. (a) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley-VCH: Weinheim, 1999; (b) Bommarius, A. S.; Riebel, B. R. *Biocatalysis*; Wiley-VCH: Weinheim, 2004; (c) Faber, K. *Biotransformations in Organic Chemistry: A Textbook*, 5th ed.; Springer: Berlin, Heidelberg, New York, 2004.
2. For a review of modern separation techniques, see: Tzschucke, C. C.; Markert, C.; Bannwarth, W.; Roller, S.; Hebel, A.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 3964–4000.
3. For enzymatic acylation with succinic acid, see: (a) Terao, Y.; Tsuji, K.; Murata, M.; Achiwa, K.; Nishio, T.; Watanabe, N.; Seto, K. *Chem. Pharm. Bull.* **1989**, *37*, 1653–1655; (b) Gutman, A. L.; Brenner, D.; Boltanski, A. *Tetrahedron: Asymmetry* **1993**, *4*, 839–844.
4. For lipase-catalyzed transformation followed by sulfation, see: Yamano, T.; Kikumoto, F.; Yamamoto, S.; Miwa, K.; Kawada, M.; Ito, T.; Ikemoto, T.; Tomimatsu, K.; Mizuno, Y. *Chem. Lett.* **2000**, 448–449.
5. For using fluorous-tagged esters with fluorous solvents, see: (a) Beier, P.; O'Hagan, D. *Chem. Commun.* **2002**, 1680–1681; (b) Luo, Z.; Swaleh, S. M.; Theil, F.; Curran, D. P. *Org. Lett.* **2002**, *4*, 2585–2587.
6. For enzymatic transesterification of esters with PEG as a nucleophile, see: (a) Wallace, J. S.; Reda, K. B.; Williams, M. E.; Morrow, C. J. *J. Org. Chem.* **1990**, *55*, 3544–3546; (b) Whalen, L. J.; Morrow, C. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1279–1288.
7. For enzyme-mediated kinetic resolution of carboxylic acids anchored on polystyrene based resin, see: Nanda, S.; Rao, B.; Yadav, J. S. *Tetrahedron Lett.* **1999**, *40*, 5905–5908.
8. For lipase-catalyzed kinetic resolution with poly(acrylamide)-ethylene glycol (PEGA)-based resin as a nucleophile, see: Ulijn, R. V.; Bisek, N.; Flitsch, S. L. *Org. Biomol. Chem.* **2003**, *1*, 621–622.
9. Córdova, A.; Tremblay, M. R.; Clapham, B.; Janda, K. D. *J. Org. Chem.* **2001**, *66*, 5645–5648.
10. Halcomb, R. L.; Huang, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 11315–11322.
11. (a) Waldmann, H.; Reidel, A. *Angew. Chem., Int. Ed.* **1997**, *36*, 647–649; (b) Sauerbrei, B.; Jungmann, V.; Waldmann, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 1143–1146.
12. (a) Ulijn, R. V.; Baragaña, B.; Halling, P. J.; Flitsch, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 10988–10989; (b) Humphrey, C. E.; Turner, N. J.; Easson, M. A. M.; Flitsch, S. L.; Ulijn, R. V. *J. Am. Chem. Soc.* **2003**, *125*, 13952–13953; (c) Basso, A.; Ulijn, R. V.; Flitsch, S. L.; Margetts, G.; Brazendale, I.; Ebert, C.; De Martin, L.; Linda, P.; Verdelli, S.; Gardossi, L. *Tetrahedron* **2004**, *60*, 589–594; (d) Basso, A.; Ebert, C.; Gardossi, L.; Linda, P.; Phuong, T. T.; Zhu, M.; Wessjohann, L. *Adv. Synth. Catal.* **2005**, *347*, 963–966.
13. (a) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489–509; (b) Wentworth, P.; Janda, K. D. *Chem. Commun.* **1999**, 1917–1924; (c) Dickerson, T. J.; Reed, N. N.; Janda, K. D. *Chem. Rev.* **2002**, *102*, 3325–3344; (d) Bergbreiter, D. E. *Chem. Rev.* **2002**, *102*, 3345–3384.
14. (a) Benaglia, M.; Celentano, G.; Cozzi, F. *Adv. Synth. Catal.* **2001**, *343*, 171–173; (b) Pozzi, G.; Cavazzini, M.; Quici, S.; Benaglia, M.; Dell'Anna, G. *Org. Lett.* **2004**, *6*, 441–443; (c) Oikawa, M.; Tanaka, T.; Kusumoto, S.; Sasaki, M. *Tetrahedron Lett.* **2004**, *45*, 787–790; (d) Reed, N. N.; Dickerson, T. J.; Boldt, G. E.; Janda, K. D. *J. Org. Chem.* **2005**, *70*, 1728–1731.
15. (a) Veronese, F. M.; Morpurgo, M. *Il Farmaco* **1999**, *54*, 497–516; (b) Greenwald, R. B. *J. Controlled Release* **2001**, *74*, 159–171.
16. Shimojo, M.; Matsumoto, K.; Nogawa, M.; Nemoto, Y.; Ohta, H. *Tetrahedron Lett.* **2004**, *45*, 6769–6773.
17. (a) Inada, Y.; Yoshimoto, T.; Matsushima, A.; Saito, Y. *Trends Biotechnol.* **1986**, *4*, 68–73; (b) Inada, Y.; Takahashi, K.; Yoshimoto, T.; Ajima, A.; Matsushima, A.; Saito, Y. *Trends Biotechnol.* **1986**, *4*, 190–194.
18. (a) Otamiri, M.; Adlerkreutz, P.; Mattiasson, B. *Biotechnol. Bioeng.* **1994**, *44*, 73–78; (b) Ruppert, S.; Gais, H. J. *Tetrahedron: Asymmetry* **1997**, *8*, 3657–3664.
19. López-Pelegrín, J. A.; Wentworth, P., Jr.; Sieber, F.; Metz, W. A.; Janda, K. D. *J. Org. Chem.* **2000**, *65*, 8527–8531.
20. Low-molecular weight PEG was used as a tag for real time reaction monitoring in the synthesis of oligosaccharides. (a) Manabe, S.; Ito, Y. *J. Am. Chem. Soc.* **2002**, *124*, 12638–12639; (b) Hanashima, S.; Manabe, S.; Ito, Y. *Synlett* **2003**, 979–982.
21. (a) Matsumoto, K.; Sato, Y.; Shimojo, M.; Hatanaka, M. *Tetrahedron: Asymmetry* **2000**, *11*, 1965–1973; (b) Matsumoto, K.; Nakamura, Y.; Shimojo, M.; Hatanaka, M. *Tetrahedron Lett.* **2002**, *43*, 6933–6936.
22. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
23. Hultin, P. G.; Jones, J. B. *Tetrahedron Lett.* **1992**, *33*, 1399.
24. (a) Casara, P.; Darwin, C.; Metcalf, B.; Jung, M. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2201–2208; (b) Cadot, C.; Dalko, P. I.; Cossy, J.; Ollivier, C.; Chuard, R.; Renaud, P. *J. Org. Chem.* **2002**, *67*, 7193–7202.
25. Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1988**, 598–600.
26. Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129–6139.

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Fluorescence study on the nyctinasty of *Phyllanthus urinaria* L. using novel fluorescence-labeled probe compounds

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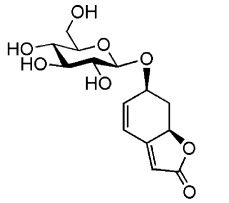
Abstract—We report the synthesis of fluorescence-labeled probes based on phyllanthurinolactone **1**, which is a leaf-closing substance of *Phyllanthus urinaria* L. The fluorescence study using biologically active probe **2** and inactive probes (*epi*-**2** and **31**) revealed that the target cell for **1** is a motor cell and suggested that some receptors, which recognize the aglycon of **1** exist on the plasma membrane of the motor cell, as with leaf-opening substances. Moreover, binding of probe **2** was specific to the plant motor cell contained in the plants belonging to the genus *Phyllanthus*. These results showed that the binding of probe **2** with a motor cell is specific to the plant genus and suggested that the genus-specific receptor for the leaf-closing substance would be involved in nyctinasty.

1. Introduction

Most leguminous plants close their leaves in the evening, as if to sleep, and open them early in the morning according to the circadian rhythm controlled by a biological clock.[1] Nyctinastic leaf-movement is induced by the swelling and shrinking of motor cells in the pulvini, a small organ located in the joint of the leaf to the stem. Motor cells play a key role in plant leaf-movement. The flux of potassium ions across the plasma membranes of the motor cells is followed by massive water flux, which results in swelling and shrinking of these cells.[2] We have revealed that nyctinasty is controlled by a pair of leaf-movement factors: leaf-opening and leaf-closing substances.[3] It is already clarified by using fluorescence-labeled leaf-opening substances that the target cell of the leaf-opening substance is a motor cell.[4] On the other hand, no attempt has been carried out to clarify the target cell of the leaf-closing substances because the structure of most leaf-closing substances is too simple[3] to develop a molecular probe, such as a fluorescence-labeled one. This is because large fluorescence dye would cause serious decrease in the bioactivity of the synthetic probe.

Phyllanthurinolactone **1** is a glycoside-type leaf-closing substance of *Phyllanthus urinaria* L.[5] (Fig. 1). The structure of **1** is comparatively large enough for the structure modification



Phyllanthurinolactone (1)

Figure 1.

that is essential for a synthetic probe. Syntheses of **1** and its analogs were completed by Mori and Audran[6] and our group.[7] The structure–activity relationship studies using them showed that the stereochemistry in aglycon of natural product **1** is important for its bioactivity and the structure modification in the sugar moiety has no effect on the bioactivity. Recently, we developed the fluorescence-labeled probe **2** based on the result of structure–activity relationship studies, and revealed that probe **2** binds to a motor cell specifically.[8] However, in order to prove the existence of a receptor on a motor cell, an appropriate control experiment using biologically inactive probe compounds such as C-4 *epi*-**2** and unsaturated derivative **31** that cannot bind to the target cell is essential. In this paper, we carried out the direct observation of the target cell for **1** using biologically active and inactive fluorescence-labeled probes and revealed that the receptor of **1** exists in a motor cell. Additionally, we found by using the biologically active probe **2** that genus-specific receptors of leaf-closing substances are involved in nyctinasty.

Keywords: Nyctinasty; Leaf-closing substance; Fluorescence; Probe compound; Motor cell; Mitsunobu reaction; Glycosidation.
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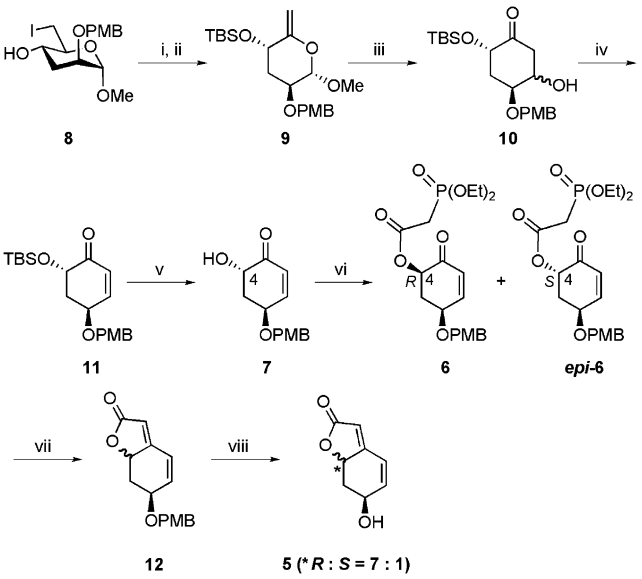
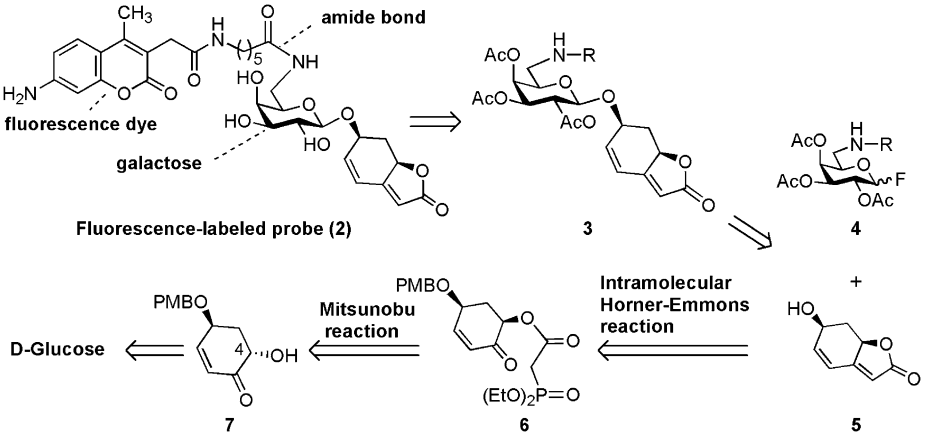
2. Results and discussion

2.1. The design and synthetic plan of fluorescence-labeled probe compounds

Based on the previously obtained result[6,7] that the structure modification of the sugar moiety did not affect the bioactivity, the big fluorescence dye was introduced into the hydroxy group at the C6′ position of the sugar moiety of **1**. Since **1** is easily hydrolyzed by β-glucosidase, the sugar moiety was changed from D-glucose into D-galactose.[9] Moreover, in order to increase the resistance against the esterase involved in a plant body, an amide bond was selected instead of ester bond connecting the fluorescence dye with **1**. The retrosynthesis of **2** is shown in Scheme 1. The fluorescence-labeled probe **2** could be synthesized from the intermediate **3**, which was bisected into the sugar moiety **4** and aglycon **5**. The lactone in aglycon **5** could be constructed by the intramolecular Horner–Emmons reaction[10] of phosphonoacetate **6**, which could be synthesized from the ketoalcohol **7** via Mitsunobu inversion[11] of the C4 stereocenter using phosphonoacetic acid. The ketoalcohol **7** could be synthesized from D-glucose.



Scheme 2. (i) TBSOTf, lutidine, CH_2Cl_2, 99%; (ii) *t*-BuOK, THF, 95%; (iii) cat. $Hg(OCOCF_3)_2$, acetone/H_2O; (vi) MsCl, Et_3N, CH_2Cl_2, 70% (two steps); (v) TBAF, AcOH, THF, quant.; (vi) see Table 1; (vii) *t*-BuOK, THF, 0 °C, 63%; (viii) DDQ, CH_2Cl_2/H_2O, 71%.



Scheme 1.

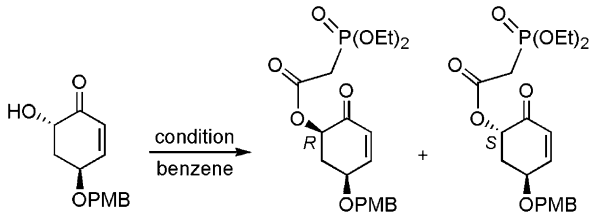
2.2. Synthesis of fluorescence-labeled probe compounds

The synthesis of aglycon is shown in Scheme 2.[8] Intermediate **8**, prepared according to the previous paper,[7] was protected by the TBS group, and then subjected to elimination of iodide to afford the enol ether **9**. A catalytic amount of $Hg(OCOCF_3)_2$-mediated Ferrier's carbocyclization[12] of **9** and following treatment of the resulting alcohol **10** with mesyl chloride and triethylamine, that is, a one-pot reaction involving mesylation and β-elimination, afforded the cyclohexanone derivative **11** in 70% overall yield in two steps. Deprotection of the TBS group gave the alcohol **7**. We considered that epimerization of the C4 stereocenter and introduction of diethyl phosphonoacetate could be carried out simultaneously using the Mitsunobu reaction[11] (Table 1). At first, the reaction did not proceed under the standard Mitsunobu reaction condition (entry 1). Tributylphosphine gave many products (entry 2). The phosphonoacetate **6** was obtained as a mixture of C4 stereoisomers (*R*:*S* = 5:1) in moderate yield by using excess amount of the Mitsunobu reagents (entry 3). However, the purification of **6** was very difficult because of a large amount of triphenylphosphine oxide, which was produced as a byproduct. Therefore, we examined phosphines **14** and **15**, which are easily removed by acid treatment (entry 4 and 5).[13] Phosphine **14** gave lower yield and stereoselectivity (*R*:*S* = 3:1). However, phosphine **15** afforded phosphonoacetate **6** in moderate yield with higher stereoselectivity (*R*:*S* = 10:1) than triphenylphosphine. Additionally, the phosphine oxide from **15** was easily removed by simple acid treatment.

Intramolecular Horner–Emmons reaction of the resulting **6** using *t*-BuOK as the base afforded lactone **12**. Finally, deprotection of the PMB group by DDQ gave the aglycon **5** as a 7:1 diastereomeric mixture.

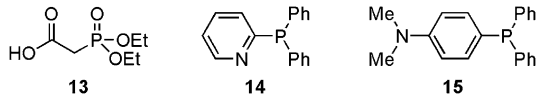
Next, we examined the conditions of the glycosidation reaction. In our previous synthesis, β-D-galactopyranosyl bromide was used for glycosidation according to the method reported by Mori and Audran.[6] In this method, the desired

Table 1. Mitsunobu reaction of alcohol **7**



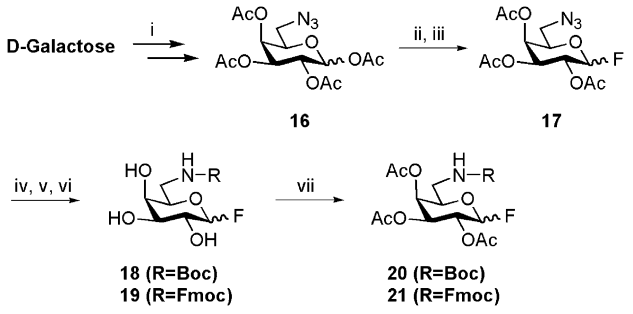
7 **6** ***epi*-6**

Entry	Phosphine (equiv)	DEAD (equiv)	**13** (equiv)	Temp (°C)	Yield % (**6**+*epi*-**6**)	Ratio (**6**:*epi*-**6**)
1	PPh_3 (2.0)	2.0	2.0	rt	0	—
2	PBu_3 (2.0)	2.0	2.0	rt	Messy	—
3	PPh_3 (8.0)	8.0	8.0	0	42	5:1
4	**14** (2.0)	8.0	8.0	0	16	3:1
5	**15** (2.0)	8.0	8.0	0	40	10:1



13 **14** **15**

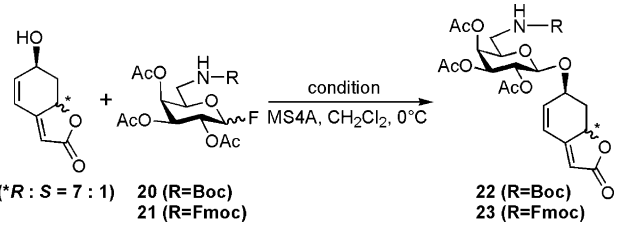
coupling product was obtained in low yield (28% yield) with a large amount of acetyl aglycon. Therefore, we examined the use of glycosyl fluoride as a glycosyl donor. The synthesis of β-D-galactopyranosyl fluoride is shown in Scheme 3. Acetate **16**, prepared from D-galactose according to the procedure in Ref. 14, was treated with hydrazine monohydrate and fluorination of the resulting hydroxy group with DAST afforded fluoride **17**. Removal of acetates and reduction of azide were followed by protection of amine with a Boc or Fmoc group to give the triols **18** and **19**. Finally, acetylation of the triols **18** and **19** yielded the 1-fluorosugars **20** and **21**.



Scheme 3. (i) See Ref. 14; (ii) H_2NNH_2/AcOH, DMF, 0 °C, 74%; (iii) DAST, CH_2Cl_2, 92%; (iv) MeONa, MeOH, 0 °C; (v) H_2, Pd/C, MeOH; (vi) $(Boc)_2O$, pyridine or FmocCl, DIPEA, THF, 0 °C; (vii) Ac_2O pyridine, 65% (four steps) (R = Boc), 28% (four steps) (R = Fmoc).

Glycosidation using β-D-galactopyranosyl fluoride is summarized in Table 2. In the case of *N*-Boc protected galactopyranosyl fluoride **20**, both Suzuki[15] and Mukaiyama methods[16] gave only a trace amount of glycoside **22** (entry 1 and 2). Under these conditions, *N*-Boc protected sugar was decomposed and the aglycon **5** was recovered. On the other hand, glycosidation of *N*-Fmoc protected galactopyranosyl fluoride **21** via the Suzuki method gave glycoside **23** in moderate yield (entry 3). Using 1.6 equiv of the sugar **21**, the yield was improved to 52% (entry 4). In this reaction condition, aglycon **5** was recovered in 15% yield with no production of acetyl aglycon.

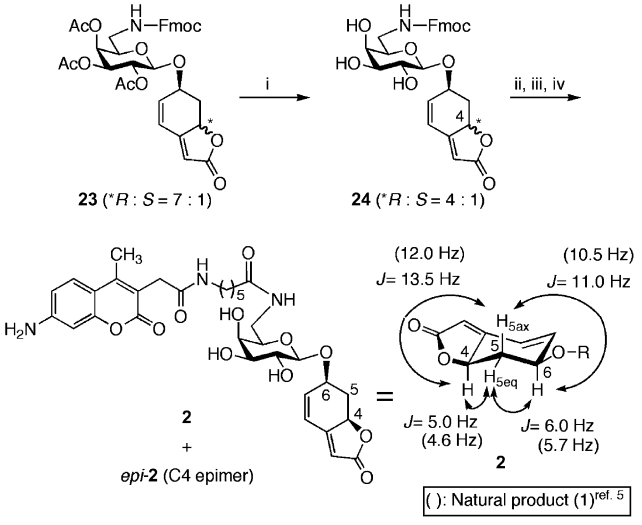
Table 2. Glycosidation



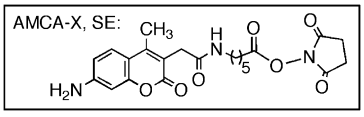
5 (*R : S = 7 : 1) **20** (R=Boc) **21** (R=Fmoc) **22** (R=Boc) **23** (R=Fmoc)

Entry	**20** or **21** (equiv)	Condition (equiv)	**22** or **23**	**5** (%)
1	**20** (2.0)	Cp_2HfCl_2 (3.0), $AgClO_4$ (3.0)	**22** (trace)	32
2	**20** (2.0)	$SnCl_2$ (5.0), $AgClO_4$ (5.0)	**22** (trace)	45
3	**21** (1.2)	Cp_2HfCl_2 (2.0), $AgClO_4$ (2.0)	**23** (26%)	40
4	**21** (1.6)	Cp_2HfCl_2 (2.0), $AgClO_4$ (2.0)	**23** (52%)	15

The synthesis of fluorescence-labeled probe **2** was then examined (Scheme 4). Acetyl groups of the glycoside **23** were removed with KCN[6,17] to give the triol **24** as a 4:1 mixture of C4 stereoisomers. At this stage, further epimerization occurred. Because the C4 proton in **24** could be easily abstracted by KCN, the resulting stable furan-type intermediate gave a mixture of **24** and its epimer by protonation. Deprotection of the Fmoc group and introduction of AMCA-X gave fluorescence-labeled probe **2** as a 4:1 diastereomeric mixture. Diastereomerically pure **2** and its epimer *epi*-**2** were obtained after purification by HPLC (COSMOSIL 5C18-AR, 20% CH_3CN aq, 260 nm). The desired **2** was confirmed by coupling constants in the ^1H NMR spectra (Scheme 4). The relation between H4 and H6 was determined to be *syn* by the coupling constants between H4 and H5ax, H4 and H5eq, H5ax and H6, and H5eq and H6.



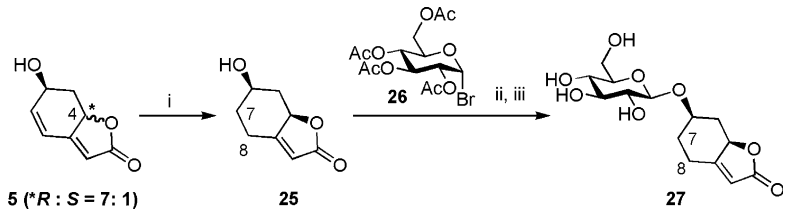
Scheme 4. (i) KCN, MeOH, 0 °C, 47%; (ii) piperidine, DMF, 0 °C; (iii) AMCA-X, SE, DMF, 0 °C, quant. (two steps); (iv) HPLC separation.



To prove the existence of a receptor, it is essential to demonstrate that biologically inactive probe compounds cannot

bind to the target cell for active compounds. Structure modification in the aglycon moiety of **2** reduces its bioactivity. Thus, we planned synthesis of fluorescence-labeled compound **31**, which has the reduced C7–C8 double bond, as a biologically inactive probe for the control fluorescence experiment.

Synthesis of 7,8-dihydro analog **27** is shown in Scheme 5. The hydrogenation of aglycon **5** using the Lindlar catalyst gave 7,8-dihydro aglycon **25**. At this stage, the reduced aglycon and its C4 epimer were easily separated by silica gel column chromatography. Glycosidation of 7,8-dihydro aglycon **25** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide



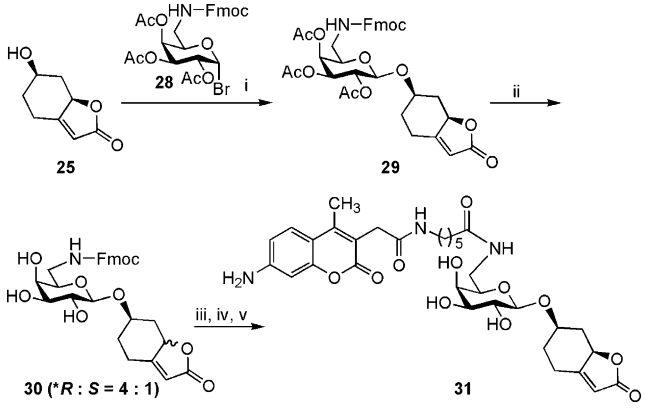
5 (*R : S = 7: 1) 25 27

Scheme 5. (i) H_2, Lindlar catalyst, AcOEt, 68%; (ii) **26**, AgOTf, Ag_2CO_3, MS4Å, CH_2Cl_2, 20%; (iii) KCN, MeOH, 31%.

26 was carried out with Ag_2CO_3 and AgOTf to afford the glycoside and subsequent deprotection of acetyl groups yielded 7,8-dihydro phyllanthurinolactone **27**.

The 7,8-dihydro analog **27** showed no leaf-closing activity against *P. urinaria* L. even at 1×10^{-4} M. From this result, we judged that the fluorescence-labeled compound with 7,8-dihydro aglycon could be used as a biologically inactive probe.

Synthesis of the biologically inactive probe **31** is shown in Scheme 6. Glycosidation of 7,8-dihydro aglycon **25** and glucopyranosyl bromide **28** via Koenigs–Knorr reaction afforded glycoside **29**. Acetyl groups of glycoside **29** were removed with KCN to give triol **30** as a 4:1 mixture of C4 stereoisomers. At this stage, epimerization of the C4 stereocenter was observed as in the case of **23**, which is the intermediate of the biologically active probe **2**. Deprotection of the Fmoc group and introduction of AMCA-X gave fluorescence-labeled probe **31** as a 4:1 diastereomeric mixture. Diastereomerically pure **31** was obtained after purification by HPLC (COSMOSIL 5C18-AR, 40% CH_3CN aq, 260 nm).



Scheme 6. (i) **28**, AgOTf, Ag_2CO_3, MS4Å, CH_2Cl_2, 48%; (ii) KCN, MeOH 61%; (iii) piperidine, DMF, 0 °C; (iv) AMCA-X, SE, DMF, 0 °C, 62% (two steps); (v) HPLC separation.

2.3. Bioassay of fluorescence-labeled probes

With the fluorescence-labeled probes **2**, *epi*-**2** and **31** were now available. Then, we examined their bioactivities. The young leaves detached from the stem of the plant *P. urinaria* with a sharp razor blade were used for bioassay. One leaf was placed in H_2O (ca. 300 μl) using a 5-ml glass tube in the biotron kept at 32 °C and allowed to stand overnight. The leaves, which opened again in the morning were used for the bioassay. The test solution was carefully poured into test tubes with a microsyringe at around 10:00 a.m. The bioactive fraction was judged by the leaf-closing activity before the leaf-closing of the plant leaf in the blank solution. Probe **2** was effective for the leaf-closing of *P. urinaria* at 1×10^{-5} M, that is, one-hundredth as effective as natural product **1**. On the other hand, *epi*-**2**, which is the C4 epimer of **2**, and 7,8-dihydro probe **31** were biologically inactive ($>1\times10^{-4}$ M).

2.4. Fluorescence study using fluorescence-labeled probes

We used fluorescence-labeled probes to seek the target cell for **1**. For this purpose, the binding experiment using a plant section was carried out. The leaf of *P. urinaria* opening in the daytime was cut into an appropriate size and fixed in agar. The agar was sliced perpendicular to the petiole by a microslicer (Dousaka EM Co., Ltd) to a thickness of 30 μm and the sections containing the pulvini were floated on distilled water. The sections were immersed in a solution containing 1×10^{-4} M of the probes (**2**, *epi*-**2**, and **31**), and allowed to stand for staining under shaded condition at room temperature for 6 h. After staining, the stained section was incubated for 15 min in equilibrium buffer (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.) to remove excess fluorescence probes. Then, the stained section was placed on a slide glass and covered by a cover glass after adding a drop of antifade reagent (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.). The observation of these sections was carried out by a fluorescence microscope (ECLIPSE E800, Nicon Co., Ltd) with an appropriate filter (B-2A, Nicon Co., Ltd; excitation wavelength 450–490 nm). At this time, the use of an antifade reagent was essential to prevent photobleaching (fading of fluorescence). Figure 2 shows photographs of plant pulvini, which contains a motor cell, under a fluorescence microscope. No stain was observed in the control section, which was treated with an aqueous solution containing no fluorescence-labeled probe (upper center). Red stains seen in the fluorescence images are due to the porphyrin in the plant tissue. The staining pattern for the fluorescence of the biologically active probe **2** was observed on the surface of the motor cell (upper right). We also carried out fluorescence studies of the interaction

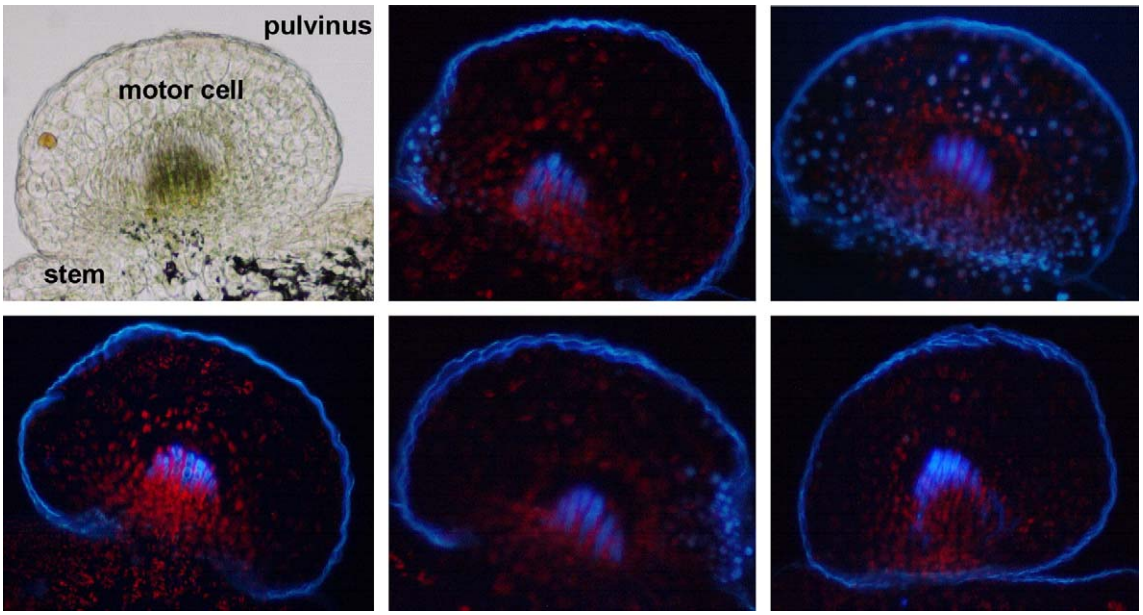


Figure 2. Fluorescence study using plant pulvini containing motor cell with fluorescence-labeled probes; upper left: Nomarskii image of plant section, upper center: fluorescence image of control section, upper right: fluorescence image of a section treated with **2**, lower left: fluorescence image of a section treated with *epi*-**2**, lower center: fluorescence image of a section treated with **31**, lower right: fluorescence image of a section treated with **2** in the presence of 100 molar excess of phyllanthurinolactone **1**.

between biologically inactive probes (*epi*-**2** and **31**) and the plant motor cell. Stains were not observed in the section treated with *epi*-**2** and **31** (lower left and lower center). Thus, it was proved that biologically inactive probe compounds cannot bind to the plant motor cell. Also, binding of probe **2** was inhibited by the coexistence of 100-fold concentration of the natural product **1**. When the section was treated by 1×10^{-4} M of **2** together with 1×10^{-2} M of **1**, no staining was observed in the plant section (lower right). Results of the structure–activity relationship were consistent with that of binding experiments. Also, it was clearly shown that the binding of biologically active fluorescence-labeled probe **2** with a motor cell is due to the specific binding of the aglycon moiety, which is the active site of this molecule, and is not a nonspecific binding due to the hydrophobicity of the fluorescence dye group (AMCA group). These results strongly suggested that some receptor for **1** exists in the motor cell, which plays a central role in the plant leaf-movement,[18] as with leaf-opening substances.[4] Along with the previous result,[6,7] some properties were revealed in a receptor molecule of **1**, that is, this receptor recognizes the precise structure of the aglycon moiety, whereas it does not recognize the sugar moiety at all.

2.5. Specific binding ability of fluorescence-labeled probe compound to *P. urinaria* L.

From our previous studies, it was revealed that each nyctinastic plant has a different leaf-movement factor whose bioactivity is specific to the plant genus.[19] Recently, we proved that the leaf-opening substances do not bind with motor cells of plants belonging to other genus by using the fluorescence-labeled probes based on the leaf-opening substances of *Cassia mimosoides* L.[4] and *Albizzia julibrissin* Durazz.[20] Thus, fluorescence-labeled probe **2** is expected to show specific leaf-closing activity to genus *Phyllanthus*, and not to be effective for other plants as well as leaf-opening substances. We examined the genus-specificity and the bioactivity of probe **2**. Probe **2** did not show leaf-closing activity against the leaves of *C. mimosoides*, *Mimosa pudica*, and *Leucaena leucocephala* at 1×10^{-4} M. From these results, the binding of probe **2** is expected to be specific to the section of plants belonging to the genus *Phyllanthus* and no binding would be observed in the experiment using the section of other plants. Then, we used probe **2** for the binding experiment with the sections of *M. pudica*, *C. mimosoides*, and *L. leucocephala*. The binding experiments were carried out according to the same method used in the case of *P. urinaria*; however, these sections gave no staining pattern resulting from **2** (Fig. 3). These results showed that the binding of probe **2** with a motor cell is specific to the genus *Phyllanthus* and suggested that a genus-specific receptor molecule for the leaf-movement factor, which is located on a motor cell would be involved in nyctinasty.

3. Conclusion

The binding of probes can be strongly correlated with leaf-closing activity. Biologically inactive *epi*-**2** and **31** did not bind to a motor cell at all. Additionally, the staining pattern resulting from the binding of probe **2** disappeared by the coexistence of an excess amount of the natural product **1**. These results strongly suggested that some receptors for **1**, which specifically recognize the stereochemistry of aglycon of **1** would be involved in the leaf-closing movement of *P. urinaria*. Moreover, since probe **2** did not show leaf-closing activity against plants belonging to other genus, it was revealed that the binding of a leaf-closing substance with a motor cell is specific to the plant genus as well as the leaf-opening substance and suggested that the genus-specific receptor for the leaf-closing substance in a motor cell would be involved in nyctinasty. In conclusion, we have succeeded in visualization of the target cell of the leaf-closing substance in the plant body by using the biologically active probe **2**

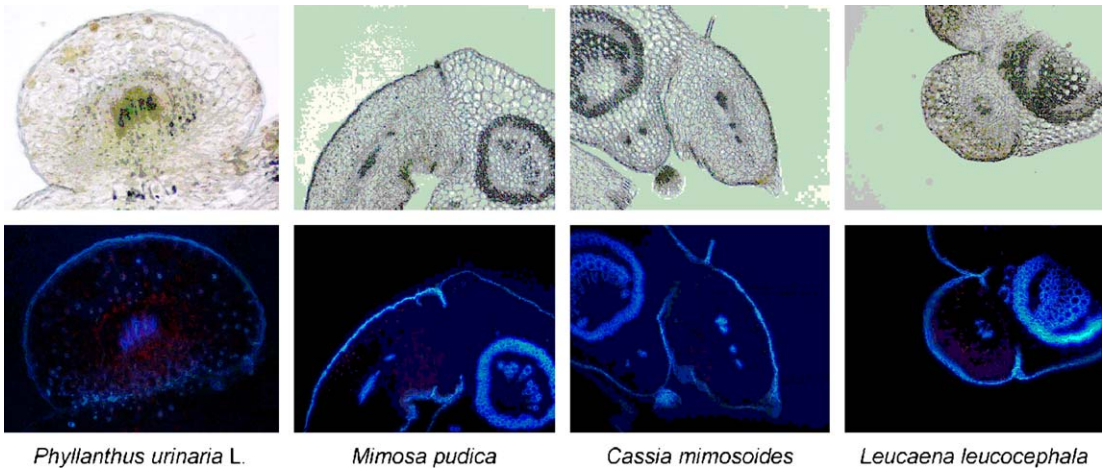


Figure 3. Photographs of plant sections in the binding experiments, which show specific binding of probe **2** with the motor cells of *Phyllanthus* plants (upper: Nomarski image of the plant section, lower: fluorescent image of the plant section treated with probe **2**.

and inactive probes *epi*-**2** and **31**, and revealed that the binding between a leaf-closing substance and its receptor in a motor cell is genus-specific. Some receptor for **1** would be involved in the leaf-closing movement in *P. urinaria* L. To reveal a receptor protein of **1**, the synthesis of photoaffinity labeling probes based on **1** is now in progress.

4. Experimental

4.1. General procedures

NMR spectra were recorded on a Jeol JNM-A600 spectrometer [^{1}H (600 MHz) and ^{13}C (150 MHz)], Jeol JNM-A400 [^{1}H (400 MHz) and ^{13}C (100 MHz)], JNM AL300 [^{1}H (300 MHz) and ^{13}C (75 MHz)], a Jeol JNM-EX 270 spectrometer [^{1}H (270 MHz) and ^{13}C (67.5 MHz)] using TMS in $CDCl_3$, CD_2HOD in CD_3OD (^{1}H; 3.33 ppm, ^{13}C; 49.8 ppm), or *t*-BuOH (^{1}H; 1.23 ppm, ^{13}C; 32.1 ppm) in D_2O as internal standards at various temperatures. The FABMS and HR-FABMS spectra were recorded on a Jeol JMS-700 or JMS-SX102 spectrometer, using glycerol or *m*-nitrobenzylalcohol as a matrix. The ESI-HRMS spectra were recorded on a Bruker APEX-III spectrometer. The IR spectra were recorded on a JASCO FT/IR-410 spectrometer. The specific rotations were measured by JASCO DIP-360 polarimeter. The HPLC purification was carried out with a Shimadzu LC-6A pump equipped with SPD-6A detector using COSMOCIL 5C_{18}-AR column (ϕ20×250 mm) (Nakalai Tesque Co., Ltd). The solvents used for HPLC were available from Kanto Chemical Co., and were filtered through a Toyo Roshi membrane filter (cellulose acetate of 0.45 mm pore size, 47 mm d.) before use. Silica gel column chromatography was performed on silica gel 60 K070 (Katayama Chemical Co., Ltd) or silica gel 60N (Kanto Chemical Co., Ltd). Reversed-phase open-column chromatography was performed on Cosmosil 75C_{18}-OPN (Nakalai Tesque Co., Ltd). TLC was performed on silica gel F_{254} (0.25 or 0.5 mm, MERCK) or RP-18F_{254S} (0.25 mm, MERCK).

4.1.1. *tert*-Butyl-[6-methoxy-5-(4-methoxy-benzyloxy)-2-methylene-tetrahydro-pyran-3-yloxy]-dimethyl-silane 9. To a solution of **8** (86.9 mg, 213 μmol) in CH_2Cl_2 (2.4 ml) was slowly added TBSOTf (72.4 μl, 320 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, to the reaction mixture was added H_2O (2 ml). The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford a TBS ether (110 mg, 99%) as a colorless oil: $[\alpha]_D^{21}$ +75.5 (*c* 1.00, MeOH). IR (film) ν 2856, 2835, 1612, 1514 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 4.60 (1H, s), 4.55 (2H, s), 3.82 (3H, s), 3.62 (1H, ddd, *J*=4.0, 8.5, 11.0 Hz), 3.50 (1H, dd, *J*=2.2, 10.1 Hz), 3.45 (1H, d, *J*=2.2 Hz), 3.40 (3H, s), 3.20 (1H, dd, *J*=8.5, 10.1 Hz), 1.90 (1H, ddd, *J*=3.6, 4.0, 13.2 Hz), 1.65 (1H, ddd, *J*=3.0, 11.0, 13.2 Hz), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 159.2, 130.1, 129.1, 113.8, 98.5, 74.6, 73.2, 70.7, 67.6, 55.2, 54.9, 33.3, 25.7, 7.7, −4.1, −4.6. ESI-HRMS (positive-ion) calcd for $C_{21}H_{35}IO_5SiNa$: $(M+Na)^+$ 545.1196; found: 545.1191.

To a solution of the above TBS ether (55.9 mg, 107 μmol) in THF (1.0 ml) was added *t*-BuOK (39.3 mg, 321 μmol) at room temperature under Ar atmosphere. After stirring for 15 h, to the reaction mixture was added 1 N HCl (2 ml). The aqueous layer was extracted with AcOEt. The combined organic layer was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford **9** (70.8 mg, 99%) as a colorless oil: $[\alpha]_D^{22}$ +11.8 (*c* 0.42, MeOH). IR (film) ν 2832, 2858, 2837, 1660, 1612 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 4.68 (1H, s), 4.70 (1H, d, *J*=1.5 Hz), 4.62 (1H, d, *J*=11.2 Hz), 4.59 (1H, d, *J*=11.2 Hz), 4.55 (1H, d, *J*=1.5 Hz), 4.59 (1H, d, *J*=11.2 Hz), 4.41 (1H, m), 3.82 (3H, s), 3.62 (1H, s), 3.45 (3H, s), 2.05 (1H, ddd, *J*=2.9, 4.0, 13.2 Hz), 1.85 (1H, ddd, *J*=2.9, 11.4, 13.2 Hz), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 159.2, 159.0, 130.0, 129.3, 113.9, 100.4, 94.0, 74.5, 70.9, 64.2, 55.2, 55.0, 34.0, 25.7, 25.6, 25.6, −4.9, −5.0. ESI-HRMS (positive-ion) calcd for $C_{21}H_{34}O_5SiNa$: $(M+Na)^+$ 417.2073; found: 417.2068.

4.1.2. 2-(*tert*-Butyl-dimethyl-silanyloxy)-5-hydroxy-4-(4-methoxy-benzyloxy)-cyclohexanone 10. To a solution of **9** (42.4 mg, 108 μmol) in acetone/H_2O (3:1, 600 μl) was slowly added $Hg(OCOCF_3)_2$ (2.3 mg, 5.4 μmol) at 0 °C under Ar atmosphere. After stirring for 9 h at 0 °C, the reaction mixture was diluted with AcOEt and washed with 10% KI aq, 20% $Na_2S_2O_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **10** (28.8 mg, 70%) as a colorless oil: $[\alpha]_D^{19}$ +27.0 (*c* 1.00, MeOH). IR (film) ν 3422, 2856, 1719 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 4.65 (1H, d, *J*=11.5 Hz), 4.57 (1H, d, *J*=11.5 Hz), 4.27 (1H, ddd, *J*=1.0, 4.8, 7.7 Hz), 4.09 (1H, m), 3.90 (3H, s), 2.76 (1H, dd, *J*=4.3, 13.8 Hz), 2.65 (1H, dd, *J*=7.0, 13.8 Hz), 2.25 (1H, m), 1.95 (1H, m), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 129.4, 114.0, 73.6, 71.9, 71.3, 55.3, 43.3, 35.0, 25.7, 25.7, 18.2, −4.8, −5.3. ESI-HRMS (positive-ion) calcd for $C_{20}H_{32}O_5SiNa$: $(M+Na)^+$ 403.1917; found: 403.1911.

4.1.3. 6-(*tert*-Butyl-dimethyl-silanyloxy)-4-(4-methoxy-benzyloxy)-cyclohex-2-enone 11. To a solution of **10** (5.8 mg, 15.3 μmol) and Et_3N (21.3 μl, 153 μmol) in CH_2Cl_2 (1.0 ml) was slowly added MsCl (3.3 μl, 42.8 μmol) at 0 °C under Ar atmosphere. After stirring for 1 h at room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with 1 N H_2SO_4, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford **11** (5.5 mg, quant.) as a colorless oil: $[\alpha]_D^{19}$ −127.5 (*c* 1.00, MeOH). IR (film) ν 3483, 2941, 2116, 1719 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, dd, *J*=2.0, 8.6 Hz), 6.89 (1H, d, *J*=10.3 Hz), 5.97 (1H, d, *J*=10.3 Hz), 4.65 (1H, d, *J*=11.5 Hz), 4.57 (1H, d, *J*=11.5 Hz), 2.25 (2H, m), 0.95 (9H, s), 0.15 (3H, s), 0.12 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 197.5, 159.4, 147.8, 129.8, 129.4, 128.1, 113.9, 71.2, 71.0, 70.2, 55.2, 37.6, 25.7, −4.7, −5.4. ESI-HRMS (positive-ion) calcd for $C_{20}H_{30}O_4SiNa$: $(M+Na)^+$ 385.1811; found: 385.1806.

4.1.4. 6-Hydroxy-4-(4-methoxy-benzyloxy)-cyclohex-2-enone 7. To a solution of **11** (10.1 mg, 27.9 μmol) and AcOH (4.8 μl, 83.7 μmol) in THF (500 μl) was slowly added TBAF (1 M in THF, 83.7 μl, 83.7 μmol) at 0 °C under Ar atmosphere. After stirring for 46 h at room temperature, the reaction mixture was diluted with AcOEt and washed with H_2O and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **7** (6.9 mg, quant.) as a colorless oil: $[\alpha]_D^{19}$ −173.1 (*c* 1.00, MeOH). IR (film) ν 3449, 2837, 1693, 1612, 1514 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.25 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 6.89 (1H, dd, *J*=5.5, 12.3 Hz), 4.53 (1H, d, *J*=11.3 Hz), 4.34 (1H, ddd, *J*=2.0, 4.0, 5.1 Hz), 3.90 (3H, s), 2.63 (1H, ddd, *J*=2.0, 5.5, 13.5 Hz), 1.98 (1H, ddd, *J*=4.0, 12.3, 13.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 200.3, 159.4, 146.7, 129.6, 129.4, 127.8, 113.9, 71.5, 69.7, 68.9, 55.2, 35.0. ESI-HRMS (positive-ion) calcd for $C_{14}H_{16}O_4Na$: $(M+Na)^+$ 271.0946; found: 271.0941.

4.1.5. (Diethoxy-phosphoryl)-acetic acid 5-(4-methoxy-benzyloxy)-2-oxo-cyclohex-3-enyl ester 6. To a solution of **7** (67.2 mg, 271 μmol) in benzene (20 ml) were slowly added DEAD (40% toluene solution, 1.0 ml, 2.17 mmol), **15** (662 mg, 2.17 mmol), and **13** (425 mg, 2.17 mmol) at 0 °C under Ar atmosphere. After stirring for 1.5 h at room temperature, the reaction mixture was diluted with AcOEt and washed with 0.5 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **6** (46.3 mg, 40%. *R*:*S* = 10:1) as a colorless oil: $[\alpha]_D^{20}$ −6.8 (*c* 1.00, $CHCl_3$). IR (film) ν 1747, 1703, 1612, 1514, 1250 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$, rt) δ 7.28 (2H, d, *J*=8.2 Hz), 6.97 (1H, d, *J*=10.6 Hz), 6.91 (2H, d, *J*=8.2 Hz), 6.06 (1H, d, *J*=10.6 Hz), 5.31 (1H, dd, *J*=5.0, 13.7 Hz), 4.62 (1H, d, *J*=11.5 Hz), 4.56 (1H, d, *J*=11.5 Hz), 4.43 (1H, m), 4.21 (2H, q, *J*=6.9 Hz), 4.18 (2H, q, *J*=6.9 Hz), 3.82 (3H, s), 3.14 (1H, d, *J*=5.0 Hz), 3.06 (1H, d, *J*=5.0 Hz), 2.75–2.67 (1H, m), 2.17 (1H, dt, *J*=8.6, 11.2 Hz), 1.36 (6H, t, *J*=6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 192.2, 164.9, 159.4, 151.8, 129.4, 129.0, 127.4, 113.6, 72.3, 72.2, 70.9, 62.9, 55.3, 36.0, 34.0. ESI-HRMS (positive-ion) calcd for $C_{20}H_{28}O_8P$: $(M+H)^+$ 427.1522; found: 427.1540.

4.1.6. 6-(4-Methoxy-benzyloxy)-7,7a-dihydro-6*H*-benzofuran-2-one 12. To a solution of **6** (4.7 mg, 11.0 μmol) in THF (500 μl) was added *t*-BuOK (1.2 mg, 11.0 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, the reaction mixture was diluted with AcOEt and washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **12** (1.9 mg, 63%. *R*:*S* = 10:1) as a colorless oil: $[\alpha]_D^{24}$ −1.1 (*c* 1.00, $CHCl_3$). IR (film) ν 1741, 1641, 1612, 1585, 1514 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$, rt) δ 7.28 (2H, d, *J*=8.6 Hz), 6.90 (2H, d, *J*=8.6 Hz), 6.57 (1H, dd, *J*=2.3, 9.9 Hz), 6.34 (1H, d, *J*=9.9 Hz), 5.80 (1H, s), 4.82 (1H, dd, *J*=5.0, 13.5 Hz), 4.61 (1H, d, *J*=11.5 Hz), 4.55 (1H, d, *J*=11.5 Hz), 4.35–4.31 (1H, m), 3.82 (3H, s), 2.94 (1H, dt, *J*=5.0, 10.9 Hz), 1.71 (1H, dt, *J*=10.9, 13.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 173.1, 162.8, 159.4, 141.5, 129.4, 129.3, 120.2, 114.0, 111.4, 78.0, 72.5, 70.8, 55.3, 37.0. ESI-HRMS (positive-ion) calcd for $C_{16}H_{17}O_4$: $(M+H)^+$ 273.1127; found: 273.1147.

4.1.7. Aglycon 5. To a solution of **12** (10.1 mg, 37.1 μmol) in CH_2Cl_2 (500 μl) were added H_2O (25 μl) and DDQ (12.6 mg, 55.7 μmol) at 0 °C under Ar atmosphere. After stirring for 4.5 h at 0 °C, to the reaction mixture was added satd $NaHCO_3$ aq. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **5** (4.0 mg, 71%) as a white powder: $[\alpha]_D^{17}$ −13.6 (*c* 0.70, $CHCl_3$). IR (film) ν 3441, 1732, 1639, 1105 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.76 (1H, s), 6.57 (1H, dd, *J*=1.8, 9.9 Hz), 6.36 (1H, d, *J*=9.9 Hz), 5.81 (1H, s), 4.90 (1H, dd, *J*=4.4, 13.2 Hz), 4.70–4.58 (1H, m), 3.40–3.50 (1H, br s, OH), 2.93 (1H, td, *J*=5.2, 10.8 Hz), 1.68 (1H, q, *J*=11.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 173.8, 163.5, 144.1, 119.6, 111.0,

78.3, 66.5, 39.7. ESI-HRMS (positive-ion) calcd for $C_8H_8O_3Na$: $(M+Na)^+$ 175.0371; found: 175.0365.

4.1.8. Acetic acid 4,5-diacetoxy-6-azidomethyl-2-fluoro-tetrahydro-pyran-3-yl ester 17. To a solution of **16** (378 mg, 1.01 mmol) in DMF (10 ml) was slowly added H_2NNH_2/AcOH (1:1, 41 μl) at 0 °C under Ar atmosphere. After stirring for 1.5 h at 0 °C, the reaction mixture was diluted with $CHCl_3$ and washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo to afford a crude mixture of anomeric galactopyranoses (268 mg) as a colorless oil. To a solution of the mixture (248 mg) in CH_2Cl_2 (7.5 ml) was added DAST (98.2 μl, 750 μmol) at 0 °°C. After stirring for 30 min at 0 °C, the reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **17** (250 mg, 80% in two steps) as a white powder. α-*anomer*: $[\alpha]_D^{20}$ +90.3 (*c* 1.00, MeOH). IR (film) *ν* 2941, 2116, 1718 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) *δ* 5.80 (1H, dd, *J*=2.2, 53.3 Hz), 5.50 (1H, d, *J*=2.5 Hz), 5.35 (1H, dd, *J*=2.5, 11.0 Hz), 5.20 (1H, ddd, *J*=2.2, 11.0, 23.4 Hz), 4.30 (1H, dd, *J*=5.1, 7.7 Hz), 3.50 (1H, dd, *J*=7.7, 12.9 Hz), 3.25 (1H, d, *J*=5.1, 12.9 Hz), 2.20 (3H, s), 2.18 (1H, s), 2.00 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) *δ* 171.5, 171.2, 171.1, 106.5, 103.4, 70.6, 70.6, 68.4, 67.9, 67.6, 67.4, 50.6, 20.8, 20.7. ESI-HRMS (positive-ion) calcd for $C_{12}H_{16}FN_3O_7Na$: $(M+Na)^+$ 356.0870; found: 356.0864. β-*anomer*: $[\alpha]_D^{20}$ +28.6 (*c* 1.00, MeOH). IR (film) *ν* 2837, 2108, 1751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) *δ* 5.50 (1H, d, *J*=3.3, Hz), 5.33 (1H, ddd, *J*=6.9, 9.9, 10.6 Hz), 5.30 (1H, dd, *J*=6.9, 52.5 Hz), 5.06 (1H, dd, *J*=3.3, 9.9 Hz), 3.95 (1H, dd, *J*=4.8, 7.6 Hz), 3.60 (1H, dd, *J*=7.6, 12.8 Hz), 3.32 (1H, d, *J*=4.8, 12.8 Hz), 2.20 (3H, s), 2.10 (3H, s), 2.00 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) *δ* 171.2, 171.1, 170.5, 109.1, 106.2, 73.0, 72.9, 70.3, 70.1, 69.2, 68.9, 67.5, 50.5, 20.7, 20.6, 20.6. ESI-HRMS (positive-ion) calcd for $C_{12}H_{16}FN_3O_7Na$: $(M+Na)^+$ 356.0870; found: 356.0864.

4.1.9. (6-Fluoro-3,4,5-trihydroxy-tetrahydro-pyran-2-yl-methyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester 19. To a solution of **17** (59.0 mg, 177 μmol) in MeOH (1.8 ml) was slowly added MeONa (31.6 mg, 585 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, Amberlite IR-120B (H^+) was added to this solution for neutralization. After filtration, the filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in MeOH (1.5 ml) was added palladium on activated carbon (17.4 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 1.5 h and the catalyst removed by filtration. The filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture and diidopropylethylamine (30.8 μl, 177 μmol) in THF (8.0 ml) was added FmocCl (45.8 mg, 177 μmol) at room temperature. After stirring for 43 h, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=5:1) to afford **19** (22.7 mg, 32% in three steps) as a white powder. α-*anomer*: $[\alpha]_D^{23}$ +88.4 (*c* 0.10, MeOH). IR (film) *ν* 3436, 1697 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, rt) *δ* 7.85 (2H, d, *J*=7.8 Hz), 7.68 (2H, d, *J*=7.3 Hz), 7.40 (4H, m), 5.50 (1H, dd, *J*=2.2, 55.5 Hz), 4.30 (2H, d, *J*=7.0 Hz), 4.21 (1H, t, *J*=7.0 Hz), 3.82 (1H, t, *J*=6.3 Hz), 3.70–3.50 (2H, m), 3.50 (1H, dd, *J*=7.7, 12.9 Hz), 3.20 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6, rt) *δ* 156.3, 143.9, 140.8, 127.7, 127.1, 125.2, 120.1, 109.7, 106.8, 71.6, 68.9, 68.7, 65.4, 46.7, 41.1. ESI-HRMS (positive-ion) calcd for $C_{21}H_{22}FNO_6Na$: $(M+Na)^+$ 426.1329; found: 426.1323. β-*anomer*: $[\alpha]_D^{22}$ −43.0 (*c* 0.10, MeOH). IR (film) *ν* 3165, 1643 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, rt) *δ* 7.85 (2H, d, *J*=7.8 Hz), 7.68 (2H, d, *J*=7.3 Hz), 7.40 (4H, m), 4.90 (1H, dd, *J*=6.9, 54.7 Hz), 4.30 (2H, d, *J*=7.0 Hz), 4.21 (1H, t, *J*=7.0 Hz), 3.60–3.10 (6H, m). ^{13}C NMR (75 MHz, DMSO-d_6, rt) *δ* 158.5, 145.5, 142.5, 129.5, 128.9, 126.8, 121.7, 113.1, 110.4, 80.0, 74.2, 74.2, 73.1, 73.0, 71.7, 71.4, 69.2, 66.7, 47.7, 41.9. ESI-HRMS (positive-ion) calcd for $C_{21}H_{22}FNO_6Na$: $(M+Na)^+$ 426.1329: found: 426.1323.

4.1.10. Acetic acid 4,5-diacetoxy-6-(*tert*-butoxycarbonyl-amino-methyl)-2-fluoro-tetrahydro-pyran-3-yl ester 20. To a solution of **17** (24.0 mg, 72.1 μmol) in MeOH (700 μl) was slowly added MeONa (12.9 mg, 238 μmol) at 0 °C under Ar atmosphere. After stirring for 30 min at 0 °C, Amberlite IR-120B (H^+) was added to this solution for neutralization. After filtration, the filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in MeOH (700 μl) was added palladium on activated carbon (8.7 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 30 min and the catalyst removed by filtration. The filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture and triethylamine (10.1 μl, 71.9 μmol) in MeOH (1.0 ml) was added Boc_2O (16.6 μl, 71.9 μmol) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in pyridine (1.0 ml) was added Ac_2O (0.5 ml) at room temperature. After stirring for 7 h, the reaction mixture was diluted with AcOEt and washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **20** (19.1 mg, 65% in four steps) as a colorless oil: $[\alpha]_D^{18}$ +51.4 (*c* 0.40, $CHCl_3$). IR (film) *ν* 2108, 1751, 1371, 1221, 1067, 1020 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) *δ* 5.78 (1H, dd, *J*=2.2, 53.4 Hz), 5.43 (1H, d, *J*=2.7 Hz), 5.36 (1H, dd, *J*=2.7, 11.0 Hz), 5.19 (1H, ddd, *J*=2.2, 11.0, 23.5 Hz), 4.20 (1H, t, *J*=6.6 Hz), 3.28 (2H, m), 2.19 (3H, s), 2.15 (3H, s), 2.02 (3H, s), 1.45 (9H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) *δ* 170.3, 169.9, 155.6, 104.3 (d, J_{C-F}=113 Hz), 80.0, 70.1, 67.9, 67.7, 67.4, 67.1, 39.9, 28.3, 20.6. ESI-HRMS (positive-ion) calcd for $C_{17}H_{26}FNO_9Na$: $(M+Na)^+$ 430.1489; found: 430.1484.

4.1.11. Acetic acid 3,5-diacetoxy-2-[(9*H*-fluoren-9-yl-methoxycarbonylamino)-methyl]-6-fluoro-tetrahydro-pyran-4-yl ester 21. To a solution of **19** (22.7 mg, 56.3 μmol) in pyridine (1.0 ml) was added Ac_2O (0.5 ml) at room temperature. After stirring for 7 h, the reaction mixture was diluted with AcOEt and washed with 1 N HCl, satd $NaHCO_3$ aq. and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **21** (25.9 mg, 87%) as a colorless oil. α-*anomer*: $[\alpha]_D^{20}$ +39.5

(*c* 0.10, MeOH). IR (film) ν 3366, 2961, 1701 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.78 (2H, d, *J*=7.3 Hz), 7.58 (2H, d, *J*=7.3 Hz), 7.35 (4H, m), 5.80 (1H, dd, *J*=2.5, 53.9 Hz), 5.50 (1H, d, *J*=2.5 Hz), 5.35 (1H, dd, *J*=2.5, 11.0 Hz), 5.18 (1H, ddd, *J*=2.5, 11.0, 23.5 Hz), 4.43 (2H, d, *J*=7.0 Hz), 4.23 (2H, m), 3.34 (2H, t, *J*=6.6 Hz), 2.20 (3H, s), 2.15 (1H, s), 2.03 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 170.3, 169.8, 156.2, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 105.7, 102.7, 69.9, 68.1, 67.6, 67.3, 67.0, 47.1, 40.5, 20.6. ESI-HRMS (positive-ion) calcd for $C_{27}H_{28}FNO_9Na$: $(M+Na)^+$ 552.1646; found: 552.1639. β-*anomer*: $[\alpha]_D^{17}$ +7.4 (*c* 0.49, MeOH). IR (film) ν 3437, 2963, 1637 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.78 (2H, d, *J*=7.3 Hz), 7.58 (2H, d, *J*=7.3 Hz), 7.35 (4H, m), 5.40–5.13 (4H, m), 4.43 (2H, d, *J*=6.6 Hz), 4.21 (1H, t, *J*=6.6 Hz), 3.88 (1H, t, *J*=6.6 Hz), 3.39 (2H, t, *J*=6.6 Hz), 2.20 (3H, s), 2.15 (1H, s), 2.03 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 172.0, 171.4, 171.1, 158.8, 145.4, 145.2, 142.6, 128.8, 128.2, 126.3, 126.2, 121.0, 109.9, 107.1, 79.5, 73.2, 73.1, 71.8, 71.7, 70.6, 70.3, 68.4, 67.9, 48.2, 41.2, 20.6, 20.5. ESI-HRMS (positive-ion) calcd for $C_{27}H_{28}FNO_9Na$: $(M+Na)^+$ 552.1646: found: 552.1639.

4.1.12. Acetic acid 3,5-diacetoxy-2-[(9*H*-fluoren-9-ylmethoxycarbonylamino)-methyl]-6-(2-oxo-2,6,7,7a-tetrahydro-benzofuran-6-yloxy)-tetrahydro-pyran-4-yl ester 23. A mixture of **5** (6.6 mg, 43.4 μmol), Cp_2HfCl_2 (32.9 mg, 86.8 μmol), $AgClO_4$ (18.0 mg, 86.8 μmol) and dried molecular sieves 4Å (5.0 mg) in anhydrous CH_2Cl_2 (500 μl) was stirred at 0 °C under Ar atmosphere for 1 h. Then a solution of **4** (36.0 mg, 68.1 μmol) in anhydrous CH_2Cl_2 (300 μl) was added to the stirred mixture. After stirring for 6 h, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (toluene/acetone = 1:3) to afford **23** (15.0 mg, 52%, *R*:*S* = 7:1) as a colorless oil: $[\alpha]_D^{18}$ +12.6 (*c* 0.10, $CHCl_3$). IR (film) ν 1747, 1221, 1155, 1070, 760 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, rt) δ 7.77 (2H, d, *J*=7.4 Hz), 7.59 (2H, d, *J*=7.4 Hz), 7.41 (2H, t, *J*=7.4 Hz), 7.33 (2H, t, *J*=7.4 Hz), 6.59 (1H, d, *J*=10.0 Hz), 6.20 (1H, d, *J*=10.0 Hz), 5.84 (1H, s), 5.35 (1H, d, *J*=2.0 Hz), 5.21 (1H, dd, *J*=7.8, 10.0 Hz), 5.12 (1H, t, *J*=6.0 Hz), 5.03 (1H, dd, *J*=2.0, 10.0 Hz), 4.83 (1H, dd, *J*=4.5, 12.0 Hz), 4.62 (1H, d, *J*=7.8 Hz), 4.59–4.54 (1H, br s), 4.44 (2H, d, *J*=6.6 Hz), 4.22 (1H, t, *J*=6.6 Hz), 3.78 (1H, t, *J*=6.8 Hz), 3.46 (1H, td, *J*=6.8, 14.0 Hz), 3.15 (1H, td, *J*=6.8, 14.0 Hz), 2.94 (1H, td, *J*=4.5, 12.0 Hz), 2.20 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 1.81 (1H, q, *J*=12.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$, rt) δ 172.9, 171.0, 170.0, 169.3, 162.0, 156.4, 143.7, 141.3, 139.6, 127.8, 127.1, 125.0, 121.1, 120.0, 115.5, 100.8, 77.9, 74.1, 71.7, 70.8, 69.0, 67.8, 67.0, 47.2, 40.4, 38.3, 20.8, 20.6. ESI-HRMS (positive-ion) calcd for $C_{35}H_{35}NO_{12}Na$: $(M+Na)^+$ 684.2057; found: 684.2051.

4.1.13. [3,4,5-Trihydroxy-6-(2-oxo-2,6,7,7a-tetrahydro-benzofuran-6-yloxy)-tetrahydro-pyran-2-ylmethyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester 24. To a solution of **23** (1.8 mg, 2.9 μmol) in MeOH (200 μl) was added KCN (6 mM in MeOH, 200 μl, 1.2 μmol) at 0 °C under Ar atmosphere. After stirring for 5 h at 0 °C, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 1:1) to afford **24** (0.7 mg, 47%, *R*:*S*=4:1) as a colorless oil: $[\alpha]_D^{20}$ −7.1 (*c* 0.10, MeOH). IR (film) ν 3533, 3308, 1719, 1686, 1638, 1535, 1448, 1263, 1078 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, rt) δ 7.77 (2H, d, *J*=7.3 Hz), 7.66 (2H, t, *J*=7.3 Hz), 7.37 (2H, t, *J*=7.3 Hz), 7.30 (2H, t, *J*=7.3 Hz), 6.63 (1H, dd, *J*=2.0, 10.1 Hz), 6.46 (1H, d, *J*=10.1 Hz), 5.83 (1H, s), 4.80 (1H, dd, *J*=4.5, 13.4 Hz), 4.57–4.62 (1H, m), 4.44 (1H, dd, *J*=6.5, 10.5 Hz), 4.39 (1H, dd, *J*=6.5, 10.5 Hz), 4.37 (1H, d, *J*=7.0 Hz), 4.38 (2H, dd, *J*=6.4, 10.4 Hz), 4.22 (1H, t, *J*=6.4 Hz), 3.70 (1H, d, *J*=2.2 Hz), 3.63 (1H, s), 3.52–3.44 (4H, m), 2.94 (1H, dt, *J*=4.5, 11.4 Hz), 1.64 (1H, dt, *J*=10.6, 13.2 Hz). ^{13}C NMR (125 MHz, CD_3OD, rt) δ 175.8, 165.9, 159.1, 145.4, 145.3, 143.2, 142.7, 128.8, 128.2, 128.1, 121.1, 120.9, 120.9, 111.7, 104.8, 79.9, 75.3, 74.9, 74.8, 72.2, 70.5, 67.6, 49.6, 42.5, 40.1. ESI-HRMS (positive-ion) calcd for $C_{29}H_{29}NO_9Na$: $(M+Na)^+$ 558.1740; found: 558.1735.

4.1.14. Biologically active fluorescence-labeled probe 2. To a solution of **24** (1.6 mg, 3.0 μmol) in DMF (300 μl) was added piperidine (1.0 μl, 10 μmol) at 0 °C under Ar atmosphere. After stirring for 4 h at 0 °C, piperidine was removed in vacuo. To the reaction mixture were added DMF (200 μl) and AMCA-X, SE (1.4 mg, 3.3 μmol) at room temperature under Ar atmosphere. After stirring for 2 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:1) and HPLC with COSMOSIL $5C_{18}$-AR column (ϕ20.0× 250 mm, H_2O/CH_3CN = 1:1) to afford **2** (0.9 mg, 48%) and *epi*-**2** (0.2 mg, 11%). Compound **2**: white powder. $[\alpha]_D^{18}$ +28.0 (*c* 1.00, MeOH). IR (film) ν 3292, 2856, 1734 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, rt) δ 7.48 (1H, d, *J*=8.5 Hz), 6.64 (1H, dd, *J*=2.5, 8.5 Hz), 6.63 (1H, dd, *J*=3.0, 10.0 Hz), 6.50 (1H, d, *J*=2.5 Hz), 6.47 (1H, d, *J*=10.0 Hz), 5.80 (1H, s), 5.00 (1H, ddd, *J*=2.0, 5.0, 13.0 Hz), 4.69 (1H, m), 4.40 (1H, d, *J*=7.5 Hz), 3.73 (1H, dd, *J*=1.0, 3.0 Hz), 3.60 (1H, ddd, *J*=1.0, 5.5, 8.0 Hz), 3.54 (2H, s), 3.50 (1H, dd, *J*=2.5, 7.0 Hz), 3.46 (1H, dd, *J*=7.5, 10.0 Hz), 3.45 (1H, dd, *J*=5.5, 13.5 Hz), 3.36 (1H, dd, *J*=8.0, 13.5 Hz), 3.18 (2H, dt, *J*=2.5, 7.0 Hz), 2.98 (1H, ddd, *J*=5.0, 6.0, 10.5 Hz), 2.38 (3H, s), 2.22 (2H, t, *J*=7.5 Hz), 1.69 (1H, ddd, *J*=10.5, 11.0, 13.0 Hz), 1.64 (2H, m), 1.52 (2H, m), 1.36 (2H, m). ^{13}C NMR (125 MHz, CD_3OD, rt) δ 176.7, 175.8, 172.9, 165.9, 164.8, 155.9, 153.9, 152.9, 143.7, 127.4, 121.1, 114.5, 113.2, 111.8, 111.7, 105.0, 100.5, 79.9, 75.7, 74.8, 74.4, 72.2, 70.6, 41.3, 40.3, 40.2, 37.0, 35.2, 30.0, 27.5, 26.6, 15.4. ESI-HRMS (positive-ion) calcd for $C_{32}H_{39}N_3O_{11}Na$: $(M+Na)^+$ 664.2478; found: 664.2478. Compound *epi*-**2**: white powder. $[\alpha]_D^{17}$ −37.3 (*c* 0.07, MeOH). IR (film) ν 3348, 2926, 1741, 1639, 1601, 1556, 1051 cm^{-1}. ^1H NMR (300 MHz, CD_3OD, rt) δ 7.49 (1H, d, *J*=7.5 Hz), 6.71 (1H, d, *J*= 8.0 Hz), 6.65 (1H, dd, *J*=2.0, 7.5 Hz), 6.48 (1H, d, *J*=2.0 Hz), 6.45 (1H, dd, *J*=4.0, 8.0 Hz), 5.85 (1H, d, *J*=1.5 Hz), 5.38 (1H, ddd, *J*=1.5, 5.5, 11.5 Hz), 4.65 (1H, m), 4.37 (1H, d, *J*=6.0 Hz), 3.62 (1H, dd, *J*=1.0, 4.0, 6.5 Hz), 3.52 (2H, s), 3.50 (1H, dd, *J*=6.5, 8.5 Hz), 3.48 (1H, dd, *J*=4.0, 12.0 Hz), 3.45 (1H, dd, *J*=3.0, 8.5 Hz), 3.38 (1H, dd, *J*=6.5, 12.0 Hz), 3.17 (1H, t, *J*=6.0 Hz), 2.82 (1H, ddd, *J*=2.0, 5.5, 11.5 Hz), 2.37 (3H, s), 2.22 (2H, t, *J*=7.5 Hz), 1.73 (1H, dt, *J*=3.0, 11.5 Hz), 1.62 (2H,

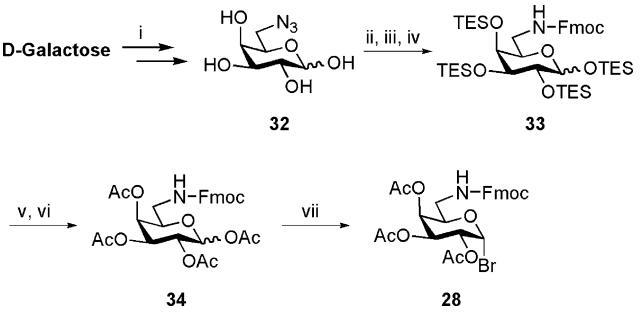
m), 1.51 (2H, m), 1.35 (2H, m). ESI-HRMS (positive-ion) calcd for $C_{32}H_{39}N_3O_{11}Na$: $(M+Na)^+$ 664.2478: found: 664.2478.

4.1.15. 7,8-Dihydro aglycon 25. To a solution of **5** (6.2 mg, 41.0 μmol) in AcOEt (800 μl) was added Lindlar catalyst (8.2 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 15 min and the catalyst removed by filtration. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20:1) to afford **25** (4.3 mg, 68%) as a colorless oil: $[\alpha]_D^{24}$ −52.1 (*c* 0.20, $CHCl_3$). IR (film) ν 3422, 1732, 1647, 1005 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.76 (1H, s), 4.73 (1H, dd, *J*=5.7, 10.8 Hz), 3.92 (1H, tt, *J*=3.0, 10.8 Hz), 2.84–2.83 (1H, m), 2.79–2.71 (1H, m), 2.33 (1H, ddd, *J*=1.8, 5.7, 14.1 Hz), 2.23–2.19 (1H, m), 1.49–1.36 (1H, m), 1.36 (1H, dd, *J*=11.4, 14.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 173.2, 169.9, 113.4, 79.5, 66.9, 42.1, 34.7, 24.2. ESI-HRMS (positive-ion) calcd for $C_8H_{10}O_3Na$: $(M+Na)^+$ 177.0528; found: 177.0521.

4.1.16. 7,8-Dihydro phyllanthurinolactone 27. A mixture of **25** (8.6 mg, 56.0 μmol), AgOTf (7.2 mg, 27.9 μmol), Ag_2CO_3 (32.3 mg, 117 μmol) and dried molecular sieves 4A (17.7 mg) in anhydrous CH_2Cl_2 (200 μl) was stirred at 0 °C under Ar atmosphere for 1 h. Then a solution of **26** (22.7 mg, 55.0 μmol) in anhydrous CH_2Cl_2 (500 μl) was added to the stirred mixture. After stirring for 2 h, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with satd $NaHCO_3$ aq and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:3) to afford a glycoside (5.4 mg, 20%) as a colorless oil: $[\alpha]_D^{16}$ −15.3 (*c* 0.10, $CHCl_3$). IR (film) ν 1751, 1225, 1038 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.79 (1H, s), 5.21 (1H, t, *J*=9.6 Hz), 5.08 (1H, t, *J*=9.6 Hz), 4.95 (1H, dd, *J*=8.1, 9.6 Hz), 4.74 (1H, dd, *J*=6.3, 12.3 Hz), 4.65 (1H, d, *J*=8.1 Hz), 4.27 (1H, dd, *J*=4.8, 12.0 Hz), 4.14 (1H, dd, *J*=2.7, 12.0 Hz), 3.88 (1H, tt, *J*=3.6, 11.1 Hz), 3.71 (1H, ddd, *J*=2.7, 4.8, 9.6 Hz), 2.93–2.83 (2H, m), 2.36–2.19 (2H, m), 2.15 (3H, s), 2.09 (3H, s), 2.03 (3H, s) 2.01 (3H, s), 1.53–1.38 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 172.9, 170.6, 170.3, 169.3, 169.1, 169.0, 113.7, 99.8, 79.2, 74.5, 72.7, 71.9, 71.3, 68.4, 62.0, 40.3, 31.6, 23.9, 20.7, 20.6. ESI-HRMS (positive-ion) calcd for $C_{22}H_{28}O_{12}Na$: $(M+Na)^+$ 507.1478; found: 507.1470.

To a solution of the above glycoside (5.4 mg, 11 μmol) in MeOH (600 μl) was added KCN (8 mM in MeOH, 100 μl, 800 nmol) at room temperature under Ar atmosphere. After stirring for 6 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:1) to afford **27** (1.1 mg, 31%) as a colorless oil: $[\alpha]_D^{18}$ −26.8 (*c* 0.05, MeOH). IR (film) ν 3393, 1738, 1072 cm^{-1}. ^1H NMR (600 MHz, CD_3OD, 40 °C) δ 5.79 (1H, s), 4.91 (1H, ddd, *J*= 1.2, 6.2, 11.8 Hz), 4.41 (1H, d, *J*= 7.9 Hz), 4.05 (1H, tt, *J*= 4.0, 11.2 Hz), 3.87 (1H, dd, *J*= 2.1, 11.8 Hz), 3.66 (1H, dd, *J*=5.6, 11.8 Hz), 3.36–2.26 (3H, m), 3.14 (1H, dd, *J*=7.9, 9.1 Hz), 2.90–2.84 (2H, m), 2.42–2.34 (2H, m), 1.44–1.38 (1H, m), 1.35 (1H, q, *J*=11.8 Hz). ^{13}C NMR (150 MHz, CD_3OD, rt) δ 176.0, 173.9, 113.4, 103.2, 81.6, 78.1, 78.0, 75.0, 74.6, 71.6, 62.8, 42.0, 32.7, 24.8. ESI-HRMS (positive-ion) calcd for $C_{14}H_{20}O_8Na$: $(M+Na)^+$ 339.1056; found: 339.1049.

4.1.17. Acetic acid 3,5-diacetoxy-2-bromo-6-[(9*H*-fluoren-9-ylmethoxycarbonyl amino)-methyl]-tetrahydropyran-4-yl ester 28. Synthesis of **33**, **34**, and **28** is shown in Scheme 7.



Scheme 7. (i) See Ref. 15; (ii) TESOTf, 2,6-lutidine, DMF, 92%; (iii) H_2/Pd–C, CH_2Cl_2; (iv) FmocCl, $NaHCO_3$, H_2O, 1,4-dioxane, 80% (two steps); (v) TFA, THF, H_2O; (vi) Ac_2O, pyridine, 80% (two steps); (vii) $BiBr_3$, TMSBr, CH_2Cl_2.

4.1.17.1. Synthesis of 33. To a solution of **32** (340 mg, 1.66 mmol) and 2,6-lutidine (1.86 ml, 15.9 mmol) in DMF (16 ml) was slowly added TESOTf (1.79 ml, 8.00 mmol) at 0 °C under Ar atmosphere. After stirring for 1 h at 0 °C, to the reaction mixture was added H_2O (2 ml). The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to afford TES ether (1.03 g, 92%, α:β = 1:1) as a colorless oil: $[\alpha]_D^{24}$ +15.3 (*c* 0.50, $CHCl_3$). IR (film) ν 2878, 2102, 1458, 1414, 1283, 1240 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 5.10 (1H, d, *J*=2.7 Hz, α), 4.47 (1H, d, *J*=6.5 Hz, β), 3.97 (1H, dd, *J*=2.0, 9.4 Hz, α), 3.96–3.91 (1H, m, α), 3.88 (1H, dd, *J*=2.7, 9.4 Hz, β), 3.82 (1H, br s, α), 3.77 (1H, br s, β), 3.63 (1H, dd, *J*=6.5, 9.0 Hz, β), 3.49 (1H, dd, *J*=7.8, 12.4 Hz, α), 3.46–3.34 (4H, m, β), 3.20 (1H, dd, *J*=5.3, 12.4 Hz, α), 1.01–0.92 (72H, m), 0.72–0.62 (48H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 103.2, 94.3, 87.3, 84.3, 78.9, 77.2, 73.9, 72.1, 70.9, 70.1, 54.2, 51.6, 7.14, 7.03, 6.99, 6.90, 6.82, 6.78, 6.72, 6.66, 5.29, 5.15, 5.12, 4.97, 4.92, 4.77, 4.67. ESI-HRMS (positive-ion) calcd for $C_{30}H_{67}N_3O_5Si_4Na$: $(M+Na)^+$ 684.4050; found: 684.4052.

To a solution of the above TES ether (63.7 mg, 94.1 μmol) in CH_2Cl_2 (2 ml) was added palladium on activated carbon (31.7 mg). Hydrogen was admitted via a balloon and the reaction mixture was stirred for 30 min and the catalyst removed by filtration. The filtrate was concentrated in vacuo to afford a crude mixture. To a solution of the mixture and 10% $NaHCO_3$ aq (118 μl, 141 μmol) in 1,4-dioxane (1.5 ml) was added FmocCl (36.5 mg, 141 μmol) at 0 °C. After stirring for 12 h, to the reaction mixture was added H_2O (2 ml). The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to afford **33** (65.8 mg, 81%, α:β = 3:2) as a colorless oil: $[\alpha]_D^{19}$ +41.2 (*c* 0.50, $CHCl_3$). IR (film) ν 2955,

2912, 2878, 1724, 1514, 1458, 1414, 1240, 1140, 1105, 1055 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.76 (4H, d, J=7.4 Hz), 7.59 (4H, d, J=7.4 Hz), 7.40 (4H, t, J=7.4 Hz), 7.30 (4H, t, J=7.4 Hz), 5.11 (1H, d, J=2.5 Hz, α), 5.06 (1H, br d, α), 4.45 (1H, d, J=7.5 Hz, β), 4.41 (1H, dd, J=6.9, 10.4 Hz, α), 4.37 (2H, d, J=6.9 Hz, β), 4.30 (1H, dd, J=6.9, 10.4 Hz, α), 4.21 (2H, t, J=6.9 Hz), 3.98 (1H, dd, J=1.8, 9.7 Hz, α), 4.00–3.88 (1H, m, β), 3.90 (1H, dd, J=2.5, 9.7 Hz, α), 3.85 (1H, br s, α), 3.75 (1H, br s, β), 3.64 (1H, dd, J=7.5, 8.7 Hz, β), 3.54–3.47 (2H, m), 3.40 (1H, br d, β), 3.29–3.22 (2H, m), 0.99–0.93 (72H, m), 0.71–0.56 (48H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 156.5, 144.0, 141.3, 128.7, 127.0, 125.1, 120.0, 99.2, 94.4, 77.2, 75.7, 74.1, 73.9, 73.8, 70.9, 70.6, 70.2, 66.9, 47.3, 42.5, 7.20, 7.07, 6.99, 6.87, 6.73, 6.61, 5.82, 5.43, 5.34, 5.25, 5.18, 4.78. ESI-HRMS (positive-ion) calcd for $C_{45}H_{79}NO_7Si_4Na$: $(M+Na)^+$ 880.4826; found: 880.4828.

4.1.17.2. Synthesis of 34. To a solution of **33** (65.8 mg, 76.6 μmol) in THF/H_2O (2:1, 900 μl) was slowly added TFA (1.5 ml) at 0 °C. After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo to afford a crude mixture. To a solution of the mixture in pyridine (1.0 ml) was added Ac_2O (1.0 ml) at room temperature. After stirring for 3 h, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to afford **34** (36.6 mg, 84%, α:β = 1:2) as a colorless oil: $[\alpha]_D^{19}$ +35.7 (c 0.50, $CHCl_3$). IR (film) ν 3382, 3020, 1751, 1526, 1450, 1369, 1221, 1074 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.77 (4H, d, J=7.2 Hz), 7.58 (4H, d, J=7.2 Hz), 7.41 (4H, t, J=7.2 Hz), 7.32 (4H, t, J=7.2 Hz), 6.37 (1H, br s, α), 5.70 (1H, d, J=8.4 Hz, β), 5.48 (1H, br s, α), 5.40 (1H, d, J=2.7 Hz, β), 5.34 (1H, dd, J=8.4, 9.8 Hz, β), 5.37–5.31 (2H, m, α), 5.08 (1H, dd, J=2.7, 9.8 Hz, β), 5.10–5.06 (1H, m, α), 4.40 (2H, d, J=6.9 Hz, α), 4.38 (2H, dd, J=6.9 Hz, β), 4.22 (2H, t, J=6.9 Hz), 3.89 (1H, t, J=6.8 Hz, β), 3.40–3.27 (4H, m), 2.19 (6H, s), 2.13 (6H, s), 2.05 (6H, s), 2.03 (3H, s, α), 2.01 (3H, s, β). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 170.5, 170.0, 169.9, 169.8, 169.4, 168.9, 156.2, 143.8, 141.3, 127.7, 127.0, 125.0, 120.0, 92.2, 89.6, 77.2, 72.8, 70.8, 69.8, 68.4, 68.0, 67.6, 67.4, 67.0, 66.5, 47.1, 40.5, 20.9, 20.8, 20.6, 20.5. ESI-HRMS (positive-ion) calcd for $C_{29}H_{31}NO_{11}Na$: $(M+Na)^+$ 592.1789; found: 592.1790.

4.1.17.3. Synthesis of 28. To a solution of **34** (27.2 mg, 47.8 μmol) in CH_2Cl_2 (500 μl) were added $BiBr_3$ (0.5 mg, 1.20 μmol) and TMSBr (24.7 μl, 191 μmol) at 0 °C under Ar atmosphere. After stirring for 1 h at 0 °C, to the reaction mixture was added satd $NaHCO_3$ aq. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:1) to afford **28** (24.8 mg, 88%) as an orange oil: $[\alpha]_D^{19}$ +137.8 (c 0.50, $CHCl_3$). IR (film) ν 3379, 3067, 2950, 1749, 1526, 1450, 1371, 1223, 1078 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.76 (2H, d, J=7.4 Hz), 7.57 (2H, d, J=7.4 Hz), 7.40 (2H, t, J=7.4 Hz), 7.31 (2H, t, J=7.4 Hz), 6.70 (1H, d, J=3.9 Hz), 5.49 (1H, d, J=2.8 Hz), 5.40 (1H, dd, J=2.8, 10.6 Hz), 5.05 (1H, dd, J=3.9, 10.6 Hz), 4.46–4.36 (2H, m), 4.33 (1H, t, J=6.8 Hz), 4.22 (1H, t, J=6.9 Hz), 3.46–3.27 (2H, m), 2.16 (3H, s), 2.11 (3H, s), 2.02 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 170.2, 170.1, 169.7, 156.2, 143.9, 141.3, 127.7, 127.1, 125.0, 120.0, 88.2, 72.2, 68.1, 67.9, 37.7, 67.0, 47.1, 40.2, 20.7, 20.6. ESI-HRMS (positive-ion) calcd for $C_{27}H_{28}BrNO_9Na$: $(M+Na)^+$ 612.0840; found: 612.0842.

4.1.18. Acetic acid 4,5-diacetoxy-2-[(9*H*-fluoren-9-yl-methoxycarbonylamino)-methyl]-6-(2-oxo-2,4,5,6,7,7a-hexahydro-benzofuran-6-yloxy)-tetrahydro-pyran-3-yl ester 29. A mixture of **25** (4.9 mg, 32.0 μmol), AgOTf (4.1 mg, 16.0 μmol), Ag_2CO_3 (18.4 mg, 67.0 μmol) and dried molecular sieves 4Å (15.2 mg) in anhydrous CH_2Cl_2 (200 μl) was stirred at 0 °C under Ar atmosphere for 1 h. Then a solution of **28** (26.5 mg, 44.9 μmol) in anhydrous CH_2Cl_2 (200 μl) was added to the stirred mixture. After stirring for 1 h, the reaction mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with satd $NaHCO_3$ aq and brine. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 1:5) to afford **29** (10.1 mg, 48%) as a colorless oil: $[\alpha]_D^{20}$ −2.25 (c 0.20, $CHCl_3$). IR (film) ν 2920, 2851, 1720, 1649, 1529, 1450, 1369, 1225 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rt) δ 7.75 (2H, d, J=7.5 Hz), 7.57 (2H, d, J=7.5 Hz), 7.39 (2H, t, J=7.5 Hz), 7.31 (2H, t, J=7.5 Hz), 5.76 (1H, s), 5.32 (1H, d, J=3.0 Hz), 5.15 (1H, t, J=8.1 Hz), 5.00 (1H, dd, J=3.0, 8.1 Hz), 4.69 (1H, dd, J=5.9, 11.3 Hz), 4.54 (1H, d, J=8.1 Hz), 4.40 (2H, d, J=6.6 Hz), 4.20 (1H, t, J=6.6 Hz), 3.84–3.65 (2H, m), 3.47 (1H, td, J=6.9, 13.7 Hz), 3.09 (1H, td, J=6.9, 13.7 Hz), 2.89–2.80 (2H, m), 2.32–2.10 (2H, m), 2.18 (3H, s), 2.02 (3H, s), 1.99 (3H, m), 1.45–1.22 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$, rt) δ 172.9, 171.1, 170.0, 169.2, 169.1, 156.4, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 113.6, 100.3, 79.2, 74.3, 71.5, 70.8, 69.1, 67.8, 67.0, 47.1, 40.4, 40.3, 31.4, 23.8, 20.7, 20.6, 20.6. ESI-HRMS (positive-ion) calcd for $C_{35}H_{37}N_1O_{12}Na$: $(M+Na)^+$ 686.2208; found: 686.2207.

4.1.19. [3,4,5-Trihydroxy-6-(2-oxo-2,4,5,6,7,7a-hexahydro-benzofuran-6-yloxy)-tetrahydro-pyran-2-ylmethyl]-carbamic acid 9*H*-fluoren-9-ylmethyl ester 30. To a solution of **29** (5.1 mg, 7.7 μmol) in MeOH (150 μl) was added KCN (8 mM in MeOH, 100 μl, 800 nmol) at room temperature under Ar atmosphere. After stirring for 4.5 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:4) to afford **30** (1.7 mg, 41%) as a colorless oil: $[\alpha]_D^{18}$ +21.4 (c 0.20, MeOH). IR (film) ν 3358, 2926, 2361, 1705, 1533, 1448, 1261, 1069 cm^{-1}. ^1H NMR (600 MHz, CD_3OD, rt) δ 7.78 (2H, d, J=7.5 Hz), 7.65 (2H, d, J=7.5 Hz), 7.38 (2H, t, J=7.5 Hz), 7.33 (2H, t, J=7.5 Hz), 5.78 (1H, s), 4.70 (1H, dd, J=6.0, 11.3 Hz), 4.41 (2H, d, J=6.6 Hz), 4.31 (1H, d, J=7.0 Hz), 4.21 (1H, t, J=6.6 Hz), 3.94 (1H, tt, J=3.6, 11.2 Hz), 3.69 (1H, d, J=2.4 Hz), 3.52–3.49 (1H, m), 3.45–3.37 (4H, m), 3.32–3.27 (2H, m), 2.86–2.80 (2H, m), 2.33–2.28 (2H, m), 1.40–1.28 (2H, m). ^{13}C NMR (150 MHz, CD_3OD, rt) δ 175.9, 173.7, 159.2, 145.4, 145.3, 142.7, 128.8, 128.2, 126.2, 126.1, 121.4, 120.9, 114.2, 113.4, 112.6, 104.0, 81.5, 75.0, 74.8, 74.7, 72.3, 70.4, 67.7, 49.6, 42.4, 42.0, 32.8, 24.8. ESI-HRMS (positive-ion) calcd for $C_{29}H_{31}N_1O_9Na$: $(M+Na)^+$ 560.1891; found: 560.1892.

4.1.20. Biologically inactive fluorescence-labeled probe 31. To a solution of **30** (3.5 mg, 6.5 μmol) in DMF

(720 μl) was added piperidine (4.8 μl, 49 mol) at 0 °C under Ar atmosphere. After stirring for 2 h at 0 °C, piperidine was removed in vacuo. To the reaction mixture was added AMCA-X, SE (3.1 mg, 7.2 μmol) at room temperature under Ar atmosphere. After stirring for 2 h, the reaction mixture was concentrated in vacuo. The residue was purified by ODS TLC (RP-18W, H_2O/MeOH = 1:1) and HPLC with COSMOSIL 5C_{18}-AR column (ϕ20.0×250 mm, H_2O/CH_3CN = 6:4) to afford **31** (1.3 mg, 62% in two steps) as a colorless oil: $[\alpha]_D^{21}$ +6.6 (*c* 0.05, MeOH). IR (film) ν 3348, 2924, 1734, 1652, 1558, 1057 cm^{-1}. ^1H NMR (600 MHz, CD_3OD, 40 °C) δ 7.48 (1H, d, *J*=8.8 Hz), 6.65 (1H, dd, *J*=2.2, 8.8 Hz), 6.51 (1H, d, *J*=2.2 Hz), 5.74 (1H, s), 4.77 (1H, dd, *J*=5.5, 11.1 Hz), 4.33 (1H, d, *J*=7.6 Hz), 3.99 (1H, tt, *J*=4.0, 11.5 Hz), 3.72 (1H, d, *J*=1.4 Hz), 3.58 (1H, dd, *J*=5.4, 7.2 Hz), 3.52 (2H, s), 3.49–3.45 (3H, m), 3.35 (1H, dd, *J*=7.8, 13.7 Hz), 3.18 (2H, t, *J*=7.5 Hz), 2.88–2.84 (2H, m), 2.37 (3H, s), 2.41–2.32 (2H, m), 2.21 (2H, t, *J*=7.5 Hz), 1.62 (2H, quintet, *J*=7.5 Hz), 1.52 (2H, quintet, *J*=7.5 Hz), 1.42 (1H, dd, *J*=5.0, 11.7 Hz), 1.36 (2H, t, *J*=7.5 Hz), 1.34 (1H, m). ^{13}C NMR (150 MHz, CD_3OD, 40 °C) δ 176.7, 175.9, 173.8, 173.0, 164.8, 155.9, 154.0, 152.9, 127.4, 114.4, 113.4, 113.2, 111.8, 104.1, 100.5, 81.6, 75.4, 74.7, 74.3, 72.3, 70.5, 42.0, 41.3, 40.3, 37.0, 35.2, 32.9, 30.0, 27.5, 26.7, 24.3, 15.4. ESI-HRMS (positive-ion) calcd for $C_{32}H_{41}N_3O_{11}Na$: $(M+Na)^+$ 666.2633; found: 666.2635.

4.1.21. Bioassay. The young leaves detached from the stem of the plant *P. urinaria* L., which was grown in the biotron of Tohoku University, with a sharp razor blade were used for bioassay. One leaf was placed in H_2O (ca. 300 μL) using a 5-ml glass tube in the greenhouse kept at 32 °C and allowed to stand overnight. The leaves, which opened again in the morning were used for the bioassay. Each test solution was carefully poured into test tubes with a microsyringe around 10:00 a.m. The bioactive fraction was judged by the leaf-closing activity before the leaf-closing of the plant leaf in the blank solution containing no sample. Other nyctinastic plants, *M. pudica*, *C. mimosoides*, and *L. leucocephala*, used in bioassay were also grown in the biotron of Tohoku University.

4.1.22. Fluorescence study using a fluorescence microscope. The leaf of *P. urinaria* L. opening in the morning was cut in an appropriate size and fixed in agar. The agar was sliced perpendicular to the petiole by a microslicer (DSK-1000, Dousaka EM Co., Ltd) to a thickness of 30 μm and the sections containing the pulvini were floated on distilled water. The sections were immersed in a solution containing various concentration of fluorescence-labeled probe compound, and allowed to stand for staining under shielded condition at room temperature for 6 h. After staining, the sections were washed by being incubated with equilibration buffer (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.) for 15 min. This section was placed on a slide glass and covered by a cover glass after adding a drop of antifade reagent (Slow Fade™ Gold Antifade Reagent, Molecular Probes Inc.). The observation of these sections was carried out by using ECLIPSE E800 microscope (Nicon) equipped with VFM fluorescence instrument. B-2A filter (Nicon Co., Ltd; excitation wavelength 450–490 nm) was used against AMCA. The plant sections of other nyctinastic plants, *M. pudica*, *C. mimosoides*, and *L. leucocephala* were prepared and treated with fluorescence-labeled probe compound in the same procedure.

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References and notes

1. Schildknecht, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 695–710.
2. Satter, R. L.; Gorton, H. L.; Vogelmann, T. C. *The Pulvinus: Motor Organ for Leaf Movement*; American Society of Plant Physiologists: 1990.
3. Ueda, M.; Yamamura, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1400–1414.
4. (a) Sugimoto, T.; Wada, Y.; Yamamura, S.; Ueda, M. *Tetrahedron* **2001**, *57*, 9817–9825; (b) Ueda, M.; Wada, Y.; Yamamura, S. *Tetrahedron Lett.* **2001**, *42*, 3869–3872.
5. Ueda, M.; Shigemori-Suzuki, T.; Yamamura, S. *Tetrahedron Lett.* **1995**, *36*, 6267–6270.
6. Audran, G.; Mori, K. *Eur. J. Org. Chem.* **1998**, 57–62.
7. Urakawa, Y.; Sugimoto, T.; Sato, H.; Ueda, M. *Tetrahedron Lett.* **2004**, *45*, 5885–5888.
8. Sato, H.; Inada, M.; Sugimoto, T.; Kato, N.; Ueda, M. *Tetrahedron Lett.* **2005**, *46*, 5537–5541.
9. (a) Ueda, M.; Sawai, Y.; Yamamura, S. *Tetrahedron Lett.* **1999**, *50*, 3757–3760; (b) Ueda, M.; Sawai, Y.; Yamamura, S. *Tetrahedron Lett.* **1999**, *55*, 10925–10936.
10. Horner, L.; Hoffmann, H.; Wippel, H. G.; Klahre, G. *Chem. Ber.* **1959**, *92*, 2499–2505.
11. Mitsunobu, O. *Synthesis* **1981**, 1–28.
12. Amano, S.; Takemura, N.; Ohtsuka, M.; Ogawa, S.; Chida, N. *Tetrahedron* **1999**, *55*, 3855–3870.
13. Itzstein, M. V.; Jenkins, M. J.; Mocerino, M. *Carbohydr. Res.* **1990**, *208*, 287–288.
14. Szarek, W. A.; Jones, J. K. N. *Can. J. Chem.* **1965**, *43*, 2345–2356.
15. Suzuki, K.; Maeta, H.; Suzuki, T.; Matsumoto, T. *Tetrahedron Lett.* **1989**, *30*, 6879–6882.
16. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431–432.
17. (a) Mori, K.; Tominaga, M.; Takigawa, T.; Matsui, M. *Synthesis* **1973**, 790–791; (b) Mori, K.; Sasaki, M. *Tetrahedron Lett.* **1979**, *20*, 1329–1332; (c) Herzig, J.; Nudelman, A.; Gottlieb, H. E.; Fischer, B. *J. Org. Chem.* **1986**, *51*, 727–730.
18. Cote, G. G. *Plant Physiol.* **1995**, *109*, 729–734.
19. (a) Ueda, M.; Takada, N.; Yamamura, S. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 2337–2347; (b) Ueda, M.; Okazaki, M.; Ueda, K.; Yamamura, S. *Tetrahedron* **2000**, *56*, 8101–8105.
20. (a) Nagano, H.; Kato, E.; Yamamura, S.; Ueda, M. *Tetrahedron Lett.* **2003**, *44*, 2953–2956; (b) Nagano, H.; Kato, E.; Yamamura, S.; Ueda, M. *Org. Biomol. Chem.* **2003**, *1*, 3186–3192.

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α-(3,7-Dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-diazines. Part 1: Synthesis and stereochemistry. Extension to *s*-triazine series

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Abstract—The general and efficient synthesis of the title compounds, consisting of the (selective) replacement of chlorine in commercial α-chlorodiazines and cyanuryl chloride by the 3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy group (Williamson method) is described. The stereochemistry of this new series of derivatives is analysed in terms of different conformational chirality exhibited in solution (^{1}H NMR) versus solid state (X-ray diffractometry), *meso* against chiral forms, respectively. In solid state, the inclusion capacity of some chiral networks as well as their supramolecular aggregation is pointed out. A good correlation between rotameric behaviour of the *c*-5-di(*s*-tri)-diazinyloxymethyl group in the two states is found.

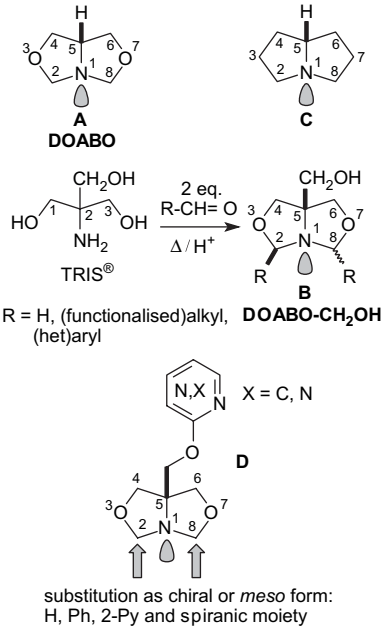
1. Introduction

The 3,7-dioxa-1-azabicyclo[3.3.0]octane heterocyclic saturated system **A** is readily available by double cyclocondensation between TRIS® (2-amino-2-hydroxymethyl-1,3-propandiol) and carbonyl compounds, yielding 5-hydroxymethyl-3,7-dioxa analogous **B** of the core alkaloid, namely *pyrrolizidine* **C** (Scheme 1).[1–4]

A series of various *C*-substituted compounds, having **A** as their basic skeleton, have been shown to have high biological interest: fertilisers, biocides, pesticides and anticancer agents.[5–14]

Although focused mainly on applied research, only few of the results reported previously validated this class as appropriate for further functionalisation.

A method for direct substitution at the carbon ring is still unknown. Functionality was ensured classically by the a priori selection of the substituted starting carbonyl compound, usually an aldehyde (Scheme 1). Thus, only compounds **A** bearing a hydroxymethyl group at C-5 were mentioned to be suitable for functionalisation at this site by acylation,[3–5,7,8,12,15] thionation[16] and, recently, by Dess–Martin oxidation.[13] Depending on the new group linked at C-5, the reported structures are of pharmaceutical[7,8,12,13] and



Scheme 1.

Keywords: Diazines; *s*-Triazines; Oxazolidines; NMR; Chirality; X-ray diffractometry.
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lately, of supramolecular interest as O-, N-, O-protected forms of TRIS®.[17]

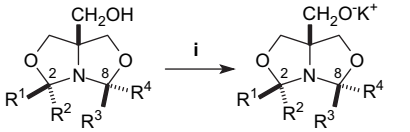
Following on from our developments in the synthesis and stereochemistry of substituted 3,7-DiOxa-*r*-1-AzaBicyclo[3.3.0]-*c*-5-Octanes (hereafter throughout abbreviated as DOABO, Scheme 1),[†,18–20] we recently established that some compounds of type **B** (Scheme 1, R=H, Ph) can be easily converted into 5-alkoxymethyl derivatives, via potassium alkoxides, in much milder conditions than those used earlier by Broadbent in 1976 (Williamson method).[7,20] Not only they were efficient nucleophiles against aliphatic halo compounds, but in a single testing example, against an α-chloro-π-deficient system such as 2,6-dichloropyrazine.[20]

An extension of this result required a larger series of competent substrates. Referring to our previous data about the selective (or exhaustive) nucleophilic replacement of chlorine in certain π-deficient systems,[20,26,27] we considered α-chlorodiazines and cyanuryl chloride as a challenging choice for investigating more elaborated building blocks with potential biological and/or supramolecular interest. Hence, we wish to report here the synthesis and stereochemistry of a new class as 3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxydi(*s*-tri)azines **D** (Scheme 1).

2. Results and discussion

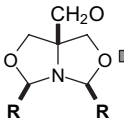
2.1. Synthesis of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-di(*s*-tri)azines

The known DOABO derivatives **1a**–**e**[19,20] were reacted with potassium hydride in conditions depicted in Scheme 2.



i: 1.05 eq. KH / THF / 40 °C / 1.0 - 1.5 hrs.

a: R^1- R^4 = H
b-*cis*: R^1 = R^3 = Ph, R^2 = R^4 = H
b-*trans*: R^1 = R^4 = Ph, R^2 = R^3 = H
c: R^1 = Ph, R^2 - R^4 = H
d-*trans* + **d**-*cis*:
R^1,R^2 = CH_2CH_2[CH(*t*-Bu)]CH_2CH_2,
R^3 = R^4 = H
e: R^1 = R^3 = 2-Py, R^2 = R^4 = H



Abbreviations:
R = H: (2H)DOABO-CH_2O
R = Ph: (2Ph)DOABO-CH_2O

Scheme 2.

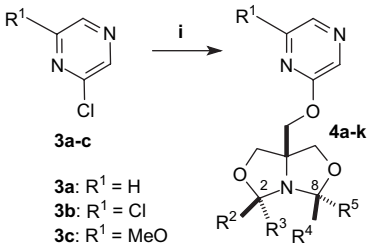
Chiral **1b**-*trans*, **1c**, **1d**-*trans* and **1d**-*cis* were used as racemates.

The study of the reaction between potassium alkoxides **2a**–**e** and α-chlorodiazines was performed using the following protocol:

(i) For exhaustive substitution of chlorine, 1.05×*n* equivalents of **2a**–**e**/equivalent of diazine possessing '*n*' chlorine atoms were used.
(ii) For selective substitution of chlorine, 1 equiv of **2a**–**e**/equivalent of chlorine to be replaced was used.

All syntheses were systematically TLC monitored.

Series of new compounds **4a**–**k** were prepared starting from the α-chloropyrazines **3a**–**c** (Scheme 3, Table 1).



i: **2a-e** / THF, *T*(°C), t (hrs.)

Scheme 3.

Only **2a** exhibited a 'methoxide-like reactivity' regarding yields and selectivity (entries 1, 7, 9 and 10). Indeed, in a competitive experiment, equimolar amounts of 2-chloropyrazine **3a**/**2a**/potassium methoxide gave, in identical conditions (entry 1), the equimolar ratio between 2-methoxypyrazine and **4a**. When **2b**-*cis*, **2b**-*trans*, **2c** and **2e** having C-2, (-8) (di)substituted DOABO units with (het)aryl groups were used as nucleophiles, the yields decreased slightly, **4a** (85%) versus **4b**-*cis* (79%) versus **4b**-*trans* (69%), or strongly, **4a** versus **4c** (48%) and **4e** (44%). The unfavourable influence of substitution at C-2, -8 was best illustrated when the results of the one-pot replacement of the two chlorine atoms in 2,6-dichloropyrazine, **2b**-*cis* versus **2a** (entries 8 and 10) were compared. Treatment of **3b** with 2.1 equiv of **2b**-*cis* yielded a complex mixture of monochloro derivative **4g**, the (2Ph)DOABO–CH_2O substituting pyrazinone **4h** (issued most probably from the partial hydrolysis of **4g** during the aqueous work-up) and, in traces only, the desired product **4i**. Using **2a** as nucleophile, compound **4k** was obtained in a clean procedure as described in a previous publication of our laboratory.[20]

The non-separable mixture of DOABO-spiranic derivatives **1d**-*trans*/**1d**-*cis* (96:4) (Scheme 2) afforded the corresponding **4d**-*trans*/**4d**-*cis* as 96:4 ratio, respectively, in the crude product and 75:25 after crystallisation from ligroin.

Next, the α-chloropyrimidines **5a**–**d** produced the series **6a**–**m** (Scheme 4, Table 2).

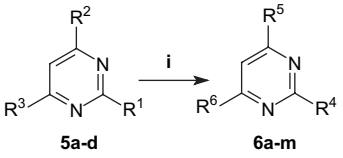
With **2a** as nucleophile, both one-pot exhaustive (entries 2, 4 and 6) and selective substitutions (entries 3, 5 and 7) were

† Stereochemical descriptor ***r*** (reference) is used in order to simplify discussion arising from the basic stereochemistry of this molecule as *cis* fused double oxazolidine system, the lone pair at N-1 being the fiducial substituent.[21] This spatial arrangement, together with the absence of pyramidal inversion at N-1 are already well documented.[7,11,18–20,22–25]

Table 1. Results in the synthesis of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-pyrazines (preparation of compounds **4a–k**)

Entry	Nucleophile→Compd	R^1	R^2	R^3	R^4	R^5	*T* (°C)	*t* (h)	Yield (%)
1	**2a**→**4a**	H	H	H	H	H	40	16	85
2	**2b**-*cis*→**4b**-*cis*	H	Ph	H	Ph	H	60	20	79
3	**2b**-*trans*→**4b**-*trans*	H	Ph	H	H	Ph	50	14	69
4	**2c**→**4c**	H	Ph	H	H	H	65	11	48
5	**2d**-*trans*→**4d**-*trans*	H	$CH_2CH_2[CH(t\text{-Bu})]CH_2CH_2$		H	H	65	2	46[a]
	2d-*cis*→**4d**-*cis*						rt	12	16[a]
6	**2e**→**4e**	H	2-Py	H	2-Py	H	rt	5	44
							35	18	
7	**2a**→**4f**	Cl	H	H	H	H	rt	6	83[b]
8	**2b**-*cis*→**4g**	Cl	Ph	H	Ph	H	65	52	34[c]
	→**4h**	OH	Ph	H	Ph	H			17[c]
	→**4i**	(2Ph)DOABO–CH_2O	Ph	H	Ph	H			6[c]
9	**2a**→**4j**	MeO	H	H	H	H	65	24	33
10	**2a**→**4k**	(2H)DOABO–CH_2O	H	H	H	H	65	3	76[d]
							rt	14	

[a] Isolated as a non-separable mixture of diastereomers **4d**-*trans*/**4d**-*cis* 75:25 (bridged N-1 and *t*-Bu groups as references) as deduced from the ^{1}H NMR spectrum of the crystallised material.
[b] Selectivity as 89:11 **4f**/**4k** in the ^{1}H NMR spectrum of the crude reaction mixture.
[c] As partial conversions of **3b** into **4g–i**.
[d] Starting directly from **3b** without isolation of the intermediate **4f**.



Scheme 4.

performed with good yields. The depicted (regio)selectivities could be ensured in very mild conditions only. Surprisingly (entry 7), the regioisomer **6h** was largely dominant against the expected **6i** as confirmed by the NMR spectra of the pure isolated **6h**, which clearly displayed equal intensity of signals for two magnetically non-equivalent DOABO–CH_2O groups. Their individual assignment, as well as for the **6b** analogous (entry 2), was performed by high-resolution ^{1}H NMR experiments, 2D ^{1}H–^{1}H (COSY and TOWNY),[28,29] ^{1}H–^{13}C (HSQC[30,31] and HMBC[32,33]), ROESY[34,35] and NOESY.[36,37]

As in the α-chloropyrazine series, the use of **2b**-*cis* gave different results (entries 8 and 9): complete replacement of chlorine in dichloropyrimidines was possible only in the 2,4-regioisomer (**5b**→**6k**) with medium yield. In identical conditions, starting from 4,6-dichloropyrimidine **5c**, the separable mixture of **6l** and **6m** was obtained, suggesting that the second substitution of chlorine in **6m** was difficult.

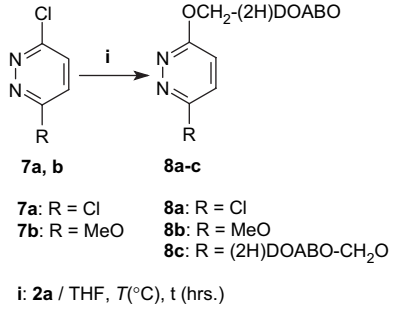
In the α-chloropyridazine series (Scheme 5, Table 3), we limited our investigation to the reactivity of **2a** exclusively.

Compounds **8a–c** were prepared, supporting the validity of our synthetic findings.

Table 2. Results in the synthesis of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-pyrimidines (preparation of compounds **6a–m**)

Entry	Reaction	R^4	R^5	R^6	*T* (°C)	*t* (h)	Yield (%)
1	**5a**→**6a**	(2H)DOABO–CH_2O	H	H	65	4	60
2	**5b**→**6b**	(2H)DOABO–CH_2O	(2H)DOABO–CH_2O	H	40	6	80
3	**5b**→**6c**	Cl	(2H)DOABO–CH_2O	H	−78→rt	24	63 (71[a])
	→**6d**	(2H)DOABO–CH_2O	Cl	H			(23[a])
4	**5c**→**6e**	H	(2H)DOABO–CH_2O	(2H)DOABO–CH_2O	45	24	81
5	**5c**→**6f**	H	(2H)DOABO–CH_2O	Cl	−78→rt	19	63 (82[b])
6	**5d**→**6g**	(2H)DOABO–CH_2O	(2H)DOABO–CH_2O	(2H)DOABO–CH_2O	65	21	58
7	**5d**→**6h**	(2H)DOABO–CH_2O	(2H)DOABO–CH_2O	Cl	−78→rt	22	76 (86[c])
	→**6i**	Cl	(2H)DOABO–CH_2O	(2H)DOABO–CH_2O			(8[c])
	→**6j**	Cl	(2H)DOABO–CH_2O	Cl			(6[c])
8	**5b**→**6k**	(2Ph)DOABO–CH_2O	(2Ph)DOABO–CH_2O	H	65	21	58
9	**5c**→**6l**	H	(2Ph)DOABO–CH_2O	(2Ph)DOABO–CH_2O	65	21	31[d]
	→**6m**	H	(2Ph)DOABO–CH_2O	Cl			23[d]

[a] Regioselectivity according to the ^{1}H NMR spectrum of the crude reaction mixture: 6% unreacted **5b**.
[b] 18% **6e** according to the ^{1}H NMR spectrum of the crude reaction mixture.
[c] Regioselectivity according to the ^{1}H NMR spectrum of the crude reaction mixture.
[d] Partial conversions of **5c**.



Scheme 5.

Table 3. Results in the synthesis of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-pyridazines (preparation of compounds **8a–c**)

Compd	R	*T* (°C)	*t* (h)	Yield (%)
8a	Cl	40	4	86 (96[a])
8b	MeO	65	18	51
8c	(2H)DOABO–CH_2O	40	3	78 (90[b])

[a] Selectivity according to the ^{1}H NMR spectrum of the crude reaction mixture: 4% **8c**.
[b] Starting directly from **7a** without isolation of the intermediate **8a**; content according to the ^{1}H NMR spectrum of the crude reaction mixture: 10% **8a**.

Finally, the nucleophilicity of the alkoxides based on **1a** and **1b**-*cis* was comparatively explored against a more π-deficient system, cyanuryl chloride. Based on the literature data reporting the reaction between alcohols and cyanuryl chloride in neutral or basic conditions,[38–41] the chemistry followed is depicted in Scheme 6. The results are summarised in Table 4.

The target compounds were the trisubstituted *s*-triazines **10a** and **10b** in a one-pot synthesis.

A much greater dependence with respect to the starting **1a** or **1b**-*cis* and their deprotonated forms was observed. Thus, **2a** was efficient only in disubstitution of chlorine with poor yield (**9a**, entry 1). No intermediate of type monoalkoxy was detected. In contrast, the use of its lithium alkoxide **2f** (entry 2) permitted rapidly the optimisation of the synthesis towards the desired **10a** in gentle and clean reproducible conditions. The mass spectra of **9a** and **10a** (ESI and FAB$^+$, respectively) fully confirmed the envisaged structures.

Table 4. Results in the synthesis of 3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy-*s*-triazines (preparation of compounds **9a**, **b**, **10a**, **b**)

Entry	Starting material	Nucleophile	*T* (°C)	*t* (h)	Results	
					Compounds	Yield (%)
1	**1a**	**2a**	65	36	**9a**	34
2		**2f**	−78→rt	20	**10a**	82
3	**1b**-*cis*	**2b**-*cis*	0	1	**10b** (51);	37
			65	40	**9b** (10);	
					1b-*cis* (39)[a]	
4		**2g**-*cis*	−60→rt	20	**10b** (46);	29
			rt	48	**9b** (8);	
			65	4	**1b**-*cis* (46)[a]	

[a] Contents according to the ^{1}H NMR spectra of the crude reaction mixtures.

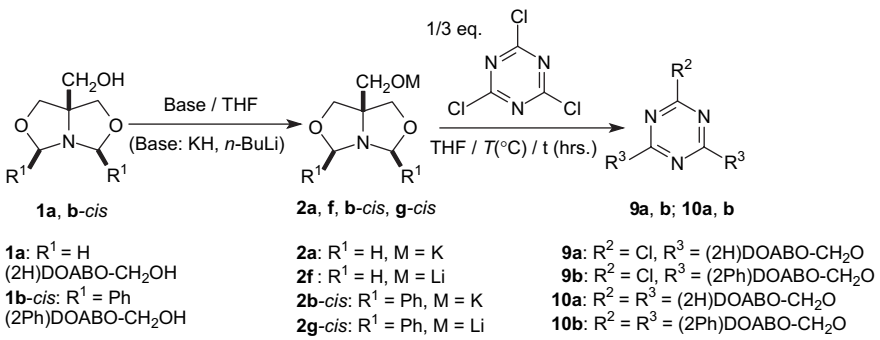
Starting from **1b**-*cis*, its potassium alkoxide **2b**-*cis* rather than **2g**-*cis* gave this time a slightly higher content of the trisubstituted product **10b** in the crude reaction mixture (entries 3 and 4). The bias between **9b** and **10b** was solved by MS-(FAB$^+$) spectrometry. With the isolated **9a**, **10a**, **9b** and **10b** in our hands, the content of the crude reaction mixtures (Table 4) based on their ^{1}H NMR spectra was determined.

2.2. Stereochemistry of α-(3,7-dioxa-*r*-1-azabicyclo-[3.3.0]oct-*c*-5-ylmethoxy)-di(*s*-tri)azines

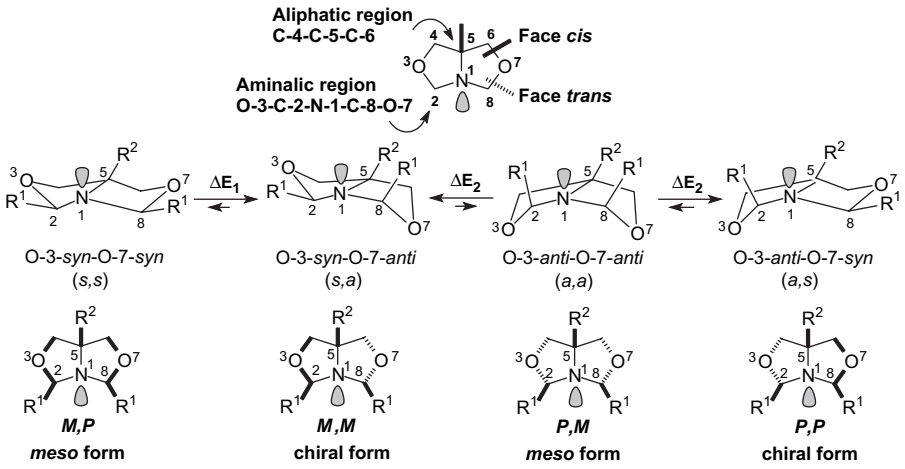
2.2.1. Conformational considerations. As pyrrolizidine **C** (Scheme 1), the skeleton of its 3,7-dioxa analogous **A** is heterofacial. All its (hetero)atoms are prostereogenic centres.[20] Except H-5, the substitution of any of the hydrogen atoms generates configurational chirality. We earlier described in detail this stereochemistry.[19,20]

The basic molecule itself **A**, a *cis* fused double oxazolidine system, as well as its *c*-C-5-achiral monosubstituted derivatives (e.g., **B**, R=H), can exist in a number of flexible conformations upon pseudorotation occurring at each oxazolidine ring. Few experimental studies confirmed this flipping,[19,20,23] presumably because determining the frozen conformation in solution is a quite difficult task, for example, in the case of compounds **1a** and **1b**-*cis* (Scheme 2).

Our previous results of the ab initio RHF/6-31G* calculations[20] in gas phase and solvation models predicted that



Scheme 6.



Type of structure	Configuration	ΔE_1 (kJ/mol)[a]	ΔE_2 (kJ/mol)[a]
R^1 = H; R^2 = CH_2OH (**1a**), MOM	Achiral	-18.87	-5.48
R^1 = Ph; R^2 = CH_2OH (**1b**-*cis*), MOM	Non-chiral (*meso* form) 1*r*,2*R*,5*s*,8*S*	-14.86	-8.54

[a]Typical ΔE values in vacuum and gas phase.[19,20]

Scheme 7.

the DOABO skeleton could be involved in three different conformational equilibriums depicted in Scheme 7.

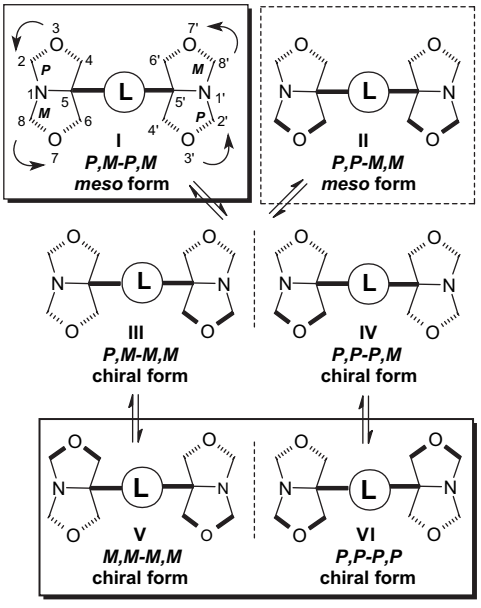
Calculation suggested an oriented flexibility of the bicycle, ascertained as a single oxazolidine ring inversion/equilibrium. It occurs regardless of the configurational nature, achiral or non-chiral, of the structure. The four stereoisomers were discriminated based on the sense of puckering in the two oxazolidine rings, *syn*/*anti* O-3/O-7, revealed as fused *O*-envelope conformers. The lone pair at N-1 was the fiducial substituent for the descriptors *syn*/*anti*. The substitution test shows that the steric relationships between homofacial protons, aminalic H-2, -8 or aliphatic H-4, -6, are different in the two types of conformers, enantiotopic in diastereomeric *meso* forms (*s*,*s*) or (*a*,*a*) but diastereotopic in chiral forms (*a*,*s*) or (*s*,*a*).

Next, in order to designate enantiomeric and *meso* form conformations, the two torsion angles in the aminalic part of the skeleton, C-5–N-1–C-2–O-3 and C-5–N-1–C-8–O-7, were selected and defined by using the helicity rules descriptors *M* and *P*.

As shown in Scheme 7, the occurrence of the *meso* (*M*,*P*) conformer can be reasonably ruled out since it was found much less stable than the alternative *meso* (*P*,*M*) diastereomer and the chiral conformers $(M,M) \equiv (P,P)$. Only the equilibriums $(M,M) \leftrightarrows (P,M) \leftrightarrows (P,P)$, consisting of two diastereomeric inversions and, overall, an enantiomeric interconversion, are to be considered. However, the magnitude of the corresponding ΔE_2 values precluded an a priori assignment of the frozen conformation in gas phase as well as in solution.[20]

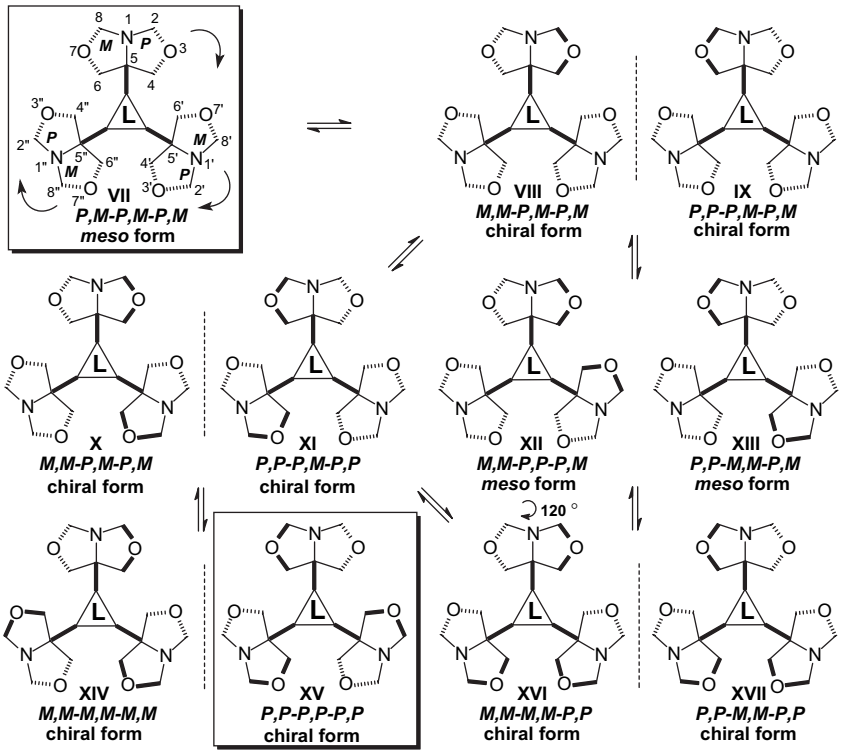
These results, issued from an apparently restrictive rotation about the C–O–C bonds only, were proved by our inspection of some earlier X-ray crystallographically determined structures in this class.[20,24,25]

For the present work, we enlarged the analysis to compounds comprising two, even three identical DOABO units tied together by an achiral linker **L** (Schemes 8 and 9).[‡] Obviously, the linker should be highly symmetric, i.e., C_{nh}, C_{nv} groups, such as di(tri)methoxy-di(*s*-tri)azine fragments. They are statistically achiral, considering the angular geometry of the –O–CH_2– sequence. The last one can promote a preferred rotamerism, as we will mention later.



Scheme 8.

[‡] In Schemes 8 and 9, the DOABO homomorphic substitution at C-2, -8 was omitted for the reason of simplicity.



Scheme 9.

The stereoisomerism depicted in Schemes 8 and 9 is exacerbated although, by neglecting all the DOABO *meso*-(*M*,*P*) type forms (Scheme 7), the conformational analysis is simplified.

In this purpose, we applied our previous proposal, namely *local stereochemistry*, referring to compounds possessing only one DOABO unit (Scheme 7) and *global stereochemistry* defining molecules built on two or three DOABO units (Schemes 8 and 9).[20] In this approach, 'dimeric' DOABO derivatives can exist as two *global meso* forms, **I** and **II**, and four *global* chiral forms, two racemates **III–IV** and **V–VI**. 'Trimeric' DOABO derivatives, the *s*-triazines **10a**, **10b**, provide three *global meso* forms, **VII**, **XII** and **XIII**, and eight *global chiral* forms, four racemates, **VIII–IX**, **X–XI**, **XIV–XV** and **XVI–XVII**. The common feature is that each conformer **I**→**VI** and **VII**→**XVII** can be generated, step by step, in a single oxazolidine ring inversion/equilibrium, following the pathways depicted in Schemes 8 and 9.

2.2.2. Determining the stereochemistry in solution by ^{1}H DNMR. A stereochemical analysis, focused on compounds **4a**, **4k**, **6e**, **6l**, **10a** and **10b**, was carried out by ^{1}H DNMR at low temperature (293–173 K) in THF-d_8 on 400 MHz timescale. The results obtained prompted us to discuss the behaviour of the two building heterocyclic systems separately.

2.2.2.1. Conformational analysis of the DOABO counterparts. In Table 5, the main chemical shifts at room temperature (T_i), at coalescence ($T_{coales.}$) and at the lowest temperature (T_{calcd}) are collected. The last one was used for calculation of the rate constant at coalescence (k_c) and the free enthalpy of activation ($\Delta G^{\neq}$) of DOABO ring inversion. These two parameters were available by applying the Eyring equations (Eqs. 1 and 2).[21,42]

$$k_c = 2.22\left(\Delta\nu^2 + 6J^2\right)^{0.5} \ [s^{-1}] \tag{1}$$

$$\Delta G^{\neq} = 19.14\, T_c(10.32 + \log T_{coales.}/k_c) \ [J/mol] \tag{2}$$

The results are listed in Table 6. They refer throughout to a single oxazolidine ring inversion/equilibrium placed in different environments, created by the number of DOABO units (1–3)/compound.

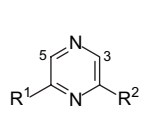
The ^{1}H DNMR behaviour of the simplest compound, **4a**, is shown in Figure 1.

We assigned the spectral shape above the coalescence point as to refer to the fast conformational interconversion involving the exchanging sites illustrated in Scheme 7 (Eq. 3):

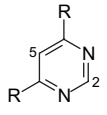
$$\mathbf{4a}\ (M,M) \leftrightarrows \mathbf{4a}\ (P,M) \leftrightarrows \mathbf{4a}\ (P,P) \tag{3}$$

Both equilibriums were seen as first-order reactions and equally populated. Consequently, the k_c value was approximated to be the same for the forward and the reverse processes. The supporting reason is that the calculated ΔE_2 values, chiral versus *meso* form (Scheme 7), were small enough. Since the temperature of coalescence was revealed to be the same for both the aminalic and aliphatic methylenes (Fig. 1), we concluded that these two 'internal clocks' were

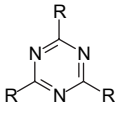
Table 5. Relevant ^{1}H DNMR data [δ (ppm) in THF-d_8] of compounds **4a**, **4k**, **6e**, **6l**, **10a** and **10b**



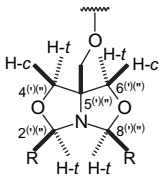
4a: R^1 = H, R^2 = (2H)DOABO-CH_2O
4k: R^1 = R^2 = (2H)DOABO-CH_2O



6e: R = (2H)DOABO-CH_2O
6l: R = (2Ph)DOABO-CH_2O



10a: R = (2H)DOABO-CH_2O
10b: R = (2Ph)DOABO-CH_2O



R = H-*c*, (2H)DOABO-CH_2O
R = Ph, (2Ph)DOABO-CH_2O

Compd	T_i (K)	δ		δ		δ[d]
	$T_{coales.}$ (K)	Aminalic methylenes[b]		Aliphatic methylenes[c]		Heteroaromatic
	T_{calcd} (K)[a]	H-2(8)$^{(r)(n)}$-*c*	H-2(8)$^{(r)(n)}$-*t*	H-4(6)$^{(r)(n)}$-*c*	H-4(6)$^{(r)(n)}$-*t*	
4a	293	4.42	4.40	3.84	3.81	H-3: 8.20
	268	4.41		3.83		H-3: 8.21
	253	4.42	4.40	3.84	3.82	H-3: 8.23
4k	293	4.42	4.40	3.84	3.81	H-3, -5: 7.78
	263	4.41		3.82		H-3, -5: 7.79
	183	4.45	4.38	3.88	3.81	H-3, -5: 7.85
6e	293	4.39	4.38	3.78	3.76	H-5: 6.12
	273	4.39		3.77		H-5: 6.15
	173	4.45	4.35	3.85	3.76	H-5: 6.35
10a	293	4.40	4.38	3.78		—
	273	4.39		3.80	3.78	—
	213	4.43	4.37	3.84	3.76	—
6l	293	—	5.59	4.00	3.91	H-5: 5.75
	173	—	5.58	3.96	3.96	H-5: 6.12
10b	293	—	5.58	3.98	3.92	—
	233	—	5.58	3.96		—
	193	—	5.58	3.99	3.94	—

[a] Temperature at which the parameters $\Delta\nu$ and ^2J were extracted from the spectrum and used for calculation of parameters k_c and $\Delta G^{\neq}$ (see Table 6).
[b] Doublets with ^2J=5.2–5.6 Hz and singlets in **6l**, **10b** above T_i and below $T_{coales.}$.
[c] Doublets with ^2J=8.4–9.0 Hz.
[d] Protons having *ortho* relationships with DOABO–CH_2O groups.

synchronised. They provided similar $\Delta G^{\neq}$ values (Table 6, entry 1).[21]

Below coalescence, we ascertained the spectral appearance to depict the (2H)DOABO unit in **4a** as frozen *meso*-(*P*,*M*) conformer (C_s symmetry, Scheme 7) because the homofacial aminalic (or aliphatic) protons were isochronous, hence enantiotopic (e.g., H-2-*c* vs H-8-*c*, etc.).

In the same way, at room temperature, the DOABO units in polysubstituted analogues **4k**, **6e**, **6l**, **10a** and **10b** were magnetically equivalent and flipping structures (Table 5). However, upon cooling, only in diazines **4k**, **6e** and *s*-triazine **10b** did the (2H)DOABO signals expose a single clear point of coalescence followed by a new relevant splitting as (AB)→(A_2)→(AB) systems. For **4k** and **6e** only, it was again possible to double-check the calculation of k_c and $\Delta G^{\neq}$, the values arising from the identical evolution of the aminalic and aliphatic methylenes (Table 6, entries 2 and 3). The calculated energetic barriers of compounds **4a**, **4k** and **6e** agree with the literature data.[43]

The ^{1}H DNMR spectra of the *s*-triazine **10a** displayed two points of coalescence, 293 K (aliphatic methylenes)

Table 6. ^{1}H DNMR data, k_c (s^{-1}) and $\Delta G^{\neq}$ (kJ/mol) values of DOABO oxazolidine ring inversion in compounds **4a**, **4k**, **6e**, **6l**, **10a** and **10b**

Entry	Compd	Oxazolidine ring inversion data											
		Aminalic zone: H-2(8)$^{(r)(n)}$-*c* versus -*t*						Aliphatic zone: H-4(6)$^{(r)(n)}$-*c* versus -*t*					
		$T_{coales.}$ (K)	T_{calcd}[a] (K)	$\Delta\nu$ (Hz)	^2J (Hz)	k_c[b] (s^{-1})	$\Delta G^{\neq}$ (kJ/mol)	$T_{coales.}$ (K)	T_{calcd}[a] (K)	$\Delta\nu$ (Hz)	^2J (Hz)	k_c[b] (s^{-1})	$\Delta G^{\neq}$ (kJ/mol)
1	**4a**	268	253	6.8	5.6	68.0	56.0	268	253	9.0	9.0	105.7	55.0
2	**4k**	263	183	28.8	5.2	139.8	53.3	263	183	29.0	8.6	159.1	53.1
3	**6e**	273	173	38.1	5.4	179.1	54.9	273	173	37.9	8.7	192.9	54.7
4	**10a**	273	213	23.0	5.4	117.3	55.8	293	213	29.4	8.9	162.5	59.3
5	**6l**	—	—	—	—	—	—	173	—	—	—	—	—
6	**10b**	—	—	—	—	—	—	233	193	19.9	9.1	132.8	47.1

[a] Temperature at which the parameters $\Delta\nu$ and ^2J were extracted from the spectrum and used for calculations.
[b] The k_c values issued by applying Eq. 1 were multiplied by 2 since the DOABO system is a double oxazolidine structure.[21,42]

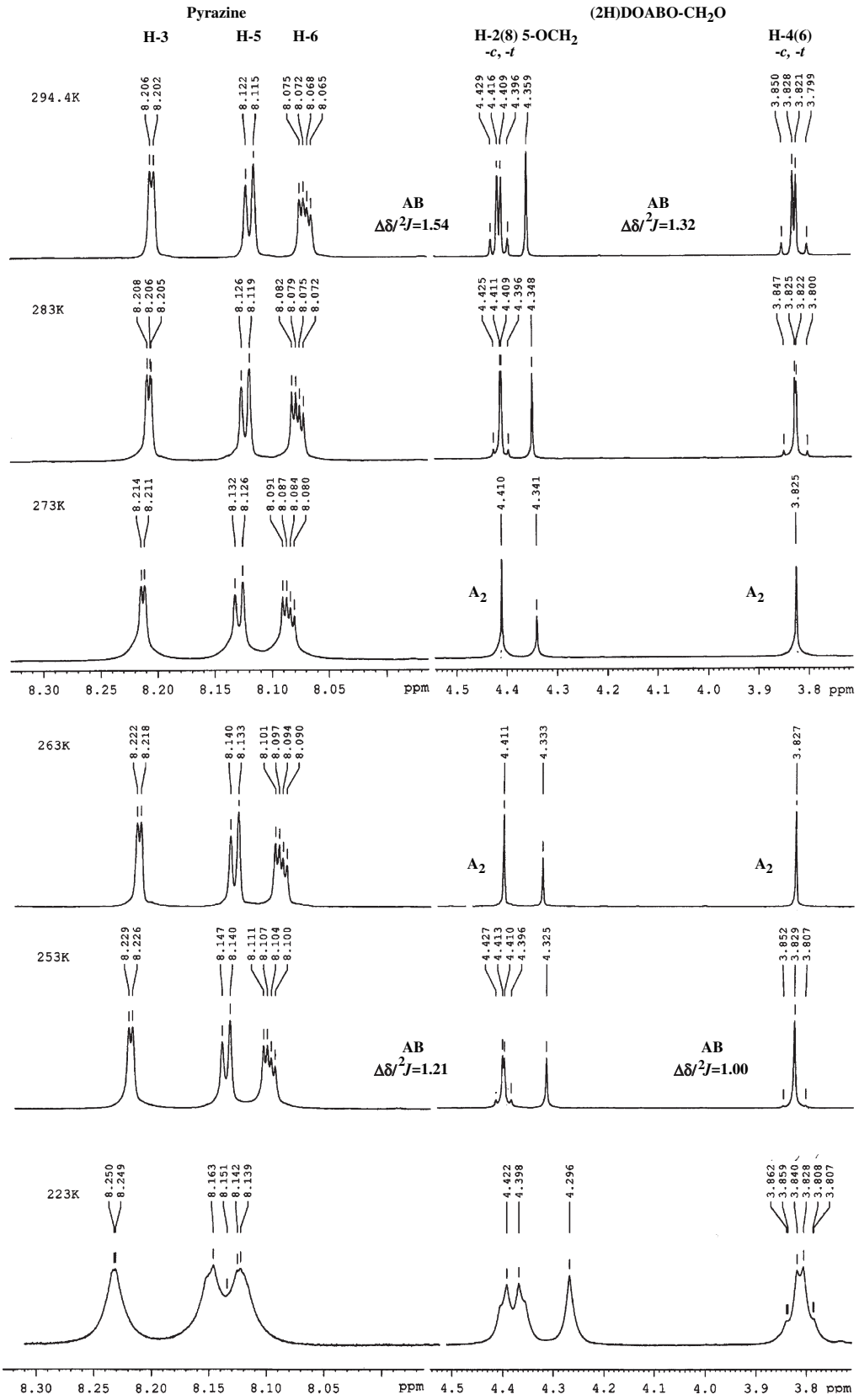


Figure 1. ^{1}H DNMR spectra of compound **4a** (400 MHz, THF-d_8).

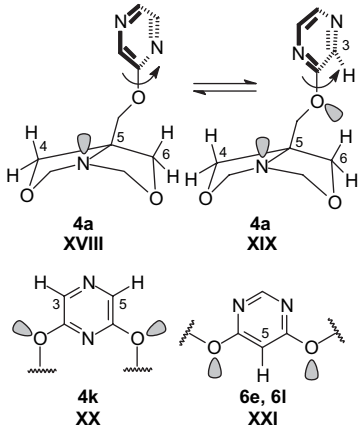
and 273 K (aminalic methylenes) providing two notably different $\Delta G^{\neq}$ values (Table 6, entry 4). The $\Delta G^{\neq}$ value issued from the analysis of the aliphatic methylenes was more credible because the difference $\Delta T = T_{\text{coales.}} - T_{\text{calcd}}$ was greater (80 K) at C-4(6)$^{(I)(II)}$ than at C-2(8)$^{(I)(II)}$ (60 K).[42] The higher $\Delta G^{\neq}$ value of the oxazolidine ring inversion in

10a should be plausible since, as a trisubstituted structure, it was the most crowded term in the series of (2H)DOABO–CH_2O group containing **4a**, **4k** and **6e**.

Nevertheless, in the case of a more crowded compound than **10a**, (2Ph)DOABO–CH_2O groups trisubstituting the *s*-triazine **10b**, the results of the DNMR experiments had to be compared with those of **6l** possessing two *meta* related (2Ph)DOABO–CH_2O groups. Thus, **6l** presented but coalescence of the methylenes C-4(6)$^{(\prime)}$ at the limit of the temperature domain, 173 K (Tables 5 and 6, entry 5) preventing us to assign its rigid conformation. That is, pyrimidine **6l** behaved like simpler (2Ph)DOABO–CH_2OR (R=H, Et, Me) derivatives.[19,20] In contrast, the *s*-triazine **10b** reached coalescence at 233 K (Table 6, entry 6).[§] The corresponding $\Delta G^{\neq}$ value was, however, the smallest in the entire series under investigation, in agreement with our earlier results referring to the faster flipping aptitude of the structures (2Ph)DOABO against (2H)DOABO, for example, **1b**-*cis* against **1a**.[20]

Just below coalescence, the compounds **4k**, **6e**, **10a** and **10b** were established as frozen double or triple *local meso* (*P*,*M*) form DOABO conformers building *global meso* forms of type **I** (Scheme 8, **4k** and **6e**) and of type **VII** (Scheme 9, **10a**, **10b**). The isochronous aminalic or aliphatic homofacial protons, which were found enantiotopic (Table 5), motivate this conclusion.

2.2.2.2. Rotameric behaviour of the *c*-5$^{(\prime)}$-di(*s*-tri)-azinyloxymethyl sequence. In the case of compound **4a** only (Table 6, entry 1), for the calculation we had to use spectral values $\Delta\nu$ and ^2J not well below the coalescence[21,42] because a subsequent process occurred (Fig. 1). Besides the broadening of all signals, an unexpected multiplicity of those of methylenes C-4(6) appeared. The slow rotation of pyrazine ring about the C-2(pyrazine)–O bond might be responsible for generating two distinct populations of rotamers **4a XVIII** and **4a XIX** (Scheme 10).



Scheme 10.

[§] The *s*-triazines **10a** and **10b** were the two cases in which, at the limit of the temperature domain, 173 K, the non-equivalence between DOABO units was displayed but the spectral appearances were not appropriate for pertinent assignments.

This rotamerism could explain the observed splitting at C-4(6) as two partially overlapping AB systems. In addition, the resonance of the pyrazine proton H-3 was significantly shifted downfield from 8.20 (294 K) to 8.29 ppm (183 K) presumably because of its statistical coplanarity with one of the lone pairs of the exocyclic oxygen (rotamer **4a XIX**). We entitled this spatial arrangement s-*trans out* bisectional rotamer with reference to the orientation of the pyrazin-2-yloxymethyl fragment against the bicycle DOABO.

By decreasing the temperature (Table 5), deshielding of the diazine protons *ortho* to the CH_2O linkage in **4k**, **6e** and even in the still flipping **6l** was observed as well. If so, the same nearly coplanar s-*trans out* bisectional conformation could expose these protons to the deshielding proximity of one of the lone pairs of the oxygen atoms in the CH_2O connectivity as rotamers of types **XX** and **XXI**. Accordingly, at low temperature, as for **4a**, our conclusion designates diazines **4k**, **6e** and **6l** to be statistically also s-*trans out* bisectional rotamers.

2.2.3. Determining the stereochemistry in solid state by X-ray diffractometry. Compounds **4b**-*cis*, **4c**, **4k**, **6l** and **10b** supplied crystals suitable for study by X-ray diffractometry. Their crystallographically determined structures are depicted in Figures 2–6. The relevant bond angles and bond lengths are collected in Tables 7 and 8, respectively.

2.2.3.1. Local stereochemistry as frozen conformation and blocked rotamerism (Scheme 11, Table 7). Inspection of all ORTEP diagrams showed exclusively the chiral O-*syn*-O-*anti* opposite orientation of the two *cis* fused oxazolidine rings as *O*-envelope conformers. Indeed, the corresponding torsion angles are small enough, ranging between 0.19 and 7.2°. The torsion angles in the aminalic zone, used to assign the conformational chirality of the DOABO skeleton (Schemes 7 and 11), are noteworthy, 16.8–28.9° in O-3$^{(\prime)(\prime\prime)}$-*syn* rings and 21.3–28.0° in O-7$^{(\prime)(\prime\prime)}$-*anti* rings.

The torsion angles describing the rotamerism of the *c*-5$^{(\prime)(\prime\prime)}$-di(*s*-tri)azinyloxymethyl motif point to its almost coplanar, bisectional, s-*trans* and *out* arrangement with respect to the medium plane of the bicycle. The most significant deviations from coplanarity, 13–17°, are observed regarding the s-*trans* conformation of the bulky substituents about the bonds C-9$^{(\prime)(\prime\prime)}$–O-10$^{(\prime)(\prime\prime)}$. The rest of deviations are considerably smaller, 0.2–6.0°.

None of the above assignments was mandatory to the presence of phenyl groups linked in positions *pseudo*-equatorial-bisectional at C-2$^{(\prime)(\prime\prime)}$ and *pseudo*-axial-orthogonal at C-8$^{(\prime)(\prime\prime)}$.

2.2.3.2. Stereoelectronic effects creating local chirality (Scheme 11, Table 8). In the O-7$^{(\prime)(\prime\prime)}$-*anti* oxazolidine rings, the contraction of the bonds N-1$^{(\prime)(\prime\prime)}$–C-8$^{(\prime)(\prime\prime)}$ versus N-1$^{(\prime)(\prime\prime)}$–C-5$^{(\prime)(\prime\prime)}$ (selected as reference), found significant in all compounds, around 0.030 Å, has been recently explained by Pavia[25] and then by us[20] in terms of the hyperconjugative interaction (*endo*-anomeric effect)[25] involving the orbitals lpN-1$^{(\prime)(\prime\prime)}$ax. (donor)→σ*C-8$^{(\prime)(\prime\prime)}$–O-7$^{(\prime)(\prime\prime)}$ (acceptor). This stereoelectronic effect is due to their near antiperiplanar position created by the frozen oxazolidine

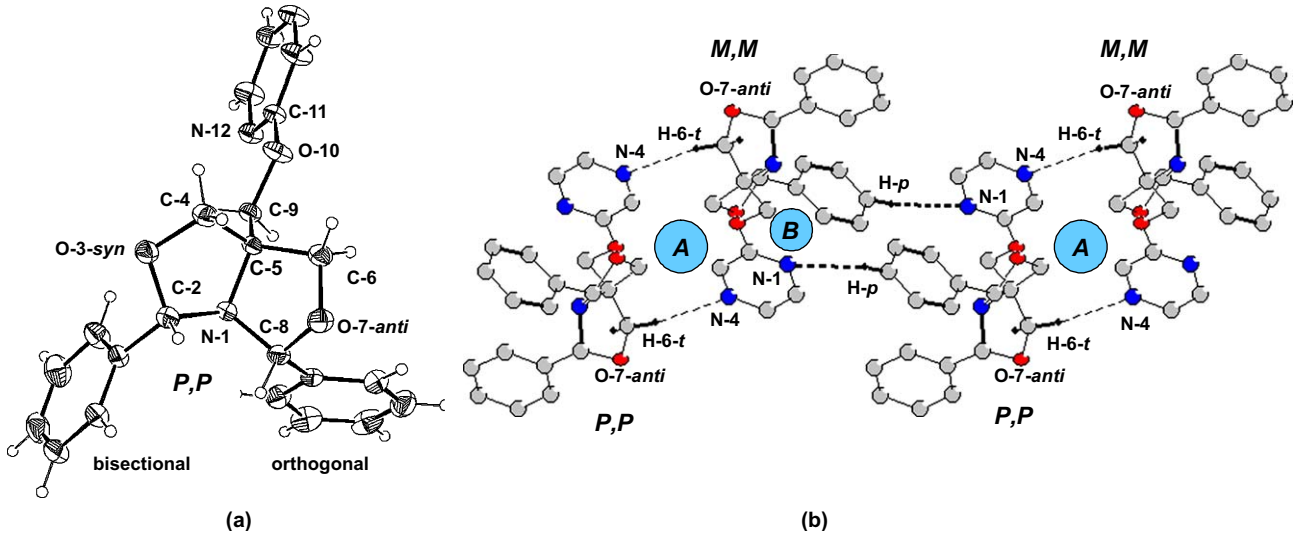


Figure 2. (a) The X-ray crystallographically determined structure of compound **4b**-*cis*; (b) the non-bonding interactions in the elementary cell.

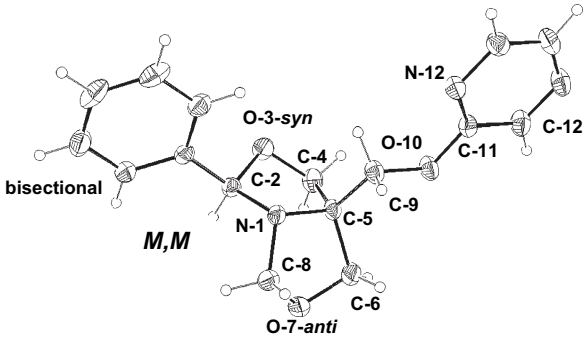


Figure 3. The X-ray crystallographically determined structure of compound **4c**.

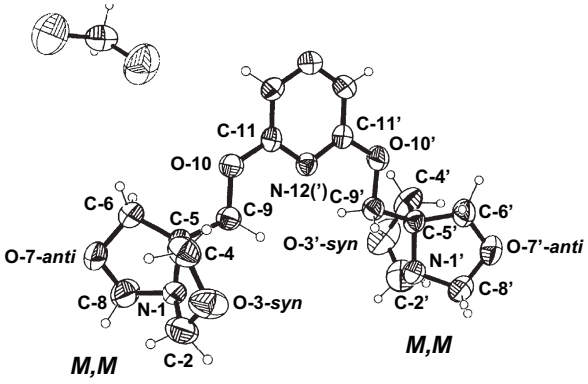
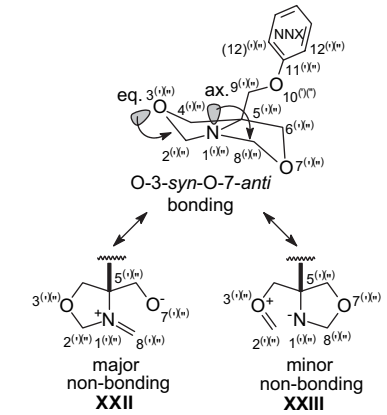


Figure 4. The X-ray crystallographically determined structure of compound *chiral* **4k**.

O-*anti*-envelope conformation. For example, in the case of compound **4k**, we lately estimated the energy of this delocalisation, $E_{del.}$=38.42 kJ/mol (NBO method).[20] The corresponding major non-bonding structure **XXII** suggests the increased basicity of the O-$7^{(\prime)(\prime\prime)}$-*anti* atom.

In the O-$3^{(\prime)(\prime\prime)}$-*syn* oxazolidine rings, a second noticeable contraction was detected this time regarding the bonds



Scheme 11.

O-$3^{(\prime)(\prime\prime)}$–C-$2^{(\prime)(\prime\prime)}$. They were shorter than O-$7^{(\prime)(\prime\prime)}$–C-$8^{(\prime)(\prime\prime)}$ with about 0.17 Å, covering however a larger domain of fluctuation, 0.05–0.050 Å. As above, this contraction originates in the O-*syn*-envelope geometry of the ring favouring the close to antiperiplanar arrangement of the orbitals lpO-$3^{(\prime)(\prime\prime)}$eq.→σ*C-$2^{(\prime)(\prime\prime)}$–N-$1^{(\prime)(\prime\prime)}$, hence the second as weaker delocalising interaction (e.g., E_{del}=30.93 kJ/mol in **4k**[20]). The matching minor non-bonding structures **XXIII** reveal a decreased basicity of the O-$3^{(\prime)(\prime\prime)}$-*syn* atom.

We concluded that the chirality of the DOABO skeleton was, in fact, the major consequence of the cross *endo*-anomeric effect, consisting in two and identically oriented delocalisation in the *syn–anti* aminalic part of the bicycle. The different basicity of the intracyclic oxygen atoms could be of practical interest, as already outlined in the literature in the case of the starting material **1b**-*cis*.[44,45]

2.2.3.3. Global stereochemistry and supramolecular interactions. In solid state, the essential characteristic of polysubstituted compounds **4k**, **6l** and **10b** was their crystallisation as *global chiral* forms. The same sense of chirality is exposed by the DOABO groups in duplicate (**4k**, **6l**), even in triplicate (**10b**) (Figs. 4–6).

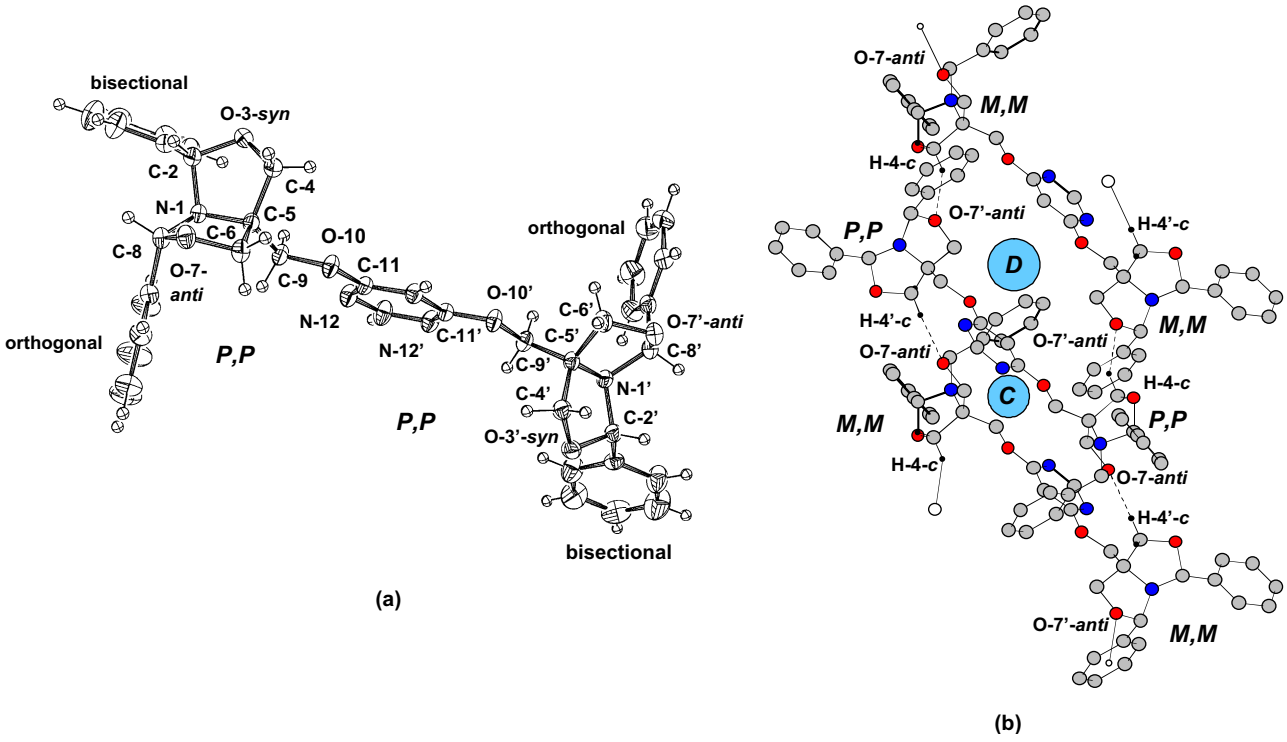


Figure 5. (a) The X-ray crystallographically determined structure of compound **6l**; (b) the non-bonding interactions in the network.

The network of **4k** consisted in *global chiral* form units of type **V** (Scheme 8) in a high occupation factor, 0.87 and *global meso* form units (not depicted, type **II**, Scheme 8) in a low occupation factor, 0.13. As shown in Figure 4, **4k** was a non-stoichiometric solvate of dichloromethane. The solvent, located in the channels of the network, had an occupation factor of 0.96. The dominant incidence of *global chiral* against *meso* form units appeared to us mandatory to the inclusion aptitude of *chiral* **4k**. Indeed, the alternative *meso* **4k** structure exhibited strong geometric distortions, discussed previously by us,[20] hence, lower inclusion ability. Moreover, the entire network was stable only in the presence of the solvent.

Stronger dichloromethane incorporating capacity manifested the network of the *s*-triazine **10b** (Fig. 6), found as triple chiral form of type **XV** (Scheme 9). It was ascertained to be a stable equimolar adduct with dichloromethane (omitted in Fig. 6 for the reason of simplicity).

Important non-bonding interactions were identified in the networks of compounds **4b**-*cis* and **6l**.

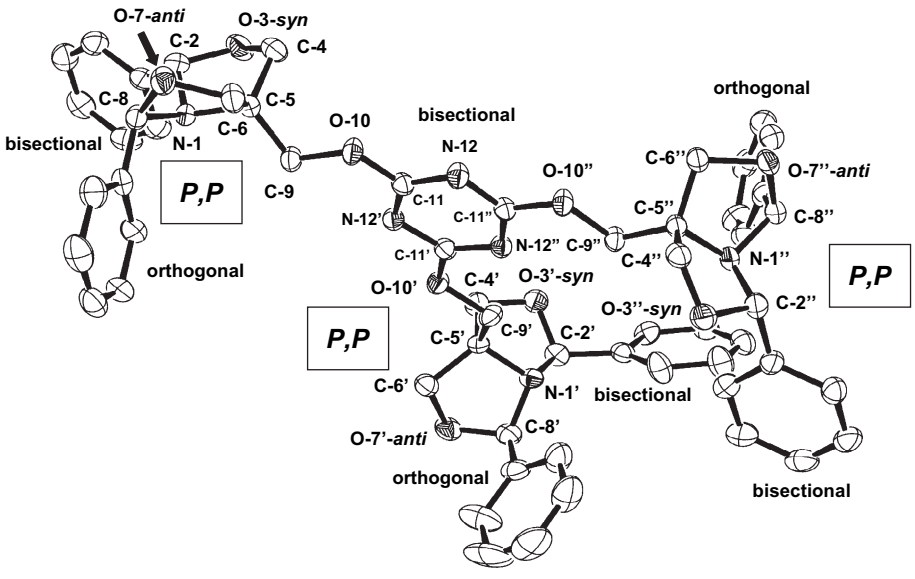


Figure 6. The X-ray crystallographically determined structure of compound **10b**.

Table 7. Relevant torsion angles (°) of compounds **4b**-*cis*, **4c**, **4k**, **6l** and **10b**

Torsion angles	Compound				
	4b-*cis*	**4c**	*chiral* **4k**[a]	**6l**	**10b**
Oxazolidines O-envelope conformation					
O-3$^{(r)(n)}$-syn rings					
C-4–C-5–N-1–C-2	+1.3(2)	−1.46(16)	−6.8(3)	−0.26(13)	−4.1(3)
C-4′–C-5′–N-1′–C-2′	—	—	−7.2(3)	−0.19(13)	+3.8(3)
C-4″–C-5″–N-1″–C-2″	—	—	—	—	+4.1(3)
O-7$^{(r)(n)}$-anti rings					
C-6–C-5–N-1–C-8	−1.6(2)	+1.17(18)	−2.8(3)	−4.07(13)	−4.5(3)
C-6′–C-5′–N-1′–C-8′	—	—	−3.8(3)	−2.54(14)	−1.6(3)
C-6″–C-5″–N-1″–C-8″	—	—	—	—	−0.9(3)
DOABO units conformational chirality					
O-3$^{(r)(n)}$-syn rings					
C-5–N-1–C-2–O-3	+23.3(2) *P*	−24.37(16) *M*	−18.6(3) *M*	+23.80(14) *P*	+28.9(3) *P*
C-5′–N-1′–C-2′–O-3′	—	—	−16.8(4) *M*	+24.09(14) *P*	+20.9(3) *P*
C-5″–N-1″–C-2″–O-3″	—	—	—	—	+22.3(3) *P*
O-7$^{(r)(n)}$-anti rings					
C-5–N-1–C-8–O-7	+24.0(2) *P*	−26.31(18) *M*	−21.6(4) *M*	+27.60(13) *P*	+28.0(3) *P*
C-5′–N-1′–C-8′–O-7′	—	—	−21.3(4) *M*	+26.24(13) *P*	+25.7(3) *P*
C-5″–N-1″–C-8″–O-7″	—	—	—	—	+24.8(3) *P*
Coplanarity of the c-5$^{(r)(n)}$-di(s-tri)azinyloxymethyl sequences					
N-1–C-5–C-9–O-10	−176.93(17)	+177.68(14)	+173.96(19)	−177.10(10)	−176.0(2)
N-1′–C-5′–C-9′–O-10′	—	—	174.78(19)	−175.54(11)	−176.9(2)
N-1″–C-5″–C-9″–O-10″	—	—	—	—	−174.2(2)
C-5–C-9–O-10–C-11	−172.91(19)	+167.01(15)	+178.57(19)	−168.55(11)	−163.9(3)
C-5′–C-9′–O-10′–C-11′	—	—	+178.2(2)	−163.21(11)	−173.4(3)
C-5″–C-9″–O-10″–C-11″	—	—	—	—	−179.3(3)
C-9–O-10–C-11–N-12	−3.3(3)	−2.5(2)	−6.1(3)	−4.77(19)	−178.0(3)
C-9′–O-10′–C-11′–N-12′	—	—	+3.2(3)	−2.82(19)	−176.7(3)
C-9″–O-10″–C-11″–N-12″	—	—	—	—	−0.2(4)

[a] For the *meso* form **4k** see text and Ref. 20.

The elementary cell of **4b**-*cis* was a tetramer (Fig. 2b), based on two different types of intermolecular interactions (**a**) and (**b**). The interatomic distances that we associated to these interactions are: (**a**) H-6-*t*(DOABO)···N-4(pyrazine) 2.550(3) Å and N-1(pyrazine)···H-*para*(C-2-*pseudo*-equatorial-bisectional phenyl ring) 2.636(2) Å. They are smaller than the corresponding sum of the van der Waals radii (ΣvdW N···H) 2.74 Å.[46] The interactions (**a**) close two

Table 8. Relevant bond lengths (Å) of compounds **4b**-*cis*, **4c**, **4k**, **6l** and **10b**

Compd	N-1–C-5	N-1–C-8		O-7–C-8	O-3–C-2	
	N-1′–C-5′	N-1′–C-8′		O-7′–C-8′	O-3′–C-2′	
	N-1″–C-5″	N-1″–C-8″		O-7″–C-8″	O-3″–C-2″	
	Length		Contraction[a]	Length		Contraction[b]
	O-7$^{(r)(n)}$-anti ring			*O-3$^{(r)(n)}$-syn ring*		
4b-*cis*	1.486(3)	1.460(3)	−0.026	1.425(3)	1.416(3)	−0.009
4c	1.491(2)	1.455(2)	−0.036	1.427(2)	1.416(2)	−0.011
chiral	1.493(3)	1.448(4)	−0.045	1.403(4)	1.398(4)	−0.005
4k[c]	1.480(3)	1.454(4)	−0.026	1.405(4)	1.355(5)	−0.050
6l	1.4890(17)	1.4579(18)	−0.0311	1.4411(18)	1.4227(17)	−0.0187
	1.4908(17)	1.4648(18)	−0.026	1.4391(17)	1.4172(17)	−0.0219
10b	1.483(4)	1.462(4)	−0.021	1.438(4)	1.421(4)	−0.017
	1.491(4)	1.465(4)	−0.026	1.437(5)	1.420(4)	−0.017
	1.486(4)	1.464(4)	−0.022	1.434(4)	1.427(4)	−0.007

[a] With respect to N-1$^{(r)(n)}$–C-5$^{(r)(n)}$.
[b] With respect to O-7$^{(r)(n)}$–C-8$^{(r)(n)}$.
[c] For the *meso* **4k** see text and Ref. 20.

identical cavities ***A***, meanwhile the interactions (**b**) lock the central cavity ***B***. Two **4b**-*cis* partners, having an opposite sense of chirality of the DOABO groups, are the building blocks of each cavity.

The network of compound **6l** was a polymeric structure (Fig. 5b) in which the non-bonding interactions between the **6l** units are of the same type H-4′-*c*···O-7-*anti* 2.464(1) Å and H-4-*c*···O-7′-*anti* 2.449(1) Å (ΣvdW O···H 2.60 Å).[46] Their magnitude is slightly different since the two DOABO groups in monomeric **6l** are geometrically not quite identical (Tables 7 and 8). Consequently, two cavities labelled ***C*** and ***D*** are observed, comprising each two **6l** units with a reverse sense of the *global chirality* one against the other.

3. Conclusions

Twenty-two examples demonstrate the Williamson procedure to be as general as simple methodology starting from *c*-5-hydroxymethyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]octanes in reaction with α-chlorodiazines and cyanuryl chloride. The nucleophilicity of the DOABO–CH_2OH reagents in alkoxide form depends on the type of substituents at positions C-2, -8 of the bicycle and the cation against the π-deficiency of the substrates. A large variety of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-di(*s*-tri)azines is available in good yields and selectivity. The conformation analysis of some structures by X-ray diffractometry and ^{1}H DNMR indicates exclusively a chiral against *meso* form frozen conformation of the DOABO skeleton in solid state versus solution, respectively. The cross *endo*-anomeric effect in the aminalic O–C–N–C–O DOABO sequence is responsible for the chiral conformation in solid state. The rotamerism of the *c*-5-di(*s*-tri)azinyloxymethyl group against bicycle is bisectional and s-*trans out* oriented both in solution and solid state. In solid state, an inclusion aptitude of the solvent by the chiral networks is found as well as non-bonding interaction creating specific self-assembly.

The attempt at exploiting these findings in synthesis will be discussed in part II of our report.

4. Experimental

Melting points are uncorrected; they were carried out on a ELECTROTHERMAL® 9100 apparatus.

Current NMR spectra were recorded on a Brucker® AM300 (300 MHz ^{1}H, 75 MHz ^{13}C) instrument. The NMR analysis of the compounds **6b** and **6h** was also carried out on a Brucker® DMX500 (500 MHz ^{1}H, 125 MHz ^{13}C) instrument. ^{1}H DNMR analysis of compounds **4a**, **4k**, **6e**, **6l**, **10a** and **10b** was carried out on a Brucker® AM400 (400 MHz ^{1}H, 100 MHz ^{13}C) instrument. TLC was performed by using aluminium sheets with silica gel 60 F_{254} (Merck®); flash column chromatography was conducted on silica gel Si 60 (40–63 μm, Merck®). IR spectra were performed on a Perkin–Elmer® Paragom FTIR spectrometer. Only relevant absorptions are listed [throughout in cm^{-1}: weak (w), medium (m) or strong (s)]. Mass spectra (MS) were recorded on an ATI-Unicam Automass® apparatus, fitted (or not) with a GC-mass coupling. Microanalyses were performed on a Carlo Erba® CHNOS 1160 apparatus. All syntheses were performed under dry nitrogen atmosphere. THF was freshly distilled from Na/benzophenone prior to use. All other solvents and starting materials were of commercial quality.

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: compound **4b**-*cis* CCDC 283623. Unit cell parameters: *a* 13.0640(11), *b* 8.8414(7), *c* 17.2561(14); space group *P*1 21/*c* 1(14). Compound **4c** CCDC 283622. Unit cell parameters: *a* 5.9105(11), *b* 18.196(3), *c* 14.199(3); space group *P*2(1)/*n*. Compound **4k** CCDC 199978. Unit cell parameters: *a* 12.251, *b* 11.072, *c* 15.243; space group *P*2(1)/*n*. Compound **6l** CCDC 238894. Unit cell parameters: *a* 27.3536(3), *b* 11.8334, *c* 23.7369(3); space group *C*2/*c*. Compound **10b** CCDC 272371. Unit cell parameters: *a* 8.9574(2), *b* 12.2323(2), *c* 24.6520(4); space group *P*-1. 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].

The synthesis of compounds **1a–e** and **4k** we discussed elsewhere.[19,20]

4.1. General procedure for the preparation of compounds 4a–j, 6a–m and 8a–c

In a 100 mL three-necked round bottom flask, potassium hydride (1.000 g as 30% oily suspension, 0.300 g 100%, 7.48 mmol) was rapidly introduced and washed with stirring three times with dry ligroin (optionally pentane, hexane) (30 mL). THF (50 mL) was then introduced with stirring to yield a fine grey suspension. Fine powdered *c*-5-hydroxymethyl-3,7-dioxa-1-azabicyclo[3.3.0]-*c*-5-octanes **1a–e** (7.12 mmol, Scheme 2) was added and the mixture was heated at 40 °C for 1.0–1.5 h (room temperature in the case of **1e**) until no more hydrogen was formed and a fine suspension was obtained. For the synthesis attempting at complete substitution of chlorine [compounds **4a**, **4b**-*cis*, **4b**-*trans*, **4c**, **4d**-*trans*, **4d**-*cis*, **4e**, **4j** (Table 1), **6a**, **6b**, **6e**, **6g**, **6k**, **6l** (Table 2), **8b**, **8c** (Table 3)] the corresponding α-chlorodiazine (6.78/*n* mmol, *n*=number of chlorine atoms to be replaced) was rapidly injected as THF (10 mL) solution, at room temperature (see Tables 1–3 for temperatures and time reaction). For selective substitution of chlorine, in the case of compounds **6c**, **6f** and **6h** the reaction mixture was cooled to −78 °C prior to the addition by injection of the corresponding stoichiometric amount of α-chloropyrimidine as THF (10 mL) solution. Then, it was allowed to slowly reach room temperature. For selective substitution of chlorine in the case of compounds **4f** and **8a**, stoichiometric amounts of α-chlorodiazine were used (conditions as temperature and time reaction are given in Tables 1 and 3). TLC monitoring was performed until the starting materials were absent or in small traces only. Double visualisation was required if **2a** was the nucleophile (Scheme 2): first UV 254 nm then I_2 bath, for the detection of **1a**. During condensation, the reaction mixture turned coloured and

potassium chloride was formed. The reaction was quenched at room temperature with water (100 mL) and dichloromethane (100 mL) with vigorous stirring. After separation, the organic layer was washed with water (about 3×50 mL) to pH=7.5–8.0 then dried over $MgSO_4$. After filtering, the organic solution was evaporated under vacuum to dryness to yield the crude product, which was directly crystallised from an appropriate solvent or purified by flash column chromatography to yield the title compounds.

4.1.1. 2-[(3,7-Dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-pyrazine (4a). Yield 85%. Yellowish crystalline powder, mp 128–129 °C (pentane) [Found: C, 53.50; H, 6.09; N, 18.55. $C_{10}H_{13}N_3O_3$ requires: C, 53.81; H, 5.87; N, 18.82%]. R_f (75% ligroin/acetone) 0.40. ν_{max} (film NaCl) 2868 (m), 1524 (s), 1465 (m), 1413 (s), 1361 (m), 1289 (s), 1134 (m), 1032 (s), 1002 (s), 915 (s), 832 (m), 692 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.19 (1H, d, *J*=1.5 Hz, H-3), 8.09 (1H, d, *J*=3.0 Hz, H-5), 8.01 (1H, dd, *J*=3.0, 1.5 Hz, H-6); *DOABO–CH₂O*: 4.47 (2H, d, *J*=5.7 Hz, H-2, -8-*c*), 4.41 (2H, d, *J*=5.7 Hz, H-2, -8-*t*), 4.33 (2H, s, 5-OCH_2), 3.83 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 160.1 (1C, C-2), 140.9 (1C, C-6), 137.5 (1C, C-3), 136.1 (1C, C-5); *DOABO–CH₂O*: 88.6 (2C, C-2, -8), 74.4 (2C, C-4, -6), 71.9 (1C, C-5), 69.0 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): 223 (6), 178 (14), 163 (13), 114 (100), 98 (17), 86 (9), 68 (26), 58 (11), 42 (18), 41 (59).

4.1.2. 2-[(*c*-2,*c*-8-Diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4b-*cis*). Yield 79%. Yellowish crystalline powder, mp 134–136 °C (flash column chromatography, eluent ligroin/AcOEt 3:1 v/v) [Found: C, 70.17; H, 5.94; N, 10.95. $C_{22}H_{21}N_3O_3$ requires C, 70.38; H, 5.64; N, 11.19%]. R_f (75% ligroin/AcOEt) 0.56. ν_{max} (film KBr) 2877 (s), 1586 (m), 1540 (s), 1418 (s), 1388 (m), 1312 (s), 1135 (s), 1065 (s), 932 (s), 834 (s), 800 (m), 763 (s), 738 (s), 696 (s), 617 (m), 537 (m), 499 (w), 465 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 8.09 (1H, d, *J*=2.6 Hz, H-5), 8.04 (1H, s, H-3), 8.01 (1H, dd, *J*=2.6, 1.3 Hz, H-6), 7.52 (4H, d, *J*=6.0 Hz, Ph), 7.36–7.30 (6H, m, Ph); *DOABO–CH₂O*: 5.61 (2H, s, H-2, -8-*t*), 4.27 (2H, s, 5-OCH_2), 4.10 (2H, d, *J*=9.0 Hz, H-4, -6-*c*), 4.00 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 160.1 (1C, C-2), 140.8 (1C, C-6), 139.7 (2C, Cq., Ph), 137.4 (1C, C-3), 136.1 (1C, C-5), 129.0 (2C, CH, Ph), 128.7 (4C, CH, Ph), 127.6 (4C, CH, Ph); *DOABO–CH₂O*: 97.8 (2C, C-2, -8), 73.6 (2C, C-4, -6), 73.3 (1C, C-5), 70.2 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^+) 375 (<1), 269 (30), 173 (100), 155 (33), 128 (21).

4.1.3. 2-[(*c*-2,*t*-8-Diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4b-*trans*). Yield 69%. Yellowish crystalline powder, mp 144–145 °C (pentane) [Found: C, 70.17; H, 5.94; N, 10.95. $C_{22}H_{21}N_3O_3$ requires C, 70.38; H, 5.64; N, 11.19%]. R_f (75% ligroin/AcOEt) 0.75. ν_{max} (film KBr) 2859 (m), 1579 (m), 1528 (s), 1412 (s), 1309 (s), 1291 (s), 1265 (m), 1090 (s), 1060 (s), 1038 (m), 1028 (w), 839 (m), 760 (s), 731 (s) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 8.48, 8.40, 8.34 (3H, s, H-3, -5, -6), 7.59–7.52 (2H, m, Ph), 7.48–7.33 (6H, m, Ph), 7.27–7.24 (2H, m, Ph); *DOABO–CH₂O*: 5.86 (1H, s, H-8-*c*), 5.49 (1H, s, H-2-*t*), 4.80 (1H, d, *J*=11.1 Hz, 5-OCH_2), 4.76 (1H, d, *J*=11.1 Hz, 5-OCH_2), 4.52 (1H, d, *J*=9.0 Hz, H-4-*c*), 4.48 (1H, d, *J*=9.0 Hz, H-6-*c*), 4.15 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 160.1 (1C, C-2), 141.0 (1C, C-6), 140.2 (1C, Cq., Ph), 137.5 (1C, C-3), 136.2 (1C, C-5), 134.5 (1C, Cq., Ph), 128.9 (1C, CH, Ph), 128.5 (1C, CH, Ph), 128.4 (2C, CH, Ph), 128.1 (2C, CH, Ph), 127.6 (2C, CH, Ph), 127.5 (2C, CH, Ph); *DOABO–CH₂O*: 94.7, 93.6 (2C, C-2, -8), 75.3, 73.6 (2C, C-4, -6), 72.9 (1C, C-5), 69.9 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^+) 375 (<1), 266 (100), 239 (40), 192 (5), 177 (10), 160 (30), 105 (50), 77 (45), 51 (20).

4.1.4. 2-[(*c*-2-Phenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4c). Yield 48%. White crystalline powder, mp 79–81 °C (flash column chromatography, eluent ligroin/AcOEt 2:1 v/v) [Found: C, 63.91; H, 6.02; N, 13.74. $C_{16}H_{17}N_3O_3$ requires C, 64.20; H, 5.72; N, 14.04%]. R_f (67% ligroin/AcOEt) 0.58. ν_{max} (film KBr) 3065 (m), 2860 (m), 1834 (w), 1580 (m), 1531 (s), 1471 (m), 1413 (s), 1305 (m), 1174 (m), 1106 (s), 1034 (m), 909 (s), 856 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 8.22 (1H, d, *J*=1.3 Hz, H-3), 8.14 (1H, d, *J*=2.6 Hz, H-5), 8.07 (1H, dd, *J*=2.6, 1.3 Hz, H-6), 7.52–7.49 (2H, m, Ph), 7.39–7.34 (3H, m, Ph); *DOABO–CH₂O*: 5.24 (1H, s, H-2-*t*), 4.57 (1H, d, *J*=7.0 Hz, H-8-*c*), 4.50 (1H, d, *J*=10.0 Hz, 5-OCH_2), 4.40 (1H, d, *J*=8.9 Hz, H-4-*c*), 4.37 (1H, d, *J*=10.0 Hz, 5-OCH_2), 4.30 (1H, d, *J*=7.0 Hz, H-8-*t*), 4.06 (1H, d, *J*=9.0 Hz, H-6-*c*), 3.83 (1H, d, *J*=8.9 Hz, H-4-*t*), 3.70 (1H, d, *J*=9.0, H-6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 160.2 (1C, C-2), 140.9 (1C, C-6), 139.5 (1C, Cq., Ph), 137.5 (1C, C-3), 136.2 (1C, C-5), 129.5 (1C, CH, Ph), 128.8 (2C, CH, Ph), 127.8 (2C, CH, Ph); *DOABO–CH₂O*: 99.2 (1C, C-2), 88.2 (1C, C-8), 75.4 (1C, C-4), 73.6 (1C, C-6), 72.5 (1C, C-5), 69.7 (1C, 5-OCH_2). MS (ES^+); *m/z* (rel int. %): (M^++1) 300 (39), 223 (2), 204 (100), 194 (36).

4.1.5. 2-{[2-(*t*-4-*tert*-Butylspirocyclohexyl)-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (4d-*trans*) (46%) and 2-{[2-(*c*-4-*tert*-butylspirocyclohexyl)-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (4d-*cis*). Yield 16%. Non-separable two-component mixture (**4d**-*trans*/**4d**-*cis* 75:25) as yellow crystalline powder, mp 103–105 °C (ligroin) [Found: C, 65.79; H, 8.19; N, 11.85. $C_{19}H_{29}N_3O_3$ requires: C, 65.68; H, 8.41; N, 12.09%]. R_f (75% ligroin/acetone) 0.60. ν_{max} (film NaCl) 2940 (s), 2857 (m), 1529 (s), 1465 (m), 1408 (s), 1284 (s), 1080 (w), 1005 (m), 909 (w) cm^{-1}. Diastereomer **4d**-*trans*: δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.21 (1H, s, H-3), 8.11 (1H, d, *J*=3.0 Hz, H-5), 8.03 (1H, d, *J*=2.6 Hz, H-6); *DOABO–CH₂O*: 4.79 (1H, d, *J*=7.5 Hz, H-8-*c*), 4.42 (1H, d, *J*=10.6 Hz, 5-OCH_2), 4.29 (1H, d, *J*=10.6 Hz, 5-OCH_2), 4.15 (1H, d, *J*=7.5 Hz, H-8-*t*), 4.04 (1H, d, *J*=9.2 Hz, H-4-*c*), 3.86 (1H, d, *J*=9.2 Hz, H-4-*t*), 3.77 (1H, d, *J*=8.7 Hz, H-6-*c*), 3.65 (1H, d, *J*=8.7 Hz, H-6-*t*); 1.97–1.79 (2H, m, spirocyclohexyl), 1.77–1.67 (1H, m, spirocyclohexyl), 1.60–1.45 (2H, m, spirocyclohexyl), 1.44–1.15 (3H, m, spirocyclohexyl), 1.03–0.89 (1H, m, spirocyclohexyl), 0.81 [9H, s, $C(CH_3)_3$]; δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 160.3 (1C, C-2), 140.8 (1C, C-6), 137.4 (1C, C-3), 136.2 (1C, C-5); *DOABO–CH₂O*: 98.1 (1C, C-2), 82.0 (1C, C-8), 73.8 (1C, C-6), 72.0 (1C, C-5),

71.6 (1C, C-4), 69.7 (1C, 5-OCH_2), 47.5 (1C, CH, spirocyclohexyl), 38.5, 32.7, 32.2, 24.7 (4C, CH_2, spirocyclohexyl), 28.0 [3C, C(CH_3)$_3$], 24.5 [1C, $C(CH_3)_3$]. Diastereomer **4d**-*cis*: δ_H (300 MHz $CDCl_3$ only distinct peaks are listed) *heteroaromatic*: 8.09 (1H, s, H-5), 8.01 (1H, s, H-6); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 136.3 (1C, C-5); *DOABO–CH_2O*: 96.2 (1C, C-2), 68.4 (1C, 5-OCH_2), 47.3 (1C, CH, spirocyclohexyl), 37.8 (1C, CH_2, spirocyclohexyl). MS (EI, 70 eV); *m*/*z* (rel int. %): (M^+) 348 (50), 334 (11), 318 (27), 292 (13), 252 (100), 234 (15), 222 (35), 194 (50), 165 (7), 152 (9), 98 (70).

4.1.6. 2-{[*c*-2,*c*-8-Bis(pyridin-2-yl)-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (4e). Yield 44%. Yellow crystalline powder, mp 89–90 °C (pentane) [Found: C, 63.44; H, 5.17; N, 18.26. $C_{20}H_{19}N_5O_3$ requires C, 63.65; H, 5.07; N, 18.56%]. R_f (100% acetone) 0.65. ν_{max} (film KBr) 3058 (m), 2863 (m), 15890 (s), 1534 (s), 1441 (m), 1414 (s), 1298 (s), 1129 (s), 1081 (s), 992 (s), 924 (m), 841 (m), 781 (s), 709 (m), 659 (m), 622 (m), 608 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.56 (2H, d, *J*=4.2 Hz, H-6, Py), 8.09 (1H, d, *J*=1.9 Hz, H-5, pyrazine), 8.05 (1H, s, H-3, pyrazine), 8.00 (1H, s, H-6, pyrazine), 7.65 (2H, dd as t, *J*=7.5, 7.5 Hz, H-4, Py), 7.54 (2H, d, *J*=7.9 Hz, H-3, Py), 7.20 (2H, dd as t, *J*=5.7, 6.0 Hz, H-5, Py); *DOABO–CH_2O*: 5.79 (2H, s, H-2, -8-*t*), 4.31 (2H, s, 5-OCH_2), 4.25 (2H, d, *J*=9.0 Hz, H-4, -6-*c*), 4.07 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 160.1 (1C, C-2, pyrazine), 159.2 (2C, C-2, Py), 149.6 (2C, C-6, Py), 140.8 (1C, C-6, pyrazine), 137.4 (1C, C-3, pyrazine), 137.0 (2C, C-4 Py), 136.1 (1C, C-5, pyrazine), 123.6 (2C, C-5, Py), 121.7 (2C, C-3, Py); *DOABO–CH_2O*: 98.4 (2C, C-2, -8), 73.5 (1C, C-5), 73.4 (2C, C-4, -6), 69.8 (1C, 5-OCH_2). MS (EI, 70 eV); *m*/*z* (rel int. %): (M^++1) 378 (62), 282 (83), 272 (19), 252 (18), 214 (13), 175 (100), 165 (23), 159 (42), 145 (37).

4.1.7. 2-Chloro-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4f). Yield 83%. Pale yellowish crystalline powder, mp 88–89 °C (flash column chromatography; eluent ligroin/acetone 3.5:1 v/v) [Found: C, 46.88; H, 4.51; N, 16.50. $C_{10}H_{12}N_3O_3Cl$ requires: C, 46.61; H, 4.69; N, 16.31%]. R_f (78% ligroin/acetone) 0.45. ν_{max} (film NaCl) 2857 (w), 2366 (w), 1563 (m), 1525 (s), 1409 (s), 1364 (m), 1309 (s), 1177 (s), 1093 (w), 1000 (m), 928 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.13 (1H, s, H-5), 8.11 (1H, s, H-3); *DOABO–CH_2O*: 4.48 (2H, d, *J*=5.7 Hz, H-2, -8-*c*), 4.42 (2H, d, *J*=5.7 Hz, H-2, -8-*t*), 4.35 (2H, s, 5-OCH_2), 3.83 (4H, s, H-4, -6-*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 159.1 (1C, C-6), 145.7 (1C, C-2), 136.3 (1C, C-3), 133.3 (1C, C-5); *DOABO–CH_2O*: 88.6 (2C, C-2, -8), 74.3 (2C, C-4, -6), 71.8 (1C, C-5), 69.8 (1C, 5-OCH_2). MS (EI, 70 eV); *m*/*z* (rel int. %): (M^+) 257 (<1), 212 (6), 197 (10), 192 (4), 169 (4), 128 (6), 114 (100), 98 (20), 86 (10), 68 (24), 58 (9), 41 (52).

4.1.8. 2-Chloro-6-[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4g). White crystalline powder (as 34% conversion of **3b**, Table 1, entry 8), mp 128–129 °C (flash column chromatography, eluent ligroin/AcOEt 2:1 v/v) [Found: C, 64.59; H, 4.60; N, 10.51. $C_{22}H_{20}N_3O_3Cl$ requires: C, 64.47; H, 4.92; N, 10.25%]. R_f (67% ligroin/AcOEt) 0.35. ν_{max} (film KBr) 3060 (m), 2990 (m), 2878 (s), 1568 (s), 1528 (s), 1435 (s), 1409 (s), 1309 (s), 1209 (s), 1179 (s), 1131 (s), 1091 (s), 1064 (s), 1006 (s), 949 (m), 923 (s), 961 (s), 762 (s), 736 (s), 697 (s), 637 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)-aromatic*: 8.14 (1H, s, H-5), 7.89 (1H, s, H-3), 7.54–7.52 (4H, m, Ph), 7.40–7.31 (6H, m, Ph); *DOABO–CH_2O*: 5.63 (2H, s, H-2, -8-*t*), 4.30 (2H, s, 5-OCH_2), 4.10 (2H, d, *J*=9.0 Hz, H-4, -6-*c*), 4.00 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 159.0 (1C, C-6), 145.7 (1C, C-2), 139.5 (2C, Cq., Ph), 136.1 (1C, C-3), 133.3 (1C, C-5), 129.0 (2C, CH, Ph), 128.8 (4C, CH, Ph), 127.5 (4C, CH, Ph); *DOABO–CH_2O*: 97.9 (2C, C-2, -8), 73.4 (2C, C-4, -6), 73.2 (1C, C-5), 70.6 (1C, 5-OCH_2). MS (EI, 70 eV); *m*/*z* (rel int. %): (M^+-1) 408 (<1), 267 (22), 266 (100), 160 (10), 105 (28).

4.1.9. 6-[(*c*-2,*c*-8-Diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-1*H*-pyrazin-2-one (4h). White crystalline powder (as 17% conversion of **3b**, Table 1, entry 8), mp 199–201 °C (flash column chromatography, eluent ligroin/AcOEt 1:1 v/v [Found: C, 67.42; H, 5.63; N, 10.46. $C_{22}H_{21}N_3O_4$ requires: C, 67.51; H, 5.41; N, 10.74%]. R_f (50% ligroin/AcOEt) 0.60. ν_{max} (film KBr) 3062 (m), 2978 (m), 2877 (s), 2442 (s), 1822 (s), 1612 (s), 1537 (s), 1449 (s), 1376 (s), 1315 (s), 1269 (s), 1188 (s), 1135 (s), 1091 (s), 921 (s), 836 (s), 757 (s), 732 (s), 695 (s) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 7.77 (1H, s, H-5), 7.62 (1H, s, H-3), 7.52–7.50 (4H, m, Ph), 7.36–7.27 (6H, m, Ph), 7.03 (1H, br s, NH); *DOABO–CH_2O*: 5.60 (2H, s, H-2, -8-*t*), 4.17 (2H, s, 5-OCH_2), 4.08 (2H, d, *J*=9.0 Hz, H-4, -6-*c*), 3.98 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 158.1 (1C, C-2), 157.0 (1C, C-6), 139.6 (2C, Cq., Ph), 129.1 (2C, CH., Ph), 128.8 (4C, CH., Ph), 127.6 (4C, CH., Ph), 125.3 (1C, C-3), 124.2 (1C, C-5); *DOABO–CH_2*: 97.7 (2C, C-2, -8), 73.6 (2C, C-4, -6), 73.2 (1C, C-5), 70.5 (1C, 5-OCH_2); MS (EI, 70 eV); *m*/*z* (rel int. %): (M^+) 391 (<5), 285 (50), 179 (15), 174 (100), 155 (13), 128 (17).

4.1.10. 2,6-Bis[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4i). White crystalline powder (as 8% conversion of **3b**); this compound was isolated only as a non-separable mixture (38%) with **4h** (62%) during the work-up by flash column chromatography of the reaction between **3b** and **2b**-*cis* (Table 1, entry 8). δ_H (300 MHz $CDCl_3$) only distinct peaks are listed as *DOABO–CH_2O*: 5.63 (4H, s, H-2, -8-*t*), 4.15 (4H, s, 5-, 5′-OCH_2), 4.07 (4H, d, *J*=9.1 Hz, H-4, -4′, -6, -6′-*c*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 139.7 (4C, Cq., Ph), 125.0 (2C, C-3, -5); *DOABO–CH_2O*: 73.2 (4C, C-4, -4′ -6, -6′). MS (EI, 70 eV); *m*/*z* (rel int. %): (M^+) 670 (<1).

4.1.11. 6-Methoxy-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (4j). Yield 33%. White crystalline powder, mp 99–100 °C (flash column chromatography, eluent ligroin/acetone 3.5:1 v/v) [Found: C, 52.30; H, 6.09; N, 16.76. $C_{11}H_{15}N_3O_4$ requires: C, 52.17; H, 5.97; N, 16.59%]. R_f (78% ligroin/acetone) 0.76. ν_{max} (film KBr) 3076 (w), 2859 (m), 1590 (m), 1540 (s), 1414 (s), 13223 (s), 1270 (s), 1182 (s), 1034 (s), 941 (s), 843 (s), 720 (w), 679 (s), 623 (w), 489 (m), 458 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 7.78, 7.75 (2H, s, H-3, -5); *DOABO–CH_2O*: 4.50 (2H, d, *J*=5.5 Hz, H-2, -8-*c*),

4.44 (2H, d, *J*=5.5 Hz, H-2, -8-*t*), 4.33 (2H, s, 5-OCH_2), 3.90 (3H, s, –OCH_3), 3.87 (4H, s, H-4, -6-*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 159.2, 158.4 (2C, C-2, -6), 125.8, 124.9 (2C, C-3, -5); *DOABO–CH_2O*: 88.5 (2C, C-2, -8), 74.6 (2C, C-4, -6), 71.9 (1C, C-5), 69.2 (1C, 5-OCH_2), 54.0 (1C, –OCH_3). MS (EI, 70 eV); *m/z* (rel int. %): (M^+) 253 (<1), 127 (100), 97 (18).

4.1.12. 2-[(3,7-Dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-pyrimidine (6a). Yield 60%. White crystalline powder, mp 107–109 °C (pentane) [Found: C, 53.59; H, 5.61; N, 19.13. $C_{10}H_{13}N_3O_3$ requires: C, 53.80; H, 5.87; N, 18.82%]. R_f (75% ligroin/acetone) 0.40. ν_{max} (film NaCl) 2858 (w), 1569 (s), 1431 (s), 1332 (s), 1300 (m), 1137 (w), 1021 (s), 925 (m), 814 (w), 682 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.44 (2H, d, *J*=4.9 Hz, H-4, -6), 6.90 (1H, dd as t, *J*=4.9, 4.9 Hz, H-5); *DOABO–CH_2O*: 4.45 (2H, d, *J*=5.5 Hz, H-2, -8-*c*), 4.38 (2H, d, *J*=5.5 Hz, H-2, -8-*t*), 4.35 (2H, s, 5-OCH_2), 3.87 (2H, d, *J*=9.4 Hz, H-4, -6-*c*), 3.84 (2H, d, *J*=9.4 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 165.2 (1C, C-2), 159.7 (2C, C-4, -6), 115.8 (1C, C-5); *DOABO–CH_2O*: 88.5 (2C, C-2, -8), 74.7 (2C, C-4, -6), 71.7 (1C, C-5), 70.6 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^+-1) 222 (10), 206 (12), 176 (14), 148 (8), 128 (100), 109 (16), 98 (11).

4.1.13. 2,4-Bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6b). Yield 80%. White crystalline powder, mp 136–137 °C (pentane) [Found: C, 52.61; H, 6.01; N, 15.58. $C_{16}H_{22}N_4O_6$ requires: C, 52.45; H, 6.05; N, 15.29%]. R_f (75% ligroin/acetone) 0.20. ν_{max} (film NaCl) 2590 (w), 2863 (w), 1585 (s), 1449 (m), 1416 (s), 1336 (m), 1274 (m), 1181 (w), 1098 (s), 1021 (m), 928 (m), 749 (w) cm^{-1}. δ_H (500 MHz benzene-d_6) *heteroaromatic*: 8.05 (1H, d, *J*=6.0 Hz, H-6), 6.15 (1H, d, *J*=6.0 Hz, H-5); *DOABO–CH_2O linked at C-2*: 4.40 (2H, s, 5-OCH_2), 4.30 (2H, d, *J*=5.3 Hz, H-2, -8-*c*), 4.05 (2H, d, *J*=5.3 Hz, H-2, -8-*t*), 3.77 (2H, d, *J*=8.7 Hz, H-4, -6-*c*), 3.64 (2H, d, *J*=8.7 Hz, H-4, -6-*t*); *DOABO–CH_2O linked at C-4*: 4.23 (2H, d, *J*=5.3 Hz, H-2, -8-*c*), 4.21 (2H, s, 5-OCH_2), 4.04 (2H, d, *J*=5.3 Hz, H-2, -8-*t*), 3.56 (2H, d, *J*=8.9 Hz, H-4, -6-*c*), 3.51 (2H, d, *J*=8.9 Hz, H-4, -6-*t*); δ_C (125 MHz benzene-d_6) *heteroaromatic*: 171.1 (1C, C-4), 165.5 (1C, C-2), 158.9 (1C, C-6), 102.3 (1C, C-5); *DOABO–CH_2O linked at C-2*: 88.1 (2C, C-2, -8), 74.3 (2C, C-4, -6), 72.7 (1C, C-5), 70.7 (1C, 5-OCH_2); *DOABO–CH_2O linked at C-4*: 88.2 (2C, C-2, -8), 73.9 (2C, C-4, -6), 71.5 (1C, C-5), 69.2. (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): 366 (<1), 238 (6), 208 (6), 128 (68), 114 (100), 98 (14), 68 (27), 42 (32), 41 (60).

4.1.14. 2-Chloro-4-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6c). Yield 63%. White crystalline powder, mp 139–140 °C (dichloromethane/pentane 1:2 v/v) [Found: C, 46.80; H, 4.81; N, 16.65. $C_{10}H_{12}N_3O_3Cl$ requires: C, 46.61; H, 4.69; N, 16.31%]. R_f (75% ligroin/acetone) 0.50. ν_{max} (film NaCl) 2857 (w), 1636 (s), 1582 (s), 1545 (m), 1446 (m), 1327 (s), 1230 (m), 1102 (w), 1017 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.30 (1H, d, *J*=5.7 Hz, H-6), 6.67 (1H, d, *J*=5.7 Hz, H-5); *DOABO–CH_2O*: 4.47 (2H, d, *J*=5.3 Hz, H-2, -8-*c*), 4.42 (2H, d, *J*=5.3 Hz, H-2, -8-*t*), 4.40 (2H, s, 5-OCH_2), 3.81 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 170.4 (1C, C-4), 160.6 (1C, C-2), 159.5 (1C, C-6), 107.4 (1C, C-5); *DOABO–CH_2O*: 88.6 (2C, C-2, -8), 74.2 (2C, C-4, -6), 71.7 (1C, C-5), 69.9 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): 257 (<1), 212 (9), 197 (12), 169 (11), 114 (100), 86 (10), 68 (14), 58 (11), 42 (16), 41 (50).

4.1.15. 4-Chloro-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6d). Yield 23%. This compound was identified as side product in the synthesis of the compound **6c** (Table 2). Its identity was established according to NMR spectra performed on the crude reaction mixture together with the residue of the column chromatography (**6c+6d**) after isolation of the pure **6c**. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.36 (1H, d, *J*=5.3 Hz, H-6), 7.00 (1H, d, *J*=5.3 Hz, H-5); *DOABO–CH_2O*: 4.49 (2H, d, *J*=5.7 Hz, H-2, -8-*c*), 4.44 (2H, d, *J*=5.7 Hz, H-2, -8-*t*), 4.40 (2H, s, 5-OCH_2), 3.88 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 165.0 (1C, C-2), 163.0 (1C, C-4), 160.4 (1C, C-6), 115.9 (1C, C-5); *DOABO–CH_2O*: 88.6 (2C, C-2, -8), 74.6 (2C, C-4, -6), 71.6 (1C, C-5), 71.2 (1C, 5-OCH_2).

4.1.16. 4,6-Bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6e). Yield 81%. White crystalline powder, mp 146–148 °C (pentane) [Found: C, 52.70; H, 5.88; N, 14.98. $C_{16}H_{22}N_4O_6$ requires: C, 52.45; H, 6.05; N, 15.29%]. R_f (75% ligroin/acetone) 0.35. ν_{max} (film NaCl) 2950 (w), 2858 (m), 1593 (s), 1563 (s), 1457 (m), 1421 (m), 1341 (m), 1195 (m), 1137 (m), 1095 (m), 1039 (s), 933 (m), 674 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.38 (1H, s, H-2), 6.08 (1H, s, H-5); *DOABO–CH_2O*: 4.49 (4H, d, *J*=5.7 Hz, H-2, -2′, -8, -8′-*c*), 4.44 (4H, d, *J*=5.7 Hz, H-2, -2′, -8, -8′-*t*), 4.38 (4H, s, 5-, 5′-OCH_2), 3.84 (8H, s, H-4, -4′, -6, -6′, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 171.0 (2C, C-4, -6), 157.8 (1C, C-2), 91.4 (1C, C-5); *DOABO–CH_2O*: 88.6 (4C, C-2, -2′, -8, -8′), 74.4 (4C, C-4, -4′, -6, -6′), 71.9 (2C, C-5, -5′), 69.4 (2C, 5-, 5′-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^++1) 367 (<1), 274 (3), 252 (2), 168 (8), 128 (100), 98 (4).

4.1.17. 4-Chloro-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6f). Yield 63%. White crystalline powder, mp 118–119 °C (flash column chromatography, eluent ligroin/acetone 3:1 v/v) [Found: C, 46.33; H, 5.02; N, 16.59. $C_{10}H_{12}N_3O_3Cl$ requires: C, 46.61; H, 4.69; N, 16.31%]. R_f (75% ligroin/acetone) 0.60. ν_{max} (film NaCl) 2956 (w), 2884 (s), 1574 (s), 1546 (s), 1454 (s), 1387 (w), 1343 (s), 1314 (m), 1264 (w), 1213 (w), 1140 (m), 1094 (s), 1040 (s), 1007 (s), 981 (m), 868 (w), 749 (s), 678 (w). δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 8.50 (1H, s, H-2), 6.74 (1H, d, *J*=0.8 Hz, H-5); *DOABO–CH_2O*: 4.44 (2H, d, *J*=5.7 Hz, H-2, -8-*c*), 4.38 (2H, d, *J*=5.7 Hz, H-2, -8-*t*), 4.38 (2H, s, 5-OCH_2), 3.78 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 170.2 (1C, C-6), 161.3 (1C, C-4), 158.5 (1C, C-2), 108.2 (1C, C-5); *DOABO–CH_2O*: 88.5 (2C, C-2, -8), 74.2 (2C, C-4, -6), 71.7 (1C, C-5), 69.8 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^+-1) 256 (2), 240 (8), 210 (7), 128 (100), 98 (7).

4.1.18. 2,4,6-Tris[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6g). Yield 58%. Yellowish crystalline powder, mp 188–189 °C (pentane/dichloromethane, 2:1 v/v) [Found: C, 51.53; H, 6.45; N, 14.11. $C_{22}H_{31}N_5O_9$

requires: C, 51.86; H, 6.13; N, 13.75%]. R_f (50% ligroin/acetone) 0.50. ν_{max} (film NaCl) 2857 (s), 1600 (s), 1405 (m), 1382 (s), 1325 (m), 1192 (w), 1095 (w), 923 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 5.74 (1H, s, H-5); *DOABO–CH_2O linked at C-2*: 4.50 (2H, d, *J*=5.5 Hz, H-2, -8-*c*), 4.42 (2H, d, *J*=5.5 Hz, H-2, -8-*t*), 4.325 (2H, s, 5-OCH_2), 3.88 (4H, s, H-4, -6, -*c*, -*t*); *DOABO–CH_2O linked at C-4, -6*: 4.48 (4H, d, *J*=5.5 Hz, H-2, -2′, -8, -8′-*c*), 4.42 (4H, d, *J*=5.3 Hz, H-2, -2′, -8, -8′-*t*), 4.331 (4H, s, 5-, 5′-OCH_2), 3.82 (8H, s, H-4, -4′, -6, -6′, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 172.4 (2C, C-4, -6), 164.3 (1C, C-2), 84.9 (1C, C-5); *DOABO–CH_2O linked at C-2*: 88.3 (2C, C-2, -8), 74.7 (2C, C-4, -6), 71.6 (1C, 5-OCH_2), 70.8 (1C, C-5); *DOABO–CH_2O linked at C-4, -6*: 88.6 (4C, C-2, -2′, -8, -8′), 74.4 (4C, C-4, -4′, -6, -6′), 71.8 (2C, 5-, 5′-OCH_2), 69.4 (2C, C-5, -5′). MS (EI, 70 eV); *m/z* (rel int. %): 510 (8), 297 (<1), 256 (<1), 197 (4), 158 (4), 128 (100), 98 (4).

4.1.19. 4-Chloro-2,6-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]-oct-*c*-5-yl)methoxy]-pyrimidine (6h). Yield 76%. White crystalline powder, mp 142–144 °C (flash column chromatography, eluent ligroin/acetone 2:1 v/v) [Found: C, 48.31; H, 4.99; N, 14.19. $C_{16}H_{21}N_4O_6Cl$ requires: C, 47.95; H, 5.28; N, 13.98%]. R_f (66% ligroin/acetone) 0.45. ν_{max} (film NaCl) 2852 (s), 1635 (w), 1577 (s), 1416 (m), 1325 (m), 1137 (m), 1093 (m), 1023 (m), 917 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 6.43 (1H, s, H-5); *DOABO–CH_2O linked at C-2*: 4.49 (2H, d, *J*=5.5 Hz, H-2, -8-*c*), 4.42 (2H, d, *J*=5.5 Hz, H-2, -8-*t*), 4.38 (2H, s, 5-OCH_2), 3.87 (4H, s, H-4, -6, -*c*, -*t*); *DOABO–CH_2O linked at C-6*: 4.48 (2H, d, *J*=5.5 Hz, H-2, -8-*c*), 4.41 (2H, d, *J*=5.5 Hz, H-2, -8-*t*), 4.35 (2H, s, 5-OCH_2), 3.81 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 171.9 (1C, C-6), 164.4 (1C, C-2), 162.4 (1C, C-4), 101.6 (1C, C-5); *DOABO–CH_2O*: 88.5 and 88.4 (4C, C-2, -8), 74.5, 74.2 (4C, C-4, -6), 71.7, 71.6 (2C, C-5), 71.2, 70.1 (2C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^+-1) 400 (5), 365 (5), 128 (100), 98 (7).

4.1.20. 2-Chloro-4,6-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]-oct-*c*-5-yl)methoxy]-pyrimidine (6i). Yield 8%. This compound was identified as side product in the synthesis of the compound **6h** (Table 2). Its identity was established according to NMR spectra performed on the crude reaction mixture together with the residue of the column chromatography (**6i+6h**) after isolation of the pure **6h**. δ_H (300 MHz $CDCl_3$ *only distinct peaks are listed*) *heteroaromatic*: 5.96 (1H, s, H-5); *DOABO–CH_2O*: 4.43 (2H, d, *J*=5.3 Hz, H-2, -2′, -8, -8′, -*c*, -*t*), 4.33 (4H, s, 5-, 5′-OCH_2), 3.77 (4H, s, H-4, -4′, -6, -6′, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 171.8.0 (2C, C-4, -6); *DOABO–CH_2*: 88.6 (4C, C-2, -2′, -8, -8′), 71.6 (2C, C-5, -5′).

4.1.21. 2,4-Dichloro-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]-oct-*c*-5-yl)methoxy]-pyrimidine (6j). White crystalline powder (6% side product in the synthesis of **6h**), mp 117–119 °C (flash column chromatography, eluent ligroin/acetone 2:1 v/v) [Found: C, 40.89; H, 4.15; N, 14.58. $C_{10}H_{11}N_3O_3Cl_2$ requires: C, 41.12; H, 3.80; N, 14.39%]. R_f (66% ligroin/acetone) 0.70. ν_{max} (film NaCl) 2857 (w), 1579 (s), 1528 (s), 1423 (w), 1367 (m), 1272 (m), 1119 (m), 1020 (s), 909 (w), 824 (m), 754 (w), 672 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 6.72 (1H, s, H-5); *DOABO–CH_2O*: 4.48 (2H, d, *J*=5.5 Hz, H-2, -8-*c*), 4.43 (2H, d, *J*=5.5 Hz, H-2, -8-*t*), 4.43 (2H, s, 5-OCH_2), 3.82 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 171.1 (1C, C-6), 162.1 (1C, C-2), 160.0 (1C, C-4), 106.7 (1C, C-5); *DOABO–CH_2O*: 88.6 (2C, C-2, -8), 74.2 (2C, C-4, -6), 71.7 (1C, C-5), 70.6 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): 292 (3), 128 (100), 98 (10).

4.1.22. 2,4-Bis[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6k). Yield 58%. White crystalline powder, mp 168–170 °C (pentane) [Found: C, 71.52; H, 5.94; N, 8.07. $C_{40}H_{38}N_4O_6$ requires C, 71.63; H, 5.71; N, 8.35%]. R_f (75% ligroin/AcOEt) 0.30. ν_{max} (film KBr) 2874 (m), 1954 (w), 1591 (s), 1571 (s), 1450 (m), 1414 (m), 1331 (m), 1210 (m), 1098 (s), 1011 (m), 927 (m), 818 (s), 698 (s), 643 (w), 522 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 8.08 (1H, d, *J*=5.8 Hz, H-6), 7.55–7.50 (8H, m, Ph), 7.36–7.28 (12H, m, Ph), 6.15 (1H, d, *J*=5.8 Hz, H-5); *DOABO–CH_2O linked at C-2*: 5.61 (2H, s, H-2, -8-*t*), 4.26 (2H, s, 5-OCH_2), 4.14 (2H, d, *J*=9.2 Hz, H-4, -6-*c*), 4.02 (2H, d, *J*=9.2 Hz, H-4, -6-*t*); *DOABO–CH_2O linked at C-4*: 5.60 (2H, s, H-2, -8-*t*), 4.23 (2H, s, 5-OCH_2), 4.04 (2H, d, *J*=9.0 Hz, H-4, -6-*c*), 3.95 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 171.0 (1C, C-4), 164.9 (1C, C-2), 158.9 (1C, C-6), 139.7, 139.6 (4C, Cq., Ph), 129.0, 128.9 (4C, CH, Ph), 128.8, 128.7 (8C, CH, Ph), 127.6, 127.5 (8C, CH, Ph), 102.6 (1C, C-5); *DOABO–CH_2O*: 97.9, 97.6 (4C, C-2, -8), 73.9, 73.5 (4C, C-4, -6), 73.10, 73.07 (2C, C-5), 71.8, 70.2 (2C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): 708 (20), 692 (100), 670 (10), 564 (5), 451 (6), 435 (22), 413 (10), 348 (5).

4.1.23. 4,6-Bis[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6l). Yield 31%. White crystalline powder, mp 176–178 °C (flash column chromatography, eluent ligroin/AcOEt 3:1 v/v) [Found: C, 71.53; H, 5.93; N, 8.07. $C_{40}H_{38}N_4O_6$ requires C, 71.63; H, 5.71; N, 8.35%]. R_f (75% ligroin/AcOEt) 0.59. ν_{max} (film KBr) 2876 (m), 1595 (s), 1455 (s), 1430 (m), 1314 (w), 1256 (s), 1166 (m), 1089 (m), 989 (w), 921 (m), 838 (s), 752 (m), 694 (m), 470 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 8.30 (1H, s, H-2), 7.52–7.49 (8H, m, Ph), 7.38–7.29 (12H, m, Ph), 5.71 (1H, s, H-5); *DOABO–CH_2O*: 5.59 (4H, s, H-2, -2′, -8, -8′-*t*), 4.23 (4H, s, 5-, 5′-OCH_2), 4.05 (4H, d, *J*=9.0 Hz, H-4, -4′, -6, -6′-*c*), 3.95 (4H, d, *J*=9.0 Hz, H-4, -4′, -6, -6′-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 170.8 (2C, C-4, -4′ -6, -6′), 157.8 (1C, C-2), 139.6 (4C, Cq., Ph), 129.0 (4C, CH, Ph), 128.8 (8C, CH, Ph), 127.6 (8C, CH, Ph); *DOABO–CH_2O*: 97.8 (4C, C-2, -2′, -8, -8′), 73.6 (4C, C-4, -4′, -6, -6′), 73.2 (2C, C-5, -5′), 70.7 (2C, 5-, 5′-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^+) 670 (31), 692 (14), 564 (9), 280 (100).

4.1.24. 4-Chloro-6-[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (6m). Yield 23%. Yellowish crystalline powder, mp 145–147 °C (flash column chromatography, eluent ligroin/AcOEt 3:1 v/v) [Found: C, 64.32; H, 5.14; N, 10.19. $C_{22}H_{20}N_3O_3Cl$ requires C, 64.47; H, 4.92; N, 10.25%]. R_f (75% ligroin/AcOEt) 0.80. ν_{max} (film KBr) 3091 (m), 2874 (m), 1573 (s), 1454 (s), 1334 (m), 1258 (m), 1213 (m), 1088 (s), 1009 (s), 931 (w), 871

(w), 804 (m), 753 (s), 696 (s), 535 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *(hetero)aromatic*: 8.50 (1H, s, H-2), 7.51–7.48 (4H, m, Ph), 7.37–7.28 (6H, m, Ph), 6.53 (1H, s, H-5); *DOABO–CH_2O*: 5.60 (2H, s, H-2, -8-*t*), 4.33 (2H, s, 5-OCH_2), 4.06 (2H, d, *J*=9.0 Hz, H-4, -6-*c*), 3.96 (2H, d, *J*=9.0 Hz, H-4, -6-*t*); δ_C (75 MHz $CDCl_3$) *(hetero)aromatic*: 170.0 (1C, C-6), 161.3 (1C, C-4), 158.5 (1C, C-2), 139.5 (2C, Cq., Ph), 129.1 (2C, CH, Ph), 128.8 (4C, CH, Ph), 127.5 (4C, CH, Ph), 108.2 (1C, C-5); *DOABO–CH_2O*: 97.8 (2C, C-2, -8), 73.4 (2C, C-4, -6), 73.1 (1C, C-5), 70.8 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): (M^++1) 410 (4), 386 (<1), 304 (100), 280 (42), 174 (98), 156 (23), 129 (11), 91 (18).

4.1.25. 3-Chloro-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (8a). Yield 86%. White crystalline powder, mp 130–132 °C (pentane) [Found: C, 46.33; H, 5.03; N, 16.13. $C_{10}H_{12}N_3O_3Cl$ requires: C, 46.61; H, 4.66; N, 16.31%]. R_f (75% ligroin/acetone) 0.50. ν_{max} (film NaCl) 2852 (w), 2082 (w), 1643 (s), 1441 (m), 1310 (w), 1101 (m), 1044 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 7.37 (1H, d, *J*=9.0 Hz, H-4), 6.97 (1H, d, *J*=9.0 Hz, H-5); *DOABO–CH_2O*: 4.54 (2H, s, 5-OCH_2), 4.50 (2H, d, *J*=5.7 Hz, H-2, -8-*c*), 4.45 (2H, d, *J*=5.7 Hz, H-2, -8-*t*), 3.86 (4H, s, H-4, -6, -*c*, -*t*). δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 164.4 (1C, C-6), 152.0 (1C, C-3), 131.4 (1C, C-4), 120.4 (1C, C-5); *DOABO–CH_2O*: 88.7 (2C, C-2, -8), 74.3 (2C, C-4, -6), 71.9 (1C, C-5), 70.4 (1C, 5-OCH_2). MS (EI, 70 eV); *m/z* (rel int. %): 257 (<1), 212 (13), 199 (8), 169 (15), 127 (15), 114 (100), 97 (19), 68 (26), 58 (13), 42 (30), 41 (76).

4.1.26. 6-Methoxy-3-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (8b). Yield 51%. White crystalline powder, mp 117–119 °C (dichloromethane/pentane 1:2 v/v) [Found: C, 51.89; H, 6.25; N, 16.91. $C_{11}H_{15}N_3O_4$ requires: C, 52.17; H, 5.97; N, 16.59%]. R_f (75% ligroin/acetone) 0.30. ν_{max} (film NaCl) 2360 (w), 1630 (s), 1476 (m), 1384 (s), 1268 (m), 1036 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 6.92 (2H, s, H-4, -5); *DOABO–CH_2O*: 4.51 (2H, d, *J*=5.7 Hz, H-2, -8-*c*), 4.48 (2H, s, 5-OCH_2), 4.46 (2H, d, *J*=5.7 Hz, H-2, -8-*t*); 4.01 (3H, s, OCH_3), 3.86 (4H, s, H-4, -6, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 162.6, 161.9 (2C, C-3, -6), 122.0, 121.6 (2C, C-4, -5); *DOABO–CH_2O*: 88.8 (2C, C-2, -8), 74.5 (2C, C-4, -6), 72.0 (1C, C-5), 69.7 (1C, 5-OCH_2), 55.0 (1C, OCH_3). MS (EI, 70 eV); *m/z* (rel int. %): 253 (<1), 223 (9), 208 (20), 195 (18), 165 (25), 140 (13), 139 (16), 128 (20), 127 (26), 114 (100), 98 (26), 97 (20), 80 (13), 68 (46), 54 (24), 42 (40), 41 (95).

4.1.27. 3,6-Bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (8c). Yield 78%. White crystalline powder, mp 195–197 °C (dichloromethane/pentane 1:2 v/v) [Found: C, 52.75; H, 5.85; N, 15.55. $C_{16}H_{22}N_4O_6$ requires: C, 52.45; H, 6.05; N, 15.29%]. R_f (75% ligroin/acetone) 0.30. ν_{max} (film NaCl) 2868 (w), 2361 (w), 1467 (m), 1446 (s), 1267 (s), 1137 (w), 1039 (s), 920 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) *heteroaromatic*: 7.24 (2H, s, H-4, -5); *DOABO–CH_2O*: 4.80 (4H, d, *J*=5.7 Hz, H-2, -2′, -8, -8′-*c*), 4.76 (4H, s, 5-, 5′-OCH_2), 4.75 (4H, d, *J*=5.7 Hz, H-2, -2′, -8, -8′-*t*), 4.16 (8H, s, H-4, -4′, -6, -6′, -*c*, -*t*); δ_C (75 MHz $CDCl_3$) *heteroaromatic*: 162.1 (2C, C-3, -6), 121.9 (2C, C-4, -5); *DOABO-CH_2O*: 88.7 (4C, C-2, -2′, -8, -8′), 74.5 (4C, C-4, -4′, -6, -6′), 71.9 (2C, 5-, 5′-OCH_2), 69.8 (2C, C-5, 5′). MS (EI, 70 eV); *m/z* (rel int. %): (M^++Na) 389 (14), (M^+−1) 365 (4), 168 (4), 128 (100), 98 (5).

4.2. Preparation of compound 9a

To a suspension in THF (50 mL) of **2a** (prepared from **1a**, 1.450 g, 10.0 mmol and potassium hydride 1.337 g as 30% KH in mineral oil suspension, 0.401 g 100%, 10.0 mmol, Scheme 2), cyanuryl chloride (0.571 g, 3.1 mmol) was added as THF (20 mL) solution. The reaction mixture was heated at 65 °C for 36 h. with vigorous stirring, until the starting **1a** was absent (TLC monitoring, eluent ligroin/acetone 2:1 v/v). The reaction was quenched with isopropanol (1 mL) with stirring for additional 30 min. The mineral compounds were filtered off and washed with excess of THF. The combined THF solution was evaporated under vacuum to dryness to provide the crude product as yellow oil. Purification by flash column chromatography (eluent ligroin/acetone 2:1 v/v visualisation in I_2-bath) afforded the desired **9a** as a yellowish crystalline powder: 0.420 g (34% yield).

4.2.1. 2-Chloro-4,6-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]-oct-*c*-5-yl)methoxy]-*s*-triazine (9a). Yield 34%. Yellowish crystalline powder, mp 91.8–93.4 °C (flash column chromatography, eluent ligroin/acetone 2:1 v/v) [Found: C, 44.91; H, 5.19; N, 17.63. $C_{15}H_{20}N_5O_6Cl$ requires: C, 44.84; H, 5.02; N, 17.43%]. R_f 0.75 (66% ligroin/acetone). ν_{max} (KBr) 2971 (m), 2868 (s), 1731 (s), 1390 (m), 1252 (s), 1138 (m), 1038 (s), 926 (s), 885 (w), 792 (m), 673 (s), 610 (s), 505 (w) cm^{-1}. δ_H (300 MHz $CDCl_3$) 4.39 (4H, s, H-2, -2′, -8, -8′-*c*), 4.37 (4H, s, H-2, -2′, -8, -8′-*t*), 4.06 (4H, s, 5-, 5′-OCH_2), 3.73 (4H, d, *J*=9.0 Hz, H-4, -4′, -6, -6′-*c*), 3.68 (4H, d, *J*=9.0 Hz, H-4, -4′, -6, -6′-*t*); δ_C (75 MHz $CDCl_3$) 171.0 (3C, C-2, -4, -6 *s*-triazine), 88.6 (4C, C-2, -2′, -8, -8′), 74.2 (4C, C-4, -4′, -6, -6′), 71.5 (2C, C-5, -5′), 66.9 (2C, 5-, 5′-OCH_2). MS (EI), *m/z* (rel int. %) (M^++1) 402 (<1), 324 (38), 256 (57), 145 (58), 127 (100).

4.3. Preparation of compound 10a

A solution of *c*-5-hydroxymethyl-3,7-dioxa-*r*-1-azabicyclo-[3.3.0]octane **1a** (0.740 g, 5.10 mmol) in THF (25 mL) was cooled at −78 °C with stirring, then *n*-BuLi (1.6 M in hexane, 3.35 mL, 5.35 mmol) was injected to provide a clear white fine suspension. After 20 min, cyanuryl chloride (0.320 g, 1.70 mmol) was injected as THF (15 mL) solution. The reaction mixture was allowed to slowly reach room temperature (20 h) with vigorous stirring then quenched with water (5 mL). The reaction mixture was evaporated to dryness, then water (50 mL) and dichloromethane (50 mL) were added with stirring. After separation, the dichloromethane solution was washed with water to neutrality and then dried over $MgSO_4$. After filtering, the organic solution was concentrated in vacuum to provide the crude product, which was taken with Et_2O to yield the compound **10a** as white crystalline powder: 0.720 g (82% yield).

4.3.1. 2,4,6-Tris[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-*s*-triazine (10a). Yield 82%. White crystalline powder, mp 238.9–239.5 °C (Et_2O) [Found: C, 49.44; H,

5.98; N, 16.44. $C_{21}H_{30}N_6O_9$ requires: C, 49.41; H, 5.92; N, 16.46%]. R_f (50% ligroin/acetone) 0.30. ν_{max} (KBr) 3444 (m), 2969 (w), 2858 (s), 1589 (s), 1414 (s), 1334 (s), 1189 (m), 1141 (m), 1096 (s), 1044 (s), 1028 (s), 943 (m), 807 (s), 750 (m), 718 (w), 676 (m), 572 (m) cm^{-1}. δ_H (300 MHz $CDCl_3$) 4.49 (6H, d, J=5.6 Hz, H-2, -2′, -2″, -8, -8′, -8″-*c*), 4.42 (6H, d, J=5.6 Hz, H-2, -2′, -2″, -8, -8′, -8″-*t*), 4.41 (6H, s, 5-, 5′-, 5″-OCH_2), 3.85 (12H, s, H-4, -4′, -4″, -6, -6′, -6″-*c*, -*t*); δ_C (75 MHz $CDCl_3$) 173.3 (3C, C-2, -4, -6 *s*-triazine), 88.5 (6C, C-2, -2′, -2″, -8, -8′, -8″), 74.3 (6C, C-4, -4′, -4″, -6, -6′, -6″), 71.5 (3C, 5-, 5′-, 5″-OCH_2), 71.3 (3C, C-5, -5′, -5″); MS (ESI), *m*/*z* (rel int. %) (M^+-1+Na^+) 532 (100), (M^+) 511 (40), 384 (10).

4.4. Preparation of compounds 9b and 10b

To a suspension in THF (50 mL) of **2b**-*cis* (prepared from **1b**-*cis*, 1.480 g, 5.0 mmol and potassium hydride 0.668 g as 30% KH in mineral oil suspension, 0.200 g 100%, 5.0 mmol, Scheme 2), cyanuryl chloride (0.302 g, 1.64 mmol) was rapidly added as THF (30 mL) solution. The reaction mixture was slowly heated at 65 °C for 40 h with vigorous stirring, until the starting **1b**-*cis* was present in traces only (TLC monitoring, eluent ligroin/acetone 3.5:1 v/v, visualisation in UV-254 nm). The reaction was quenched with water (50 mL) and dichloromethane (125 mL) with stirring for additional 30 min. After separation, the aqueous layer was extracted with dichloromethane (3×25 mL) and the combined dichloromethane solution was washed with water to neutrality. After drying on $MgSO_4$, the organic solution was evaporated under vacuum to yield 1.10 g of the crude reaction mixture. Purification by flash column chromatography (eluent ligroin/acetone 3.5:1 v/v visualisation in UV-254 nm) afforded the following fractions: 0.137 g recovered **1b**-*cis*; 0.370 g desired **10b** as a white crystalline powder. The column was then completely eluted with pure acetone to afford 0.310 g mixture **10b** (66%)+**9b** (34%), according to the ^1H NMR spectrum.

4.4.1. 2-Chloro-4,6-bis[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-triazine (9b). Yield 8%. δ_H (300 MHz $CDCl_3$) as detected from the mixture with **10b**: 5.59 (4H, s, H-2, -8-*t*), 4.31 (4H, s, 5-, 5′-OCH_2), 4.06 (4H, d, J=9.2 Hz, H-4, -4′, -6, -6′-*c*), 3.98 (4H, d, J=9.2 Hz, H-4, -4′, -6, -6′-*t*); δ_C (75 MHz $CDCl_3$), 171.8 (3C, C-2, -4, -6 *s*-triazine), 139.3 (4C, Cq., Ph), 127.5 (8C, CH, Ph). MS (FAB^+), *m*/*z* (rel int. %) (M^+-1) 704 (20).

4.4.2. 2,4,6-Tris[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-*s*-triazine (10b). Yield 37%. White crystalline powder, mp 162.5–164.2 °C (flash column chromatography, eluent ligroin/acetone 3.5:1 v/v) [Found: C, 70.61; H, 5.70; N, 8.44. $C_{57}H_{54}N_6O_9$ requires: C, 70.80; H, 5.63; N, 8.69%] R_f (78% ligroin/acetone) 0.40. ν_{max} (KBr) 3063 (w), 2871 (m), 1571 (s), 1417 (s), 1334 (s), 1210 (m), 1131 (s), 1088 (m), 1068 (m), 922 (m), 820 (w), 762 (m), 735 (s), 698 (s) cm^{-1}. δ_H (300 MHz $CDCl_3$) 7.51 (12H, m, Ph), 7.32–7.26 (18H, m, Ph), 5.59 (6H, s, H-2, -2′, -2″, -8, -8′, -8″-*t*), 4.24 (6H, s, 5-, 5′-, 5″-OCH_2), 4.06 (6H, d, J=9.2 Hz, H-4, -4′, -4″, -6, -6′ -6″-*c*), 3.98 (6H, d, J=9.2 Hz, H-4, -4′, -4″, -6, -6′, -6″-*t*); δ_C (75 MHz $CDCl_3$) 172.9 (3C, C-2, -4, -6 *s*-triazine), 139.5 (6C, Cq., Ph), 129.1 (6C, CH, Ph), 128.8 (12C, CH, Ph), 127.5 (12C, CH, Ph), 97.6 (6C, C-2, -2′, -2″, -8, -8′, -8″), 73.6 (6C, C-4, -4′, -4″, -6, -6′, -6″), 72.8 (3C, 5-, 5′-, 5″-OCH_2), 72.2 (3C, C-5, -5′, -5″); MS (FAB^+), *m*/*z* (rel int. %) (M^++1) 967.9 (100).

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References and notes

1. Senkus, M. *J. Am. Chem. Soc.* **1945**, *67*, 1515–1519.
2. Senkus, M. U.S. Patent 2,401,196; *Chem. Abstr.* **1946**, *40*, P5446. See Ref. 4 and related patents.
3. Pierce, S.; Lunsford, D. C.; Raiford, R. W., Jr.; Rush, J. L.; Riley, D. W. *J. Am. Chem. Soc.* **1951**, *73*, 2595–2596.
4. Pierce, S.; Lunsford, D. C. *J. Am. Chem. Soc.* **1951**, *73*, 2596–2598.
5. Tilford, C. H.; Van Campen, M. G., Jr.; Shelton, R. S. *J. Am. Chem. Soc.* **1947**, *69*, 2902–2906.
6. Darabantu, M.; Mager, S.; Plé, G.; Puscas, C. *Heterocycles* **1995**, *41*, 2327–2356 and the references cited therein.
7. Broadbent, H. S.; Burnham, W. S.; Sheely, R. M.; Olsen, R. K. *J. Heterocycl. Chem.* **1976**, *13*, 337–348.
8. Barbulescu, N.; Moga, S. G.; Sintamarian, A.; Cuza, O.; Vasilescu, V. *Rom. Patent* 83,939; *Chem. Abstr.* **1985**, *102*, P149252r and related patents.
9. Nouguier, R.; Crozet, M.; Vanelle, P.; Maldonado, J. *Tetrahedron Lett.* **1985**, *26*, 5523–5524.
10. Zayed, S. E. *Pak. J. Sci. Ind. Res.* **1987**, *30*, 432–438; *Chem. Abstr.* **1988**, *108*, 94446y.
11. Vanelle, P.; De Meo, M. P.; Maldonado, J.; Nouguier, R.; Crozet, M. P.; Laget, M.; Dumenil, G. *Eur. J. Med. Chem.* **1990**, *25*, 241–250.
12. Mattson, A.; Norin, T. *Synth. Commun.* **1994**, *24*, 1489–1491.
13. Bonnet, D.; Pascal, J.; Grass-Masse, H.; Melnyk, O. *Tetrahedron Lett.* **2001**, *42*, 1875–1877.
14. Japan, Jpn. Tokkyo Koho JP 08,325,147, 1996; *conf.* C. A. 1997, 126, 139880
15. Laurent, P. A.; Riehl, M.; Frazao, C. S. *Bull. Soc. Chim. Fr.* **1967**, *10*, 3868–3872.
16. Cobb, R. L. U.S. Patent 3,843,726; *Chem. Abstr.* **1975**, *82*, P861193t.
17. Cardona, C.; Gawley, R. E. *J. Org. Chem.* **2002**, *67*, 1411–1413.
18. Darabantu, M.; Plé, G.; Mager, S.; Gaina, L.; Cotora, E.; Mates, A.; Costas, L. *Tetrahedron* **1997**, *53*, 1891–1908.
19. Darabantu, M.; Plé, G.; Maiereanu, C.; Silaghi-Dumitrescu, I.; Ramondenc, Y.; Mager, S. *Tetrahedron* **2000**, *56*, 3799–3816.
20. Darabantu, M.; Maiereanu, C.; Silaghi-Dumitrescu, I.; Toupet, L.; Condamine, E.; Ramondenc, Y.; Berghian, C.; Plé, G.; Plé, N. *Eur. J. Org. Chem.* **2004**, *12*, 2644–2661.
21. Eliel, E. L.; Wilen, H. S. *Stereochemistry of the Organic Compounds*; Wiley: New York, NY, 1994; pp 221–239, 488–507, 1017, 1199.
22. Cookson, R. C.; Crabb, T. A. *Tetrahedron* **1968**, *24*, 2385–2397.
23. Crabb, T. A.; Hall, M. J.; Williams, R. O. *Tetrahedron* **1973**, *29*, 3389–3398.

24. Brush, J. R.; Magee, R. J.; O'Connor, M. J.; Teo, S. B.; Geue, R. J.; Snow, M. R. *J. Am. Chem. Soc.* **1973**, 2034–2035.
25. Monge, S.; Sélambaron, J.; Carré, F.; Verducci, J.; Roque, J. P.; Pavia, A. A. *Carbohydr. Res.* **2000**, *328*, 127–133.
26. Darabantu, M.; Lequeux, T.; Pommelet, J. C.; Plé, N.; Turck, A.; Toupet, L. *Tetrahedron Lett.* **2000**, *41*, 6763–6767.
27. Darabantu, M.; Lequeux, T.; Pommelet, J. C.; Plé, N.; Turck, A. *Tetrahedron* **2001**, *57*, 739–750.
28. Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246.
29. Hurd, R. E. *J. Magn. Reson.* **1990**, *87*, 422–425.
30. Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301–315.
31. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565–569.
32. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
33. Willker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287–292.
34. Bothner-By, A. A.; Stephens, R. L.; Lee, J. M.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.
35. Bax, A. A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207–213.
36. Jeener, J.; Meier, B. H.; Bachman, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4553.
37. Parella, T.; Sanchez-Ferrando, F.; Virgili, A. *J. Magn. Reson.* **1997**, *125*, 145–148.
38. Dudley, J. R.; Thuyrston, J. T.; Schaefer, F. C.; Holm-Hansen, D.; Hull, C. J.; Adams, P. *J. Am. Chem. Soc.* **1951**, *73*, 2986–2990.
39. Weber, A. J. M.; Huysmans, W. G. B.; Mijs, W. J.; Bovee, W. M. M. J.; Smidt, J.; Vriend, J. *Recl. Trav. Chim. Pays-Bas* **1978**, *97*, 107–109.
40. Menicagli, R.; Malanga, C.; Peluso, P. *Synth. Commun.* **1994**, *24*, 2153–2158.
41. Cronin, J. S.; Ginah, F. O.; Murray, R. A.; Copp, D. J. *Synth. Commun.* **1996**, *26*, 3491–3494.
42. Friebolin, H. *Basic One- and Two Dimensional NMR Spectroscopy*; VCH Verlagsgesellschaft/VCH: Weinheim/New York, NY, 1991; p 271.
43. Riedell, F. G. *Cyclic Organonitrogen Stereodynamics*; Lambert, J. B., Takeuchi, Y., Eds.; VCH: New York, NY, 1992; p 159.
44. Sélambarom, J.; Monge, S.; Carré, F.; Roque, P. J.; Pavia, A. A. *Tetrahedron* **2002**, *58*, 9559–9566.
45. Sélambarom, J.; Carré, F.; Fruchier, A.; Roque, P. J.; Pavia, A. A. *Tetrahedron* **2002**, *58*, 4439–4444.
46. Emsley, J. *Die Elemente*; Walter de Gruyter: Berlin, New York, NY, 1994; pp 166, 178, 206.

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α-(3,7-Dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-diazines. Part 2: Functionalisation via directed *ortho*-metallation and cross-coupling reactions

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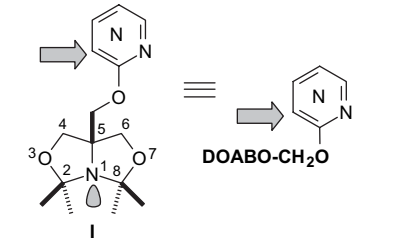
Abstract—The functionalisation of the title compounds via regioselective Directed *ortho*-Metallation (DoM) and cross-coupling reactions is studied. The compatibility of the 3,7-DiOxa-*r*-1-AzaBicyclo[3.3.0]Oct-*c*-5-ylmethoxy system (DOABO–CH_2O) to typical reaction conditions is established. Its role as Directed *ortho*-Metallation Group (DoMG) is examined, including competition with classical DoMGs, chlorine and methoxy. The chelating ability of some functionalised terms such as DOABO–CH_2O substituting chiral diarylmethanols and polyaza analogues of 2,6-terpyridine is discussed as intramolecular steric relationships determining configuration and aptitude to bind selectively transition metals, respectively.

1. Introduction

Advances in the field of Directed *ortho*-Metallation with organolithium reagents of azines and diazines have been recently reviewed.[1,2] This methodology, the so-called DoM reaction, allows access to functionalised π-deficient systems in clean, rapid, selective and high yielding transformations. In this context, of crucial relevance are the Directed *ortho*-Metallation Groups (DoMGs) whose increasing diversity makes the method overall attractive.

Few examples are known in which the DoMG is a heterocyclic saturated system: 1,3-diox-2-yl (in pyrazine and pyridine series),[3,4] 1,3-dioxol-2-yl,[5] pyrrolidin-1-yl[6] and piperidin-1-yl[7] (in pyridine series). However, their role appeared to us as protecting groups of the carbonyl and amino functionality linked *ortho* to the reaction site rather than connected to a peculiar stereochemistry of the DoMGs of this type.

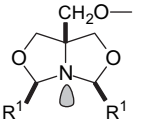
Hence, the objective of the present report is based on our previous acquired knowledge about the synthesis and stereochemistry of a new series of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-diazines **I** (Scheme 1),[8–11] aiming at their further functionalisation via DoM reactions.



I

3,7-**DiO**xa-*r*-1-**AzaB**icyclo[3.3.0]**O**ct-*c*-5-ylmethoxy

Abbreviations:



R¹ = H: (2H)DOABO-CH_2O
R¹ = Ph: (2Ph)DOABO-CH_2O

Scheme 1.

We have recently reported the synthesis by double cross-coupling under Stille conditions[12,13] of a new class of polyaza analogues of 2,6-terpyridine possessing the

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2,6-disubstituted pyrazine as a central unit linked to various heteroaromatic systems as subunits.[14] Our ongoing efforts to prepare new aza polydentate architectures prompted us, as another objective, to test the 3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy fragments α-substituting the building-blocks, pyrazines, pyrimidines and pyridazines.

No such chemistry assisted by the DOABO heterocyclic system has been reported so far.

2. Results and discussion

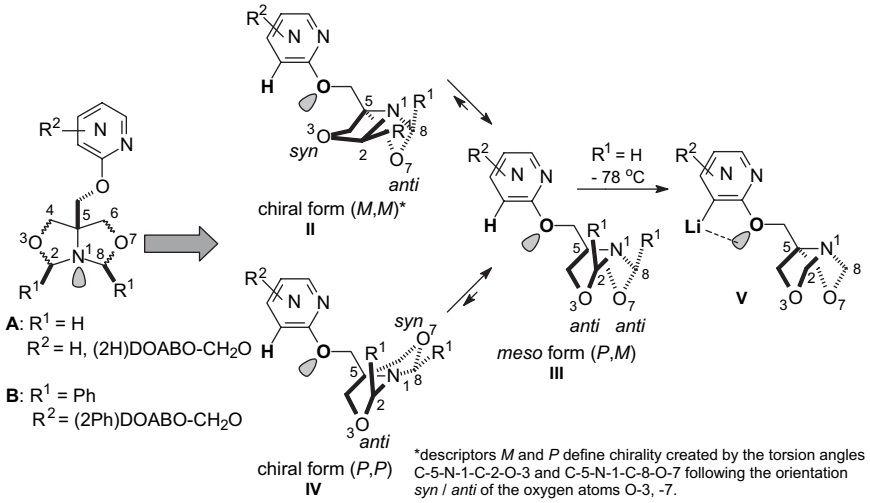
2.1. Functionalisation via directed *ortho*-metallation of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-diazines

Our study started from the stereochemistry, supported by ^{1}H DNMR performed in [D_8]THF and X-ray diffractometry,[8] of the DOABO group attached at the α-position of a diazine by a methoxy like unit, e.g., compounds of type **A** and **B** (Scheme 2).

With these premises, we investigated the metallation in three series, pyrazine, pyrimidine and pyridazine, bearing the DOABO–CH_2O group at the α-position. Whenever available, a comparison with the behaviour in DoM conditions of the methoxy group α-substituting diazines was made.

2.1.1. Functionalisation of α-(3,7-dioxa-*r*-1-azabicyclo-[3.3.0]oct-*c*-5-ylmethoxy)-pyrazines. The testing experiments were carried out on compound **1a** (Scheme 3, Table 1).

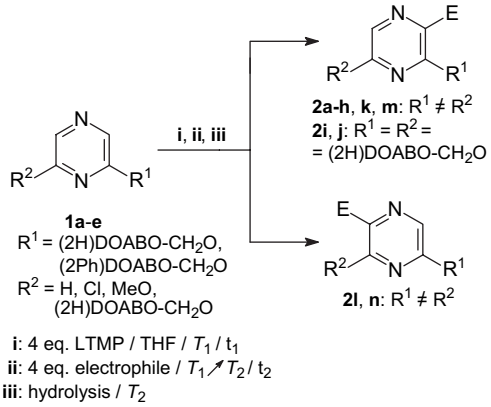
Treatment of **1a** with 1.1 equiv of LTMP (lithium-2,2,6,6,-tetramethylpiperidide) at −78 °C for 60 min as for 2-methoxypyrazine[17,18] followed by quenching with 20% DCl/D_2O (at −78 °C) afforded the starting material in 99% yield. Deuteriation was 84% at C-3′ if 2.1 equiv of LTMP were used. The best result, 98% deuterium incorporation in the crude product, was obtained with 4 equiv of LTMP (compound **2a**, Table 1). This excess could be explained by the high chelating ability at −78 °C of the frozen *meso* form (*P*,*M*) conformation of the (2H)DOABO–CH_2O group of **1a** (R^1=R^2=H, **III**→**V**, Scheme 2).



Scheme 2.

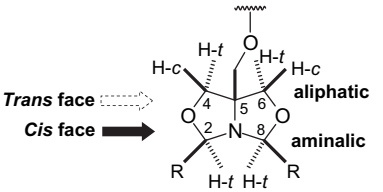
As seen previously,[8] the heterofacial cis fused double oxazolidine part of the compounds **A** (R^1=H) was flipping at room temperature (conformers **II**–**III**–**IV**) in an overall enantiomeric inversion. It became rigid on a large domain of low temperatures (273–173 K) with the potential chelating sites O-3, N-1 and O-7 orientated as a frozen nonchiral conformation **III**. In contrast, the *all-cis* C-2, C-5 and C-8 trisubstituted DOABO analogue **B** (R^1=Ph) was flipping still at 173 K. By lowering the temperature, the common conformational feature for structures **A** and **B** was the progressive orientation of the *c*-5-diazinyloxymethyl moiety in a near coplanar *s-trans out* arrangement, bisecting the DOABO skeleton. Consequently, coplanarity also involved the *ortho* diazine proton and the lone pair of the oxygen atom in the CH_2O linkage, as also proved by the crystallographically-determined structures of type **A** and **B**.[8] So, the CH_2O connection could coordinate the lithium atom to the lone pair of its oxygen atom to bring the base into close vicinity of the *ortho* diazine hydrogen and to provoke its removal ('Complex Induced Proximity Effect', CIPE,[15,16] e.g., conformer **V**, Scheme 2).

Keeping in mind these experimental conditions, the lithiated compound **1a**, upon treatment with various electrophiles, afforded the products **2b**–**g** (Table 1, entry 1) with satisfactory



Scheme 3.

Table 1. Results of the functionalisation via directed *ortho*-metallation of achiral α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-pyrazines. Preparation of compounds **2a–n**



R = H-*c*: (2H)DOABO-CH_2O
R = Ph: (2Ph)DOABO-CH_2O

Entry	Starting material	R^1 / R^2	E	Products: Compd (%)	Products: Compd (%)	Conditions: t_1 (h)	T_1 (°C)	T_2 (°C)	t_2 (h)
1	**1a**	(2H)DOABO–CH_2O / H	D[a]	**2a** (98)[b]	—	1	−78	−78	—
			Ph–CH–OH	**2b** (79)	—	1	−78	rt	12
			Me–CH–OH	**2c** (41)	—	1	−78	−78	4.5
			Et[c]	**2d** (80)	—	1	−78	−78	1.5
			I	**2e** (68)	—	1	−78	−78	2
			Sn(*n*-Bu)$_3$[d]	**2f** (73)	—	1	−78	−78	2
			SPh[d]	**2g** (73)	—	1	−78	−78	2
2	**1b**	(2Ph)DOABO–CH_2O / H	Ph–CH–OH	**2h** (98)	—	1	−78	rt	12
3	**1c**	(2H)DOABO–CH_2O	D[a]	**2i** (71)[e]		0.5	0	—	—
		(2H)DOABO–CH_2O	Ph–CH–OH	**2j** (61)		2	−78	−50	10
4	**1d**	(2H)DOABO–CH_2O / Cl	Ph–CH–OH	**2k** (52) [67][f]	**2l** (26) [33]	1	−78	rt	12
5	**1e**	(2H)DOABO–CH_2O / MeO	Ph–CH–OH	**2m** (29) [41]	**2n** (41) [59]	1	−78	rt	12

[a] DCl 20% g/g (8 equiv) in D_2O solution were used.
[b] In round brackets, yields as isolated compounds after flash column chromatography; for **2a**, as deuterium incorporation in quantitatively isolated crude product (^{1}H NMR monitoring); for **2k–n**, as partial yields.
[c] MeI as electrophile; less than 10% of the intermediate methyl derivative was detected in the crude reaction mixture (^{1}H NMR monitoring).
[d] ClSn(*n*-Bu)$_3$ (for **2f**) and Ph_2S_2 (for **2g**) as electrophiles.
[e] Incorporation (50%) of deuterium with respect to the starting **1c**.
[f] In square brackets: contents according to the ^{1}H NMR spectra of the crude reaction mixtures.

to good yields and complete *ortho* regioselectivity indicating that the (2H)DOABO–CH_2O moiety acted as an effective DoMG.

The flipping (2Ph)DOABO–CH_2O fragment (Scheme 2) was also an authentic DoMG, as proved by the excellent result in the synthesis of compound **2h** (Table 1, entry 2).

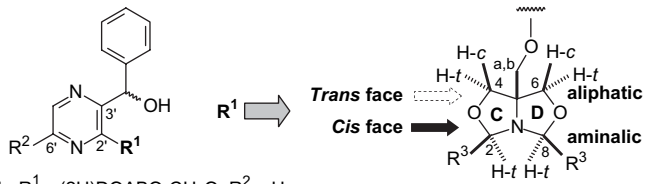
Products **2b**, **2c**, **2h**, **2j**, **2k** and **2m** have on *ortho* vicinity of a stereogenic centre versus the heterofacial DOABO skeleton. The ^{1}H NMR spectrum of **2b** showed the expected diastereotopic positions of the bicycle: C-2 versus C-8 and C-4 versus C-6. However, in the ^{1}H NMR spectrum of **2c** these relevant positions appeared almost undifferentiated. For this reason, we considered it of interest to continue our study by using mainly benzaldehyde as the electrophile and the structure of the chiral diarylmethanols of type **2b** closer to the DOABO–CH_2O group intimate stereochemistry.[8]

The deuteriation of compound **1c**, possessing twice the (2H)DOABO–CH_2O environment (Table 1, entry 3) yielded **2i** as product, but fast decomposition of the reaction mixture was observed. Consequently, no reaction of the metallated compound **1c** occurred at 0 °C with benzaldehyde. In turn, at −78 °C, in contrast with the result observed with 2,6-dimethoxypyrazine,[19,20] the reaction gave the compound **2j** in a satisfactory yield. Taking into account that 4 equiv of LTMP were necessary (vs 2.2 equiv LTMP reported for 2,6-dimethoxypyrazine), we considered that these conditions are also associated with the increased chelating ability of the double frozen *meso* form (*P,M*) of **1c** (R^1=H, R^2=(2H)DOABO–CH_2O, **III**→**V**, Scheme 2).

Compound **2j** was investigated by high resolution NMR in order to discriminate the two regioisomeric *ortho*-(C-2′) and *para*-(C-6′) (2H)DOABO–CH_2O groups with respect to the C-3′-α-hydroxybenzyl chiral centre. Individual ^{1}H–^{13}C heteronuclear correlations (HSQC[21,22] and HMBC[23,24] experiments) and the assignment of the homofacial bicyclic protons as -*c*(*cis*) or -*t*(*trans*) with respect to the lone pair at N-1 in each (2H)DOABO environment (2D ^{1}H–^{1}H NOESY[25,26] experiments) were established. The results are collected in Table 2 together with those supporting two other terms of the series, compounds **2b** and **2h**.

Only the *ortho*-linked DOABO–CH_2O groups exhibited significant ^{1}H magnetic nonequivalence as diastereotopicity (Δδ values) between the homofacial aminalic (or aliphatic)

Table 2. Discriminating ^{1}H NMR assignments in the case of compounds **2b**, **2h** and **2j**



2b: R^1 = (2H)DOABO-CH_2O, R^2 = H
2h: R^1 = (2Ph)DOABO-CH_2O, R^2 = H
2j: R^1 = R^2 = (2H)DOABO-CH_2O

R^3 = H-*c*: (2H)DOABO-CH_2O
R^3 = Ph: (2Ph)DOABO-CH_2O

Compd	Position of DOABO–CH_2O linkage	Solvent	δ (ppm)					Diastereotopicity as $\Delta\delta$ values (ppm) between labelled positions	
			Positions						
			Aminalic protons		Aliphatic protons		5-CH_aH_bO		
			H-2-*c*	H-8-*c*	H-4-*c*	H-6-*c*	H-a	On *cis* face	
			H-2-*t*	H-8-*t*	H-4-*t*	H-6-*t*	H-b	(2)–(8)	(4)–(6)
			$\Delta\delta$ (*c*-*t*)	$\Delta\delta$ (*c*-*t*)	$\Delta\delta$ (*c*-*t*)	$\Delta\delta$ (*c*-*t*)	$\Delta\delta$ (a–b)	On *trans* face	
			Ring	Ring	Ring	Ring		(2)–(8)	(4)–(6)
2b	C-2′	$CDCl_3$	4.36	4.29	3.64	3.28	4.26[a]	+0.07	**+0.36**
			4.31	4.25	3.64	3.38	4.14[a]	+0.06	+0.26
			+0.05	+0.04	0.00	−0.10	**0.12**		
			C	**D**	**C**	**D**			
2h	C-2′	$CDCl_3$	—	—	3.91	3.46	4.17	—	**+0.45**
			5.50	5.41	3.81	3.32	4.00	+0.09	+0.49
			—	—	+0.10	+0.14	**0.17**		
			C	**D**	**C**	**D**			
2j	C-2′	$CDCl_3$	4.39	4.34	3.69	3.33	4.23	+0.05	**+0.36**
			4.35	4.30	3.69	3.48	4.15	+0.05	+0.21
			+0.04	+0.04	0.00	−0.15	**0.08**		
			C	**D**	**C**	**D**			
		[D_6]benzene	4.23	4.16	3.52	3.28	3.99	+0.07	**+0.24**
			4.02	3.98	3.52	3.38	3.89	+0.04	+0.14
			+0.21	+0.18	0.00	−0.10	**0.10**		
			C	**D**	**C**	**D**			
	C-6′	$CDCl_3$	4.46	4.45	3.83	3.82	4.27	+0.01	+0.01
			4.40	4.39	3.83	3.82	4.23	+0.01	+0.01
			+0.06	+0.06	0.00	0.00	0.04		
		[D_6]benzene	4.28	4.27	3.67	3.65	4.08	+0.01	+0.02
			4.05	4.05	3.59	3.58	3.98	0.00	+0.01
			+0.23	+0.22	+0.08	+0.07	0.10		

[a] Assigned arbitrarily.

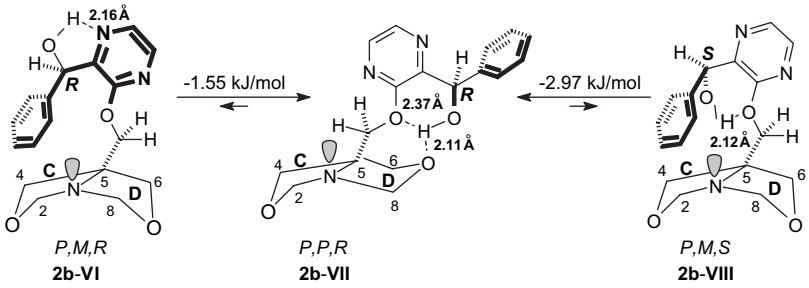
protons. It must be observed that it was more important in the *cis* aliphatic part of the bicycle, and, on the whole, even with respect to the $\Delta\delta$ (a–b) value revealed by the exocyclic methylene *c*-5-OCH-a, H-b. The presence of the phenyl groups at C-2 and C-8 in **2h** strongly increased the diastereotopicity.

In compound **2j**, for the remote *para* (2H)DOABO–CH_2O group, the discussed diastereotopicity was negligible. Therefore, besides NOESY experiments, the diastereotopicity criterion appeared to us as a useful and rapid tool to discriminate regioisomers in this class of compounds.

The study of the *ortho* (2H)DOABO–CH_2O group in compound **2j** by 2D ^{1}H–^{1}H NOESY experiments made it possible to differentiate the two oxazolidine environments, labelled arbitrarily **C** and **D**. The geminal anisochrony at C-6 $\Delta\delta$ (*c*-*t*) (ring **D**) was significant in comparison with C-4 (ring **C**) where no geminal anisochrony was found even at 500 MHz resolution. The same finding was valid for **2b** but not for **2h**. In compounds **2b** and **2j**, by neglecting the absence of the geminal anisochrony at C-4, all *cis* orientated protons were more deshielded than the corresponding *trans* ones, except the reverse situation observed at C-6. Hence, we assigned ring **D** to be sterically closer to the anisotropy created by the *ortho* α-hydroxybenzyl group. The same discrimination in the case of **2h** might be envisaged cautiously.

For the simplest term **2b**, the assignment of a favoured configuration of the C-3′-chiral centre, associated to an appropriate conformation of the adjacent DOABO skeleton was attempted by means of ab initio molecular orbital calculation with full geometry optimisation (level RHF/6-31G*, Scheme 4).

Three distinct conformers were found, **2b-VI**, **2b-VII** and **2b-VIII**. They were all orthogonal rotamers regarding the



Scheme 4.

orientation of the pyrazine ring. The magnitudes of the total ΔE values (<3 kJ mol^{-1}) were too small to predict the most stable spatial arrangement but each of them was in agreement with two remarks, supported by ^{1}H NMR data (Table 2).

(i) In conformers **2b-VI** and **2b-VIII**, the geometry of the two aromatic rings provided a 'cage' with a more deshielding influence on both faces of the bicycle faces with respect to oxazolidine **C** against **D**, e.g., H-4 versus H-6 and H-2 versus H-8. Comparable NMR data applied for the *ortho* (2H)DOABO–CH_2O group in compound **2j** were observed (Table 2). This stereochemistry (*P*,*M*,*R*) and (*P*,*M*,*S*) also facilitated the development of the expected intramolecular hydrogen bonds (benzyl)O–H…N-4′(pyrazine) and (benzyl)O–H…O–CH_2(DOABO).

(ii) In conformer **2b-VII**, the chiral centre was, this time, closer to oxazolidine unit **D**. Hence, the geminal anisochrony at C-6 was noticeable; meanwhile almost no geminal anisochrony was exhibited by the methylene C-4. Moreover, the conformational chirality *P*,*P* of the DOABO skeleton appeared in relationship with the *R* configuration of the chiral centre since they together made possible two six-membered chelate intramolecular hydrogen bonds.

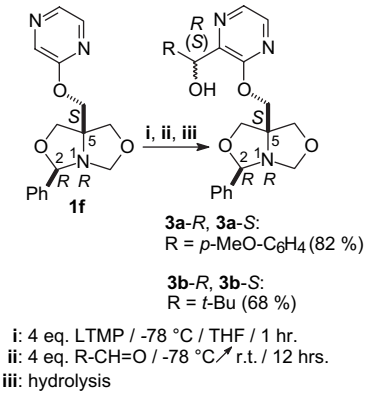
The NMR based stereochemical assignments for compounds **2b**, **2j** could be extrapolated for all synthesised chiral diarylmethanols having an *ortho* correspondence between the α-hydroxybenzyl and (2H)DOABO–CH_2O groups. Indeed, they all exhibited only small fluctuations of the DOABO chemical shifts in comparison with **2b** and **2j**.

The competition as DoMGs, (2H)DOABO–CH_2O against chlorine and methoxy, respectively, was also investigated (Table 1).

Starting from **1d** (entry 4), no trace of a deuteriated derivative was detected when the reaction mixture was quenched with DCl/D_2O, after several attempts: 4 equiv of LTMP (lithiation at −78 °C for 1, 6 even 14 h). In contrast, upon treatment of the reaction mixture with benzaldehyde at −78 °C, and slow evolution to room temperature, the regioisomers **2k** and **2l** were obtained in good yield with the competitive regioselectivity similar to 2-chloro-6-methoxypyrazine.[18] Thus, the synthesis of compounds **2k** and **2l** proved the in situ trapping when benzaldehyde was used as an electrophile. The easily separable compounds **2k** and **2l** were also easily discriminated based on ^{1}H NMR diastereotopicity criterion: it was around 0.31 ppm on the aliphatic motif of the DOABO *cis* face in **2k** but negligible in **2l** (0.01 ppm).

The competitive metallation of **1e** (entry 5) indicated comparable powers of orientation of the methoxy versus (2H)DOABO–CH_2O groups. The result was also reliable with the role played by the 5-OCH_2 group in creating CIPE, similar to an authentic DoMG, methoxy. In order to discriminate the nonseparable regioisomeric products **2m**+**2n**, the variable diastereotopicity in the aliphatic (2H)DOABO sequences was crucial. It was 0.38 versus 0.25 ppm (*cis* vs *trans* face) in **2m** but undetectable in **2n**.

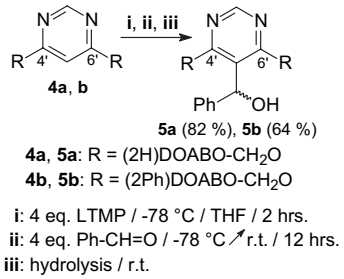
The lithiation of chiral **1f** (depicted as 1*R*,2*R*,5*S* enantiomer in Scheme 5), followed by quenching with anisaldehyde or pivalaldehyde resulted in the absence of diastereoselectivity, the equimolar nonseparable mixture of diastereomers **3a**-*R*+**3a**-*S* or **3b**-*R*+**3b**-*S* being isolated in each case (50:50, 1*R*,2*R*,5*S*,*R*:1*R*,2*R*,5*S*,*S*).



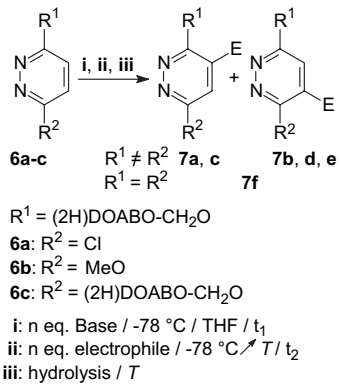
Scheme 5.

We explained this lack of diastereoselectivity by the stabilisation of the lithiated **1f** as *s-trans out* bisectional rotamer (**II**–**IV**, R^1(C-2)=Ph, R^1(C-8)=H, Scheme 2). Hence, the reaction site was significantly remote from the DOABO chiral centres N-1, C-2 and C-5.

2.1.2. Functionalisation of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-pyrimidines. The DoMG aptitude of the DOABO–CH_2O group substituting a diazine ring was also investigated in the pyrimidine series (Scheme 6).



Scheme 6.



Scheme 7.

The behaviour of 4′,6′-disubstituted compounds **4a** and **4b** was compared.

Starting from **4a**, having, at −78 °C, the two (2H)DOABO units blocked as *meso* form (*P,M*) conformers (**III**, Scheme 2),[8] no reaction was observed at this temperature. A slow progress was detected by TLC monitoring only if the reaction mixture was gently warmed up to room temperature. The NMR spectrum of the crude reaction mixture indicated a content of about 66% **5a** and 34% **4a**.

In the case of **4b**, whose (2Ph)DOABO units were still flipping at −78 °C (R^1=Ph, **II**–**III**–**IV**, Scheme 2),[8] the reaction reached completion at this temperature. The smaller yield (64%) was due to the partial decomposition of the product **5b** during isolation by flash column chromatography.

We rationalised these results as correlated with the different conformational behaviour of the DOABO systems in metallation conditions (**4a** vs **4b**) and to the in situ trapping of the electrophile in the case of **4a**.

2.1.3. Functionalisation of α-(3,7-dioxa-*r*-1-azabicyclo-[3.3.0]oct-*c*-5-ylmethoxy)-pyridazines. In the pyridazine series, the competitive *ortho*-metallation assisted by the (2H)DOABO–CH_2O group versus chlorine and methoxy, respectively, was studied.

Lithium-*N*,*N*-*tert*-butyl-(1-isopropylpentyl)amide (called 'LB_1', p*K*a=38.3 in THF), more hindered and basic than LDA and LTMP (p*K*a=35.7 and 37.3, respectively), was previously used for the claimed improved regioselective metallation of 3-chloro-6-methoxypyridazine.[27] Therefore, we tested the deuteriation of the analogous **6a** in identical conditions (2.2 equiv LB_1 at −78 °C, reaction time 30 min). The unreacted **6a** was recovered in almost quantitative yield. By using 4 equiv LB_1, the regioselectivity was poor (entry 1, compounds **7a**, and **7b**). The content of the crude reaction mixture was calculated based on the ^{1}H NMR spectrum, which displayed well-separated signals in the aromatic part: H-4′ and H-5′ at 7.37 6.97 ppm (d, *J*=9.2 Hz), respectively, in **6a**; H-5′ at 7.37 ppm (s) in **7a**; H-5′ at 6.97 ppm (s) in **7b**. Then, the global composition was checked and established in proportional correlation with the intensity of the singlet located at 3.86 ppm, assigned to DOABO methylenes C-4, C-6 in all **6a**, **7a** and **7b** environments (Scheme 7, Table 3).

Hence, the same metallating reagent, LTMP, as in the pyrazine and pyrimidine series, was again used (Table 3).

Compounds **7c** and **7d** were obtained with good global yield (entry 1), but with low *ortho* regioselectivity mandatory to the (2H)DOABO–CH_2O group in competition with chlorine. It was rather comparable with the already reported competition of methoxyethoxy versus chloro (*ortho* to chloro:*ortho* to methoxyethoxy as 32:48),[28] than methoxy versus chloro in the pyridazine series (*ortho* to chloro:*ortho* to methoxy as 20:80).[27,29] The individual assignment of the regioisomers **7c** and **7d** was unproblematic since the DOABO group exhibited different ^{1}H NMR diastereotopicity between homofacial aliphatic protons only in **7c**: $\Delta\delta$= 0.33 ppm (*cis* face) and 0.26 ppm (*trans* face). These $\Delta\delta$ values were 0.00 ppm in **7d**.

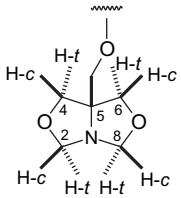
The functionalisation of the pyridazine **6b** was seen as the most illustrative competition between the methoxy against (2H)DOABO–CH_2O group because the sites to be deprotonated had *ortho* relationship (entry 2). The reaction occurred with complete *ortho* to methoxy regioselectivity. Identification of the product **7e** was based on its 2D ^{1}H–^{1}H NOESY[25,26] spectrum supporting the observation that, in the alicyclic region of the spectrum, poor or no diastereotopicity was evidenced in [D_6]benzene and $CDCl_3$. That is, the chiral centre was linked farther from the *ortho* position with respect to the (2H)DOABO–CH_2O group. In this case, the different steric hindrance against the bulky base LTMP, promoted by the two chelating oxygen-fragments, MeO and 5-OCH_2 in (2H)DOABO–CH_2O, became noteworthy.

We expected to better estimate this effect by the metallation of the symmetric 3′,6′-disubstituted pyridazine **6c** (entry 3). Unfortunately, despite dilution (more than 10^{-2} M in THF) and progressive increasing of the molar ratio **6c**:LTMP (4→8 equiv), the reaction mixture constantly was a very fine suspension of **6c**, from −78 °C to room temperature. For the largest excess of the metallating reagent, after column chromatography, a nonseparable mixture **6c+7f** was isolated.

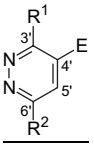
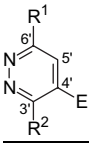
2.2. Functionalisation of α-(3,7-dioxa-*r*-1-azabicyclo-[3.3.0]oct-*c*-5-ylmethoxy)-α-chlorodiazines by cross-coupling reactions under Stille conditions

In this section, our preliminary results concerning the synthesis and coordination ability of four polyaza heterocycles possessing the (2H)DOABO–CH_2O group as peripheral sites is described (Scheme 8, Table 4).

Table 3. Results of the functionalisation via directed *ortho*-metallation of α-(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxy)-pyridazines. Preparation of compounds **7a–f**



(2H)DOABO-CH_2O

Entry	Starting material	R^1 / R^2	E	Products: Compd ()	Products: Compd ()	*n*	Base	t_1 (min)	*T* (°C)	t_2 (h)
1	**6a**	(2H)DOABO–CH_2O Cl	D[a] Ph–CH–OH	**7a** [15][b] **7c** (53)[d] [63]	**7b** [32] **7d** (31) [37]	4 4	LB_1[c] LTMP	60 90	−78 rt	— 14
2	**6b**	(2H)DOABO–CH_2O MeO	Ph–CH–OH	—	**7e** (78)	4	LTMP	70	rt	16
3	**6c**	(2H)DOABO–CH_2O (2H)DOABO–CH_2O	Ph–CH–OH	**7f** [36]		8	LTMP	120	rt	24

[a] DCl 20% g/g (8 equiv) in D_2O solution was used.
[b] In square brackets: contents according to the ^1H NMR spectra of the crude reaction mixtures; 53% of starting **6a** in the mixture **7a+7b**: 64% of starting **6c** as a mixture with **7f**.
[c] Lithium-*N,N-tert*-butyl-(1-isopropylpentyl)amide.
[d] In round brackets, yields as isolated compounds after flash column chromatography; for **7c** and **7d** as partial yields.

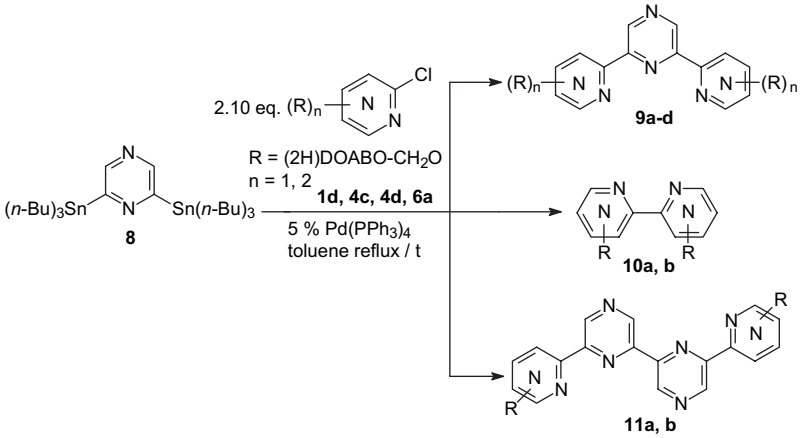
Thus, the 2,6-bis(tri-*n*-butylstannyl)pyrazine **8** was reacted under Stille conditions as double cross-coupling with the (2H)DOABO–CH_2O fragments α-substituting the α-chloro-diazines **1d**, **4c**, **4d** and **6a**. Their syntheses, together with that of the starting **8** were previously reported by us.[8,14]

The target compounds were 2,6-bis(diazinyl)pyrazines **9a–d**.

Very clean reactions accompanied the preparation of the compounds **9a** and **9c**. In turn, separation of **9b** and **9d** as pure analytical samples was more cumbersome than expected because they were contaminated by side products, homocoupling **10a** and **10b** and hetero–homocoupling derivatives **11a** and **11b**. As shown in Table 4, the compounds **10a**, **10b**, **11a** and **11b** were assigned on the basis of their well-separated signals in the ^1H NMR (aromatic zone) and MS spectra of the crude reaction mixtures.

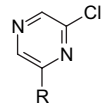
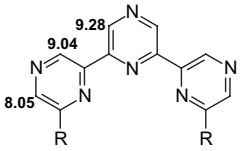
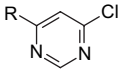
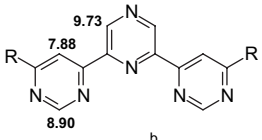
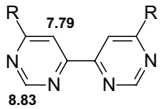
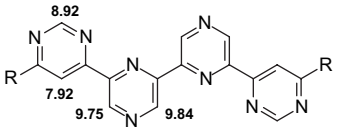
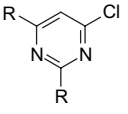
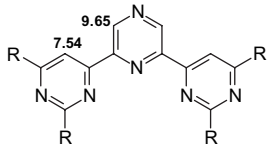
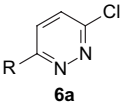
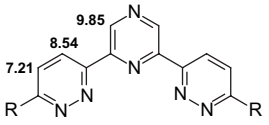
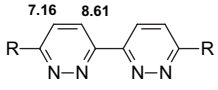
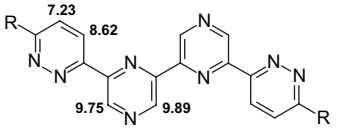
With the pure **9a–d** in our hands, their coordination ability against two transition metals, Zn^{2+} and Eu^{3+}, was examined. The first results of this exploratory study, carried out by means of UV spectroscopy, are collected in Table 5.

The measurements were performed in acetonitrile by using 4×10^{-5} M as initial concentration of the compounds **9a–d**. UV spectra were recorded for each 0.2 equiv of the salt



Scheme 8.

Table 4. Results in the cross-coupling reactions of compounds **1d**, **4c**, **4d** and **6a**. Preparation of compounds **9a–d**

Entry	Starting material	t (h)	Yield (%)	Main products	Side products	
1	 **1d**	22	65	 **9a**[a]		
2	 **4c**	48	60	 **9b** (82%)[b]	 **10a** (6%)	 **11a** (12%)
3	 **4d**	27	70	 **9c**		
4	 **6a**	48	22	 **9d** (50%)	 **10b** (35%)	 **11b** (15%)

[a] R=(2H)DOABO–CH_2O.
[b] Contents issued from the ^{1}H NMR spectra of the crude reaction mixtures.

added as 3×10^{-4} M solution, the final number of equivalents of the salt being 2.0 in all cases. Successive high dilutions were required by the very low solubility of our compounds in acetonitrile.

The UV data indicated that the terpyrazine **9a** was inert against both cations (entries 1–5).

Compound **9b** was an efficient ligand for both Zn^{2+} and Eu^{3+}. The consecutive UV spectra showed a relevant bathochromic effect as $\Delta\lambda_{max.}=26$ nm for Zn^{2+}(entry 7 vs 6) and 16 nm for Eu^{3+} (entry 9 vs 6) until 1 equiv of M^{n+} was added and the saturation was reached (entries 8 and 10). Two isosbestic points were displayed in each case. Accordingly, two successive equilibriums including the

Table 5. Relevant UV data about the coordination ability of compounds **9a–d**

Entry	Ligand L	Salt	Metal (equiv M^{n+})	Absorptions as λ (nm) (log ε)	Isosbestic points λ (nm)		Proposed stoichiometry L:M
1	**9a**	**9a** only	0.0	242 (4.10); 323 (4.27)			
2		+$Zn(BF_4)_2$	1.0	242 (4.10); 323 (4.25)			
3			2.0	242 (4.11); 324 (4.26)	—		—
4		+$EuCl_3\times6H_2O$	1.0	242 (4.11); 323 (4.25)			
5			2.0	241 (4.13); 322 (4.25)	—		—
6	**9b**	**9b** only	0.0	209 (4.63); 246 (4.17); 293 (4.18)			
7		+$Zn(BF_4)_2$	1.0	211 (4.65); 319 (4.23)			1:1
8			2.0	211 (4.67); 319 (4.24)	226	305	
9		+$EuCl_3\times6H_2O$	1.0	210 (4.72); 309 (4.22)			1:1
10			2.0	211 (4.75); 310 (4.26)	230	311	
11	**9c**	**9c** only	0.0	203 (4.68); 250 (4.25); 298 (4.27)			
12		+$Zn(BF_4)_2$	1.0	205 (4.66); 335 (4.33); 347 (4.33)			1:1
13			2.0	205 (4.66); 335 (4.33); 345 (4.41)	236	318	
14		+$EuCl_3\times6H_2O$	1.0	203 (4.68); 250 (4.25); 298 (4.24)	—		—
15			2.0	203 (4.68); 250 (4.24); 298 (4.24)			
16	**9d**	**9d** only	0.0	209 (4.48); 252 (4.39); 297 (4.24)			
17		+$Zn(BF_4)_2$	1.0	212 (4.45); 234 (4.41); 257 (4.36)			
18			1.6	213 (4.46); 234 (4.40); 263 (4.36); 347 (4.04)	263	280	1:1.5
19			2.0	213 (4.47); 234 (4.45); 265 (4.37); 347 (4.05)	334		
20		+$EuCl_3\times6H_2O$	1.0	209 (4.48); 252 (4.41); 285 (4.26)			
21			2.0	209 (4.48); 252 (4.41); 283 (4.26)	—		—

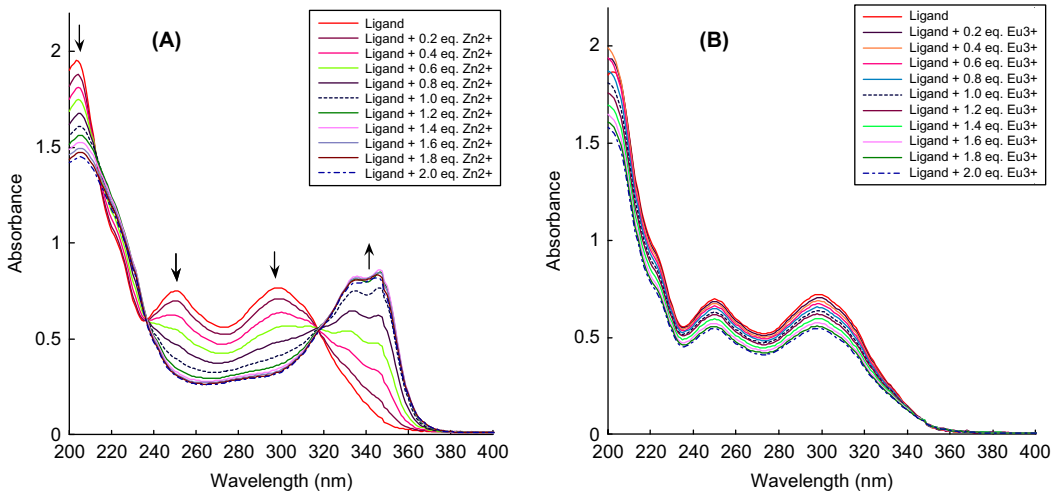


Figure 1. UV spectra of compound **9c** in the presence of progressive increased concentration of Zn^{2+} as $Zn(BF_4)_2$ (**A**) and Eu^{3+} as $EuCl_3 \cdot 6H_2O$ (**B**).

free **9b** and its complex [**9b**]:[M^{n+}] (1:1 stoichiometry) were proposed.

Compound **9c**, possessing twice the number of (2H)DOABO–CH_2O groups, was a selective ligand (Fig. 1).

The UV monitoring of its behaviour in the presence of increased amounts of M^{n+}, indicated only with Zn^{2+} a strong bathochromic effect at $\Delta\lambda_{max.}$=49 nm (entry 12 vs 11) and two isosbestic points located at 236 and 318 nm. The same stoichiometry of the complex as above, [**9c**]:[Zn^{2+}]=1:1 in equilibrium with the free **9c**, via an intermediate, was plausible. No modification of the UV spectrum was observed in the presence of Eu^{3+} (entries 14 and 15).

Finally, the bis(pyridazinyl)pyrazine **9d** was not only a selective ligand but the stoichiometry of its coordination with Zn^{2+} showed an increased chelating ability as [**9d**]:[Zn^{2+}]=1:1.5 (entry 18). Consequently, besides the bathochromic effect at $\Delta\lambda_{max.}$=50 nm (entries 16–19), three isosbestic points were found, consistent with the occurrence of three successive equilibriums.

3. Conclusions

The 3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-ylmethoxydiazines can be highly functionalised on the diazine site via directed *ortho*-metallation reaction. The DOABO–CH_2O architecture, alone or in competition with chloro or methoxy groups, acts as a DoMG since the 5-OCH_2 moiety creates CIPE. The latter is favoured by the orientation of the diazinyloxymethyl fragment as bisectional and coplanar *s-trans out* rotamer. Although the metallation conditions are very different from those previously reported for methoxydiazines, the results appear quite similar. By appropriate choice of the electrophile, elaborated chiral diarylmethanols are prepared. They exhibit stereospecific relationships between configurational and conformational chirality of the molecule creating internal six-membered chelate hydrogen bonds. The DOABO–CH_2O group α-substituting diazines is also compatible with cross-coupling reactions providing aza analogues of terpyridine with selective coordinating ability against transition metals.

4. Experimental

4.1. General

Melting points are uncorrected; they were carried out on an ELECTROTHERMAL® 9100 instrument. Current NMR spectra were recorded on a Brucker® AM300 (300 MHz ^1H, 75 MHz ^{13}C). NMR analysis for the compound **2j** was performed on a Brucker® DMX500 (500 MHz ^1H, 125 MHz ^{13}C). TLC was performed by using aluminium sheets with silica gel 60 F_{254} (Merck®); flash column chromatography was conducted on silica gel Si 60 (40–63 μm, Merck®). IR spectra were performed on a Perkin–Elmer® Paragom FTIR spectrometer. Only relevant absorptions are listed [throughout in cm^{-1}: weak (w), medium (m) or strong (s)]. Mass spectra (MS) were recorded on an ATI-Unicam Automass® apparatus, fitted (or not) with a GC-mass coupling. UV spectra were measured on a VARIAN® CARY 100 SCANS instrument. Microanalyses were performed on a Carlo Erba® CHNOS 1160 apparatus. All synthesis was performed under dry nitrogen atmosphere. THF was freshly distilled from Na/benzophenone prior to use. All solvents and starting materials were of commercial quality.

UV spectra were compiled by using SPECFIT/32® and Varian Carry Winuv® programs.

Molecular orbital calculations for the compound **2b**: the conformational space of these systems has been investigated by using the 'Conformer Distribution' facility (MMFF force field) from Spartan'o2. [Spartan'o2, Wavefunction, Inc. Irvine, CA]. The set of conformers thus generated has been subjected, within the same package, to full geometry optimisation at the RHF/6-31G* ab initio level. The default convergence criteria (Energy = 0.000001 hartrees, rms gradient = 0.000450 hartrees/bohr) have been imposed throughout for all the ab initio computations.

The syntheses of the starting materials **1a–f**, **4a–d** and **6a–c** was described elsewhere.[8,11]

4.2. General procedure for the preparation of compounds 2a–j, 3a, 3b, 5a, 5b and 7a–f by Directed *ortho*-Metallation methodology

In 25–50 mL (Note 1) THF and with vigorous stirring, 2,2,6,6-tetramethylpiperidine (HTMP) (0.688 mL, 0.565 g 100%, 0.576 g 98%, 4 mmol) was injected. The solution was cooled at −10 to −15 °C and then *n*-BuLi (2.50 mL as 1.6 M solution in hexane, 4.00 mmol, optionally 1.54 mL as 2.6 M in hexane) was injected. The clear yellowish solution was stirred at −10 to −15 °C for additional 15 min and then cooled to −78 °C. The starting DOABO–CH_2O substituting diazine (1.00 mmol) as a THF solution (2–10 mL, Note 1) was introduced. Specific conditions to perform the reaction are presented in Tables 1 and 3 and Schemes 5 and 6 (Note 2). TLC (UV 254 nm) monitored all syntheses as follows: 0.2–0.3 mL from the reaction mixture were rapidly quenched with 2 mL 1:1 v/v mixture ethyl acetate (optionally ether):water. The sample was collected from the organic layer after vigorous stirring and separation. If no reaction occurred at −78 °C or very slow evolution was observed, the reaction mixture was let to reach very gently the room temperature.

The reaction mixture was quenched according to one of the following variants:

A In the case of deuteriated compounds **2a** and **7a**, **7b**, the reaction was quenched at −78 °C (0 °C in the case of **2i**) with 8 equiv of DCl as 20% g/g solution in D_2O. Then it was allowed to reach the room temperature. The next work up was made according to variant **C**.

B In the case of compounds **2c–g**, the reaction was quenched at −78 °C with 10 mL 1:1 v/v THF:EtOH. Then it was allowed to reach room temperature. The next work up was made according to variant **C**.

C For the rest of the compounds, the reaction mixture was quenched at room temperature with 100 mL 1:1 v/v dichloromethane:water. After separation, the aqueous layer was extracted with dichloromethane (2×15 mL) and then the combined organic solution was washed with water (×25 mL) to neutrality. After drying on $MgSO_4$ and filtering, the dichloromethane solution was evaporated under vacuum to dryness. The obtained oily residue was analysed by ^1H NMR as a crude reaction mixture. For deuteriated compounds **2a**, **2i** and **7a**, **7b** conclusions were provided at this stage (Note 3). For the rest of the compounds, the mixtures were purified by column chromatography to yield the title compounds (Note 4).

Note 1 THF 25 (50) mL for diazines possessing one (two, respectively) DOABO fragment(s).

Note 2 After the accumulation time, usually clear coloured solutions were obtained as follows: **1a**, **1e** (yellow → reddish-brown), **1b** (yellow → orange), **1c** (orange at −78 °C and brown at 0 °C), **1d** (reddish orange), **1f** (yellow → reddish-yellow), **4a**, **4b** (no change), **6a** (bright yellow → reddish violet with LiB_1, reddish orange with LTMP), **6b** (red) and **6c** (pale reddish).

Note 3 For mono deuteriated compounds **2a** and **2i** the magnitude of the corresponding integral is given in each case as percentages with respect to the most intense signal. For **7a** and **7b**, see the text.

Note 4 CARE! After column chromatography, almost all compounds were preliminarily obtained as viscous pale yellowish oils. They were then crystallised from ligroin:ether mixtures (about 1:1 v/v) or pentane. Crystallisations take place over a long time (1–7 days). All melting points, elementary analysis, NMR, IR and MS spectra refer to crystalline isolated structures. The TLC monitoring of all reactions and separations by column chromatography evidenced very weak absorption in UV (254 nm). Concentrated samples were used.

4.2.1. 3-[^2H]-2-[(3,7-Dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2a). Yield 98%. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 8.23 (1H, d, *J*=1.1 Hz, H-3, 1.7%), 8.13 (1H, d, *J*=3.0 Hz, H-5, 93%), 8.06 (1H, d, *J*=2.8 Hz, H-6, 100%). MS (EI, 70 eV) *m/z* (rel int. %): (M^++1) 225 (5), 207 (5), 177 (4), 128 (100%), 98 (9).

4.2.2. *rac*-3-(α-Hydroxybenzyl)-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2b). Yield 79%. Yellowish crystalline powder, mp 84–85 °C (pentane) (column chromatography, eluent AcOEt:ligroin 20:1 v/v). [Found: C, 61.79; H, 6.10; N, 12.45. $C_{17}H_{19}N_3O_4$ requires: C, 61.99; H, 5.81; N, 12.76%]. R_f (95% AcOEt:ligroin) 0.40. ν_{max} (film NaCl): 3600 (s), 2863 (w), 2356 (w), 1540 (w), 1419 (s), 1320 (w), 1176 (m), 1093 (w), 1042 (s), 925 (m), 700 (s) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.07 (1H, d, *J*=2.8 Hz, H-5), 7.96 (1H, d, *J*=2.8 Hz, H-6), 7.30–7.10 (5H, m, Ph), 5.71 (1H, d, *J*=4.7 Hz, C*H*OH), 5.05 (1H, d, *J*=4.7 Hz, OH); *DOABO–CH_2O*: 4.36 (1H, d, *J*=5.7 Hz, H-2-*c*), 4.31 (1H, d, *J*=5.7 Hz, H-2-*t*), 4.29 (1H, d, *J*=6.4 Hz, H-8-*c*), 4.26 (1H, d, *J*=10.9 Hz, 5-OCH_aH$_b$), 4.25 (1H, d, *J*=6.4 Hz, H-8-*t*), 4.14 (1H, d, *J*=10.9 Hz, 5-OCH$_a$$H_b$), 3.64 (2H, s, H-4-*c*, H-4-*t*), 3.38 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.28 (1H, d, *J*=9.0 Hz, H-6-*c*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 156.8 (1C, C-2), 146.2 (1C, C-3), 141.9 (1C, Cq., Ph), 140.3 (1C, C-6), 135.5 (1C, C-5), 128.9 (2C, CH, Ph), 128.5 (1C, CH, Ph), 127.6 (2C, CH, Ph); *DOABO–CH_2O*: 88.4, 88.3 (2C, C-2, C-8), 74.32, 74.28 (2C, C-4, C-6), 71.9 (1C, CHOH), 71.6 (1C, C-5), 69.3 (1C, 5-OCH_2). MS (EI, 70 eV) *m/z* (rel int. %): (M^+−1) 328 (3), 312 (100), 281.9 (13), 254.8 (10), 211.7 (11), 186.8 (8), 128 (75), 98 (32).

4.2.3. *rac*-3-(1-Hydroxyeth-1-yl)-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2c). Yield 41%. White crystalline powder, mp 79–80 °C (Et_2O:ligroin 1:1 v/v) (column chromatography, eluent AcOEt:ligroin 20:1 v/v). [Found: C, 53.94; H, 6.41; N, 15.39. $C_{12}H_{17}N_3O_4$ requires: C, 53.92; H, 6.41; N, 15.72%]. R_f (95% AcOEt:ligroin) 0.19. ν_{max} (film KBr): 3414(s), 2853 (s), 1544 (m), 1417 (s), 1342 (s), 1306 (s), 1271 (m), 1175 (s), 1136 (s), 1103 (s), 1064 (s), 1038 (s), 1004 (s), 944 (m), 916 (s), 854 (w), 765 (w), 678 (m), 617 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 8.05 (1H, d, *J*=2.3 Hz, H-5), 7.97 (1H, d, *J*=2.3 Hz, H-6), 4.94 (1H, q, *J*=6.4 Hz, C*H*OH), 3.83 (1H, br s, OH); *DOABO–CH_2O*: 4.49 (2H,

d, J=5.5 Hz, H-2, H-8-*c*), 4.43 (2H, d, J=5.5 Hz, H-2, H-8-*t*), 4.40 (1H, d, J=10.7 Hz, 5-OCH_aH$_b$), 4.35 (1H, d, J=10.7 Hz, 5-OCH$_a$$H_b$), 3.85 (2H, d, J=9.4 Hz, H-4, H-6, H-*c*), 3.82 (2H, d, J=9.4 Hz, H-4, H-6, H-*t*), 1.42 (3H, d, J=6.4 Hz, CH$_3$); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 156.8 (1C, C-2), 148.5 (1C, C-3), 139.8 (1C, C-6), 135.8 (1C, C-5); *DOABO–CH$_2$O*: 88.6 (2C, C-2, C-8), 74.2 (2C, C-4, C-6), 72.0 (1C, C-5), 69.0 (1C, 5-OCH$_2$), 65.7 (1C, CHOH), 22.6 (1C, CH$_3$). δ_H (300 MHz, [D$_6$]bezene) *DOABO–CH$_2$O*: 4.27 (1H, d, J=5.3 Hz, H-2-*c*), 4.25 (1H, d, J=5.3 Hz, H-8-*c*), 4.19 (1H, d, J=10.9 Hz, 5-OCH_aH$_b$), 4.10 (1H, d, J=10.9 Hz, 5-OCH$_a$$H_b$), 4.06 (2H, d, J=5.5 Hz, H-2, H-8-*t*), 3.50 (2H, d, J=9.4 Hz, H-4, H-6-*c*), 3.45 (2H, d, J=9.4 Hz, H-4, H-6-*t*). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 267 (6), 222 (12), 207 (15), 128 (18), 114 (100), 98 (20), 86 (10), 68 (21).

4.2.4. 3-Ethyl-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2d). Yield 80%. Yellowish crystalline powder, mp 31–35 °C (pentane), (column chromatography, eluent AcOEt:ligroin 20:1 v/v). [Found: C, 57.09; H, 7.15; N, 16.55. $C_{12}H_{17}N_3O_3$ requires: C, 57.36; H, 6.82; N, 16.72%]. R_f (95% AcOEt:ligroin) 0.40. ν_{max} (film KBr): 2976.1, 2858 (s), 1548 (s), 1418 (s), 1334 (s), 1184 (s), 1147 (s), 1099 (s), 1043 (s), 1020 (w), 1003 (s), 925 (s), 846 (m), 748 (m), 665 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 7.98 (1H, d, J=2.6 Hz, H-5), 7.83 (1H, d, J=2.6 Hz, H-6); *DOABO–CH$_2$O*: 4.46 (2H, d, J=5.5 Hz, H-2, H-8-*c*), 4.42 (2H, d, J=5.5 Hz, H-2, H-8-*t*), 4.32 (2H, s, 5-OCH$_2$), 3.83 (2H, d, J=9.0 Hz, H-4, H-6-*c*), 3.81 (2H, d, J=9.0 Hz, H-4, H-6-*t*), 2.73 (2H, q, J=7.5 Hz, CH_2CH$_3$), 1.19 (3H, t, J=7.5 Hz, CH$_2$CH_3); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 158.0 (1C, C-2), 149.1 (1C, C-3), 138.2 (1C, C-6), 136.6 (1C, C-5); *DOABO–CH$_2$O*: 88.8 (2C, C-2, C-8), 74.4 (2C, C-4, C-6), 72.0 (1C, C-5), 68.9 (1C, 5-OCH$_2$), 26.1 (1C, *C*H$_2$CH$_3$), 11.6 (1C, CH$_2$*C*H$_3$). MS (CI) *m/z* (rel int. %): (M^++14) 256 (5), 251 (<1), 235 (9), 221 (18), 141 (100), 128 (24), 115 (14).

4.2.5. 3-Iodo-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2e). Yield 68%. Yellow crystalline powder, mp 71–72 °C (pentane) (column chromatography, eluent AcOEt:pentane 4:1 v/v). [Found: C, 34.55; H, 3.45; N, 11.97. $C_{10}H_{12}N_3O_3I$ requires: C, 34.40; H, 3.46; N, 12.04%]; R_f (75% AcOEt:pentane) 0.55. ν_{max} (film KBr): 2980 (w), 2872 (s), 1510 (s), 1443 (m), 1404 (s), 1354 (s), 1344 (s), 1330 (s), 1161 (s), 1092 (s), 1041 (s), 1018 (m), 988 (m), 931 (m), 913 (m), 851 (m), 713 (m), 631 (w), 454 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 7.94 (2H, s, H-5, H-6); *DOABO–CH$_2$O*: 4.56 (2H, d, J=5.3 Hz, H-2, H-8-*c*), 4.46 (2H, d, J=5.3 Hz, H-2, H-8-*t*), 4.36 (2H, s, 5-OCH$_2$), 3.93 (2H, d, J=9.0 Hz, H-4, H-6-*c*), 3.89 (2H, d, J=9.0 Hz, H-4, H-6-*t*); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 158.9 (1C, C-2), 139.8 (1C, C-6), 138.4 (1C, C-5), 108.0 (1C, C-3); *DOABO–CH$_2$O*: 88.9 (2C, C-2, C-8), 74.3 (2C, C-4, C-6), 71.8 (1C, C-5), 70.4 (1C, 5-OCH$_2$). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 349 (4), 304 (5), 289 (15), 261 (5), 222 (40), 205 (5), 128 (14), 114 (100), 98 (28), 86 (10), 68 (30).

4.2.6. 3-Tri-*n*-butylstannyl-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2f). Yield 73%. Yellow oil (column chromatography, eluent ligroin:AcOEt 2:1). [Found: C, 51.29; H, 7.95; N, 8.25. $C_{22}H_{39}N_3O_3Sn$ requires: C, 51.58; H, 7.67; N, 8.20%]. R_f (66% ligroin: AcOEt) 0.60. ν_{max} (film NaCl): 2955 (s), 2928 (s), 2855 (s), 1559 (w), 1503 (m), 1463 (w), 1389 (s), 1342 (s), 1326 (s), 1294 (m), 1155 (s), 1100 (s), 1088 (s), 1047 (m), 1025 (m), 1002 (m), 931 (m), 865 (w), 843 (w), 749 (w), 693 (w), 601 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 8.26 (1H, d, J=2.6 Hz, H-5), 7.84 (1H, dd, J=2.6, 5.1 Hz, H-6); *DOABO–CH$_2$O*: 4.47 (2H, d, J=5.3 Hz, H-2, H-8-*c*), 4.42 (2H, d, J=5.3 Hz, H-2, H-8-*t*), 4.31 (2H, s, 5-OCH$_2$), 3.84 (4H, dd as t, J=8.7 Hz, H-4, H-6, H-c, H-*t*), 1.54–1.44 (6H, m, CH$_2$CH_2CH$_2$CH$_3$), 1.32–1.20 (6H, m, CH$_2$CH$_2$CH_2CH$_3$), 1.14–1.07 (6H, m, CH_2CH$_2$*C*H$_2$CH$_3$), 0.82 (9H, t, J=7.3 Hz, CH$_2$CH$_2$CH$_2$CH_3); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 164.5 (1C, C-3), 160.8 (1C, C-2), 139.7 (1C, C-6), 138.9 (1C, C-5); *DOABO–CH$_2$O*: 88.6 (2C, C-2, C-8), 75.0 (2C, C-4, C-6), 71.9 (1C, C-5), 69.6 (1C, 5-OCH$_2$), 29.4 (3C, CH$_2$*C*H$_2$CH$_2$CH$_3$), 27.7 (3C, CH$_2$CH$_2$*C*H$_2$CH$_3$), 14.1 (3C, CH$_2$CH$_2$CH$_2$*C*H$_3$), 10.4 (3C, *C*H$_2$CH$_2$CH$_2$CH$_3$). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 512 (<1), 456 ($M^+-C_4H_9$, 100), 329 (5), 229 (5), 215 (16), 177 (15), 128 (36), 114 (30), 98 (75), 86 (<5), 68 (35).

4.2.7. 2-[(3,7-Dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-3-thiophenylpyrazine (2g). Yield 73%. Yellow crystalline powder, mp 102–103 °C (ligroin) (column chromatography, eluent AcOEt:ligroin 20:1 v/v). [Found: C, 57.91; H, 5.24; N, 12.64. $C_{16}H_{17}N_3SO_3$ requires: C, 58.00; H, 5.17; N, 12.68%]. R_f (95% AcOEt:ligroin) 0.70. ν_{max} (film KBr): 2871 (m), 1517 (s), 1476 (m), 1440 (w), 1409 (s), 1360 (s), 1175 (s), 1130 (w), 1105 (s), 1059 (m), 1046 (s), 1022 (m), 932 (s), 916 (s), 897 (w), 750 (s), 693 (m), 681 (w), 457 (w). δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.85 (1H, d, J=2.6 Hz, H-5), 7.74 (1H, d, J=2.6 Hz, H-6), 7.53–7.50 (2H, m, Ph), 7.40–7.39 (3H, m, Ph); *DOABO–CH$_2$O*: 4.57 (2H, d, J=5.5 Hz, H-2, H-8-*c*), 4.47 (2H, d, J=5.5 Hz, H-2, H-8-*t*), 4.43 (2H, s, 5-OCH$_2$), 3.92 (2H, d, J=8.9 Hz, H-4, H-6-*c*), 3.87 (2H, d, J=8.9 Hz, H-4, H-6-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 155.8 (1C, C-2), 146.8 (1C, C-3), 137.1 (1C, C-6), 136.3 (1C, C-5), 135.5 (2C, CH, Ph), 129.64 (2C, CH, Ph), 129.55 (1C, CH, Ph), 128.7 (1C, Cq., Ph); *DOABO–CH$_2$O*: 88.9 (2C, C-2, C-8), 74.4 (2C, C-4, C-6), 71.9 (1C, C-5), 69.5 (1C, 5-OCH$_2$). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 331 (15), 222 (20), 203 (9), 187 (5), 160 (7), 128 (100), 121 (5), 114 (33), 98 (20), 86 (7), 77 (10), 68 (28).

4.2.8. *rac*-3-(α-Hydroxybenzyl)-2-[(*c*-2,*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2h). Yield 98%. Yellow crystalline powder, mp 88–90 °C (column chromatography, eluent ligroin:AcOEt 2:1 v/v). [Found: C, 72.47; H, 5.52; N, 8.81. $C_{29}H_{27}N_3O_4$ requires: C, 72.33; H, 5.65; N, 8.73%]. R_f (67% ligroin:AcOEt) 0.60. ν_{max} (film KBr): 3401 (m), 2875 (m), 1547 (s), 1423 (m), 1211 (m), 831 (s), 739 (s), 696 (s), 634 (w), 534 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.03 (1H, d, J=2.4 Hz, H-5), 7.92 (1H, d, J=2.4 Hz, H-6), 7.43–7.38 (4H, m, Ph), 7.24–7.15 (9H, m, Ph), 7.10–7.08 (2H, m, Ph), 5.55 (1H, d, J=3.8 Hz, C*H*–OH), 4.99 (1H, d, J=3.8 Hz, OH); *DOABO–CH$_2$O*: 5.50 (1H, s, H-2-*t*), 5.41 (1H, s, H-8-*t*), 4.17 (1H, d, J=10.2 Hz, 5-OCH_aH$_b$), 4.00 (1H, d, J=10.2 Hz, 5-OCH$_a$$H_b$), 3.91 (1H, d, J=8.9 Hz, H-4-*c*), 3.81 (1H, d, J=9.4 Hz, H-4-*t*); 3.46 (1H, d,

J=9.2 Hz, H-6-*c*), 3.32 (1H, d, *J*=9.2 Hz, H-6-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 156.7 (1C, C-2), 145.8 (1C, C-3), 141.8 (1C, Cq., Ph), 140.4 (1C, C-6), 139.6 (1C, Cq., Ph), 139.4 (1C, Cq., Ph), 135.2 (1C, C-5), 129.1 (1C, CH, Ph), 129.0 (2C, CH, Ph), 128.9 (1C, CH, Ph), 128.8 (4C, CH, Ph), 128.6 (1C, CH, Ph), 127.7 (2C, CH, Ph), 127.6 (2C, CH, Ph), 127.5 (2C, CH, Ph); *DOABO–CH_2O*: 98.0, 97.2 (2C, C-2, C-8), 73.7 (1C, C-5), 73.3, 72.9 (2C, C-4, C-6), 71.8 (1C, CHOH), 70.5 (1C, 5-OCH_2). MS (EI, 70 eV) *m/z* (rel int. %): (M^+-1) 480 (<1), 464 (4), 376 (22), 358 (34), 281 (70), 174 (100), 156 (11).

4.2.9. 3-[^2H]-2,6-Bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2i). Yield 71%. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 7.79 (1H, s, H-5, 50%). MS (EI, 70 eV) *m/z* (rel int. %): (M^++1) 368 (10), 279 (<1), 212 (<1), 128 (100), 98 (4).

4.2.10. *rac*-3-(α-Hydroxybenzyl)-2,6-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2j). Yield 61%. White crystalline powder, mp 172–174 °C (Et_2O: pentane 1:1) (column chromatography, eluent ligroin: acetone 1:1 v/v). [Found: C, 58.80; H, 5.69; N, 11.86. $C_{23}H_{28}N_4O_7$ requires: C, 58.47; H, 5.97; N, 11.86%]. R_f (50% ligroin:acetone) 0.75. ν_{max} (film NaCl): 3600 (s), 2852 (s), 1537 (m), 1452 (s), 1413 (s), 1315 (s), 1142 (m), 1039 (m), 925 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.40 (1H, s, H-5), 7.28–7.16 (5H, m, Ph); 5.69 (1H, br s, C*H*OH), 4.81 (1H, br s, OH); *DOABO–CH_2O linked at C-2*: 4.39 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.35 (1H, d, *J*=5.5 Hz, H-2-*t*), 4.34 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.30 (1H, d, *J*=5.5 Hz, H-8-*t*), 4.23 (1H, d, *J*=10.6 Hz, 5-OC$H_a$$H_b$), 4.15 (1H, d, *J*=10.6 Hz, 5-OCH_aH_b), 3.69 (2H, s, H-4-c, H-4-*t*), 3.48 (1H, d, *J*=9.4 Hz, H-6-*t*), 3.33 (1H, d, *J*=9.4 Hz, H-6-*c*); *DOABO–CH_2O linked at C-6*: 4.46 (1H, d, *J*=5.3 Hz, H-2-*c*), 4.45 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.40 (1H, d, *J*=5.3 Hz, H-2-*t*), 4.39 (1H, d, *J*=5.3 Hz, H-8-*t*), 4.27 (1H, d, *J*=10.6 Hz, 5-OC$H_a$$H_b$), 4.23 (1H, d, *J*=10.6 Hz, 5-OCH_aH_b), 3.83 (2H, s, H-4, H-*c*, H-*t*), 3.82 (2H, s, H-6, H-*c*, H-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 158.1 (1C, C-6), 154.3 (1C, C-2), 142.7 (1C, Cq., Ph), 136.4 (1C, C-3), 128.8 (2C, CH, Ph), 128.3 (1C, CH, Ph), 127.3 (2C, CH, Ph), 123.2 (1C, C-5), 71.5 (1C, CHOH); *DOABO–CH_2O linked at C-2*: 88.3, 88.2 (2C, C-2, C-8), 74.3 (2C, C-4, C-6), 71.5 (1C, C-5), 69.3 (1C, 5-OCH_2); *DOABO–CH_2O linked at C-6*: 88.4 (2C, C-2, C-8), 74.4 (2C, C-4, C-6), 71.8 (1C, C-5), 69.7 (1C, 5-OCH_2). δ_H (500 MHz, [D_6]benzene) *(hetero)aromatic*: 7.62 (1H, s, H-5), 7.35 (2H, d, *J*=7.2 Hz, *ortho*-Ph), 7.09 (2H, m, *meta*-Ph), 7.02 (1H, m, *para*-Ph), 5.86 (1H, d, *J*=7.2 Hz, C*H*OH), 4.99 (1H, d, *J*=7.2 Hz, OH); *DOABO–CH_2O linked at C-2*: 4.23 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.16 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.02 (1H, d, *J*=5.5 Hz, H-2-*t*), 3.99 (1H, d, *J*=10.6 Hz, 5-OC$H_a$$H_b$), 3.98 (1H, d, *J*=5.5 Hz, H-8-*t*), 3.89 (1H, d, *J*=10.6 Hz, 5-OCH_aH_b), 3.52 (2H, s, H-4-*c*, H-4-*t*), 3.38 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.28 (1H, d *J*=9.0 Hz, H-6-*c*); *DOABO–CH_2O linked at C-6*: 4.28 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.27 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.08 (1H, d, *J*=11.3 Hz, 5-OC$H_a$$H_b$), 4.05 (2H, d, *J*=5.5 Hz, H-2, H-8-*t*), 3.98 (1H, d, *J*=11.3 Hz, 5-OCH_aH_b), 3.67 (1H, d, *J*=9.0 Hz, H-4-*c*), 3.65 (1H, d *J*=9.0 Hz, H-6-*c*), 3.59 (1H, d, *J*=9.0 Hz, H-4-*t*), 3.58 (1H, d, *J*=9.0 Hz, H-6-*t*). δ_C (125 MHz, [D_6]benzene) *(hetero)aromatic*: 158.0 (1C, C-6), 154.3 (1C, C-2), 143.5 (1C, Cq., Ph), 137.1 (1C, C-3), 128.6 (2C, CH, *meta*-Ph), 127.9 (1C, CH, *para*-Ph), 127.4 (2C, CH, *ortho*-Ph), 122.6 (1C, C-5), 71.6 (1C, CHOH); *DOABO–CH_2O linked at C-2*: 87.88 (1C, C-8), 87.82 (1C, C-2), 73.96 (1C, C-6), 73.90 (1C, C-4), 71.4 (1C, C-5), 69.3 (1C, 5-OCH_2); *DOABO–CH_2O linked at C-6*: 88.0 (2C, C-2, C-8), 74.00 (1C, C-4), 73.96 (1C, C-6), 71.6 (1C, C-5), 69.7 (1C, 5-OCH_2). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 472 (<1), 344 (3), 212 (4), 128 (100), 98 (7).

4.2.11. *rac*-6-Chloro-3-(α-hydroxybenzyl)-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2k). Yield 52%. White crystalline powder, mp 76–78 °C (Et_2O: pentane 1:1 v/v) (column chromatography, eluent ligroin: AcOEt 1:1 v/v). [Found: C, 55.89; H, 5.25; N, 11.81. $C_{17}H_{18}N_3O_4Cl$ requires: C, 56.13; H, 4.99; N, 11.55%]. R_f (50% ligroin:AcOEt) 0.50. ν_{max} (film NaCl): 3416 (s), 2857 (m), 1540 (m), 1422 (s), 1351 (s), 1173 (m), 1129 (m), 1041 (s), 930 (w), 749 (w), 700 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.15 (1H, s, H-5), 7.32–7.20 (5H, m, Ph), 5.76 (1H, br s, C*H*OH), 4.76 (1H, br s, OH); *DOABO–CH_2O*: 4.41 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.36 (1H, d, *J*=5.5 Hz, H-2-*t*), 4.33 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.32 (1H, d, *J*=5.5 Hz, H-8-*t*), 4.30 (1H, d, *J*=10.9 Hz, 5-OC$H_a$$H_b$), 4.21 (1H, d, *J*=10.9 Hz, 5-OCH_aH_b), 3.68 (2H, s, H-4-c, H-4-*t*), 3.47 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.36 (1H, d, *J*=9.0 Hz, H-6-*c*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 155.6 (1C, C-2), 144.7 (1C, C-3), 144.2 (1C, C-6), 141.4 (1C, Cq., Ph), 134.3 (1C, C-5), 129.0 (2C, CH, Ph), 128.7 (1C, CH, Ph), 127.5 (2C, CH, Ph); *DOABO–CH_2O*: 88.40, 88.35 (2C, C-2, C-8), 74.11, 74.06 (2C, C-4, C-6), 71.8 (1C, CHOH), 71.5 (1C, C-5), 70.0 (1C, 5-OCH_2). δ_H (300 MHz, [D_6]benzene) *(hetero)aromatic*: *DOABO–CH_2O*: 4.28 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.17 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.10 (1H, d, *J*=5.5 Hz, H-2-*t*), 4.06 (1H, d, *J*=5.5 Hz, H-8-*t*), 3.97 (1H, d, *J*=10.6 Hz, 5-OC$H_a$$H_b$), 3.88 (1H, d, *J*=10.6 Hz, 5-OCH_aH_b), 3.51 (1H, d, *J*=9.0 Hz, H-4-*t*), 3.48 (1H, d *J*=9.0 Hz, H-4-*c*), 3.35 (1H, d *J*=9.0 Hz, H-6-*t*), 3.18 (1H, d, *J*=9.0 Hz, H-6-*c*). MS (EI, 70 eV) *m/z* (rel int. %): (M^+-1) 362.6 (3), 346 (100), 318 (6), 178 (6), 128 (93), 98 (65).

4.2.12. *rac*-2-Chloro-3-(α-hydroxybenzyl)-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2l). Yield 26%. White crystalline powder, mp 117–119 °C (Et_2O:pentane 1:1 v/v) (column chromatography, eluent ligroin:AcOEt 1:1 v/v). [Found: C, 56.44; H, 5.30; N, 11.29. $C_{17}H_{18}N_3O_4Cl$ requires: C, 56.13; H, 4.99; N, 11.55%]. R_f (50% ligroin:AcOEt) 0.35. ν_{max} (film NaCl): 3419 (s), 2868 (m), 1640 (w), 1566 (w), 1526 (w), 1446 (s), 1328 (s), 1169 (s), 1041 (s), 930 (w), 751 (s), 700 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.16 (1H, s, H-5), 7.35–7.20 (5H, m, Ph), 5.98 (1H, s, C*H*OH), 5.05 (1H, br s, OH); *DOABO–CH_2O*: 4.48 (1H, d, *J*=5.7 Hz, H-2-*c*), 4.47 (1H, d, *J*=5.7 Hz, H-8-*c*), 4.42 (2H, d, *J*=5.7 Hz, H-2, H-8-*t*), 4.35 (1H, s, 5-OC$H_a$$H_b$), 4.33 (1H, s, 5-$OCH_aH_b$), 3.84 (2H, s, H-4, H-*c*, H-*t*), 3.83 (2H, s, H-6, H-*c*, H-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 158.5 (1C, C-6), 146.3 (1C, C-3), 143.1 (1C, C-2), 141.7 (1C, Cq., Ph), 132.1 (1C, C-5), 128.9 (2C, CH, Ph), 128.4 (1C, CH, Ph), 127.4 (2C, CH, Ph); *DOABO–CH_2O*:

88.6 (2C, C-2, C-8), 74.3 (2C, C-4, C-6), 71.9 (1C, CHOH), 71.8 (1C, C-5), 70.1 (1C, 5-OCH_2). δ_H (300 MHz, [D_6]benzene) *(hetero)aromatic*: 7.60 (1H, s, H-5), 7.43 (2H, d, *J*=7.2 Hz, Ph), 7.20–7.00 (3H, m, Ph), 6.10 (1H, s, C*H*OH), 4.35 (1H, br s, OH); *DOABO–CH_2O*: 4.24 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.23 (1H, d, *J*=5.5 Hz, H-8-*c*), 4.05 (2H, d, *J*=5.5Hz, H-2, H-8-*t*), 3.98 (1H, s, *J*=10.7 Hz, 5-OC*H_a*H_b), 3.89 (1H, d, *J*=10.7 Hz, 5-OCH_aH_b), 3.51 (2H, s, H-4, H-*c*, H-*t*), 3.50 (2H, s, H-6, H-*c*, H-*t*). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 363.6 (3), 346 (8), 128 (100), 98 (15).

4.2.13. *rac*-3-(α-Hydroxybenzyl)-6-methoxy-2-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2m) and *rac*-3-(α-hydroxybenzyl)-2-methoxy-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazine (2n). Nonseparable mixture as white crystalline powder, mp 114–119 °C (Et_2O) (column chromatography, eluent ligroin: AcOEt 1:1 v/v). [Found: C, 60.30; H, 5.62; N, 11.80. $C_{18}H_{21}N_3O_5$ requires: C, 60.16; H, 5.89; N, 11.69%]. R_f (50% ligroin:AcOEt) 0.32. ν_{max} (film KBr): 3224 (m), 2886 (w), 2863 (m), 1583 (m), 1410 (m), 1184 (m), 922 (m), 703 (s), 561 (m), 491 (w) cm^{-1}. *Regioisomer* ***2m*** (29%): δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.77 (1H, s, H-5), 7.32–7.16 (5H, m, Ph), 5.68 (1H, d, *J*=6.4 Hz, C*H*OH), 4.78 (1H, d, *J*=6.4 Hz, OH); *DOABO–CH_2O*: 4.41 (1H, d, *J*=4.5 Hz, H-2-*c*), 4.37 (1H, d, *J*=4.5 Hz, H-2-*t*), 4.36 (1H, d, *J*=4.5 Hz, H-8-*c*), 4.29 (1H, d, *J*=4.5 Hz, H-8-*t*), 4.26 (1H, d, *J*=10.6 Hz, 5-OC*H_a*H_b), 4.19 (1H, d, *J*=10.6 Hz, 5-OCH_aH_b), 3.88–3.83 (3H, m, OCH_3), 3.73 (1H, d, *J*=9.8 Hz, H-4-*c*), 3.70 (1H, d, *J*=9.8 Hz, H-4-*t*), 3.45 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.35 (1H, d, *J*=9.0 Hz, H-6-*c*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 159.0 (1C, C-6), 154.4 (1C, C-2), 142.9 (1C, Cq., Ph), 135.5 (1C, C-3), 128.8 (2C, CH, Ph), 128.2 (1C, CH, Ph), 127.3 (2C, CH, Ph), 123.2 (1C, C-5); *DOABO–CH_2O*: 88.3, 88.2 (2C, C-2, C-8), 74.3 (2C, C-4, C-6), 71.6 (1C, C-5), 71.5 (1C, CHOH), 69.3 (1C, 5-OCH_2), 54.4 (1C, OCH_3). *Regioisomer* ***2n*** (41%): δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.71 (1H, s, H-3), 7.32–7.16 (5H, m, Ph), 5.82 (1H, d, *J*=7.4 Hz, C*H*OH), 4.55 (1H, d, *J*=7.4 Hz, OH); *DOABO–CH_2O*: 4.48 (1H, d, *J*=4.9 Hz, H-2-*c*), 4.47 (1H, d, *J*=4.9 Hz, H-8-*c*), 4.41 (2H, d, *J*=4.9 Hz, H-2, H-8-*t*), 4.31 (1H, d, *J*=8.5 Hz, 5-OC*H_a*H_b), 4.28 (1H, d, *J*=8.5 Hz, 5-OCH_aH_b), 3.88–3.83 (3H, OCH_3; 4H, H-4, H-6, H-*c*, H-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 158.1 (1C, C-6), 155.3 (1C, C-2), 142.9 (1C, Cq., Ph), 137.1 (1C, C-5), 128.6 (2C, CH, Ph), 127.8 (1C, CH, Ph), 127.1 (2C, CH, Ph), 122.6 (1C, C-3); *DOABO–CH_2O*: 88.4 (2C, C-2, C-8), 74.5 (2C, C-4, C-6), 71.9 (1C, C-5), 70.8 (1C, CHOH), 69.5 (1C, 5-OCH_2), 54.1 (1C, OCH_3). MS (EI, 70 eV) *m/z* (rel int. %): 342 (9), 312 (4), 217 (5), 128 (100), 98 (10).

4.2.14. 3-[(*R)-4-Methoxy-α-hydroxybenzyl]-2-{[(1*R**,2*R**,5*S**)-2-phenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (3a-*R*) and 3-[(*S**)-4-methoxy-α-hydroxybenzyl]-2-{[(1*R**,2*R**,5*S**)-2-phenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (3a-*S*).** Nonseparable equimolar component mixture (82%) was obtained as yellow crystalline powder, mp 84–88 °C (column chromatography, eluent ligroin: AcOEt 2:1 v/v). [Found: C, 66.07; H, 5.76; N, 9.48. $C_{24}H_{25}N_3O_5$ requires: C, 66.19; H, 5.79; N, 9.65%]. R_f (67% ligroin:AcOEt) 0.20. ν_{max} (film KBr): 3449 (m), 2860 (m), 1611 (s), 1512 (s), 1418 (m), 1349 (w), 1256 (m), 1177 (m), 1028 (m), 911 (m), 853 (s), 754 (s), 701 (s), 615 (w), 571 (m), 494 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.17–8.15 (2H, m, H-5), 8.06–8.04 (2H, m, H-6), 7.50–7.43 (4H, m, Ph), 7.37–7.32 (6H, m, Ph), 7.18–7.12 (4H, m, Ph), 6.84–6.80 (4H, m, Ph), 5.75 (1H, d, *J*=6.4 Hz, C*H*–OH), 5.71 (1H, d, *J*=6.4 Hz, C*H*–OH), 5.00 (1H, d, *J*=6.4 Hz, OH), 4.94 (1H, d, *J*=6.4 Hz, OH); *DOABO–CH_2O*: 5.21 (1H, s, H-2-*t*), 5.16 (1H, s, H-2-*t*), 4.53 (1H, d, *J*=7.1 Hz, H-8-*c*), 4.49 (1H, d, *J*=7.1 Hz, H-8-*c*), 4.44 (1H, d, *J*=10.2 Hz, 5-OC*H_a*H_b), 4.41 (1H, d, *J*=10.2 Hz, 5-OCH_aH_b), 4.30 (1H, d, *J*=10.2 Hz, 5-OC*H_a*H_b), 4.296 (1H, d, *J*=9.0 Hz, H-4-*c*), 4.25 (1H, d, *J*=7.1 Hz, H-8-*t*), 4.22 (1H, d, *J*=10.2 Hz, 5-OCH_aH_b), 4.16 (1H, d, *J*=7.1 Hz, H-8-*t*), 3.95 (1H, d, *J*=9.0 Hz, H-4-*c*), 3.75 (2H, br s, H-4-*t*, H-6-*c*), 3.75 (3H, s, –OCH_3), 3.74 (3H, s, –OCH_3), 3.67 (1H, d, *J*=9.0 Hz, H-6-*c*), 3.62 (1H, d, *J*=9.0 Hz, H-4-*t*), 3.50 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.26 (1H, d, *J*=9.0 Hz, H-6-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 159.9, 159.8 (2C, 2C-2), 156.8 (2C, Cq., Ph), 146.4, 146.2 (2C, C-3), 140.3, 140.2 (2C, C-6), 139.5, 139.3 (2C, Cq., Ph), 135.4, 135.3 (2C, C-5), 134.1 (2C, Cq., Ph), 129.5, 128.9, 128.88, 128.8, 127.8, 127.78 (14C, CH, Ph), 114.4, 114.3 (4C, CH, Ph); *DOABO–CH_2O*: 99.2, 99.1 (2C, C-2), 85.1 (2C, C-8), 75.1 (2C, C-6), 73.5, 73.3 (2C, C-4), 72.3, 72.2 (2C, C-5), 71.3, 71.0 (2C, CHOH), 70.1, 69.6 (2C, 5-OCH_2), 55.7 (2C, –OCH_3). MS (ES^+) *m/z* (rel int. %): 422 (23), 313 (2), 295 (15), 237 (21), 202 (20), 146 (12).

4.2.15. 3-[(1*R)-2,2-Dimethyl-1-hydroxyprop-1-yl]-2-{[(1*R**,2*R**,5*S**)-2-phenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (3b-*R*) and 3-[(1*S**)-2,2-dimethyl-1-hydroxyprop-1-yl]-2-{[(1*R**,2*R**,5*S**)-2-phenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl]methoxy}-pyrazine (3b-*S*).** Yield 68%. Nonseparable equimolar component mixture was obtained as white crystalline powder, mp 140–142 °C (column chromatography, eluent ligroin:AcOEt 2:1 v/v). [Found: C, 65.61; H, 7.21; N, 11.05. $C_{21}H_{27}N_3O_4$ requires: C, 65.45; H, 7.01; N, 10.91%]. R_f (67% ligroin:AcOEt) 0.48. ν_{max} (KBr film): 3436 (m), 2956 (m), 2868 (m), 1161 (m), 1585 (w), 1511 (s), 1418 (m), 1315 (w), 1028 (m), 917 (m), 830 (m), 755 (s), 700, (m), 576 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.16 (2H, d, *J*=2.6 Hz, H-5), 8.03–8.02 (2H, m, H-6), 7.52–7.49 (4H, m, Ph), 7.36–7.34 (6H, m, Ph); *DOABO–CH_2O*: 5.26 (1H, s, H-2-*t*), 5.25 (1H, s, H-2-*t*), 4.64 (1H, d, *J*=9.7 Hz, C*H*–OH), 4.61 (1H, d, *J*=9.7 Hz, C*H*–OH), 4.59 (1H, d, *J*=7.1 Hz, H-8-*c*), 4.58 (1H, d, *J*=7.1 Hz, H-8-*c*), 4.56 (1H, d, *J*=10.6 Hz, 5-OC*H_a*H_b), 4.48 (1H, d, *J*=10.6 Hz, 5-OC*H_a*H_b), 4.37 (1H, d, *J*=9.0 Hz, H-4-*c*), 4.35 (1H, d, *J*=7.1 Hz, H-8-*t*), 4.34 (2H, d, *J*=10.6 Hz, 5-OCH_aH_b), 4.33 (1H, d, *J*=7.1 Hz, H-8-*t*), 4.32 (1H, d, *J*=9.0 Hz, H-4-*c*), 4.06 (1H, d, *J*=9.0 Hz, H-6-*c*), 4.04 (1H, d, *J*=9.0 Hz, H-6-*c*), 3.83 (2H, d, *J*=9.0 Hz, H-4-*t*), 3.73 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.70 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.58 (1H, d, *J*=9.7 Hz, OH), 3.56 (1H, d, *J*=9.7 Hz, OH), 0.905, 0.900 (18H, s, CH_3); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 157.0 (2C, C-2), 146.8 (2C, C-3), 139.9 (2C, C-6), 139.4 (2C, Cq., Ph), 136.1 (2C, C-5), 129.5, 128.8, 127.9, 127.8 (10C, CH,

Ph); *DOABO–CH_2O*: 99.3, 99.2 (2C, C-2), 85.3 (2C, C-8), 75.5, 75.4 (2C, C-4), 75.0, 74.9 (2C, CHOH), 73.6 (2C, C-6), 72.5 (2C, C-5), 70.2, 69.9 (2C, 5-OCH_2), 37.9 (2C, Cq., *t*-Bu), 26.3, 26.2 (6C, CH_3). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 385 (<1), 328 (23), 222 (30), 204 (100), 190 (83), 105 (13), 98 (60), 91 (20), 68 (54), 57 (26).

4.2.16. *rac*-5-(α-Hydroxybenzyl)-4,6-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidine (5a). Yield 82% taking into account the recovered starting material **4a**; total conversion: 71%. White crystalline powder, mp 121–122 °C (Et_2O:pentane 1:1 v/v) (column chromatography, eluent acetone). [Found: C, 58.71; H, 6.02; N, 11.87. $C_{23}H_{28}N_4O_7$ requires: C, 58.47; H, 5.97; N, 11.86%]. R_f (100% acetone) 0.80. ν_{max} (film NaCl): 3431 (s), 2925 (m), 2857 (s), 1574 (s), 1442 (m), 1300 (w), 1101 (s), 1011 (w), 1023 (w), 925 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.34 (1H, s, H-2), 7.30–7.23 (5H, m, Ph), 6.05 (1H, s, C*H*OH), 4.20 (1H, br s, OH); *DOABO–CH_2O*: 4.42 (4H, d, *J*=5.3 Hz, H-2, H-8-*c*), 4.41 (2H, d, *J*=10.9 Hz, 5-OCH_aH_b), 4.35 (2H, d, *J*=10.9 Hz, 5-OCH_aH_b), 4.35 (2H, d, *J*=5.3 Hz, H-2-*t*), 4.34 (2H, d, *J*=5.3 Hz, H-8-*t*), 3.70 (2H, d, *J*=9.0 Hz, H-4-*c*), 3.66 (2H, d, *J*=9.0 Hz, H-4-*t*), 3.65 (2H, d, *J*=9.4 Hz, H-6-*t*), 3.61 (2H, d, *J*=9.0 Hz, H-6-*c*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 167.4 (2C, C-4, C-6), 156.4 (1C, C-2), 143.2 (1C, Cq., Ph), 128.8 (2C, CH, Ph), 127.7 (1C, CH, Ph), 125.5 (2C, CH, Ph), 107.4 (1C, C-5); *DOABO–CH_2O*: 88.34, 88.26 (2C, C-2, C-8), 73.8, 73.7 (2C, C-4, C-6), 71.9 (2C, C-5), 69.5 (2C, 5-OCH_2); 67.0 (1C, CHOH). MS (EI, 70 eV) *m/z* (rel int. %): (M^+-1) 471 (<1), 455 (50), 328 (8), 128 (100), 98 (5).

4.2.17. *rac*-5-(α-Hydroxybenzyl)-4,6-bis[(*c*-2-*c*-8-diphenyl-3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-pyrimidine (5b). Yield 64%. White crystalline powder, mp 165–167 °C (flash column chromatography, eluent ligroin:AcOEt 3.5:1 v/v). [Found: C, 72.50; H, 5.90; N, 7.45. $C_{47}H_{44}N_4O_7$ requires: C, 72.66; H, 5.71; N, 7.21%]. R_f (77% ligroin:AcOEt) 0.51. ν_{max} (film KBr): 3580 (w), 2866 (m), 1578 (s), 1442 (s), 1304 (m), 1106 (s), 1064 (m), 945 (w), 916 (m), 718 (w), 699 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 8.31 (1H, s, H-2), 7.51–7.49 (8H, m, Ph), 7.34–7.30 (15H, m, Ph), 7.18 (2H, d, *J*=7.2 Hz, Ph), 5.93 (1H, d, *J*=10.8 Hz, C*H*OH), 3.08 (1H, d, *J*=10.8 Hz, OH); *DOABO–CH_2O*: 5.57, (2H, H-8-*t*), 5.55 (2H, s, H-2-*t*), 4.31 (2H, d, *J*=10.4 Hz, 5-OCH_aH_b), 4.25 (2H, d, *J*=10.4 Hz, 5-OCH_aH_b), 3.92 (2H, d, *J*=8.9 Hz, H-6-*c*), 3.81 (2H, d, *J*=8.9 Hz, H-6-*t*), 3.73 (2H, d, *J*=9.2 Hz, H-4-*c*), 3.69 (2H, d, *J*=9.2 Hz, H-4-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 167.3 (2C, C-4, C-6), 156.5 (1C, C-2), 143.2 (1C, Cq., Ph), 139.34, 139.3 (4C, Cq., Ph), 129.1, 129.0, 128.8, 127.7, 127.6, 125.5, 125.2 (25C, CH, Ph), 107.2 (1C, C-5); *DOABO–CH_2O*: 97.6, 97.59 (4C, C-2, C-8), 73.3, 73.2 (4C, C-4, C-6), 73.0 (2C, C-5), 71.0 (2C, 5-OCH_2), 66.7 (1C, CHOH). MS (FAB^+) *m/z* (rel int. %): (M^++1) 778 (100), 760 (25).

4.2.18. 6-Chloro-4-[^{2}*H*]-3-[(3,7-dioxa-*r*-1-azabicyclo-[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (7a) and 3-chloro-4-[^{2}*H*]-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-pyridazine (7b). Yield 47%. *Regioisomer* ***7a*** (15%): δ_H (300 MHz, $CDCl_3$) *aromatic*: 7.37 (1H, s, H-5). *Regioisomer* ***7b*** (32%): δ_H (300 MHz, $CDCl_3$) *aromatic*: 6.97 (1H, s, H-5). MS (EI, 70 eV) *m/z* (rel int. %): (M^++1) 259.6 (<1), 258 (<1), 257 (4), 196 (3), 157.5 (5), 128 (100), 98 (3).

4.2.19. *rac*-6-Chloro-4-(α-hydroxybenzyl)-3-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (7c) and *rac*-3-chloro-4-(α-hydroxybenzyl)-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (7d). Nonseparable component mixture was obtained as white crystalline powder, mp 154–167 °C (Et_2O:pentane 1:1 v/v) (column chromatography, eluent ligroin:acetone 1.8:1.0 v/v). [Found: C, 55.89; H, 5.21; N, 11.49. $C_{17}H_{18}N_3O_4Cl$ requires: C, 56.13; H, 4.99; N, 11.55%]. R_f (65% ligroin: acetone) 0.75. ν_{max} (film NaCl): 3417 (m), 3379 (s), 2868 (w), 1635 (m), 1586 (w), 1416 and 1414 (s), 1359 (s), 1095 (m), 1044 and 1041 (m), 930 and 928 (w), 754 and 749 (w), 703 (m) cm^{-1}. *Regioisomer* ***7c*** (53%): δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.69 (1H, s, H-5), 7.40–7.31 (3H, m, Ph), 7.28–7.20 (2H, m, Ph), 5.78 (1H, s, C*H*OH), 5.05 (1H, br s, OH); *DOABO–CH_2O*: 4.45 (1H, d, *J*=11.3 Hz, 5-OCH_aH_b), 4.44 (1H, d, *J*=5.5 Hz, H-2-*c*), 4.40 (1H, d, *J*=11.3 Hz, 5-OCH_aH_b), 4.39 (1H, d, *J*=5.5 Hz, H-2-*t*), 4.38 (1H, d, *J*=5.7 Hz, H-8-*c*), 4.34 (1H, d, *J*=5.7 Hz, H-8-*t*), 3.71 (2H, s, H-4-*c*, H-4-*t*), 3.45 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.38 (1H, d, *J*=9.0 Hz, H-6-*c*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 161.6 (1C, C-3), 152.6 (1C, C-4), 140.2 (1C, Cq., Ph), 136.2 (1C, C-6), 129.4 (2C, CH, Ph), 128.0 (1C, C-5), 127.7 (1C, CH, Ph), 127.5 (2C, CH, Ph); *DOABO–CH_2O*: 88.4, 88.3 (2C, C-2, C-8), 74.1, 73.9 (2C, C-4, C-6), 71.7 (1C, C-5), 70.7 (1C, CHOH), 70.3 (1C, 5-OCH_2). *Regioisomer* ***7d*** (31%): δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.47 (1H, s, H-5), 7.40–7.31 (3H, m, Ph), 7.28–7.20 (2H, m, Ph), 5.90 (1H, s, C*H*OH), 5.05 (1H, br s, OH); *DOABO–CH_2O*: 4.53 (1H, d, *J*=5.7 Hz, H-2-*c*), 4.53 (2H, m, 5-OCH_aH_b), 4.52 (1H, d, *J*=5.7 Hz, H-8-*c*), 4.46 (2H, d, *J*=5.7 Hz, H-2, H-8-*t*), 3.90 (4H, s, H-4, H-6, H-*c*, H-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 165.4 (1C, C-6), 150.5 (1C, C-4), 146.0 (1C, Cq., Ph), 140.4 (1C, C-3), 129.2 (2C, CH, Ph), 128.0 (2C, CH, Ph), 127.7 (1C, CH, Ph), 117.3 (1C, C-5); *DOABO–CH_2O*: 88.7 (2C, C-2, C-8), 74.4, 74.3 (2C, C-4, C-6), 72.1 (1C, CHOH), 71.9 (1C, C-5), 70.3 (1C, 5-OCH_2). MS (EI, 70 eV) *m/z* (rel int. %): (M^+) 363.6 (5), 346 (7), 128 (100), 98 (14).

4.2.20. *rac*-4-(α-Hydroxybenzyl)-3-methoxy-6-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazine (7e). Yield 78%. Yellowish crystalline powder, mp 44–46 °C (pentane) (column chromatography, eluent ligroin:acetone 1.5:1 v/v). [Found: C, 60.40; H, 5.99; N, 11.79. $C_{18}H_{21}N_3O_5$ requires: C, 60.16; H, 5.89; N, 11.69%]; R_f (60% ligroin:acetone) 0.40. ν_{max} (film NaCl): 3297 (s), 2945 (s), 2866 (s), 1620 (w), 1461 (s), 1412 (s), 1381 (s), 1227 (w), 1133 (w), 1023 (s), 928 (m), 751 (s), 700 (m) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic*: 7.28–7.15 (6H, m, pyridazine, Ph), 5.74 (1H, br s, C*H*OH), 4.28 (1H, br s, OH); *DOABO–CH_2O*: 4.37 (2H, d, *J*=5.7 Hz, H-2, H-8-*c*), 4.32 (2H, s, 5-OCH_aH_b), 4.31 (2H, d, *J*=5.7 Hz, H-2, H-8-*t*), 3.86 (3H, s, OCH_3), 3.76 (4H, s, H-4, H-6, H-c, H-*t*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 162.6 (1C, C-6), 159.9 (1C, C-3), 141.3 (1C, Cq., Ph), 138.5 (1C, C-4), 128.9 (2C, CH, Ph), 128.6 (1C, CH, Ph), 127.4

(2C, CH, Ph), 117.2 (1C, C-5); *DOABO–CH_2O*: 88.5 (2C, C-2, C-8), 74.4 (2C, C-4, C-6), 71.9 (1C, 5-OCH_2), 70.0 (1C, CHOH), 69.4 (1C, C-5), 55.0 (1C, OCH_3). δ_H (300 MHz, [D_6]benzene) *DOABO–CH_2O*: 4.46 (1H, d, *J*=10.7 Hz, 5-OC$H_a$$H_b$), 4.39 (1H, d, *J*=10.7 Hz, 5-OC$H_a$$H_b$), 4.26 (1H, d, *J*=5.3 Hz, H-2-*c*), 4.24 (1H, d, *J*=5.3 Hz, H-8-*c*), 4.054 (1H, d, *J*=5.3 Hz, H-2-*t*), 4.046 (1H, d, *J*=5.5 Hz, H-8-*t*), 3.66 (1H, d, *J*=8.7 Hz, H-4-*c*), 3.62 (1H, d, *J*=8.7 Hz, H-6-*c*), 3.57 (1H, d, *J*=8.7 Hz, H-4-*t*), 3.55 (1H, d, *J*=8.7 Hz, H-6-*t*). MS (EI, 70 eV) *m/z* (rel int. %): (M^+−1) 358 (10), 342 (20), 245 (15), 128 (100), 98 (9).

4.2.21. *rac*-4-(α-Hydroxybenzyl)-3,6-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]octane-*c*-5-yl)methoxy]-pyridazine (7f). This compound was only identified in both the NMR spectra of the crude reaction mixture (together with the starting **6c**) and after unsuccessful attempt of separation by column chromatography (as mixture 36% **7f**+64% **6c**). δ_H (300 MHz, $CDCl_3$) *(hetero)aromatic, only distinct peaks are listed*: 7.35–7.20 (5H, m, Ph), 6.96 (1H, H-5), 5.72 (1H, br s, C*H*OH), 4.81 (1H, br s, OH); *DOABO–CH_2O C-3, C-6*: 4.52–4.30 (12H, irresolvable multiplet, H-2, H-8, H-*c*, H-*t*, 5-OCH_2); *DOABO–CH_2O linked at C-6*: 3.86 (4H, s, H-4, H-6, H-*c*, H-*t*); *DOABO–CH_2O linked at C-3*: 3.68 (2H, s, H-4-*c*, H-4-*t*), 3.43 (1H, d, *J*=9.0 Hz, H-6-*t*), 3.35 (1H, d, *J*=9.0 Hz, H-6-*c*); δ_C (75 MHz, $CDCl_3$) *(hetero)aromatic*: 162.9 (1C, C-3), 162.1 (1C, C-6), 159.4 (1C, C-4), 140.9 (1C, Cq., Ph), 129.2 (2C, CH, Ph), 129.1 (1C, CH, Ph), 127.5 (2C, CH, Ph), 117.9 (1C, C-5), 70.8 (1C, CHOH); *DOABO–CH_2O linked at C-3*: 88.4, 88.3 (2C, C-2, C-8), 74.3, 74.1 (2C, C-4, C-6), 71.8 (1C, 5-OCH_2), 69.7 (1C, C-5); *DOABO–CH_2O linked at C-6*: 88.7 (2C, C-2, C-8), 74.4 (2C, C-4, C-6), 71.9 (1C, 5-OCH_2), 69.8 (1C, C-5).

4.3. General procedure for the preparation of compounds 9a–d by cross-coupling under Stille conditions

In dry toluene (25 mL) and under a dry nitrogen atmosphere, 2,6-bis(tri-*n*-butylstannyl)pyrazine **8** 0.493 g (0.75 mmol) and chlorodiazine **1d**, **4c** and **6a** 0.405 g (1.575 mmol, 2.10 equiv) (0.630 g, 1.575 mmol, 2.10 equiv in the case of **4d**) were dissolved with stirring. $Pd(PPh_3)_4$ 0.091 g (0.079 mmol, 5% with respect to chlorodiazine) was rapidly added. The solution was heated at reflux for 22–48 h (Table 4) until the TLC monitoring (UV 254 nm) indicated the starting materials in traces only (compounds **9a**–**c**) or no more significant evolution of the reaction (in the case of compound **9d**), **8** (ligroin:AcOEt 50:1 v/v), **1d** (ligroin: AcOEt 2:1 v/v), **4c** and **6a** (ligroin:acetone 3:1 v/v), **4d** (ligroin:acetone 2:1 v/v). A second elution system was used to detect the desired products **9a**–**d** as shown below. During all the syntheses, Pd metal precipitated abundantly. The reaction mixture was filtered hot (100 °C) and the solids were washed (×50 mL) several times with hot EtOH. The combined organic filtrate was evaporated under vacuum and the solid residue was directly crystallised from an appropriate solvent or subjected to column chromatography to yield the title compounds **9a**–**d**.

4.3.1. 2,6-Bis{6′-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrazin-2′-yl}-pyrazine (9a). Yield 65%. Grey crystalline powder, mp 218 °C (dec, EtOH). [Found: C, 55.25; H, 5.15; N, 21.55. $C_{24}H_{26}N_8O_6$ requires: C, 55.17; H, 5.02; N, 21.45%]. R_f (95% dichloromethane–ethanol) 0.52. ν_{max} (film KBr): 3401 (m), 2875 (m), 1539 (s), 1402 (s), 1369 (m), 1215 (s), 939 (s), 898 (w), 782 (m), 730 (w) cm^{-1}. δ_H (300 MHz, CF_3CD_2OD) *heteroaromatic*: 9.28 (2H, s, H-3, H-5), 9.04 (2H, s, H-3′), 8.05 (2H, s, H-5′); *DOABO–CH_2O*: 4.40 (4H, s, 5-OCH_2), 4.36 (4H, d, *J*=6.2 Hz, H-2, H-8-*c*), 4.33 (4H, d, *J*=6.2 Hz, H-2, H-8-*t*), 3.80 (8H, s, H-4, H-6, H-*c*, H-*t*); δ_C (75 MHz, CF_3CD_2OD) *heteroaromatic*: 157.0 (2C, C-6′), 146.4 (2C, C-2′), 143.5 (2C, C-2, C-6), 139.3 (2C, C-3, C-5), 133.1 (2C, C-5′), 131.2 (2C, C-3′); *DOABO–CH_2O*: 85.1 (4C, C-2, C-8), 71.2 (4C, C-4, C-6), 69.1 (2C, C-5), 65.1 (2C, 5-OCH_2). MS (FAB^+) *m/z* (rel int. %): (M^++1) 523 (14), 289 (100), 235 (30), 165 (48).

4.3.2. 2,6-Bis{6′-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidin-4′-yl}-pyrazine (9b). Yield 60%. Yellowish crystalline powder, mp 222–225 °C (flash column chromatography, AcOEt 100%). [Found: C, 55.10; H, 5.10; N, 21.50. $C_{24}H_{26}N_8O_6$ requires: C, 55.17; H, 5.02; N, 21.45%]; R_f (100% AcOEt) 0.42. ν_{max} (film KBr): 3468 (w), 2864 (m), 1598 (s), 1538 (s), 1427 (s), 1346 (m), 1316 (w), 1096 (s), 753 (m), 680 (w), 569 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 9.73 (2H, s, H-3, H-5), 8.90 (2H, s, H-2′), 7.88 (2H, s, H-5′); *DOABO–CH_2O*: 4.57 (4H, d, *J*=5.5 Hz, H-2, H-8-*c*), 4.55 (4H, s, 5-OCH_2), 4.50 (4H, d, *J*=5.5 Hz, H-2, H-8-*t*), 3.93 (8H, s, H-4, H-6, H-*c*, H-*t*); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 170.6 (2C, C-6′), 162.1 (2C, C-4′), 158.8 (2C, C-2′), 148.1 (2C, C-2, C-6), 145.2 (2C, C-3, C-5), 105.4 (2C, C-5′); *DOABO–CH_2O*: 88.6 (4C, C-2, C-8), 74.3 (4C, C-4, C-6), 72.0 (2C, C-5), 69.3 (2C, 5-OCH_2). MS (FAB^+) *m/z* (rel int. %): (M^++1) 523 (10), 283 (<1), 136 (35), 128 (100).

4.3.3. 2,6-Bis{2′,6′-bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]-oct-*c*-5-yl)methoxy]-pyrimidin-4′-yl}-pyrazine (9c). Yield 70%. Grey crystalline powder, mp 190 °C (dec). [Found: C, 53.35; H, 5.37; N, 17.50. $C_{36}H_{44}N_{10}O_{12}$ requires: C, 53.46; H, 5.48; N, 17.32%]. R_f (50% acetone–dichloromethane) 0.56. ν_{max} (film KBr): 3477 (m), 2954 (m), 2867 (s), 1572 (s), 1440 (w), 1407 (m), 1329 (s), 1118 (m), 1043 (m), 929 (m), 750 (m), 676 (w) cm^{-1}. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 9.65 (2H, s, H-3, H-5), 7.54 (2H, s, H-5′); *DOABO–CH_2O*: 4.56 (8H, d, *J*=5.3 Hz, H-2, H-8-*c*), 4.51 (8H, s, 5-OCH_2), 4.49 (4H, d, *J*=5.3 Hz, H-2, H-8-*c*), 4.47 (4H, d, *J*=5.3 Hz, H-2, H-8-*t*), 3.98 (8H, d, *J*=9.6 Hz, H-4, H-6-*c*), 3.89 (8H, d, *J*=9.6 Hz, H-4, H-6-*t*); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 172.6 (2C, C-6′), 165.2 (2C, C-2′), 163.6 (2C, C-4′), 147.9 (2C, C-2, C-6), 145.2 (2C, C-3, C-5), 100.0 (2C, C-5′); *DOABO–CH_2O*: 88.6, 88.4 (8C, C-2, C-8), 74.7, 74.3 (8C, C-4, C-6), 71.9, 71.8 (4C, C-5), 71.0, 69.6 (4C, 5-OCH_2). MS (FAB^+) *m/z* (rel int. %): (M^++1) 809.8 (93), 459.9 (53), 391 (100).

4.3.4. 2,6-Bis{6′-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazin-3′-yl}-pyrazine (9d). Yield 22%. Yellow crystalline powder, mp 190 °C (dec) (flash column chromatography, eluent ligroin:acetone:ethanol 2:1:1 v/v/v). [Found: C, 55.32; H, 4.97; N, 21.30. $C_{24}H_{26}N_8O_6$ requires: C, 55.17; H, 5.02; N, 21.45%]. R_f (ligroin:acetone: ethanol 50:25:25) 0.48. ν_{max} (film KBr): 3400 (m), 2871 (m), 1593 (m), 1417 (s), 1378 (w), 1310 (s), 1134 (m), 1098 (m), 930 (m), 860 (w), 753 (w) cm^{-1}. δ_H (300 MHz,

$CDCl_3$) *heteroaromatic*: 9.85 (2H, s, H-3, H-5), 8.54 (2H, d, J=9.2 Hz, H-4′), 7.21 (2H, d, J=9.2 Hz, H-5′); *DOABO–CH_2O*: 4.73 (4H, s, 5-OCH_2), 4.59 (4H, d, J=5.7 Hz, H-2, H-8-*c*), 4.53 (4H, d, J=5.7 Hz, H-2, H-8-*t*), 3.96 (8H, s, H-4, H-6, H-*c*, H-*t*); δ_C (75 MHz, $CDCl_3$) *heteroaromatic*: 165.4 (2C, C-6′), 153.9 (2C, C-3′), 147.6 (2C, C-2, C-6), 143.3 (2C, C-3, C-5), 128.3 (2C, C-4′), 118.4 (2C, C-5′); *DOABO–CH_2O*: 88.8 (4C, C-2, C-8), 74.4 (4C, C-4, C-6), 72.0 (2C, C-5), 70.4 (2C, 5-OCH_2). MS (FAB^+) *m/z* (rel int. %): ($M+Na^+$) 545 (2), (M^++1) 523 (39), 283 (100), 128 (55), 95 (20).

4.3.5. 6,6′-Bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-4,4′-bipyrimidine (10a). This compound was identified as side product in the crude reaction mixture of the synthesis of the compound **9a** in 6% occurrence. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 8.83 (2H, s, H-2, H-2′), 7.79 (2H, s, H-5, H-5′). MS (FAB^+) *m/z* (rel int. %): (M^++1) 475 (<1), 289 (29).

4.3.6. 6,6′-Bis[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)-methoxy]-3,3′-bipyridazine (10b). This compound was identified as side product in the crude reaction mixture of the synthesis of the compound **9d** in 35% occurrence. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 8.61 (2H, d, J=9.3 Hz, H-4, H-4′), 7.16 (2H, d, J=9.3 Hz, H-5, H-5′). MS (FAB^+) *m/z* (rel int. %): (M^++1) 445 (6).

4.3.7. 6,6′-Bis{6″-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyrimidin-4″-yl}-2,2′-bipyrazine (11a). This compound was identified as side product in the crude reaction mixture of the synthesis of the compound **9b** in 12% occurrence. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 9.84 (2H, s, H-3, H-3′), 9.75 (2H, s, H-5, H-5′), 8.92 (2H, s, H-2″), 7.92 (2H, s, H-5″). MS (FAB^+) *m/z* (rel int. %): (M^++1) 630 (<1), 588 (<1), 564 (3).

4.3.8. 6,6′-Bis{6″-[(3,7-dioxa-*r*-1-azabicyclo[3.3.0]oct-*c*-5-yl)methoxy]-pyridazin-3″-yl}-2,2′-bipyrazine (11b). This compound was identified as side product in the crude reaction mixture of the synthesis of the compound **9d** in 15% occurrence. δ_H (300 MHz, $CDCl_3$) *heteroaromatic*: 9.89 (2H, s, H-3, H-3′), 9.75 (2H, s, H-5, H-5′), 8.62 (2H, d, J=9.2 Hz, H-4″), 7.23 (2H, d, J=9.2 Hz, H-5″). MS (FAB^+) *m/z* (rel int. %): 585 (<1), 564 (4).

Acknowledgements

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References and notes

1. Mongin, F.; Quéguiner, G. *Tetrahedron* **2001**, *57*, 4059–4090.
2. Turck, A.; Plé, N.; Mongin, F.; Quéguiner, G. *Tetrahedron* **2001**, *57*, 4489–4505.
3. Zhang, C. Y.; Tour, J. M. *J. Am. Chem. Soc.* **1999**, *121*, 8783–8790.
4. Lavergne, O.; Lesueur-Ginot, L.; Pla Rodas, F.; Kasprzyk, P. G.; Pommier, J.; Demarquay, D.; Prévost, G.; Ulibarri, G.; Rolland, A.; Schiano-Liberatore, A.-M.; Harnett, J.; Pons, D.; Camara, J.; Bigg, D. C. H. *J. Med. Chem.* **1998**, *41*, 5410–5419.
5. Henegar, K. E.; Ashford, S. W.; Baughman, T. A.; Sih, J. C.; Gu, R.-L. *J. Org. Chem.* **1997**, *62*, 6588–6597.
6. Sammakia, T.; Hurley, T. B. *J. Org. Chem.* **1999**, *64*, 4652–4664.
7. Remuzon, P.; Bouzard, D.; Jacquet, J.-P. *Heterocycles* **1993**, *36*, 431–434.
8. Part I of our report and the literature cited therein.
9. Darabantu, M.; Maiereanu, C.; Silaghi-Dumitrescu, I.; Toupet, L.; Condamine, E.; Ramondenc, Y.; Berghian, C.; Plé, G.; Plé, N. *Eur. J. Org. Chem.* **2004**, *12*, 2644–2661.
10. Maiereanu, C.; Darabantu, M.; Plé, G.; Berghian, C.; Condamine, E.; Ramondenc, Y.; Silaghi-Dumitrescu, I.; Mager, S. *Tetrahedron* **2002**, *58*, 2681–2693.
11. Darabantu, M.; Plé, G.; Silaghi-Dumitrescu, I.; Maiereanu, C.; Turos, I.; Silberg, I. A.; Mager, S. *Tetrahedron* **2000**, *56*, 3785–3798.
12. Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508–524.
13. Labadie, J. W.; Stille, J. K. *J. Am. Chem. Soc.* **1983**, *105*, 6129–6137.
14. Darabantu, M.; Boully, L.; Turck, A.; Plé, N. *Tetrahedron* **2005**, *61*, 2897–2905.
15. Snieckus, V. *Chem. Rev.* **1990**, *90*, 879–933.
16. Queguiner, G.; Marsais, F.; Sniekus, V.; Epsztajn, J. *Adv. Heterocycl. Chem.* **1991**, *52*, 187–304.
17. Ward, J. S.; Merritt, L. *J. Heterocycl. Chem.* **1991**, *28*, 765–768.
18. Turck, A.; Trohay, D.; Mojovic, L.; Plé, N.; Quéguiner, G. *J. Organomet. Chem.* **1991**, *412*, 301–310.
19. Turck, A.; Plé, N.; Quéguiner, G. *Heterocycles* **1994**, *37*, 2149–2172.
20. Turck, A.; Plé, N.; Dognon, D.; Harmoy, C.; Quéguiner, G. *J. Heterocycl. Chem.* **1994**, *31*, 1449–1453.
21. Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301–315.
22. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565–569.
23. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
24. Willker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287–292.
25. Jeener, J.; Meier, B. H.; Bachman, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4553.
26. Parella, T.; Sanchez-Ferrando, F.; Virgili, A. *J. Magn. Reson.* **1997**, *125*, 145–148.
27. Mojovic, L.; Turck, A.; Plé, N.; Dorsy, M.; Ndzi, B.; Quéguiner, G. *Tetrahedron* **1996**, *52*, 10417–10426.
28. Turck, A.; Plé, N.; Ndzi, B.; Quéguiner, G.; Haider, N.; Schuller, H.; Heinisch, G. *Tetrahedron* **1993**, *49*, 599–606.
29. Turck, A.; Plé, N.; Mojovic, L.; Quéguiner, G. *Bull. Soc. Chim. Fr.* **1993**, *130*, 488–492.

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Azedaralide: total synthesis, relative and absolute stereochemical assignment

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Abstract—Azedaralide, a potentially advanced intermediate for the total synthesis of various tetranortriterpenes, was constructed utilising the Fernández-Mateos protocol and assigned both relative and absolute stereochemistries. Both asymmetric aldol and classical chiral resolution attempts failed to deliver pure enantiomers whereas preparative chiral chromatography resolved racemic azedaralide with ease.

1. Introduction

Azedaralide **1**, isolated from *Melia azedarach* (1.7 mg from 1.5 kg root bark) by Nakatani et al.,[1] displays antifeedant and ichthyotoxic properties, and is to date the only δ-lactone degraded limonoid isolated from *M. azedarach*.[1] In addition, azedaralide **1** appears to have the required connectivity to act as an advanced intermediate for total syntheses studies of the tetranortriterpenes (limonoids) andirobin **2** and mexicanolide **3** (Fig. 1).[2] In this regard two distinctly different approaches to viable quantities of azedaralide **1** were investigated, the results of which are described herein.

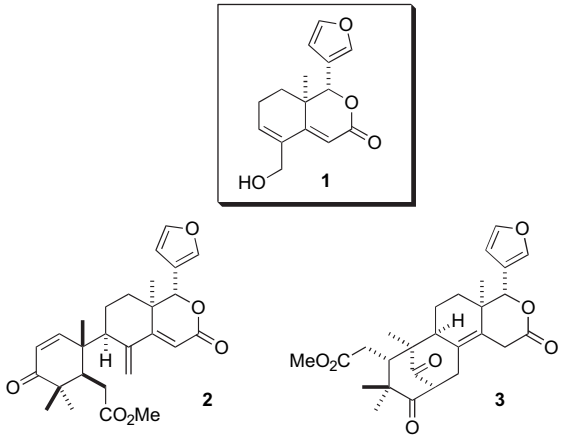

Figure 1.

2. Results and discussion

Due to the large amount of work already conducted on the total synthesis of *dl*-pyroangolensolide[3–7] **4**, the most simplistic, yet logically direct approach to azedaralide **1**, that being exocyclic allylic oxidation was examined in the first instance. Numerous methods to effect allylic oxidation were exhaustively investigated, for example, SeO_2/*t*-BuO_2H,[8] $Pd(OAc)_2$[9] and $Hg(OAc)_2$,[10] but in all cases these procedures provided epoxide **5** and/or starting material **4**. However, selenium dioxide or selenious acid in dioxane[11] afforded azedaralide **1**[12] in 17% yield along with the corresponding aldehyde **6** (28%), resulting from over oxidation (Fig. 2).

Although access to **1** using the selenium dioxide protocol was rapid, the disappointing yield and purity indicated that an alternative strategy was required. Of the procedures

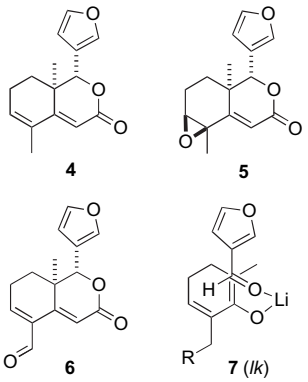

Figure 2.

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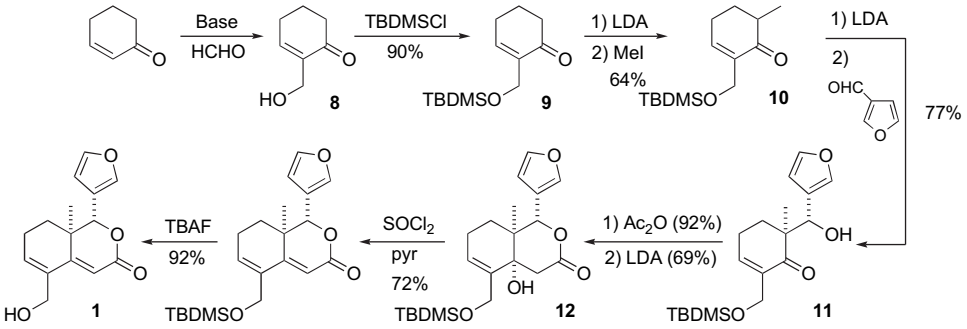

Scheme 1.

available for the construction of *dl*-pyroangolensolide[3–7] **4**, Fernández-Mateos[7] maintains an unrivalled sequence (stereospecific, four steps, 62% overall yield). On closer inspection of the proposed transition state[13] [*lk* approach, **7** (R=H)] the allylic methyl group seems not to play a role in the stereochemical outcome of the reaction, hence, additional functionality, for example, an ether unit (**7**, R=OR′) should be easily accommodated without transition state disruption (Fig. 2).

Installation of the oxygen functionality was best achieved using the El Gaïed (DMAP or imidazole)[14] Baylis–Hillman variants (Scheme 1). Alcohol **8** was then protected as a *t*-BDMS ether (i.e., **9**) (90%) and methylated (i.e., **10**) (64%). The Fernández-Mateos stereospecific aldol proceeded without incident, as predicted, giving **11** in 77% yield, which was routinely acetylated (92%) and cyclised affording the hydroxylactone **12** (69%). Elimination (72%) and deprotection (92%) revealed azedaralide **1** [19% overall yield, eight steps], as confirmed by X-ray crystal structure analysis of the racemate (Fig. 3).

In an attempt to introduce asymmetry into the racemic sequence seen in Scheme 1, both asymmetric aldol and classical chiral resolution type protocols were investigated. For example, asymmetric aldol reactions with **10** and 3-furaldehyde failed (e.g., DIP-Cl[15]), and difficulties were also encountered in converting **10** into the SAMP derivative.[16] Classical chiral resolution using covalent chiral auxiliaries was attempted by converting **11** to the (–)-menthoxy acetate

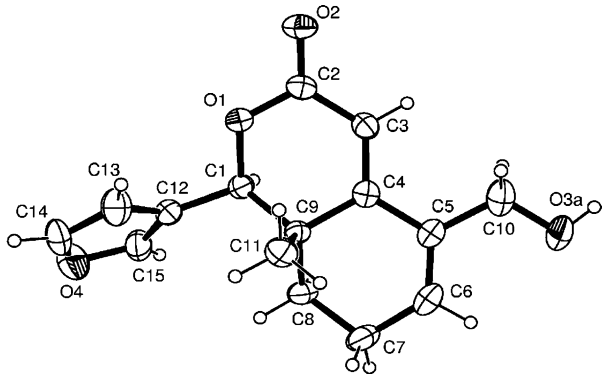

Figure 3. ORTEP3 view of **1** crystallised as a racemate (30% probability ellipsoids).

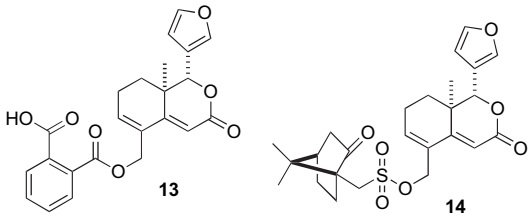

Figure 4.

and (+)-Mosher's ester[17] derivatives. Although the diastereomers could be easily seen by NMR analysis separation was not achieved. Non-covalent methods, for example, treating the phthalate derivative **13** with α-methylbenzylamine[18] (and brucine) did not yield crystals (Fig. 4). Lipases, both acetylase (lipase PS) and deacetylase (lipase PS)[19,20] protocols failed to react with either the alcohol **11** or corresponding acetate.

Preparative chiral chromatography on the other hand completely resolved the mixture of azedaralide enantiomers. This task including transpacific transportation required only days in comparison to months investigating failed asymmetric and classical protocols described above. The (+)-enantiomer was then converted to the crystalline sulfonate **14** (Fig. 4) using 1*S*-(+)-10-camphorsulfonyl chloride,[21] which gave the X-ray crystal structure shown in Figure 5. Considering the predetermined absolute stereochemistry of the camphorsulfonyl chloride [via the sulfonic acid (*R*,*S*)] the absolute stereochemistry of the (+)-enantiomer, as demonstrated in the X-ray crystal structure, must be *R*,*R*. The (+)-enantiomer has a rotation of +385.0, which is at variance with that reported rotation (+165)[1] for the isolated natural product. Considering that only 1.7 mg of the isolated natural product were obtained and that the ^{1}H NMR of the material revealed impurities,[12] we are inclined to accept our optical rotation value as more accurate.

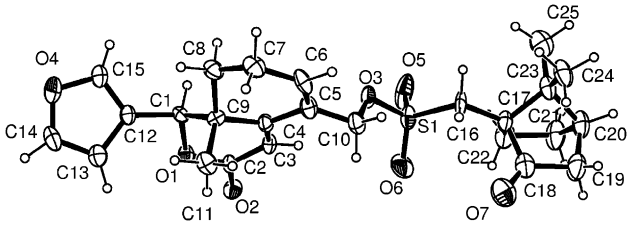

Figure 5. ORTEP3 view of **14** (30% probability ellipsoids shown).

3. Conclusion

In conclusion, capitalising on the ingenious stereospecific Fernández-Mateos protocol has allowed a direct synthesis of azedaralide **1** in viable quantities. Furthermore, the current sequence (eight steps, 19% overall yield) is a near 70 fold improvement in overall yield on the only tetranortriterpene advanced intermediate **15** reported[5] so far (15 steps, 0.27% overall yield) (Fig. 6). Chiral chromatography in conjunction with X-ray crystal structure analysis was paramount for elucidating and confirming Nakatani's proposed configuration of azedaralide **1**.

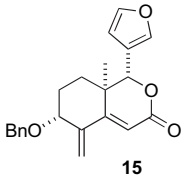

Figure 6.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on Bruker AV400 (400.13 MHz; 100.62 MHz), AV300 (300.13 MHz; 75.47 MHz) and DRX (or AV) 500 (500.13 MHz; 125.77 MHz) instruments in deuteriochloroform ($CDCl_3$). Coupling constants are given in Hertz and chemical shifts are expressed as δ values in parts per million. High and low resolution EI mass spectral data were obtained on a KRATOS MS 25 RFA. Electrospray mass spectrometry was performed on a Finnigan MAT 900 XL-Trap. Microanalyses were performed by the University of Queensland Microanalytical Service. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Chiral chromatography was performed on a Berger Multigram SFC (Mettler–Toledo) using a Chiralpak AS-H-SFC column 2.1 dia×25 cm long (Chiral Technologies) and a K-2501 UV detector (Knauer) with an eluent mixture of CO_2/MeOH 80/20 flowing at 25 mL/min. Injection, detection and collection were controlled by AutoPrep software (PDR-Chiral). IR spectra were obtained on a Perkin Elmer FTIR Spectrometer, Spectrum 2000.

4.2. X-ray crystallography

Data for all compounds were collected at 293 K on an Enraf–Nonius CAD4 diffractometer. Data reduction, direct methods structure solution and full least squares refinement (SHELX97[22]) were performed with the WINGX package.[23] Drawings of all molecules were created with ORTEP3.[24] Data in CIF format have been deposited with the Cambridge Crystallographic Data Centre (CCDC deposition numbers 601060 and 601061). Copies of the data can be obtained free of charge upon request to deposit@ccdc.cam.ac.uk.

4.2.1. Reaction of pyroangolensolide 4 with selenium dioxide. *dl*-Pyroangolensolide **4** (500 mg, 2.05 mmol) and freshly sublimed selenium dioxide (341 mg, 3.07 mmol) were heated at reflux in 1,4-dioxane (34 mL) for 20 h. A colour change from pale yellow to dark brown was observed after 5–10 min. On cooling the solvent was removed under high vacuum and the residue was redissolved in dichloromethane and filtered. The filtrate was washed with 2 M hydrochloric acid, water and brine followed by drying (Na_2SO_4) and evaporation in vacuo. The residue was then reacted under the same conditions as above and after work up was subjected to column chromatography (2:1 diethyl ether/petroleum spirit) which afforded two fractions.

Fraction 1 afforded aldehyde **6** (148 mg, 28%) as white crystals. mp 147–149 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.02 (s, 3H), 1.42–1.51 (m, 1H), 1.56–1.62 (m, 1H), 2.47–2.58 (m, 1H), 2.62–2.75 (m, 1H), 5.14 (s, 1H), 6.43–6.44 (m, 1H), 7.17–7.19 (m, 1H), 7.21 (s, 1H), 7.41–7.42 (m, 1H), 7.46–7.48 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.0, 23.5, 29.1, 36.9, 80.2, 109.9, 115.1, 120.0, 133.1, 141.2, 143.1, 151.4, 158.4, 165.1, 191.7; MS (EI) *m*/*z* (%): 258 ($M^{+\bullet}$, 2), 214 (1), 178 (1), 162 (100), 147 (14), 134 (27), 119 (21), 105 (16), 91 (25). Anal. Calcd for $C_{15}H_{14}O_4$: C, 70.03; H, 5.09. Found: C, 70.08; H, 5.45%.

Fraction 2 afforded azedaralide **1**, which contained an unidentified impurity (<10%) (92 mg, 17%). Characterisation data below.

4.2.2. 2-[(*tert*-Butyldimethylsilyloxy)methyl]-2-cyclohexenone 9. 2-Hydroxymethyl-2-cyclohexenone **8** (5 g, 40.32 mmol) was dissolved in anhydrous dichloromethane (100 mL) and anhydrous triethylamine (13.25 mL) under an argon atmosphere. To this was added in one portion, *tert*-butyldimethylsilyl chloride (7.17 g, 47.56 mmol) and the mixture was allowed to stir for 24 h at room temperature. The reaction mixture was poured into a separatory funnel containing saturated sodium hydrogen carbonate (25 mL), and the organic layer partitioned. The aqueous layer was extracted with dichloromethane (3×30 mL) and the combined organic phases were washed with water, dried (Na_2SO_4), evaporated and the residue subjected to column chromatography (2:1 petroleum spirit/diethyl ether), yielding the titled compound **9** as a pale yellow oil (8.56 g, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.05 (s, 6H), 0.90 (s, 9H), 1.98 (p, J=6.3 Hz, 2H), 2.37–2.41 (m, 4H), 4.33 (AB, 2H), 6.97–6.99 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: −5.5, −3.6, 18.3, 22.9, 25.55, 25.6, 25.9, 38.3, 60.0, 138.3, 143.9, 199.0; Near IR (Neat) ν (cm^{-1}) 1671, 1461, 1399; MS (EI) *m*/*z* (%): 240 ($M^{+\bullet}$, 1), 225 (4), 193 (3), 183 (100), 167 (1), 151 (9), 142 (1), 127 (2), 117 (1), 109 (1), 101 (1), 91 (3); HRMS calcd for $C_{13}H_{24}O_2Si$ 240.1545, Found 240.1548.

4.2.3. 2-[(*tert*-Butyldimethylsilyloxy)methyl]-6-methyl-2-cyclohexenone 10. To a cold (0 °C) stirring solution of diisopropylamine (6.16 mL, 43.7 mmol) in anhydrous tetrahydrofuran (50 mL) under an argon atmosphere, was added *n*-butyl lithium (1 M in hexanes, 31.5 mL, 41.6 mmol) over a period of 10 min. After a further 45 min, 2-[(*tert*-butyldimethylsilyloxy)methyl]-2-cyclohexenone **9** (10 g, 41.6 mmol) in tetrahydrofuran (50 mL) was added dropwise via

cannula. The reaction was stirred at 0 °C for a further 60 min, before dropwise addition of iodomethane (7.8 mL, 125 mmol). Saturated sodium hydrogen carbonate (40 mL) was added to the cold solution after 30 min, and the suspension was allowed to warm to room temperature (2 h). Extraction with petroleum spirit (4×100 mL), followed by washing with brine, drying (Na_2SO_4) and evaporation resulted in an oily residue. Column chromatography (5:1 petroleum spirit/diethyl ether) afforded **10** as an orange-brown oil (6.8 g, 64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.05 (s, 6H), 0.90 (s, 9H), 1.11 (d, J=6.83 Hz, 3H), 1.66–1.74 (m, 1H), 2.00–2.06 (m, 1H), 2.37–2.41 (m, 2H), 4.29–4.35 (m, 2H), 6.91–6.92 (br m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: −5.4, 1.0, 15.0, 18.4, 25.0, 25.9, 29.7, 31.0, 41.7, 60.2, 137.7, 143.0, 201.6; MS (EI) m/z (%): 254 ($M^{+\bullet}$, 1), 239 (4), 225 (5), 211 (2), 207 (6), 197 (99), 193 (6), 183 (100), 167 (6), 155 (7), 153 (9), 151 (12), 127 (5), 117 (2), 105 (7), 91 (8); HRMS calcd for $C_{14}H_{26}O_2Si$ 254.1702, Found 254.1699.

4.2.4. 2-[(*tert*-Butyldimethylsilyloxy)methyl]-6-[(furan-3-yl)hydroxymethyl]-6-methyl-2-cyclohexenone 11. To a stirred solution of diisopropylamine (5 mL, 35.7 mmol) in anhydrous tetrahydrofuran (100 mL) at 0 °C under an argon atmosphere, was added *n*-butyl lithium (1.32 M in hexanes, 25.8 mL, 34.1 mmol) dropwise over a period of 5 min. After 25 min at 0 °C, the solution was cooled to −78 °C and a solution of 2-[(*tert*-butyldimethylsilyloxy)methyl]-6-methyl-2-cyclohexenone **10** (5 g, 19.7 mmol) in anhydrous tetrahydrofuran (25 mL) was added via cannula (3 min). The reaction was stirred for 3 h at −78 °C before quenching with saturated ammonium chloride solution (30 mL) and slow warming to room temperature (12 h). The organic layer was partitioned and the aqueous layer extracted with dichloromethane (4×50 mL). The combined organic layers were washed with water and brine, dried (Na_2SO_4) and evaporated. Column chromatography of the residue (5:1 petroleum spirit/diethyl ether) afforded the desired product **11** as a pale yellow oil (5.3 g, 77%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.06 (s, 6H), 0.90 (s, 9H), 1.17 (s, 3H), 1.49–1.53 (m, 1H), 1.69–1.75 (m, 1H), 2.37–2.41 (br m, 2H), 4.25–4.39 (m, 2H), 4.89 (s, 1H), 6.36 (s, 1H), 6.97 (br s, 1H), 7.35 (d, J=1.5 Hz, 1H), 7.36 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: −5.5, 14.5, 18.3, 22.3, 25.9, 31.1, 47.5, 60.1, 71.5, 110.1, 123.9, 136.6, 140.5, 142.5, 144.1, 206.2; Near IR (Neat) ν (cm^{-1}) 3444, 1651, 1503, 1461; HRMS ESI calcd for $C_{19}H_{30}O_4NaSi$ 373.1811, Found 373.1809.

4.2.5. 2-[(*tert*-Butyldimethylsilyloxy)methyl]-6-[(furan-3-yl)acetoxymethyl]-6-methyl-2-cyclohexenone. Acetic anhydride (8.7 mL) was added dropwise to a cold (0 °C) solution of **11** (2.5 g, 7.14 mmol) in pyridine (8.7 mL) under an argon atmosphere. The cold bath was removed and stirring continued at room temperature for 4 h, followed by addition of iced water (20 mL). On warming to room temperature, the mixture was transferred to a separatory funnel and extracted with dichloromethane (4×20 mL). The combined extracts were washed successively with sodium hydrogen carbonate, water and brine, dried (Na_2SO_4) and evaporated. Excess pyridine was removed in vacuo prior to column chromatography (2:1 petroleum spirit/diethyl ether) affording the titled compound as a yellow oil (2.59 g, 92%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.05 (s, 6H), 0.89 (s, 9H), 1.16 (s, 3H), 1.79–1.91 (m, 2H), 2.05 (s, 3H), 2.39 (br s, 2H), 4.21–4.32 (m, 2H), 6.28 (s, 1H), 6.33 (s, 1H), 6.87 (br s, 1H), 7.28 (s, 1H), 7.30 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: −5.53, −5.49, 18.3, 18.6, 20.9, 22.1, 25.9, 29.2, 48.9, 60.1, 71.6, 110.1, 122.2, 137.0, 140.6, 142.0, 142.5, 169.7, 200.4; HRMS calcd for $C_{21}H_{32}O_5NaSi$ 415.1916, Found 415.1923.

4.2.6. 5-[(*tert*-Butyldimethylsilyloxy)methyl]-1-(furan-3-yl)-4a-hydroxy-8a-methyl-4,4a,8,8a-tetrahydro-1*H*-isochromen-3(7*H*)-one 12. To a stirred solution of diisopropylamine (8.67 mL, 6.18 mmol) in anhydrous tetrahydrofuran (15 mL) at 0 °C under an argon atmosphere, was added *n*-butyl lithium (1.32 M in hexanes, 4.46 mL) dropwise over a period of 4 min. After 30 min at 0 °C, the solution was cooled to −78 °C and a solution of 2-[(*tert*-butyldimethylsilyloxy)methyl]-6-[(furan-3-yl)-acetoxymethyl]-6-methyl-2-cyclohexenone (2 g, 5.10 mmol) in tetrahydrofuran (15 mL) was added dropwise. The reaction was stirred at −78 °C for 5 h, before quenching with saturated ammonium chloride solution (15 mL). After warming to room temperature (12 h), the mixture was transferred to a separatory funnel, extracted with dichloromethane (4×20 mL) and washed successively with water and brine. The extracts were then dried (Na_2SO_4), evaporated and subjected to column chromatography (2:1 diethyl ether/petroleum spirit) affording the titled compound **12** (1.37 g, 69%) as a white, crystalline solid.

Mp 121–123 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.10 (s, 3H), 0.12 (s, 3H), 0.90 (s, 9H), 1.01 (s, 3H), 1.31–1.36 (m, 1H), 1.88–1.93 (m, 1H), 2.09–2.16 (m, 2H), 3.02 (AB, 2H), 3.69 (br s, 1H), 4.10 (d, J=11.6 Hz, 1H), 4.47 (d, J=11.6 Hz, 1H), 5.21 (s, 1H), 5.87 (br s, 1H), 6.43 (s, 1H), 7.40 (d, J=1.6 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: −5.57, −5.58, 15.0, 18.0, 21.8, 25.8, 27.3, 39.5, 39.9, 66.4, 71.5, 77.7, 109.8, 121.2, 128.5, 137.0, 140.6, 143.0, 170.3; HRMS calcd for $C_{21}H_{32}O_5NaSi$ 415.1916, Found 415.1919.

4.2.7. 5-[(*tert*-Butyldimethylsilyloxy)methyl]-1-(furan-3-yl)-8a-methyl-8,8a-dihydro-1*H*-isochromen-3(7*H*)-one. To a solution of **12** (1.00 g, 2.55 mmol) in anhydrous dichloromethane (15 mL), was added anhydrous pyridine (825 μL, 10.2 mmol) under an argon atmosphere. The reaction flask was cooled in an ice-bath, and thionyl chloride (372 μL, 5.10 mmol) added dropwise. After 20 min, water (10 mL) was added and the mixture was allowed to warm to room temperature over 1 h. The reaction mixture was extracted with dichloromethane (2×30 ml), washed with saturated sodium hydrogen carbonate and brine then dried (Na_2SO_4). Evaporation followed by column chromatography of the residue (1:1 petroleum spirit/diethyl ether) gave the titled compound as a white, crystalline solid (690 mg, 72%).

Mp 93.5–94.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.07 (s, 6H), 0.90 (s, 9H), 1.01 (s, 3H), 1.41–1.50 (m, 2H), 2.28–2.37 (m, 2H), 4.30 (AB, 2H), 5.11 (s, 1H), 5.79 (s, 1H),

6.43 (br s, 2H), 7.40 (d, J=1.5 Hz, 1H), 7.46 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: −5.38, −5.35, 15.9, 18.3, 22.0, 25.9, 29.9, 37.1, 62.4, 80.7, 109.3, 110.1, 120.2, 132.2, 134.7, 141.1, 142.9, 157.3, 165.8; Near IR (Neat) ν (cm^{-1}) 1704, 1635, 1597; HRMS calcd for $C_{21}H_{30}O_4NaSi$ 397.1811, Found 397.1817; Anal. Calcd for $C_{21}H_{30}O_4Si$: C, 67.34; H, 8.07. Found: C, 67.37; H, 8.26%.

4.2.8. 1-(Furan-3-yl)-5-hydroxymethyl-8a-methyl-8,8a-dihydro-1*H*-isochromen-3(7*H*)-one (azedaralide) 1. Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 2.67 mL, 2.67 mmol) was added dropwise to a 0 °C solution of 5-[(*tert*-butyldimethylsilyloxy)methyl]-1-(furan-3-yl)-8a-methyl-8,8a-dihydro-1*H*-isochromen-3(7*H*)-one (500 mg, 1.34 mmol) in anhydrous tetrahydrofuran (25 mL). The solution was stirred at this temperature for 20 min, before dilution with ethyl acetate (10 mL) and hydrochloric acid (1 M, 10 mL). The reaction was extracted with ethyl acetate (3×30 mL). The combined organic phases were washed with brine (40 mL) and dried (Na_2SO_4). Evaporation and column chromatography of the residue (diethyl ether) afforded azedaralide (**1**) as a pale yellow, crystalline solid (320 mg, 92%). After chiral chromatography (+)-azedaralide $[\alpha]_D$ +385.0 (*c* 1.59, MeOH) and (−)-azedaralide $[\alpha]_D$ −391.9 (*c* 1.47, MeOH) at 27 °C.

Mp 108–108.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.02 (s, 3H), 1.41–1.51 (m, 3H), 2.25–2.39 (m, 2H), 4.33 (q, J=12.8 Hz, 2H), 5.13 (s, 1H), 5.94 (s, 1H), 6.43 (br s, 1H), 7.41 (t, J=1.5 Hz, 1H), 7.47 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 16.0, 22.0, 29.7, 37.1, 62.8, 80.7, 110.0, 110.2, 120.1, 132.5, 136.7, 141.2, 143.0, 157.3, 165.8; MS (EI) *m/z* (%): 260 ($M^{+\bullet}$, 2), 216 (1), 183 (2), 173 (2), 164 (100), 149 (15), 135 (3), 119 (38), 105 (11), 99 (13), 91 (14); Near IR [(+)-azedaralide] (Neat) ν (cm^{-1}) 3429, 1676, 1629, 1591; HRMS calcd for $C_{15}H_{16}O_4$ 260.1049, Found 260.1050; Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.37; H, 6.32%.

4.2.9. [(1*R*,8a*R*)-1-(furan-3-yl)-8a-methyl-3-oxo-3,7,8,8a-tetrahydro-1*H*-isochromen-5-yl]methyl (1*S*,4*R*)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl methanesulfonate 14. (+)-Azedaralide (20 mg, 0.077 mmol) was dissolved in anhydrous tetrahydrofuran (5 mL) under an atmosphere of argon. Anhydrous triethylamine (25 μL, 0.18 mmol) was added and the reaction vessel cooled in an ice-bath, prior to dropwise addition of (+)-camphorsulfonyl chloride (29 mg, 0.12 mmol). The solution was then allowed to warm slowly to room temperature. After 21 h the solvent was removed in vacuo, and the residue taken up into dichloromethane (10 mL), before washing with hydrochloric acid (2 M), and water. The organic phase was dried (Na_2SO_4), and evaporated. The residue was subjected to column chromatography (diethyl ether) to yield the desired sulfonate (32 mg, 88%) as an amorphous solid, which was recrystallised (ethyl acetate) affording white needles. $[\alpha]_D$ +260.1 (*c* 1.45, $CDCl_3$) at 27 °C.

Mp 160.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (s, 3H), 1.03 (s, 3H), 1.09 (s, 3H), 1.43–1.50 (m, 3H), 1.60–1.65 (m, 1H), 1.92–2.12 (m, 3H), 2.34–2.44 (m, 4H), 3.01 (d, 1H, J=15 Hz), 3.57 (dd, 1H, J=15, 1 Hz), 4.93 (AB, m, 2H), 5.14 (s, 1H), 5.95 (s, 1H), 6.43 (s, 1H), 6.59–6.60 (m, 1H), 7.41–7.42 (m, 1H), 7.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 15.9, 19.7, 19.8, 22.4, 24.9, 26.9, 29.3, 37.2, 42.5, 42.7, 48.0, 48.1, 58.0, 69.4, 80.5, 110.0, 111.3, 119.9, 127.8, 141.2, 142.5, 143.0, 156.0, 165.1, 214.5; MS (EI) *m/z* (%): 474 ($M^{+\bullet}$, 2), 410 (1), 378 (1), 294 (1), 260 (9), 242 (3), 215 (15), 178 (3), 164 (85), 146 (46), 118 (100), 109 (34), 91 (29); HRMS calcd for $C_{25}H_{33}O_7S$ 474.1712, Found 474.1712.

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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.2006.05.030.

References and notes

1. Nakatani, M.; Huang, R. C.; Okamura, H.; Iwagawa, T.; Tadera, K. *Phytochemistry* **1998**, *49*, 1773–1776.
2. Mulholland, D. A.; Parel, B.; Coombes, P. H. *Curr. Org. Chem.* **2000**, *4*, 1011–1054; (b) Taylor, D. A. H. *Fortschr. Chem. Org. Naturst.* **1984**, *45*, 1–102; (c) Deyer, D. L. *Fortschr. Chem. Org. Naturst.* **1968**, *26*, 190–244.
3. Fukuyama, Y.; Tokoroyama, T.; Kubota, T. *Tetrahedron Lett.* **1973**, 4869–4872.
4. Drewes, S. E.; Grieco, P. A.; Huffman, J. C. *J. Org. Chem.* **1985**, *50*, 1309–1311.
5. Liu, H.-J.; Dieck-Abularach, T. *Heterocycles* **1987**, *25*, 245–249.
6. Fernández Mateos, A.; de la Fuente Blanco, J. A. *J. Org. Chem.* **1991**, *56*, 7084–7092.
7. Fernández-Mateos, A.; Grande Benito, M.; Pascual Coca, G.; Rubio González, R.; Tapia Hernández, C. *Tetrahedron* **1995**, *51*, 7521–7526.
8. (a) Warpehoski, M. A.; Chabaud, B.; Sharpless, K. B. *J. Org. Chem.* **1982**, *47*, 2897–2900; (b) Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526–5528.
9. Kitching, W.; Rappoport, Z.; Winstein, S.; Young, W. G. *J. Am. Chem. Soc.* **1966**, *88*, 2054–2055.
10. Rappoport, Z.; Sleezer, P. D.; Winstein, S.; Young, W. G. *Tetrahedron Lett.* **1965**, 3719–3728.
11. (a) Connolly, J. D.; MacLellan, M.; Okorie, D. A.; Taylor, D. A. H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1993–1996; (b) Adesogan, E. K.; MacLachlan, L. K.; Taylor, D. A. H. *S. Afr. J. Chem.* **1987**, *40*, 25–29.
12. The ^{1}H NMR spectrum of azedaralide **1** provided by M. Nakatani, et al.[1] is identical in all respects to our data. The ^{13}C NMR spectrum is in slight disagreement; we observed an additional peak at 110.0 ppm, whereas M. Nakatani, et al.[1] reports overlapping peaks at 157.2 ppm. A visual comparison of the ^{1}H NMR spectra can be made from the supporting information.

13. Fernández-Mateos, A.; Pascual Coca, G.; Pérez Alonso, J. J.; Rubio González, R.; Tapia Hernández, C. *Tetrahedron Lett.* **1995**, *36*, 961–964.
14. (a) Gatri, R.; El Gaïed, M. M. *Tetrahedron Lett.* **2002**, *43*, 7835–7836; (b) Rezgui, F.; El Gaied, M. M. *Tetrahedron Lett.* **1998**, *39*, 5965–5966.
15. Mickel, S. J.; Daeffler, R.; Prikoszovich, W. *Org. Prog. Res. Dev.* **2005**, *9*, 113–120.
16. Lio, H.; Monden, M.; Okada, K.; Tokoroyama, T. *J. Chem. Soc., Chem. Commun.* **1987**, 358–359.
17. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.
18. Sato, K.; Nakayama, T.; Mori, K. *Agric. Biol. Chem.* **1979**, *43*, 1571–1575.
19. Takanami, T.; Tokoro, H.; Kato, D.-I.; Nishiyama, S.; Sugai, T. *Tetrahedron Lett.* **2005**, *46*, 3291–3295.
20. Luna, A.; Astorga, C.; Fülöp, F.; Goto, V. *Tetrahedron: Asymmetry* **1998**, *9*, 4483–4487.
21. Brown, R. J.; Annis, G.; Casalnuovo, A.; Chan, D.; Shapiro, R.; Marshall, W. J. *Tetrahedron* **2004**, *60*, 4361–4375.
22. Sheldrick, G. M. *SHELX97. Programs for Crystal Structure Analysis*; University of Göttingen: Germany, 1997.
23. Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837–838.
24. Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.

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New microviridins from a water bloom of the cyanobacterium *Microcystis aeruginosa*

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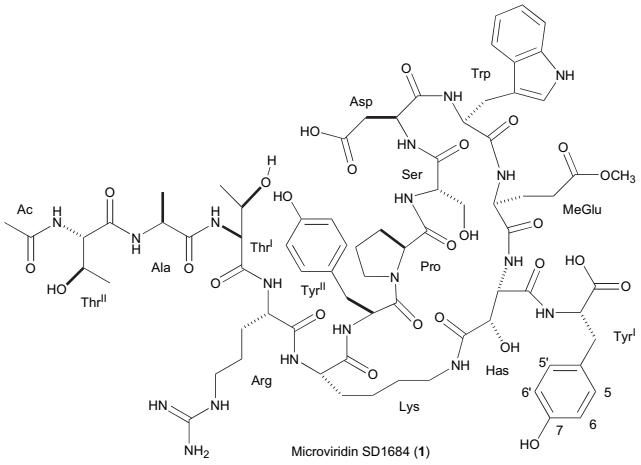
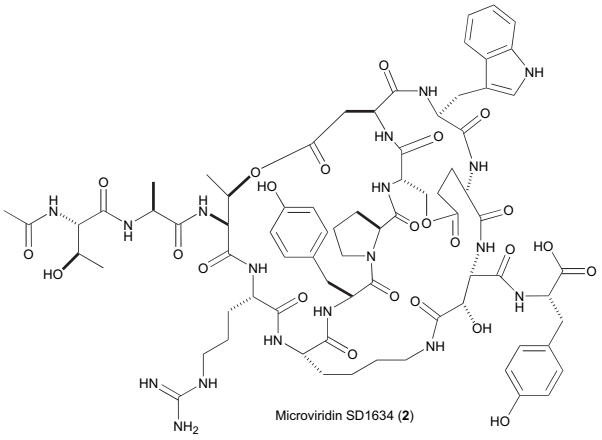
Abstract—Three new microviridins namely, SD1684 (**1**), SD1634 (**2**), and SD1652 (**3**), were isolated from the hydrophilic extract of *Microcystis aeruginosa*. The planar structures of compounds **1**–**3** were determined by homonuclear and inverse-heteronuclear 2D-NMR techniques as well as by high-resolution mass spectrometry. The absolute configuration of the asymmetric centers was studied using Marfey's method for HPLC. Compounds **1**–**3** contain L-*threo*-β-hydroxy aspartic acid as a building block of the peptide chain. This is the first example where microviridins contain non-proteinogenic amino acid in their structure. Compound **2** is a mild serine protease inhibitor.

1. Introduction

Microviridins are cyclic peptides produced by strains of cyanobacteria (*Microcystis*, *Nostoc*, and *Oscillatoria* spp.) that produce massive water blooms and have the capacity to synthesize hepatotoxins of the microcystins family.[1] They contain either 13 or 14 L-amino acids, nine of which are conserved: Tyr$^{(1)}$, Glu$^{(3)}$, Trp$^{(4)}$, Asp$^{(5)}$, Ser$^{(6)}$, Pro$^{(7)}$, Tyr$^{(8)}$, Lys$^{(9)}$, and Thr$^{(11)}$. They consist of a monocyclic peptide structure with zero to two lactone bridges, which produce a rigid core structure between positions 2 and 11. Ten cyclic peptides of this type have thus far been characterized from extracts of water-bloom-forming cyanobacteria. Microviridin A was isolated from *Microcystis viridis* as tyrosinase inhibitor.[2] Microviridins B and C were isolated from *Microcystis aeruginosa* as elastase inhibitors.[3] Microviridins D, E, and F were isolated from *Oscillatoria* (*Planktothrix*) *agardhii* as an elastase and chymotrypsin inhibitors.[4] Microviridins G and H were isolated from *Nostoc minutum* as elastase inhibitors.[5] Microviridin I was isolated from *O. (P.) agardhii* as an elastase, chymotrypsin, and trypsin inhibitor.[6] Microviridin J was isolated from a cultured *M. aeruginosa* and possesses potent toxicity to *Daphnia* and inhibits serine proteases.[7] Here we report the isolation and structure elucidation of three new microviridins.



Microviridin SD1684 (**1**)


Microviridin SD1634 (**2**)

Keywords: Natural products; Cyanobacteria; *Microcystis aeruginosa*; Protease inhibitors.
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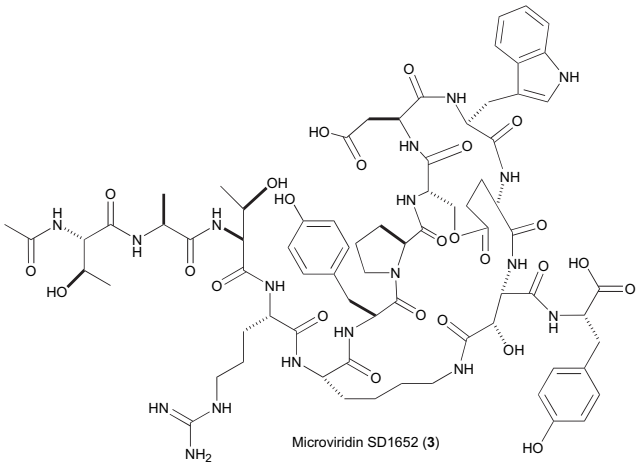

A non-toxic strain of the cyanobacterium *M. aeruginosa* (strain IL-215) was collected in the summer of 1998, from a pond in the Dan District sewage treatment plant, the Shofdan. The freeze-dried sample of the cyanobacterium was extracted with 70% MeOH in H_2O. The extract was found to inhibit several serine and amino proteases. The active extract was flash-chromatographed on an ODS column. Two fractions eluted from the column, with 60 and 70% MeOH in H_2O, exhibited protease inhibitory activity and were further purified on a reversed-phase HPLC column. Seven serine- and amino-protease inhibitors were isolated from these fractions and their structures were published.[8] The fractions eluted from an ODS flash-column with 30%–50% methanol, exhibit serine–protease inhibitory activity but were insoluble in chloroform/methanol solutions, like the less polar protease inhibitors. Repeated preparative reversed-phase HPLC afforded seven pure compounds with molecular weights between 1634 and 1715 mass units. The composition of the amino acids of all seven compounds was found to be identical by amino acid analysis of the hydrolyzed peptides. The structures of three of these compounds, microviridins SD1684 (**1**), SD1634 (**2**), and SD1652 (**3**), were determined by NMR and MS techniques. The other four compounds exist, in solution, as a complex mixture of conformers, and their structure could not be solved by NMR.[†] An attempt to solve the structure of the latter four compounds by MS–MS measurements, failed due to the uninterpretable fragmentation pattern of the cyclic portion, of either **1**–**3** or the four unsolved ones. The fragmentation pattern of the side chains of all compounds, on the other hand, was interpretable and consistent with the proposed structures of **1**–**3**.

Microviridin SD1684 (**1**) was isolated as a glassy solid material that exhibited a negative-FAB quasi-molecular ion at *m/z* 1683.6 but failed to give a positive-FAB molecular ion. Its molecular formula, $C_{76}H_{104}N_{18}O_{26}$, is based on HR MALDI-TOF MS measurements. The structure elucidation of **1** was complicated due to the overlapping of several protons and carbons in the NMR spectra. The NMR spectra of **1** were examined in several deuterated solvents. The best resolved spectra were obtained in methanol-d_3 and pyridine-d_5. Even in these two solvents at 500 MHz, the overlapping of too many signals did not allow the unambiguous assignment of all of the amino acid subunits. To overcome this problem we measured the 1-D and 2-D NMR spectra of **1** in CD_3OH on an 800 MHz spectrometer. Sixteen exchangeable protons were observed in the proton NMR spectrum (δ_H 10.20–6.50 ppm). Two *para*-substituted phenol moieties (δ_H 7.10 d, 2H; 7.06 d, 2H; 6.79 d, 2H, and 6.71 d, 2H, ppm) and five vinylic protons (δ_H 7.61 d, 7.36 d, 7.12 t, 7.08 s, and 7.04 t, ppm) of the tryptophan moiety were observed in the aromatic region. Upfield from the aromatic region (δ_H 5.50–3.30 ppm), sixteen methine protons, six protons of three methylenes next to nitrogen and oxygen, and one *O*-methoxyl group, were observed. Protons of six methylenes located next to a nitrogen and a carbonyl resonate between δ_H 3.30 and 2.20 ppm. A singlet of acetyl protons and three doublet methyls along with protons of eight aliphatic methylenes appear at the high-field end of the spectrum. In the carbon NMR spectrum, 17 carboxyl-carbons, a guanidine carbon, two phenol-carbons, and 18 vinyl carbons (13 methines and five quaternary) were observed at the low field end of the spectrum. Sixteen methines, two methylenes, and a methyl were detected in the region between 75 and 45 ppm. Fourteen methylene and four methyl carbons appear in the aliphatic region of the ^{13}C NMR spectrum. H–H COSY and TOCSY experiments allowed the assignment of 17 fragments, which account for 87 of the 104 protons of **1**. The fragments are: two *para*-substituted phenol moieties of tyrosine, a 1,2-disubstituted phenyl moiety, a three substituted vinyl imide moiety, three moieties of α-NH to the β-methylene of aromatic amino acids, α-NH to the β-methine of an unidentified amino acid, α-NH to the γ-methylene of glutamic acid, α-NH to the β-methylene of aspartic acid, α-NH to the β-methylene of serine, α-methine to the δ-methylene of proline, α-NH to ε-NH of an ε-*N*-substituted lysine, α-NH to δ-NH of arginine, two moieties of α-NH to γ-methyl of threonine and α-NH to the β-methyl of alanine. The correlations from the HMQC spectrum (see Table 1) reinforced these assignments and confirmed the existence of the *O*-methyl and *N*-acetyl moieties, in **1**. HMBC correlations (see Table 1) allowed full assignment of the 14 acid units of **1**: two Tyr, β-hydroxy aspartic acid (Has), 5-*O*-methyl glutamic acid, Trp, Asp, Ser, Pro, ε-*N*-substituted lysine, Arg, two Thr units, Ala, and an acetyl amide. The amino acid sequence of microviridin SD1684 (**1**) was determined from HMBC correlations of the NH proton of an amino acid with the carbonyl of an adjacent amino acid (Tyr^I-Has, Glu-Trp, Trp-Asp, Asp-Ser, Ser-Pro, Tyr^{II}-Lys, Lys-Arg, Arg-Thr^I, Thr^I-Ala, Ala-Thr^{II}, and Thr^{II}-Ac) and *O*Me with a 5-carbonyl of Glu. These correlations established all but three amide connectivities of the cyclopeptide, the connectivity between Pro-N and Tyr^{II}-carbonyl, the connectivity between ε-NH of Lys and 4-carbonyl of Has, and the connectivity between Has-NH and the Glu 1-carbonyl. Has 4-carbonyl and Tyr^{II}-carbonyl (δ_C 172.8 ppm), as well as Pro-carbonyl and Glu 1-carbonyl (δ_C 174.0 ppm),

[†] The proton NMR spectrum of each of these compounds was measured in several deuterated solvents and solvent mixtures at room temperature and if possible in higher temperatures. All seven compounds that were homogenous by HPLC, presented a MALDI-TOF mass spectrum, which indicated that it contains a single component (only one set of MH^+, MNa^+, and MK^+ ions). Compounds **1**–**3**, which presented a set of two or three NMR signals for each chemical unit (easily observed on methyl groups and aromatic protons) gave one sharp set of signals in the solvent that were used for structure elucidation. For the other four compounds such a solvent could not be obtained.

Table 1. NMR data of microviridin SD1684 (**1**) in CD_3OH[a]

Position		$\delta_{C/N}$, Mult.[b]	δ_H, Mult., *J* (Hz)	HMBC correlations[c]	ROESY correlations[d]
Tyr^I	1	174.9 s		Tyr^I-2,3,3′	
	2	55.9 d	4.65 dt 5.8, 7.5	Tyr^I-3,3′	
	3	37.7 t	2.98 dd 13.9, 7.5; 3.09, dd, 13.9, 5.8	Tyr^I-2,5,5′	
	4	128.7 s		Tyr^I-2,3,3′,6,6′	
	5,5′	131.3 d×2	7.10 d 8.4	Tyr^I-3,3′,5,5′	
	6,6′	116.3 d×2	6.71 d 8.4	Tyr^I-5,5′,6,6′	
	7	157.3 s		Tyr^I-5,5′,6,6′	
	NH	115.9 d	8.25 d 7.2		Has-3
Has	1	171.3 s		Has-2, Tyr^I-NH	
	2	56.8 d	5.03 br s		
	3	73.4 d	4.77 br s		Lys-ε-NH, Tyr^I-NH
	4	172.8 s		Has-3	
	NH	111.1 d	8.67 br s		Glu-2,4, Tyr^I-2
Glu	1	174.0 s		Glu-2,3,3′	
	2	52.2 d	5.29 ddd 8.2, 8.4, 7.8	Glu-3,3′,4,4′	Has-NH,3
	3	30.5 t	1.92 dq 14.8, 7.3; 1.88 dq 14.8, 7.3	Glu-4,4′	
	4	29.5 t	2.33 dt 16.9, 7.2; 2.49 dt 16.9, 7.2	Glu-3,3′	Glu-NH, Has-NH
	5	175.0 s		Glu-3,3′,4,4′,*O*Me	
	*O*Me	52.1 q	3.57 s		
	NH	123.8 d	8.32 d 7.8		Glu-4
Trp	1	173.4 s		Trp-2,3, Glu-NH	
	2	55.8 d	4.61 m	Trp-5, NH	Ser-NH, Glu-NH
	3	28.6 t	3.14 dd 14.7, 7.8; 3.09 dd 14.7, 5.4	Trp-2	Trp-NH,5
	4	110.5 s		Trp-3,5,9,NH(9)	
	4a	128.5 s		Trp-3,5,8,9,NH(9)	
	5	119.2 d	7.61 d 8.0	Trp-7	Trp-2,3
	6	119.7 d	7.12 t 7.5	Trp-8	
	7	122.3 d	7.04 t 7.5	Trp-5	Trp-NH(9)
	8	112.3 d	7.36 d 8.1	Trp-5,6	Trp-NH(9)
	8a	138.0 s		Trp-5,7,9,NH(9)	
	9	124.7 d	7.08 br s	Trp-3,NH(9)	
	NH(9)	127.1 d	10.12 br s		Trp-7,8
	NH	119.2 d	8.02 d 6.5		Trp-2,3, Asp-NH,2
Asp	1	173.1 s		Trp-NH, Asp-2,3,3′	
	2	51.8 d	4.68 dt 6.3, 8.0	Asp-3,NH	Asp-NH, Trp-NH
	3	36.1 t	2.70 dd 17.5, 7.9; 3.07 dd 17.5, 6.3	Asp-2	Asp-NH
	4	174.4 s		Asp-2,3,3′	
	NH	115.9 d	8.69 d 8.0		Asp-2,3, Ser-2, Trp-NH
Ser	1	171.8 s		Ser-3, Asp-NH	
	2	58.2 d	4.29 br s		Asp-NH
	3	63.0 t	3.85 br s (2H)		Ser-NH, Trp-NH
	NH	122.1 d	8.27 m		Ser-3, Trp-2
Pro	1	174.0 s		Pro-2,3, Ser-NH	
	2	62.1 d	3.78 br d 8.3	Pro-4′	Pro-5, Tyr^{II}-2,5,5′,6,6′
	3	32.1 t	1.52 m; 2.05 m	Pro-2,5′	Tyr^{II}-6,6′
	4	22.6 t	1.62 m; 1.80 m	Pro-5	Tyr^{II}-6,6′
	5	48.0 t	3.47 dt 9.6, 7.2; 3.57 m	Pro-2,3,3′	Pro-2, Tyr^{II}-5,5′
Tyr^{II}	1	172.8 s		Tyr^{II}-3,3′	
	2	55.4 d	4.62 m	Tyr^{II}-3	Tyr^{II}-NH, Pro-2
	3	38.6 t	2.89 dd 13.1, 6.1; 3.00 dd 13.1, 9.1	Tyr^{II}-5,5′	Tyr^{II}-NH
	4	127.0 s		Tyr^{II}-3,3′,6,6′	
	5,5′	131.5 d×2	7.06 d 8.4	Tyr^{II}-3,3′,5,5′	Pro-2,5
	6,6′	116.9 d×2	6.79 d 8.4	Tyr^{II}-5,5′,6,6′	Pro-2,3,4′
	7	158.1 s		Tyr^{II}-5,5′,6,6′	
	NH	118.8 d	8.29 m		Tyr^{II}-2,3
Lys	1	174.5 s		Lys-3′, Tyr^{II}-NH	
	2	53.3 d	4.54 m	Lys-3′	Lys-α-NH
	3	29.9 t	1.69 m; 1.79 m		Lys-α-NH; Lys-α-NH
	4	25.0 t	1.44 m; 1.42 m		Lys-α,ε-NH; Lys-α,ε-NH
	5	30.0 t	1.44 m; 1.42 m		Lys-α,ε-NH; Lys-α,ε-NH
	6	40.6 t	3.70 br m; 2.81 br m		Lys-ε-NH; Lys-ε-NH
	α-NH	127.0 d	8.83 d 7.7		Lys-2,3,3′,4,4′,5,5′, Arg-2
	ε-NH	118.6 d	8.08 t 6.6		Lys-4,4′,5,5′,6,6′, Has-3
Arg	1	174.7 s		Arg-2, Lys-α-NH	
	2	53.4 d	4.78 q 7.5	Arg-3,4	Lys-α-NH
	3	29.7 t	1.80 m (2H)		Arg-NH
	4	25.9 t	1.48 m; 1.57 m	Arg-2,5	Arg-6(NH)
	5	42.0 t	3.19 br m; 3.15 br m	Arg-4,4′	Arg-6(NH)

(*continued*)

Table 1. (*continued*)

Position		$\delta_{C/N}$, Mult.[b]	δ_H, Mult., J (Hz)	HMBC correlations[c]	ROESY correlations[d]
	6(NH)	83.7 d	7.34 t 4.1		Arg-4,5
	7	158.5 s		Arg-5	
	8,9	22.0 t; 74.0 d	7.25 br m; 6.55 br m		
	NH	121.1 d	8.54 br s		Arg-3, Thr^I-2,3
Thr^I	1	172.0 s		Arg-NH, Thr^I-2	
	2	58.8 d	4.59 m	Thr^I-4,NH	Arg-NH
	3	69.9 d	4.25 dq 4.4, 6.3	Thr^I-4	Arg-NH
	4	20.4 q	1.17 d 6.3		Thr^I-NH
	NH	110.9 d	8.07 d 7.9		Thr^I-4, Ala-NH
Ala	1	174.8 s		Ala-2,3, Thr^I-2,NH	
	2	51.0 d	4.43 dq 6.3, 6.8	Ala-3,NH	Thr^I-NH
	3	17.7 q	1.38 d 6.8	Ala-2,NH	Ala-NH
	NH	125.4 d	8.19 d 6.3		Ala-3, Thr^{II}-3, Thr^I-NH
Thr^{II}	1	172.5 s		Thr^{II}-2, Ala-NH	
	2	60.3 d	4.33 dd 4.7, 8.0	Thr^{II}-4,NH	Ala-NH
	3	68.5 d	4.17 dq 4.7, 6.3	Thr^{II}-2,4,NH	Thr^{II}-NH, Ala-NH
	4	19.9 q	1.20 d 6.3		Thr^{II}-NH
	NH	117.3 d	8.17 d 8.0		Thr^{II}-3,4, Ac-2
Ac	1	173.9 s		Ac-2, Thr^{II}-2,NH	
	2	22.5 q	2.06 s		Thr^{II}-NH

[a] Carried out on an DMX-800 Bruker instrument.
[b] Multiplicity and assignment from HMQC experiment.
[c] Determined from HMBC experiment, $^nJ_{CH}$=8 Hz, recycle time 1 s, the HMBC correlations are reported as correlations of the protons printed in the column with the carbons in the rows.
[d] By ROESY experiment, mixing time 400 ms.

resonate at the same chemical shift and thus introduce some ambiguity to the assignment and correlations of these amino acids. The connectivity between these amino acids was established on the basis of NOE correlations from ROESY experiments. The experiment in methanol-d_3 presents an NOE between Pro H-2 and Tyr^{II} H-2, suggesting a cis conformation of the Pro amide bond (reinforced by the ^{13}C chemical shifts difference between positions 3 and 4 of proline, >9 ppm[9]), between Has H-3 and ε-NH of Lys, confirming the cyclic structure of the peptide, and between Has NH and Glu H-2. The ROESY experiment in pyridine-d_5 (see Table 4) presents an additional NOE between Ser H-2 and Pro H-4 confirming the correlation between Ser-NH and Pro-carbonyl that was established from the HMBC experiment in methanol-d_3. The rest of the amino acid sequence could also be assembled from the ROESY data (see Table 1). A set of NOE's between Asp-NH and Trp-NH, Ser-NH and Trp-H-2 as well as Ser-H_2-3 and Trp-NH suggest that the peptide has a β-turn around the Asp residue. The ^1H–^{15}N HSQC experiment allowed the assignment of the ^{15}N signals of the various amino acids of compound **1** other than that of Pro (see Table 1). All the secondary amide nitrogen signals resonated in the expected region (δ_N 105–120 ppm, relative to ammonia).[10] Acid hydrolysis of microviridin SD1684 (**1**) and derivatization with Marfey's reagent,[11] followed by HPLC analysis, demonstrated the L-stereochemistry of all amino acids. The stereochemistry of the Has residue was established by this method as L-*threo*-β-hydroxy aspartic acid.

Microviridin SD1634 (**2**) was isolated as a glassy solid material that exhibits a negative-FAB quasi-molecular ion at *m/z* 1633.9. Its molecular formula, $C_{75}H_{98}N_{18}O_{24}$, is based on HR MALDI-TOF MS measurements. This molecular formula corresponds to a loss of a molecule of water and a molecule of methanol from compound **1**. The NMR spectra of **2** were examined in several deuterated solvents. The best resolved spectra were obtained in 10:1 methanol-d_3/aq TFA at pH 3.3. When the proton NMR spectrum of **2** is compared with that of **1** some differences are encountered: (i) the methoxyl signal is missing in the spectrum of **2**; (ii) one of the two threonine methyl groups is downfield shifted and a quartet methine proton appears at 5.54 ppm; (iii) there is a dramatic change in the chemical shifts of the amide protons that resonate between 8.85 and 8.00 ppm in the spectrum of **1** and between 9.11 and 6.30 ppm in the spectrum of **2**. Combining these observations with the mass-spectral data and the structure of previously known microviridins suggested that **2** contains two lactone bridges that cause conformational changes in the peptide backbone. The conformational changes were also reflected in ^{15}N chemical shifts of the amide nitrogen atoms (see Tables 1 and 2). Analyses of the NMR spectra and Marfey's analysis[11] revealed that **2** contains the same 13 amino acids (L-Ala, L-Arg, L-Asp, L-Glu, L-Has, L-Lys, L-Pro, L-Ser, 2×L-Thr, L-Trp, and 2×L-Tyr) and acetate as **1**. The assignment of the NMR signals further suggested that the serine methylene (δ_H 4.90 m and 2.77 br d in **2**, relative to δ_H 3.85 br s in **1**) and a methine next to oxygen of one of the two threonine moieties (δ_H H-3: 5.56 q and H_3-4 1.39 d in **2**, relative to δ_H H-3: 4.25 dq and H_3-4 1.17 d in **1**) are esterified. Seven of the carbonyl carbons overlapped in the ^{13}C NMR spectrum (Ser, Lys, and Thr^{II}, resonate at 172.9 ppm and Asp, Pro, Tyr^{II}, and Ac, at 173.7 ppm) and thus prevented the assignment of the amino acid sequence solely on the basis of HMBC correlation between the amide NH proton of an amino acid with the carbonyl of an adjacent amino acid. Based on the HMBC connectivity two fragments could be assigned (see Table 2): Tyr^I-Has-Glu-Trp and Lys-Arg-Thr^I-Ala. The following NOE's between: Trp-NH and Asp-2 and NH; Asp-NH and Ser-2; Ser-NH and Pro-4 and 5′; Pro-2 and Tyr^{II}-2, 5 and 5′; Tyr^{II}-NH and Lys-2; Ala-NH and Thr^{II}-2

Table 2. NMR data of microviridin SD1634 (**2**) in 10:1 CD_3OH/aq TFA pH 3.3[a]

Position		$\delta_{C/N}$, Mult.[b]	δ_H, Mult., J (Hz)	HMBC correlations[c]	ROESY correlations[d]
Tyr^I	1	174.4 s		Tyr^I-3	
	2	55.7 d	4.58 m	Tyr^I-3	Tyr^I-3,NH
	3	37.4 t	3.09 m	Tyr^I-2,5,5′	Tyr^I-2,NH
	4	128.8 s		Tyr^I-3,5,5′,6,6′	
	5,5′	131.4 d×2	7.11 d 8.3	Tyr^I-3,5,5′	
	6,6′	116.3 d×2	6.73 d 8.3	Tyr^I-5,5′,6,6′	
	7	157.2 s		Tyr^I-5,5′	
	NH	118.6 d	7.67 d 8.0		Tyr^I-2,3, Has-2,3
Has	1	171.2 s		Has-2, Tyr^I-NH	
	2	57.2 d	4.74 br d 9.5	Has-NH	Tyr^I-NH, Has-NH
	3	72.1 d	4.54 br s		Lys-ε-NH, Tyr^I-NH
	4	173.3 s		Has-3, Lys-ε-NH	
	NH	104.2 d	7.02 d 8.6		Glu-2,3, Has-2
Glu	1	172.0 s		Glu-2, Has-NH	
	2	56.8 d	4.09 m	Glu-NH	Glu-4,4′,NH, Has-NH
	3	30.6 t	1.37 m	Glu-NH	Glu-4,4′,NH, Has-NH
	4	31.6 t	2.09 m; 1.07 m		Glu-2,3; Glu-2,3,NH
	5	172.6 s		Ser-3,3′, Glu-3,3′,4	
	NH	116.8 d	6.54 d 7.5		Glu-2,3,4′, Trp-2
Trp	1	174.3 s		Trp-2,3, Glu-NH	
	2	55.5 d	4.79 m	Trp-3,3′	Trp-3,3′,NH, Glu-NH
	3	26.6 t	3.23 m; 3.58 dd 4.5, 15.5		Trp-3′,NH; Trp-3,9
	4	109.8 s		Trp-3,3′,9,NH(9)	
	4a	128.8 s		Trp-3,3′,8,9,NH(9)	
	5	118.8 d	7.58 d 8.0	Trp-6,7,8	
	6	120.7 d	7.10 t 7.9	Trp-8	
	7	122.9 d	7.11 t 7.9	Trp-5	
	8	113.0 d	7.30 d 7.5	Trp-6	Trp-NH(9)
	8a	138.4 s		Trp-5,9,NH(9)	
	9	125.0 d	7.18 s	Trp-3,3′,NH(9)	
	NH(9)	129.5 d	10.62 s		Trp-8,9
	NH	111.3 d	6.90 d 7.5		Trp-2,3, Asp-NH,2
Asp	1	173.7 s		Asp-2, Trp-NH	
	2	51.4 d	4.47 br d 10.0	Asp-3,NH	Asp-3′,NH, Trp-NH
	3	35.4 t	3.04 m; 2.94 m	Asp-NH	Asp-NH; Asp-2
	4	172.1 s		Asp-2,3,3′, Thr(2)-3	
	NH	116.9 d	9.11 br s		Asp-2,3, Ser-2,3′, Trp-NH
Ser	1	172.9 s		Ser-2,3,3′, Asp-NH	
	2	55.0 d	4.30 br s		Ser-NH, Asp-NH
	3	62.5 t	4.90 m; 2.78 br d 11.5		Lys-ε-NH; Asp-NH
	NH	109.0 d	6.33 d 2.9		Ser-2, Pro-4,5′
Pro	1	173.7 s		Pro-2, Ser-NH	
	2	62.2 d	3.38 m		Pro-3′,4,4′, Tyr^{II}-2,5,5′
	3	31.7 t	1.63 m; 1.33 m	Pro-2	Pro-2
	4	22.6 t	1.33 m; 1.59 m	Pro-2	Pro-2,5′, Ser-NH; Pro-2,5′
	5	47.4 t	3.20 m; 3.38 m		Pro-4,4′, Ser-NH
Tyr^{II}	1	173.7 s		Tyr^{II}-2,3	
	2	54.1 d	4.35 m	Tyr^{II}-3,NH	Tyr^{II}-3,5,5′,NH, Pro-2
	3	38.6 t	2.84 d 8.2	Tyr^{II}-2,5,5′,NH	Tyr^{II}-2,5,5′,NH
	4	127.5 s		Tyr^{II}-3,6,6′	
	5,5′	131.3 d×2	6.96 d 8.2	Tyr^{II}-3,5,5′	Tyr^{II}-2,3, Pro-2
	6,6′	116.6 d×2	6.70 d 8.2	Tyr^{II}-5,5′,6,6′	
	7	157.9 s		Tyr^{II}-5,5′	
	NH	125.4 d	8.48 d 7.0		Tyr^{II}-2,3, Lys-2
Lys	1	172.9 s		Lys-2, Tyr^{II}-NH	
	2	55.4 d	3.95 m	Lys-α-NH	Lys-3,4,α-NH, Tyr^{II}-NH
	3	32.6 t	1.70 m; 1.57 m		Lys-2; Lys-α-NH,4
	4	23.0 t	1.18 m; 1.44 m		Lys-2,3′
	5	29.4 t	1.68 m; 1.24 m		
	6	39.9 t	2.97 m; 3.21 m		Lys-ε-NH
	α-NH	120.9 d	7.54 d 6.5		Lys-2,3′, Arg-NH, Asp-2, Thr^I-3
	ε-NH	118.6 d	7.61 t 5.7		Lys-6, Has-3, Ser-3
Arg	1	173.0 s		Arg-2, Lys-α-NH	
	2	53.3 d	4.46 m	Arg-NH	Arg-3,3′,4,4′,5,NH
	3	28.9 t	1.56 m; 2.11 m		Arg-2,NH,6(NH); Arg-2,6(NH)
	4	25.9 t	1.56 m; 1.67 m	Arg-5	Arg-2,6(NH); Arg-2,5
	5	41.9 t	3.17 m		Arg-2,4′,6(NH)
	6(NH)	83.3 d	7.34 t 5.0		Arg-3,3′,4,5

(*continued*)

Table 2. (*continued*)

Position		$\delta_{C/N}$, Mult.[b]	δ_H, Mult., *J* (Hz)	HMBC correlations[c]	ROESY correlations[d]
	7	158.7 s		Arg-6(NH)	
	8,9	22.1 t	7.23 br m		
	NH's	74.3 d	6.40 br m		
	NH	118.8 d	8.74 d 9.0		Arg-2,3, Lys-α-NH, Thr^{I}-2,3
Thr^{I}	1	172.8 s		Arg-NH, Thr^{I}-2	
	2	57.8 d	4.58 br d 11.5	Thr^{I}-4,NH	Thr^{I}-NH, Arg-NH
	3	73.2 d	5.56 q 6.5	Thr^{I}-4	Thr^{I}-2,4,NH, Arg-NH, Lys-α-NH
	4	18.3 q	1.39 d 6.6	Thr^{I}-3	Thr^{I}-3
	NH	106.0 d	7.95 d 7.8		Thr^{I}-2,3, Ala-2
Ala	1	175.6 s		Ala-2,3, Thr^{I}-NH	
	2	50.4 d	4.59 q 7.0	Ala-3,NH	Ala-NH, Thr^{I}-NH
	3	17.6 q	1.45 d 7.0	Ala-2,NH	Ala-2,NH
	NH	122.7 d	8.26 d 6.5		Ala-2,3, Thr^{II}-2,3
Thr^{II}	1	172.9 s		Thr^{II}-2, Ala-NH	
	2	60.3 d	4.43 dd 4.0, 8.5	Thr^{II}-4,NH	Thr^{II}-4,NH, Ala-NH
	3	68.3 d	4.24 dq 4.0, 6.5	Thr^{II}-4	Thr^{II}-4,NH, Ala-NH
	4	20.0 q	1.24 d 6.4		Thr^{II}-2,3
	NH	115.3 d	7.94 d 8.5		Thr^{II}-2,3, Ac-2
Ac	1	173.7 s		Ac-2, Thr^{II}-2,NH	
	2	22.6 q	2.08 s		Thr^{II}-2,NH

[a] Carried out on an ARX-500 Bruker instrument.
[b] Multiplicity and assignment from HMQC experiment.
[c] Determined from HMBC experiment, $^{n}J_{CH}$=8 Hz, recycle time 1 s, the HMBC correlations are reported as correlations of the protons printed in the column with the carbons in the rows.
[d] By ROESY experiment, mixing time 400 ms.

and 3; Thr^{II}-NH and Ac-2, allowed the assignment of the whole molecule backbone, Tyr^{I}-Has-Glu-Trp-Asp-Ser-Pro-Tyr^{II}-Lys-Arg-Thr^{I}-Ala-Thr^{II}-Ac. The amide bond between Lys-ε-NH and Has 4-carbonyl could be assigned on the basis of an HMBC correlation and NOE between Has-3 and Lys-ε-NH. The ester bond between Ser-3 oxygen and Glu-5 carbonyl was assigned on the basis of the HMBC correlation of Ser-3 and 3′ protons with Glu-5 carbonyl. Finally, the ester bond between Thr^{I}-3 oxygen and Asp-4 carbonyl was assigned on the basis of the HMBC correlation of Thr^{I}-3 proton with Asp-4 carbonyl. The discussion above led to the assignment of structure **2** to microviridin SD1634.

Microviridin SD1652 (**3**) is a glassy solid material that exhibits a negative-FAB quasi-molecular ion at *m/z* 1651.7. Its molecular formula, $C_{75}H_{100}N_{18}O_{25}$, is based on HR MALDI-TOF MS measurements. This molecular formula corresponds to the loss of a molecule of methanol from compound **1**. The NMR spectra of **3** were examined in several deuterated solvents. The best resolved spectra were obtained in DMSO-d_6 at 330 K. Examination of the proton NMR spectrum of **3** (see Table 3) revealed that: (i) the methoxyl signal is missing in the spectrum of **3**; (ii) the two threonine methyls resonate at a similar chemical shift; (iii) the serine methyleneoxy protons resonate in a relatively low field suggesting the involvement of the oxygen in an ester bond. Combining this data with the mass spectral data suggested that compound **3** contains only one lactone bridge, most probably between the Glu carbonyl and the serine methyleneoxy. Analyses of the COSY, TOCSY, HMQC, and HMBC revealed the structures of the same 13 amino acids (Ala, Arg, Asp, Glu, Has, Lys, Pro, Ser, 2×Thr, Trp, and 2×Tyr) and acetyl residues while Marfey's analysis[11] revealed that they posses the same absolute stereochemistry as that of **1** and **2**. Four of the amide carbonyl signals were not assigned because they did not show any correlation in the HMBC spectrum, probably due to the relatively small amount of material available. The amide backbone of the peptide was determined on the basis of HMBC and NOE correlations. Tyr^{I} was connected to Has on the basis of the HMBC correlation of Tyr^{I}-NH and Has-CO. An NOE between Has-NH and Glu-2 proton connected the two acids. Glu-NH correlates in the HMBC map with Trp-carbonyl. The sequence Trp-Asp-Ser-Pro-Tyr^{II} was connected on the basis of an NOE between Trp-NH and Asp-NH, Asp-NH and Ser-2, Ser-NH and Pro-2, and Pro-5,5′ and Tyr^{II}-2. An HMBC correlation between the amide NH proton of an amino acid and the carbonyl of an adjacent amino acid established the sequence: Tyr^{II}-Lys-Arg-Thr^{I}-Ala. An NOE between Ala-NH and Thr^{II}-2 and an HMBC correlation between Thr^{II}-NH and the acetyl carbonyl established the sequence Ala-Thr^{II}-Ac. An NOE between Has-3 and Lys-ε-NH established the cyclization of the peptide through the Has and Lys side chains. The lactone bridge between Glu-5 carbonyl and Ser-methyleneoxy was established by the HMBC correlation of Ser-3′ proton and Glu-5 carbonyl. On the basis of the arguments discussed above, the structure **3** was assigned to microviridin SD1652.

Compounds **1**–**3** were isolated through a serine protease (chymotrypsin and trypsin) inhibition-guided separation. The final fractions that yielded compounds **1**–**3**, inhibited both chymotrypsin and trypsin. Pure **1** and **3** were found to be inactive in the assay, while **2** completely inhibited the proteolytic activity of chymotrypsin and trypsin at a concentration of 45 μg/mL. The IC_{50} values were determined only for compound **2**, against the serine proteases trypsin and chymotrypsin. Microviridin SD1634 (**2**) inhibited trypsin activity with an IC_{50} value of 13.4 μg/mL (8.2 μM) and chymotrypsin with an IC_{50} value of 25.7 μg/mL (15.7 μM). These IC_{50}

Table 3. NMR data of microviridin SD1652 (**3**) in DMSO-d_6 at 330 K[a]

Position		$\delta_{C/N}$, Mult.[b]	δ_H, Mult., J (Hz)	HMBC correlations[c]	ROESY correlations[d]
Tyr^I	1	171.9 s		Tyr^I-2,3	
	2	54.3 d	4.37 m	Tyr^I-3,3′,NH	Tyr^I-3,5,5′,NH, Has-NH
	3	36.3 t	2.95 dd 7.1, 13.7; 3.00 dd 6.3, 13.8	Tyr^I-2,5,5′	Tyr^I-2,5,5′,NH; Tyr^I-NH
	4	127.3 s		Tyr^I-2,3,6,6′	
	5,5′	129.8 d×2	7.01 d 8.1	Tyr^I-3,3′,5,5′	Tyr^I-2,3, Has-2,3,NH
	6,6′	114.9 d×2	6.64 d 8.2	Tyr^I-5,5′,6,6′	
	7	155.7 s		Tyr^I-5,5′,6,6′	
	NH	116.8 d	7.13 d 7.4		Tyr^I-2,3,3′, Has-2,3
Has	1	168.2 s		Has-2, Tyr^I-NH	
	2	55.5 d	4.73 m		Has-NH, Tyr^I-5,5′,NH
	3	70.5 d	4.48 br s	Has-2	Has-NH, Lys-ε-NH, Tyr^I-5,5′NH
	4	170.3 s[e]			
	NH	100.0 d	7.00 d 7.9		Has-2,3, Tyr^I-2
Glu	1	171.0 s		Has-NH	
	2	55.5 d	3.99 m		Has-NH
	3	25.6 t	1.87 m; 2.09 m	Glu-4′	Glu-NH
	4	30.7 t	1.47 m; 2.68 m	Glu-3′	Glu-NH
	5	170.6 s		Glu-4,4′, Ser-3′	
	NH	112.3 d	6.89 d 7.9		Glu-3′,4, Ser-3
Trp	1	172.0 s		Trp-2,3,3′, Glu-NH	
	2	53.8 d	4.56 m	Trp-3,3′,NH	Trp-3,3′,NH
	3	26.2 t	3.15 m; 3.17 m	Trp-2	Trp-2,9,NH; Trp-2,9,NH
	4	109.3 s		Trp-3,3′,5,9,NH(9)	
	4a	127.1 s		Trp-3,3′,5,6,8,9,NH(9)	
	5	117.9 d	7.45 d 7.9	Trp-7	
	6	118.3 d	6.95 t 7.5	Trp-8	
	7	121.0 d	7.06 t 7.7	Trp-5	
	8	111.6 d	7.30 d 8.1	Trp-5,6	Trp-NH(9)
	8a	136.2 s		Trp-5,7,9,NH(9)	
	9	123.1 d	7.17 s	Trp-3,3′,NH(9)	Trp-3,3′,NH(9)
	NH(9)	131.5 d	10.69 s		Trp-8,9
	NH	111.5 d	7.50 d 6.8		Trp-2,3,3′, Asp-3,3′,NH, Ser-3
Asp	1	171.1 s[e]			
	2	53.0 d	4.31 m	Asp-3,3′	Asp-3,3′
	3	34.5 t	2.60 m; 2.67 m		Asp-2,NH, Trp-NH
					Asp-2,NH, Trp-NH
	4	171.5 s[e]			
	NH	122.6 d	9.12 s		Asp-2,3,3′, Ser-2,3,NH, Trp-NH
Ser	1	170.6 s		Ser-2,3′	
	2	51.1 d	4.72 br s	Ser-3′,NH	Ser-3, Asp-NH
	3	63.4 t	4.11 br d 10.7; 4.57 br d 10.7		Ser-2, Asp-NH, Trp-NH, Glu-NH
	NH	109.8 d	8.09 d 7.3		Asp-NH, Pro-2,5′
Pro	1	171.2 s		Ser-NH	
	2	59.1 d	4.59 m		Pro-3,3′,5, Ser-NH
	3	28.0 t	1.80 m; 1.87 m	Pro-5′	Pro-2; Pro-2
	4	24.8 t	1.85 m; 1.91 m		Pro-5′; Pro-5′
	5	46.5 t	3.00 m; 3.68 m		Tyr^{II}-2; Pro-4,4′, Tyr^{II}-2, Ser-NH
Tyr^{II}	1	170.8 s[e]			
	2	52.4 d	4.49 m	Tyr^{II}-3,3′	Tyr^{II}-3,3′, Pro-5,5′, Lys-NH
	3	35.8 t	2.62 m; 2.76 m	Tyr^{II}-2,5,5′	Tyr^{II}-2,NH; Tyr^{II}-2,NH
	4	127.5 s		Tyr^{II}-2,3,3′,6,6′	
	5,5′	129.8 d×2	7.01 d 8.1	Tyr^{II}-3,3′,5,5′	Tyr^{II}-3,3′
	6,6′	115.0 d×2	6.65 d 8.2	Tyr^{II}-5,5′,6,6′	
	7	155.8 s		Tyr^{II}-5,5′,6,6′	
	NH	114.2 d	8.06 d 8.1		Tyr^{II}-3,3′
Lys	1	170.7 s		Lys-2, Tyr^{II}-NH	
	2	51.3 d	4.38 m	Lys-α-NH	Lys-3,4,4′
	3	31.9 t	1.56 m	Lys-4	Lys-2,α-NH,ε-NH
	4	21.4 t	0.80 m; 1.17 m		Lys-2; Lys-2,α-NH,ε-NH
	5	28.9 t	1.26 m; 1.17 m		Lys-ε-NH
	6	39.5 t	2.85 m; 3.00 m		Lys-ε-NH; Lys-ε-NH
	α-NH	114.6 d	7.48 d 8.0		Lys-3,4,4′, Arg-3, Tyr^{II}-2
	ε-NH	121.0 d	8.05 t 6.2		Lys-3,4′,5′,6,6′, Has-2
Arg	1	170.2 s		Arg-2, Lys-α-NH	
	2	51.8 d	4.32 m	Arg-NH	Arg-3,3′,4,4′,5,NH
	3	29.0 t	1.50 m; 1.71 m	Arg-2,4,4′,5	Arg-2,NH,6(NH), Lys-α-NH; Arg-2,NH,6(NH)
	4	24.7 t	1.48 m; 1.69 m	Arg-2,3,3′,5	Arg-2,6(NH); Arg-2,NH,6(NH)
	5	40.5 t	3.09 m	Arg-4	Arg-2,6(NH)

(*continued*)

Table 3. (*continued*)

Position		$\delta_{C/N}$, Mult.[b]	δ_H, Mult., J (Hz)	HMBC correlations[c]	ROESY correlations[d]
	6(NH)	85.0 d	7.33 t 5.5		Arg-3,3′,4,4′,5
	7	156.8 s		Arg-6(NH)	
	8,9	22.0 d	7.10 m		
	NH's	74.0 t	6.95 m (2H)		
	NH	118.6 d	7.69 d 8.0		Arg-2,3,4,4′, Thr^{I}-2,3
Thr^{I}	1	169.7 s		Arg-NH, Thr^{I}-2	
	2	58.2 d	4.20 dd 4.2, 8.2	Thr^{I}-4,NH	Thr^{I}-NH, Arg-NH
	3	66.5 d	3.98 m	Thr^{I}-2,4,NH	Thr^{I}-4,NH, Arg-NH
	4	19.3 q	1.04 d 6.5	Thr^{I}-2	Thr^{I}-3,NH, Ala-3
	NH	109.2 d	7.53 d 8.2		Thr^{I}-2,3,4
Ala	1	172.1 s		Ala-3, Thr^{I}-NH	
	2	48.3 d	4.35 m	Ala-3,NH	Ala-3,NH
	3	17.6 q	1.25 d 6.9	Ala-2,NH	Ala-2,NH, Thr^{I}-NH
	NH	121.5 d	7.83 d 7.0		Ala-2,3, Thr^{II}-2,3
Thr^{II}	1	170.0 s		Ala-NH	
	2	58.4 d	4.21 dd 4.2, 8.1	Thr^{II}-4,NH	Thr^{II}-NH, Ala-NH
	3	66.5 d	3.98 m	Thr^{II}-2,4,NH	Thr^{II}-4,NH, Ala-NH
	4	19.5 q	1.05 d 6.4	Thr^{II}-2	Thr^{II}-3
	NH	115.4 d	7.56 d 8.1		Thr^{II}-2,3, Ac-2
Ac	1	169.6 s		Ac-2, Thr^{II}-2,NH	
	2	22.4 q	1.91 s		Thr^{II}-NH

[a] Carried out on an ARX-500 Bruker instrument.
[b] Multiplicity and assignment from HMQC experiment.
[c] Determined from HMBC experiment, $^{n}J_{CH}$=8 Hz, recycle time 1 s, the HMBC correlations are reported as correlations of the protons printed in the column with the carbons in the rows.
[d] By ROESY experiment, mixing time 400 ms.
[e] The assignment of these carbon signals may be interchange.

values are mild relative to the standard inhibitors used for comparison: antipain,[12] for trypsin (IC_{50} 0.39 μM) and nostopeptin BN920[13] for chymotrypsin (IC_{50} 0.11 μM).

2. Experimental

2.1. General

High-resolution MS were recorded on a Fisons VG Auto-SpecQ M 250 instrument and an Applied Biosystems Voyager System 4312 instrument. UV spectra were recorded on a Kontron 931 plus spectrophotometer. Optical rotation values were obtained on a Jasco P-1010 polarimeter at the sodium D line (589 nm). NMR spectra were recorded on a Bruker Avance 800 Spectrometer at 800.13 MHz for ^{1}H and 201.19 MHz for ^{13}C, a Bruker ARX-500 spectrometer at 500.136 MHz for ^{1}H and 125.76 MHz for ^{13}C, and a Bruker Avance 400 spectrometer at 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C and 40.55 MHz for ^{15}N. ^{1}H, ^{13}C, DEPT, gCOSY, gTOCSY, gROESY, gHMQC, and gHMBC spectra were recorded using standard Bruker pulse sequences. HPLC separations were performed on an ISCO HPLC system (model 2350 pump and model 2360 gradient programer) equipped with an Applied Biosystems Inc. diode-array detector and Merck–Hitachi HPLC system (model L-4200 UV–VIS detector and model L-6200A Intelligent pump).

2.1.1. Water bloom material. *M. aeruginosa*, TAU strain IL-215, was collected, in July 1998, from a pond in the Dan District sewage treatment plant, the Shofdan, in Israel. Morphological classification of the preserved field sample was done under a microscope using the morphological criteria proposed by Komárek and Anagnostidis.[14] A preserved sample of the bloom material is kept in our laboratory labeled as IL-215.

2.1.2. Isolation procedure. The freeze-dried cells (131 g) were extracted with 7:3 MeOH/H_2O. The crude extract (21.2 g) was evaporated and separated on an ODS (YMC-GEL, 120A, 4.4×6.4 cm) flash-column with increasing amounts of MeOH in water. Fractions 4–6 (3:7, 6:4, and 1:1 MeOH/H_2O, 353 mg) were subjected to a reversed-phase HPLC (YMC-ODS-A 5 mm, 250 mm×20.0 mm, DAD at 238 nm, flow rate 5.0 mL/min) in 6:4 water/methanol to obtain three fractions: fraction 1 (71.9 mg, retention time of 20.0 min), fraction 2 (42.8 mg, retention time of 23 min), and fraction 3 (74.5 mg, retention time of 35 min). Fraction 3 was subjected to a reversed-phase HPLC (YMC-ODS-A 5 mm, 250 mm×20.0 mm, DAD at 238 nm, 3:2 0.1% TFA in water/acetonitrile, flow rate 5.0 mL/min). Compound **1** (26.1 mg, 0.02% yield based on the dry weight of the bacteria) was eluted from the column with a retention time of 36.5 min, while compound **2** (13.1 mg, 0.01% yield based on the dry weight of the bacteria) was eluted from the column with a retention time of 29.3 min. Fraction 2 was subjected to the same column and conditions to afford semi-pure **3** (22.3 mg, retention time 28.3 min), which was further purified on the same column with 7:3 0.1% TFA in water/acetonitrile as eluent, to afford pure **3** (10.2 mg, 0.008% yield based on the dry weight of the bacteria), which was eluted from the column with a retention time of 40.6 min.

2.1.2.1. Microviridin SD1684 (1). $[\alpha]_D^{21}$ −21.0 (*c* 16.0, MeOH); UV λ_{max} (MeOH) 224 nm (ε 14,300), 281 nm

(ε 2500). For NMR data see Table 1. Negative FABMS *m/z* 1683.6 $[M-H]^-$; HR MALDI-TOF MS *m/z* 1685.7370 (MH^+, calcd for $C_{76}H_{105}N_{1826}$ *m/z* 1685.7441).

2.1.2.2. Microviridin SD1634 (2). $[\alpha]_D^{21}$ −4.9 (*c* 6.7, MeOH); UV λ_{max} (MeOH) 224 nm (ε 13,500), 281 nm (ε 2600). For NMR data see Table 2. Negative FABMS *m/z* 1633.9 $[M-H]^-$; HR MALDI-TOF MS *m/z* 1635.7208 (MH^+, calcd for $C_{75}H_{99}N_{18}O_{24}$ *m/z* 1635.7074).

2.1.2.3. Microviridin SD1652 (3). $[\alpha]_D^{21}$ −7.3 (*c* 6.8, MeOH); UV λ_{max} (MeOH) 227 nm (ε 22,900), 280 nm (ε 6800). For NMR data see Table 3. Negative FABMS *m/z* 1651.7 $[M-H]^-$; HR MALDI-TOF MS *m/z* 1653.7134 (MH^+, calcd for $C_{75}H_{101}N_{18}O_{25}$ *m/z* 1653.7179).

2.1.3. Determination of the absolute configuration of the amino acids. Portions of compounds **1**–**3** (0.5 mg) were dissolved in 6 M HCl (1 mL). The reaction mixture was then placed in a sealed glass bomb at 110 °C for 20 h. After removal of HCl, by repeated evaporation in vacuo, the hydrolysate was resuspended in water (40 mL). A solution of (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (FDAA) (4.2 mmol) in acetone (150 mL) and 1 M $NaHCO_3$ (20 mL) was added to each reaction vessel and the reaction mixture was stirred at 40 °C for 1 h. A 2 M HCl solution (10 mL) was added to each reaction vessel and the solution was evaporated in vacuo. The *N*-[(-dinitrophenyl)-5-L-alanine amide]-amino acid derivatives, from hydrolysates, were compared with similar derivatized standard amino acids by HPLC analysis: Knauer GmbH Eurospher 100 C18, 10 μm, 4.6×300 mm, flow rate: 1 mL/min, UV detection at 340 nm, linear gradient elution from 9:1 50 mM triethylammonium phosphate (TEAP) buffer (pH 3)/acetonitrile to 1:1 TEAP/acetonitrile within 60 min. The determination of the absolute configuration of each amino acid was confirmed by spiking the derivatized hydrolysates with the derivatized authentic amino acids. Retention times of the derivatized amino acids were: L-Ala, 36.1 min; D-Ala, 41.3 min; L-Arg, 24.5 min; D-Arg, 26.0 min; L-Asp, 30.6 min; D-Asp, 32.3 min; L-Glu, 32.0 min; D-Glu, 34.5 min; D-*threo*-β-Has, 21.7 min; L-*threo*-β-Has, 22.4 min; L-Lys, 53.9 min; D-Lys, 56.7 min; L-Pro, 38.5 min; D-Pro, 41.5 min; L-Ser, 29.0 min; D-Ser, 29.0 min; L-Thr, 29.7 min; D-Thr, 35.0 min; L-Trp, 54.0 min; D-Trp, 57.1 min; L-Tyr, 63.0 min; and D-Tyr, 67.6 min. HPLC analysis of Marfey's derivatives of **1**, **2**, and **3** established: L-*threo*-β-Has, 22.4 min; L-Arg, 24.5 min; L-Ser, 29.0 min; L-Thr, 29.7 min; L-Asp, 30.6 min; L-Glu, 32.0 min; L-Ala, 36.1 min; L-Pro, 38.5 min; L-Trp, 54.0 min; L-Lys, 53.9 min; and L-Tyr, 63.0 min, for the three compounds.

2.1.4. Protease inhibition assays. Trypsin and chymotrypsin were purchased from Sigma Chemical Co. Trypsin was dissolved in 50 mM Tris–HCl/100 mM NaCl/1 mM $CaCl_2$ to prepare a 1 mg/mL solution. Chymotrypsin was dissolved in 50 mM Tris–HCl/100 mM NaCl/1 mM $CaCl_2$/1 mM HCl to prepare a 1 mg/mL solution. A 2 mM solution of *N*-benzoyl-D,L-arginine-*p*-nitroanilide (for trypsin) and Suc-Gly-Gly-*p*-nitroanilide (for chymotrypsin) in the appropriate buffer solution was used as substrate solution. The test sample was dissolved in ethanol and diluted with the same buffer solution used for the enzyme and substrate. A 100 mL buffer solution, 10 mL enzyme solution, and 10 mL of test solution were added to each microtiter plate well and pre-incubated at 37 °C for 5 min. Then 100 mL of substrate solution was added to begin the reaction. The absorbance of the well was immediately measured at 405 nm. The developed color was measured after incubation at 37 °C for 30 min.

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Supplementary data

^1H, ^{13}C NMR, COSY, TOCSY, ROESY, HMQC, and HMBC spectra of microviridins SD1684, SD1634, and SD1652 and Table 4, presenting NMR data of microviridin SD1684 in pyridine-d_5, are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.05.028.

References and notes

1. Rinehart, K. L.; Namikoshi, M. *J. Ind. Microbiol.* **1996**, *17*, 373–384.
2. Ishitsuka, M. O.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1990**, *112*, 8180–8182.
3. Okino, T.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron* **1995**, *51*, 10679–10686.
4. Shin, H. J.; Murakami, M.; Matsuda, H.; Yamaguchi, K. *Tetrahedron* **1996**, *52*, 8159–8168.
5. Murakami, M.; Sun, Q.; Ishida, K.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Phytochemistry* **1997**, *45*, 1197–1202.
6. Fujii, K.; Sivonen, K.; Naganawa, E.; Harada, K. *Tetrahedron* **2000**, *56*, 725–733.
7. Rohrlack, T.; Christoffersen, K.; Hansen, P. E.; Zhang, W.; Czarnecki, O.; Henning, M.; Fastner, J.; Erhard, M.; Neilan, B. A.; Kaebernick, M. *J. Chem. Ecol.* **2003**, *29*, 1757–1770.
8. Reshef, V.; Carmeli, S. *Tetrahedron* **2001**, *57*, 2885–2894.
9. Slemion, I. Z.; Wieland, T.; Pook, K. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702–703.
10. Williamson, R. T.; Marques, B. L.; Gerwick, W. H. *Tetrahedron* **1999**, *55*, 2881–2888.
11. Marfey's reagent: 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
12. Suda, H.; Aoyagi, T.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1972**, *25*, 263–266.
13. Ploutno, A.; Carmeli, S. *Tetrahedron* **2002**, *58*, 9949–9957.
14. Komárek, J.; Anagnostidis, K. In *Süsswasserflora von Mitteleuropa*; Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D., Eds.; Cyanoprokaryota, Vol. 1. Teil Chroococcales; Gustav Fischer: Jena, 1999; pp 225–236.

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Isoxazoline-carbocyclic aminols for nucleoside synthesis through aza-Diels–Alder reactions

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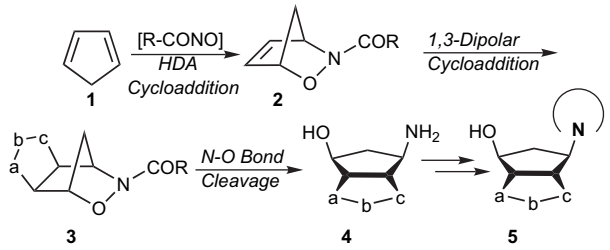
Abstract—A novel approach to useful aminols for the synthesis of carbocyclic nucleosides is reported starting from a convenient source, the 2-azanorborn-5-enes. These are readily available through the Grieco cycloaddition of cyclopentadiene with iminium salts and are reactive dipolarophiles toward nitrile oxides. The prolific elaboration of the isoxazoline cycloadducts allowed preparation of the target aminols through the unmasking of the hydroxymethylene group at the C3 level of the azanorbornene structure.

1. Introduction

The preparation of carbocyclic and heterocyclic nucleoside analogues has been extensively pursued due to the importance in the development of new anti-viral drugs.[1] Most of the nucleoside analogues refer to a general structure defined by the presence of a five-membered ring (furanose ring in natural compounds) of a carbocyclic or heterocyclic nature, which holds a hydroxymethylene group cis-related to the heterobase needed for enzyme or nucleic acid recognition.[2] Great interest has also risen in the so-called nor-derivatives where the hydroxymethylene substituent is replaced by an OH function.[3]

In this context, we have recently developed a synthesis of the isoxazoline-carbocyclic nucleosides **5** by the linear construction of the desired purine and pyrimidine bases on the regioisomeric aminols **4** (Scheme 1) obtained through elaboration of the hetero-Diels–Alder (HDA) cycloadducts **2** of cyclopentadiene **1** with the nitrosocarbonyl intermediates (RCONO).[4] These fleeting intermediates are generated traditionally by periodate oxidation of hydroxamic acids[5] or by oxidation of nitrile oxides with *N*-methylmorpholine *N*-oxide (NMO),[6] and are promptly trapped with dienes to afford HDA cycloadducts in high yields. The HDA cyclopentadiene cycloadducts **2** have proved to be highly reactive dipolarophiles toward nitrile oxides, affording the 1,3-dipolar cycloadducts of type **3**, which are converted quantitatively by detachment of the acyl moiety and reductive cleavage of the N–O bond into the stereodefined *anti* aminols **4**.[7] Starting from these, by the linear construction of the heterobases, we have detailed the first synthesis of a class of racemic purine- and pyrimidine-carbocyclic nucleosides **5** containing a fused isoxazolinic ring and lacking a methylene group in the side chain in the carbocyclic unit.[4]



Scheme 1.

For direct access to the nucleoside analogues carrying the hydroxymethylene moiety on the carbocyclic unit, in order to compare the role of the elongation of the side chain on the biological activity of isoxazoline-nucleosides, the 2-azabicyclo[2.2.1]hept-5-en-3-one **6** looked promising for this purpose (Fig. 1). This lactam **6** has found wide applications in carbocyclic nucleoside synthesis[2,8] because of the easy access to the required aminols by easy cleavage of the lactam moiety. The preparation of the starting azaheptenone **6** is however affected by some problems; it can be prepared through cycloaddition of cyclopentadiene to tosyl cyanide or methanesulfonyl cyanide, whose preparations require a potentially hazardous starting material (cyanogen chloride).[9] Although available from various chemical suppliers,

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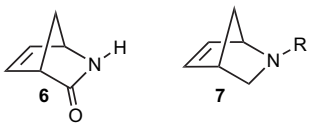


Figure 1.

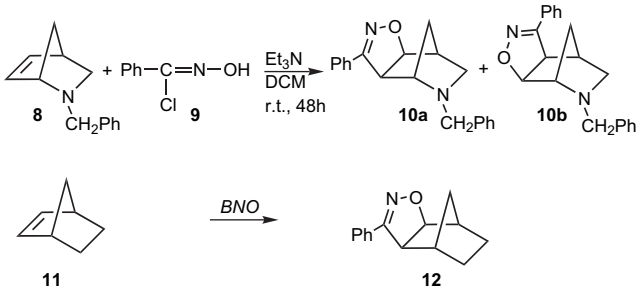
the cost of the racemic compound is too high for large-scale synthesis, and even more is that of the enantiomerically pure forms.[10]

Structurally similar to lactam **6** are Grieco's 2-azanorbornenes **7** that are derived from the cycloaddition of cyclopentadiene with iminium salts generated in situ under Mannich-like conditions, in a mild and convenient aqueous aza-Diels–Alder (ADA) reaction.[11] Despite their easy availability, no attempts have been made for their use in nucleosidic syntheses. To this purpose the aza-methylene bridge has to be modified, precisely the carbon C3 must be oxidized by appropriate unmasking procedures. Here we report the synthetic approach to novel isoxazoline-carbocyclic aminols bearing an hydroxymethylene functionality starting from the easy available 2-azanorbornenes of type **7**.

2. Results and discussion

2.1. The *N*-benzyl-2-azanorborn-5-ene 8 as dipolarophile

The *N*-benzyl-2-azanorborn-5-ene **8** was prepared by addition of freshly distilled cyclopentadiene to an aqueous solution of benzylamine hydrochloride and 37% aqueous formaldehyde in an ADA reaction according to the well-known procedure.[12] The 1,3-dipolar cycloaddition of benzonitrile oxide (BNO) with **8** was performed with the in situ procedure,[13] by adding the benzhydroximoyl chloride **9** to a dichloromethane (DCM) solution of a slight excess of *N*-benzyl-2-azanorborn-5-ene **8** (1.2 equiv) and a slight excess of Et_3N (1.1 equiv) (Scheme 2).



Scheme 2.

After stirring at room temperature for 48 h, the two regioisomeric isoxazoline cycloadducts **10a** and **10b** were isolated in 49% and 43% yields, respectively.

The structures rely upon their analytical and spectroscopic data. The ^{1}H NMR spectrum of **10a** showed the isoxazoline protons as doublets (J=8 Hz) at δ 4.03 and 4.83, while in regioisomer **10b** the isoxazoline doublets (J=8 Hz) were found at δ 3.74 and 4.94. The absence of appreciable coupling between the isoxazoline and bridge-head protons fully support the *exo* structures[7a,14] and is in line with the *exo*-selective addition to these dipolarophiles reported in literature for the dihydroxylation reactions of **8**.[15] The regiochemistry is however not clearly indicated by the spectra and an X-ray analysis of single crystal of **10b** allowed us to unequivocally attribute the correct regioisomeric structure. Figure 2 reports the ORTEP view of **10b**.

We previously found that 2-oxa-3-azanorbornenes of type **2** add BNO *exo*-selectively, and were 1.7 times more reactive than norbornene as a consequence of the higher relief of strain.[7,16] Similarly, we have performed a few competition experiments with *N*-benzyl-2-azanorbornene **8** and norbornene **11** in the cycloaddition with BNO. Table 1 gives the product distributions for the reactions conducted in a few representative solvents. These results show that the 2-azanorbornene **8** still remains a highly reactive dipolarophile but less than norbornene **11**. As an average, the reactivity of the 2-azanorbornene **8** is half that of norbornene **11** in apolar solvents (entries 1 and 2) and decreases to one third in more polar (entry 8) or polarizable (entries 5 and 6) solvents or in alcohols (entries 9 and 10). The lesser reactivity of the 2-azanorbornene **8** with respect to norbornene **11** could be attributed to the reduced strain in **8**. Replacement of the eclipsed dimethylene bridge of norbornene with the aminomethylene moiety causes a decrease in strain, in keeping with the reduced torsional barriers of amines with respect to alkanes.[17] The further decrease in reactivity in

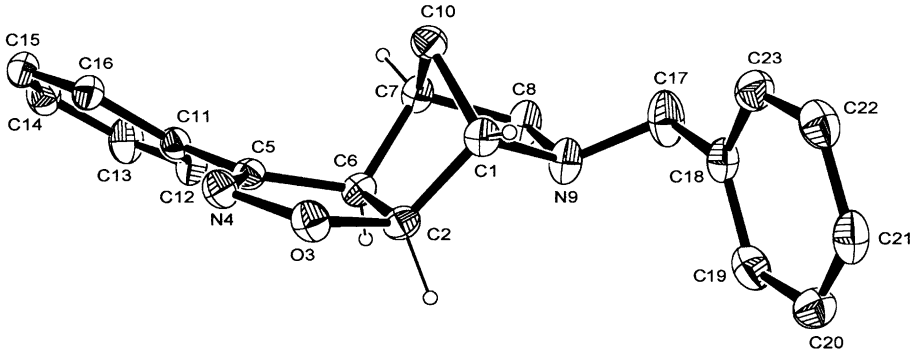


Figure 2. ORTEP plot of cycloadduct **10b** with atom labeling (ellipsoid at 25% probability). Hydrogen atoms are omitted for clarity with exception of C1, C2, C6, and C7.

Table 1. Competition experiments with *N*-benzyl-2-azanorbornene **8** and norbornene **11** in the cycloaddition to BNO and regioisomeric distribution[a,b]

No.	Solvent	**12** (%)	**10a** (%)	**10b** (%)	**10a+10b/12**	**10a/10b**
1	*n*-Hexane	66	19	13	0.48	59/41
2	Cyclohexane	65	17	12	0.45	59/41
3	THF	68	14	12	0.38	54/46
4	Acetone	73	13	11	0.33	54/46
5	C_6H_6	74	13	10	0.31	57/43
6	DCM	76	11	10	0.28	52/48
7	AcOEt	78	11	9	0.26	55/45
8	MeCN	76	10	8	0.24	56/44
9	MeOH	76	13	8	0.28	62/38
10	EtOH	75	13	8	0.28	62/38

[a] BNO generated in situ in the presence of a mixture of dipolarophiles **8** and **11**, 5 equiv each.
[b] Quantitative data are the average of HPLC analyses performed in duplicate from independent experiments.

polar or alcoholic solvents may be attributed to some complexation or hydrogen-bonding involving the nitrogen lone pair that increases the electron attracting power of the nitrogen and lowers the π orbital of the nearby C=C double bond. A decrease in reactivity in the cycloadditions of the mildly electrophilic nitrile oxides is then expected.[13]

The slight regiochemical preference of cycloadduct **10a** over **10b** compares well with previous results on the cycloadditions of BNO with 3-substituted cyclopentenes and can be attributed to the polarization of the double bond caused by the electronegative nitrogen allylic substitution.[13] Solvents affect slightly only the regiochemistry, which increases in alcohols but unexpectedly decreases in polar and polarizable solvents.

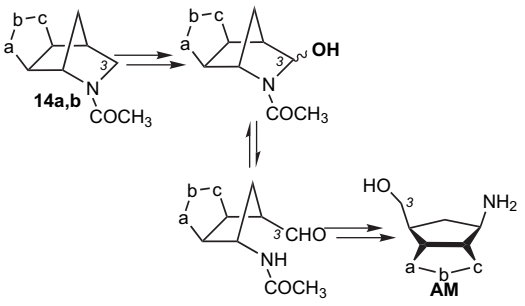
2.2. Synthesis of stereoisomeric aminols

Before attempting the cleavage step we have transformed the adducts into more convenient derivatives. Since the hydrogenolytic detachment of the *N*-benzyl moiety performed poorly, we found a convenient and high yielding alternative. The first step was the oxidation of **10a** and **10b**, which were converted into the corresponding *N*-oxides **13a** and **13b** by treating with 1.2 equiv of mCPBA in DCM at room temperature.[18] Conversion of the *N*-oxides into the amides **14a** and **14b** was achieved through the mild Polonovski rearrangement[19] of the amine *N*-oxides promoted by Ac_2O to afford the *N*-acetyl derivatives (Scheme 3).

The *N*-oxides **13a** and **13b** were isolated in quantitative yields and fully characterized spectroscopically. The *exo* position of the N–O bond was inferred by significative shifts of various signals due to the deshielding effect produced by the *N*-oxide group. In particular, the C3-H_{exo}, cis-related to the oxygen, is deshielded and occurs at δ 3.79 (dd, *J*=12, 5 Hz) in **13a** and at δ 3.75 (dd, *J*=12, 4 Hz) in **13b**, a typical behavior of *N*-oxides as reported in literature.[20]

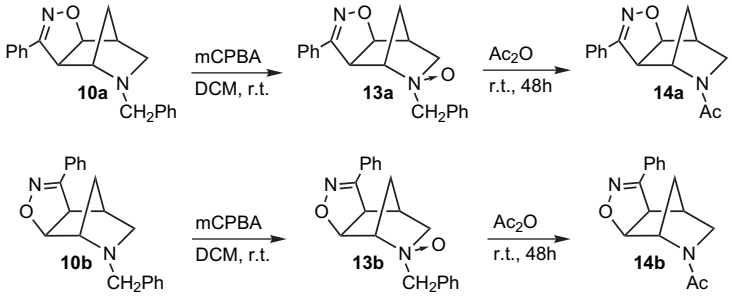
The Polonovski rearrangement of the *N*-oxides **13a** and **13b** was performed by dissolving the oxidized compounds in Ac_2O and leaving to react at room temperature for 48 h. The excess of Ac_2O was then decomposed with water overnight and, after adjustment of pH to 8, the water solutions were extracted with DCM. From the dried organic phase the *N*-acetyl derivatives **14a** and **14b** were isolated upon evaporation of the solvent as solid compounds in 94% and 85% yields, respectively. The structures rely upon the collected analytical and spectroscopic data. Besides little differences in the chemical shifts relative to the isoxazoline protons and those of the azanorbornene structure, the most relevant changes referred to the presence of the acetyl groups at δ 2.04 and 2.16, respectively for **14a** and **14b**.

Starting from the amides **14a** and **14b** we planned the oxidative strategy shown schematically in Scheme 4 for unmasking a hydroxymethylene group at the C3 of the amides.

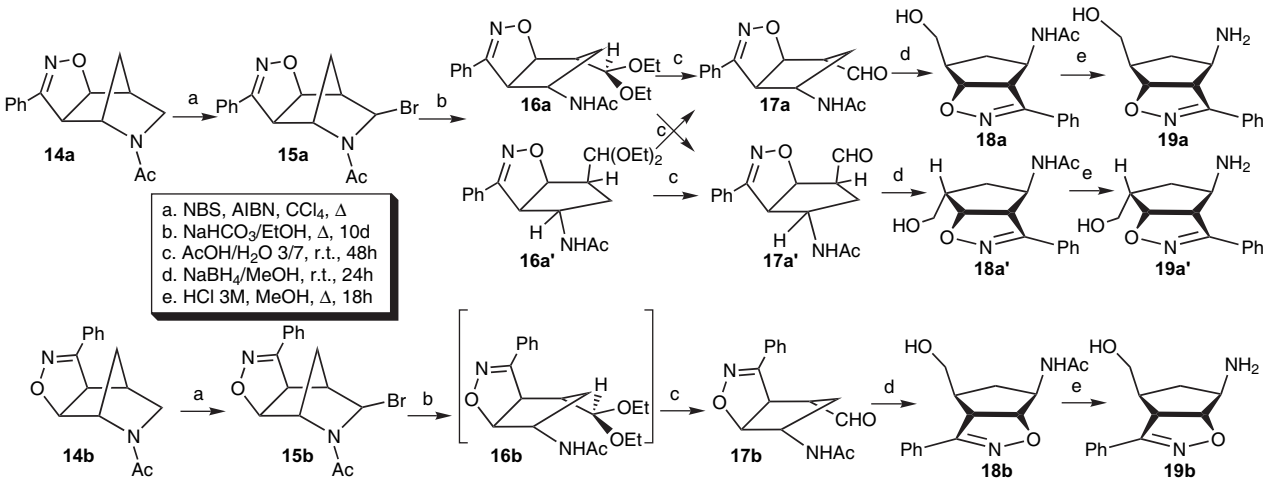


Scheme 4.

After a few attempts at a direct conversion of the C3 into a carbonyl function,[21] we found a convenient procedure for the oxidation of the regioisomeric *N*-acetyl derivatives **14a** and **14b** through NBS/AIBN bromination reaction (Scheme 5). Bromination of the cycloadducts **14a** and **14b** takes place easily in refluxing CCl_4 for 1–2 h. After filtration of succinimide and evaporation of the solvent, the residues were submitted to column chromatography on silica gel eluting with $CHCl_3$. The chromatographic separations afforded the expected *exo*-bromo derivatives **15a** and **15b** as the



Scheme 3.



Scheme 5.

major constituents, both obtained in 47% yield. Their structures rely upon the ^{1}H NMR spectra, which show the CH–Br signals as singlets at δ 5.37 and 5.45, respectively, for **15a** and **15b**. The lack of coupling with the bridge-head protons is consistent with the *exo* stereochemistry of bromine atom.[14]

In the case of the bromination of **14a**, chromatographic separation of the reaction mixtures also afforded more advanced intermediates toward the final aminol targets. Two epimeric diethoxy acetals **16a** and **16a′** and the corresponding aldehydes **17a** and **17a′** could be isolated, while in the case of the bromination of **14b** only the aldehyde **17b** was characterized. The formation of the acetals and the aldehydes can be attributed to the silica gel-promoted transformation of the bromo derivatives **15** due to the presence of ethanol as stabilizing agent in the chloroform used for the chromatographic separation. From **14a** in particular, two epimeric acetals **16a** (mp 140–142 °C) and **16a′** (mp 110–112 °C) were isolated as white solids in fair yields (16% and 12%, respectively). The structures rely upon careful spectroscopic analyses. The ring opening of the azanorbornene skeleton was indicated by the presence of a neat NH–Ac absorption in the FTIR spectrum (in **16a**, band at 3260 cm^{-1}; in **16a′**, band at 3271 cm^{-1}) and in the ^{1}H NMR spectra by the signals of two different OEt groups attributable to diastereotopic diethylacetal moieties. Moreover in these cleavage products, the flexible cyclopentane moiety allows couplings between the isoxazoline protons and the adjacent methines. In the retained product **16a** the H5-isoxazolinic proton occurs as a double doublet at δ 5.16 because of coupling with the H4-isoxazolinic proton at δ 4.09 (J=10 Hz) and the adjacent *trans* methine (J_{trans}=3 Hz) while in the epimeric product **16a′** the H5-isoxazolinic proton is a double doublet at δ 5.12 owing to the coupling with the H4-isoxazolinic proton at δ 4.23 (J=9 Hz) and the adjacent *cis* methine (J_{cis}=6 Hz).

NOESY experiments support the attributions showing diagnostic cross-peaks between the acetal proton and the H5-isoxazoline one in isomer **16a**. In the epimer **16a′** the NOESY cross-peaks occur between the acetal proton and one of the two different cyclopentane methylene protons and between the other methylene protons with the amide NH. By adapting the same procedure the bromide **15b** could be converted into the aldehyde **17b**. From the chromatographic separation an oily fraction was isolated containing presumably the acetal **16b** and related hemiacetal structures. The oil could not be purified but was directly hydrolyzed to the aldehyde. The formation of a single aldehyde **17b** in this case is probably due to the severe steric hindrance for substituents cis-located to the phenyl isoxazoline moiety, which offsets the epimerization.

The last compounds eluted are the aldehydes **17**. From **14a**, the two epimeric aldehydes **17a** and **17a′** were isolated as an oily mixture in comparable amounts in a combined yield of 18%. In the ^{1}H NMR spectrum of the mixture two singlets corresponding to the aldehyde groups were detected at δ 9.97 and 10.01, while the signal at δ 6.25 corresponded to the NH group, as the FTIR spectrum confirmed (NH band at 3297 cm^{-1} and the prominent aldehyde absorption). Since chromatographic separation of the two aldehydes was unsatisfactory, the mixture was submitted to the subsequent reductive step. From **14b** a single aldehyde **17b** was obtained as a crystalline compound (20% yield). It shows a neat NH absorption at 3520 cm^{-1} and the C=O band at 1716 cm^{-1} in the FTIR spectrum. In the ^{1}H NMR spectrum the aldehyde signal occurs as a sharp singlet at δ 9.79 while the NH is a broad singlet at δ 7.07.

The transformation of the bromides **15** into the acetals **16** and then into the aldehyde **17** could be optimized by adapting reported procedures. Thus refluxing the bromide **15a** in ethanol in the presence of an excess of $NaHCO_3$[22] allowed the complete conversion of **15a** into a 1:1 mixture of the two epimeric acetals **16a** and **16a′**, which were quantitatively hydrolyzed to the corresponding aldehydes **17a** and **17a′** upon treatment with AcOH/H_2O 3:7 at room temperature for 48 h.[23] Noteworthy and in accordance with expected enolization in acidic medium, from any single epimeric acetal **16a** or **16a′** the two epimeric aldehydes were obtained in a nearly 1:1 ratio and, as before, not separated but submitted to subsequent reduction. By applying the same procedure to the bromide **15b** a single aldehyde **17b** was obtained.

The reduction of the aldehydes **17** to the desired primary alcohols **18** was performed with $NaBH_4$ in MeOH solution. From the diastereomeric aldehydes **17a** and **17a′**, the

corresponding alcohols **18a** and **18a′** were obtained in quantitative yields and could be easily separated by column chromatography as colorless solids. The structures rely upon their analytical and spectroscopic data. Besides the signals attributable to the cyclopenta-isoxazoline skeleton, the ^{1}H NMR spectrum of the alcohols **18** showed the significant presence of the OH and the diastereotopic protons of the hydroxymethylene group as multiplets at δ 3.73 and 3.84 in **18a** and at δ 3.71 and 3.86 in **18a′**. The stereochemistry relies upon the vicinal coupling of the H5-isoxazolinic protons and the methines. In the retained product **18a** the H5-isoxazolinic proton at δ 5.13 (dd, J=9.6, 4 Hz) is coupled with the smaller J_{trans} with the adjacent *trans* methine while in the epimeric product **18a′** the H5-isoxazolinic proton at δ 5.20 (dd, J=9, 5 Hz) is coupled with the larger J_{cis} with the analogous type of proton.

The NOESY experiment carried out on **18a** confirmed the stereochemistry due to the existence of cross-peaks correlating the hydroxymethylene protons with the H5-isoxazoline proton at δ 5.13 and the NH proton with the H4-isoxazoline one at δ 4.11. In the epimer **18a′** the NOESY experiment confirmed the *anti* relationship between the alcoholic function and the acetamide group because of the sole presence of a cross-peak correlating the H5-isoxazoline proton at δ 5.20 and the *CH*–CH_2OH proton, while the CH_2OH shows no NOE cross-peaks. The spectra of the regioisomeric alcohol **18b** show the hydroxymethylene protons at δ 3.74 and 3.83; the H5-isoxazolinic proton at δ 4.99 (dd, J=9 Hz with the H4-isoxazolinic proton at δ 4.18) is trans coupled with the *CH*–NHAc (J=2 Hz) at δ 4.39.

Finally, the three regio- and stereoisomeric alcohols **18** were definitively and quantitatively hydrolyzed to the corresponding aminols **19** by boiling a methanolic solution with HCl 3 M overnight (Scheme 5). Table 2 summarizes the main physical and spectroscopic data of the target compounds. Very sharp bands were found in the FTIR spectra between 3250–3370 cm^{-1} corresponding to the NH_2 groups while the OH bands are somewhat broad and at lower frequencies because of strong intra- and inter-molecular hydrogen bonds. In the ^{1}H NMR spectra the hydroxymethylene groups gave AB systems at the expected chemical shifts and the isoxazoline protons were also in their typical range.

Table 2. Physical and spectroscopic data of the aminols **19**

Entry	**19**	Mp °C[a]	FTIR (cm^{-1})		^{1}H NMR (δ)		
			ν_{OH}	ν_{NH_2}	CH_2OH	H4-isoxaz.	H5-isoxaz.
1	**a**	116–118	3176	3334, 3266	3.70	4.02	5.21
2	**a′**	154–156	3158	3330, 3257	3.67, 3.87	4.03	5.28
3	**b**	130–131	3176	3370, 3291	3.70	4.26	4.79

[a] White solids from benzene/ethanol.

3. Conclusions

In the search of a new and convenient synthetic route to isoxazoline-carbocyclic aminols with a primary hydroxy group useful for nucleoside preparations, we took advantage of the easily available *N*-benzyl-2-azanorbornene, which underwent 1,3-dipolar cycloaddition with BNO to afford the regioisomeric cycloadducts **10a** and **10b** in good yields. Replacement of the benzyl group was easily achieved through *N*-oxidation and a successive Polonovski rearrangement leading to the *N*-acetyl derivatives, which proved to be convenient intermediates for the activation of the C3 position of the azanorbornene structure. Upon treatment with NBS and solvolysis[24] of the bromo derivatives **15a** and **15b**, the aldehydes **17a** and **17b** were obtained. The straightforward reduction of the latters gave the target aminols.

In conclusion, we have proposed a novel approach to useful aminols for the synthesis of carbocyclic nucleosides starting from the readily available 2-azanorbornenes and unmasking the hydroxymethylene group at the C3 level of the azanorbornene structure.

The aminols will be used for the linear construction of purine and pyrimidine carbocyclic nucleosides whose activities as anti-viral agents will be compared with those of the previously synthesized compounds.

4. Crystal structure analyses

The ORTEP view of compound **10b** with the atomic numbering is shown in Figure 2. The crystal and collection data as well as the structure refinements of the cycloadduct are given in Table 3. Table 4 reports the bond lengths and angles while Table 5 the torsion angles.

Table 3. Crystal data, collection data, and structure refinements of cycloadduct **10b**

	10b
Formula	$C_{20}H_{20}N_2O$
CCD deposit no.	291,467
MW	304.38
Crystal size, mm	0.52×0.42×0.28
Temperature, K	293(2)
Crystal system	Monoclinic
Space group	$P2_1/n$
a, Å	12.622(2)
b, Å	9.687(1)
c, Å	13.348(1)
β	96.01(1)
V, Å^3	1623.1(3)
Z	4
D_{calcd}, g/cm^3	1.246
Abs coeff., μ, cm^{-1}	0.774
Radiation	Mo Kα
λ	0.071073
F(000)	648
Range (°) for data coll.	$2.1\leq\theta\leq30.0$
Index range	$-17<h<17$, $0<k<13$, $0<l<18$
Reflection no.	4723
Unique refl.	4656
Correction applied	Lorentz polarization
Absorption correction	Semi-empirical from psi-scan
Absorption factors T	0.998, 0.973
No. of obsd refl. $I>2\sigma(I)$	1610
Refinement method	Full-matrix least-squares on F^2
Variables no.	288
Weights	0.0474, 0.003
Goodness-of-fit	0.923 (No refl. 4656)
R_1	0.064 (No refl. 1610)
wR_2	0.143 (No refl. 4656)
($\Delta\rho$) max, min, eÅ^{-3}	0.220, −0.110

Table 4. Bond lengths (Å) and angles (°) with Esd's in parentheses of cycloadducts **10b**

	10b
Bond lengths	
C1–C2	1.521(3)
C1–N9	1.481(3)
C1–C10	1.526(4)
C2–O3	1.454(3)
C2–C6	1.534(4)
O3–N4	1.420(3)
N4–C5	1.278(3)
C5–C6	1.496(3)
C5–C11	1.464(3)
C6–C7	1.542(3)
C7–C8	1.524(4)
C7–C10	1.522(4)
C8–N9	1.493(4)
N9–C17	1.430(4)
Bond angles	
C2–C1–N9	103.9(2)
C2–C1–C10	102.8(2)
N9–C1–C10	104.6(2)
C1–C2–O3	112.5(2)
C1–C2–C6	102.7(2)
O3–C2–C6	105.2(2)
C2–O3–N4	109.5(2)
O3–N4–C5	109.6(2)
N4–C5–C6	114.5(2)
N4–C5–C11	120.0(2)
C6–C5–C11	125.5(2)
C2–C6–C5	101.2(2)
C2–C6–C7	102.9(2)
C5–C6–C7	114.4(2)
C6–C7–C8	107.1(2)
C6–C7–C10	101.9(2)
C8–C7–C10	100.8(2)
C7–C8–N9	103.4(2)
C1–N9–C8	104.0(2)
C1–N9–C17	116.1(2)
C8–N9–C17	111.7(1)
C1–C10–C7	93.0(2)

In the azanorbornane ring the sum of the angles at N9 [331.8(2)°] is consistent with sp^3 hybridization. The heights of the pyramids with the nitrogen atom at the apex and the three atoms connected to it at the base is 0.459(2) Å. In the isoxazoline rings the angle at N4 of **10b** is consistent with sp^2 hybridization of N4 whereby the 2pz lone pair takes part in π-bonding within a part of the heterocyclic rings. Indeed the bond distances N4–C5 show a double bond character: 1.278(3) Å. The internal angles at the nitrogen atoms, O3–N4–C5 109.6(2)°, C1–N9–C8 104.0(2)° differ by 5.6°, justified by the different types of nitrogen; the narrower angle C1–N9–C8 is caused by the lone pair.

Bond lengths and angles in the condensed phenyl rings are reasonable, on weighted average,[25] 1.374(3) Å and 119.9(4)°. The phenyl ring attached to isoxazoline is nearly planar with maximum deviation 0.008(3) Å; this ring is tilted with respect to the isoxazoline moiety by 26.2(1)°, implying only a small conjugation between the double bond N4–C5 and the phenyl. The orientation is expressed by torsion angles given in Table 5. The deviations of atoms from the least-squares plane of the isoxazoline rings are in the range from −0.005(2) Å to 0.006(2) Å consistent with conformation *E* puckering parameters:[26] Q=0.010, ϕ=144.8° (ideal value 144°). The interplanar angle between the isoxazoline and the moiety C1–C2–C6–C7 is 117.98(9)°.

Table 5. Torsion angles (°) in compound **10b** with Esd's in parentheses

	10b
N4–C5–C11–C12	24.8(3)
N4–C5–C11–C16	−154.0(2)
C6–C5–C11–C12	−153.9(2)
C6–C5–C11–C16	27.3(3)

The six-membered ring of the azanorbornane is puckered with deviations by least-squares plane in the range −0.614(2) Å to 0.342(3) Å. These rings adopt boat conformation with parameters: Q=0.979, ϕ=62.4°, and θ=88.5° (ideal conformation ϕ=60° and θ=90°). The four chiral atoms C1, C2, C6, and C7 have the following configuration specified by the sequence rule:[27] *S*, *S*, *R*, *R* or *R*, *R*, *S*, *S* because of the centric space group. The molecular packing in the crystal is determined by van der Waal's contacts.

5. Experimental

All melting points are uncorrected. Elemental analyses were done on a C. Erba 1106 elemental analyzer. IR spectra (Nujol mulls) were recorded on an FTIR Perkin–Elmer RX-1. ^{1}H and ^{13}C NMR spectra and NOESY experiments were recorded on a Bruker AVANCE 300 in the specified deuterated solvents. Chemical shifts are expressed in parts per million from internal tetramethylsilane (δ). UV–vis spectra were recorded on an UV Perkin–Elmer LAMBDA 16 spectrophotometer using acetonitrile as solvent. HPLC analyses were carried out by means of a WATERS 1525 instrument equipped with an UV 2487 detector (λ=263 nm) both controlled by Breeze™ software and a RP C-18 Intersil ODS-2 column; a mixture of H_2O/CH_3CN 60:40 (1.0 mL/min) was used as an eluant. Column chromatography and TLC: silica gel 60 (0.063–0.200 mm) (Merck), eluants as specified. The identification of samples from different experiments was secured by mixed mps and superimposable IR spectra.

Materials. Benzhydroximoyl chloride **9** was prepared according to the well-known procedures.[28]

5.1. Cycloaddition of BNO with *N*-benzyl-2-azanorbornene

To *N*-benzyl-2-azanorbornene **8** (61.0 g, 327 mmol) dissolved in DCM (130 mL), Et_3N (42 mL, 300 mmol) was added and a solution of benzhydroximoyl chloride **9** (42.4 g, 273 mmol) in 150 mL of DCM was added dropwise under stirring at 0 °C. The reaction was continued for 48 h. The organic solution was washed with water and dried over anhydrous Na_2SO_4. The crude residue was then submitted to column chromatography to separate the cycloadducts **10a** and **10b**, which were isolated in 49% and 43% yields, respectively.

10a (33.8 g, 49%): As straw colored crystals from ethanol, mp 116–118 °C; [Found C, 78.9; H, 6.6; N, 9.1. $C_{20}H_{20}N_2O$ (MW=304.38) requires C, 78.92; H, 6.62; N, 9.20%]; ν_{max} (Nujol) 1590 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.25–7.56 (10H, m, Ph), 4.83 (1H, dt, *J* 8, 1 Hz, H5-isoxaz.),

4.03 (1H, d, *J* 8 Hz, H4-isoxaz.), 3.79 (2H, AB syst., *J* 13 Hz, C*H*$_2$–Ph), 3.51 (1H, s, CH–N), 2.94 (1H, dd, *J* 10, 4 Hz, H3-*exo*), 2.78 (1H, br s, CH), 2.12 (1H, d, *J* 10 Hz, H3-*endo*), 1.64 (2H, m, CH$_2$); δ_C (75 MHz, $CDCl_3$) 156.4, 138.8, 129.7, 128.8, 128.6, 128.5, 128.4, 127.1, 126.6, 86.2, 61.8, 58.0, 53.3, 52.3, 44.4, 30.4.

10b (29.7 g, 43%): As straw colored crystals from ethanol, mp 119–121 °C; [Found C, 78.9; H, 6.7; N, 9.2. $C_{20}H_{20}N_2O$ (MW=304.38) requires C, 78.92; H, 6.62; N, 9.20%]; ν_{max} (Nujol) 1592 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.74 (4H, m, Ph), 7.25–7.56 (6H, m, Ph), 4.94 (1H, dt, *J* 8, 1.4 Hz, H5-isoxaz.), 3.78 (2H, s, C*H*$_2$–Ph), 3.74 (1H, d, *J* 8 Hz, H4-isoxaz.), 3.50 (1H, s, CH–N), 2.69 (1H, br s, CH); 2.63 (2H, m, H3); 1.58 (2H, m, CH$_2$); δ_C (75 MHz, $CDCl_3$) 156.7, 139.2, 129.8, 128.9, 128.7, 128.3, 127.0, 126.7, 85.8, 64.4, 59.5, 57.3, 55.7, 40.6, 28.8.

5.2. Competition experiments between norbornenes 8 and 11, and regioisomeric distribution of cycloadducts 10a and 10b

For the competition experiments, benzhydroximoyl chloride **9** (15 mg, 9.6 mmol) was added to a solution of *N*-benzyl-2-azanorbornene **8** and norbornene **11** in equimolecular amounts in 25 mL of the desired solvent along with 1.1 equiv of Et_3N. The mixtures were allowed to react at room temperature for 48 h. The solvents were evaporated and the crude residue was taken up in 25 mL of acetonitrile to be submitted to HPLC analyses.

For the determination of the regioisomeric distribution, the same quantities and procedure were followed in the absence of norbornene **11**.

The results are reported in Table 2.

5.3. Oxidation of the cycloadducts 10a and 10b with mCPBA

To DCM solutions of cycloadducts **10a** and **10b**, a slight excess (1.1 equiv) of mCPBA was added portionwise under stirring, cooling with a water bath the solutions if the temperature rises. The reactions were continued until the starting cycloadducts are consumed (TLC monitoring). Once the reactions were over, the solutions were neutralized with K_2CO_3 and were washed with water with final drying over anhydrous Na_2SO_4. Upon evaporation of the solvent, the crude *N*-oxides **13a** and **13b** were obtained in quantitative yields.

13a: White solid from ethanol, mp 122–124 °C; [Found C, 74.8; H, 6.1; N, 8.7. $C_{20}H_{20}N_2O_2$ (MW=320.38) requires C, 74.97; H, 6.29; N, 8.74%]; ν_{max} (Nujol) 1654 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.98 (2H, m, Ph), 7.00–7.60 (8H, m, Ph), 5.05 (1H, d, *J* 13 Hz, H5-isoxaz.), 4.97 (1H, d, *J* 8 Hz, C*H*$_2$–Ph), 4.45 (1H, d, *J* 13 Hz, H4-isoxaz.), 4.23 (1H, d, *J* 8 Hz, C*H*$_2$–Ph), 3.94 (1H, s, CH–N), 3.79 (1H, dd, *J* 12, 5 Hz, H3-*exo*), 3.56 (1H, dd, *J* 12, 1 Hz, H3-*endo*), 3.11 (1H, d, *J* 4 Hz, CH), 2.98 (1H, d, *J* 12 Hz, CH$_2$), 1.84 (1H, d, *J* 12 Hz, CH$_2$); δ_C (75 MHz, $CDCl_3$) 153.7, 131.2, 130.8, 130.4, 129.6, 128.9, 128.7, 127.1, 126.4, 84.5, 76.2, 74.8, 69.4, 52.9, 43.1, 29.9.

13b: White solid from ethanol, mp 144–146 °C; [Found C, 74.8; H, 6.2; N, 8.6. $C_{20}H_{20}N_2O_2$ (MW=320.38) requires C, 74.97; H, 6.29; N, 8.74%]; ν_{max} (Nujol) 1654 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.62 (4H, m, Ph), 7.35 (6H, m, Ph), 5.83 (1H, d, *J* 8 Hz, H5-isoxaz.), 4.64 (1H, d, *J* 12 Hz, C*H*$_2$–Ph), 4.37 (1H, d, *J* 12 Hz, C*H*$_2$–Ph), 4.12 (1H, d, *J* 8 Hz, H4-isoxaz.), 3.86 (1H, s, CH–N), 3.75 (1H, dd, *J* 12, 4 Hz, H3-*exo*), 3.30 (1H, d, *J* 12 Hz, H3-*endo*), 2.80 (1H, d, *J* 14 Hz, CH), 2.78 (1H, d, *J* 12 Hz, CH$_2$), 1.69 (1H, d, *J* 12 Hz, CH$_2$); δ_C (75 MHz, $CDCl_3$) 156.8, 132.0, 130.6, 130.4, 129.4, 128.9, 128.4, 127.7, 126.8, 81.8, 76.0, 73.0, 67.8, 54.4, 40.3, 31.4.

5.4. Polonovski rearrangement

N-Oxides **13a** and **13b** were dissolved in pure Ac_2O (2.5 equiv) by controlling the temperature with an ice-bath. Stirring was continued at room temperature for 48 h. After this period of time, excess Ac_2O was decomposed by addition of water (leaving the mixtures overnight). To the solutions $NaHCO_3$ was added up to pH=8 and the solutions extracted with DCM. The organic phases were dried over anhydrous Na_2SO_4 and evaporation of the solvent afforded the crude *N*-acetyl derivatives **14a** and **14b** in nearly quantitative yields.

14a (94%): As straw colored crystals from benzene/*n*-hexane/drops of ethanol, mp 154–156 °C; [Found C, 70.3; H, 6.3; N, 10.9. $C_{15}H_{16}N_2O_2$ (MW=256.29) requires C, 70.29; H, 6.29; N, 10.93%]; ν_{max} (Nujol) 1630 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.85 (2H, m, Ph), 7.45 (3H, m, Ph), 4.91 (1H, m, H5-isoxaz.), 4.89 (1H, m, CH–N), 3.98 (1H, m, H4-isoxaz.), 3.47 (1H, dd, *J* 10, 4 Hz, H3-*endo*), 3.07 (1H, dd, *J* 10, 2 Hz, H3-*exo*), 3.04 (1H, s, CH), 2.04 (3H, s, CH$_3$), 1.76 (2H, m, CH$_2$); δ_C (75 MHz, $CDCl_3$) 168.9, 155.3, 130.2, 129.1, 128.9, 126.8, 85.5, 57.8, 56.4, 47.6, 44.4, 30.7, 21.7.

14b (92%): As straw colored crystals from benzene/*n*-hexane/drops of ethanol, mp 180–182 °C; [Found C, 70.2; H, 6.3; N, 11.0. $C_{15}H_{16}N_2O_2$ (MW=256.29) requires C, 70.29; H, 6.29; N, 10.93%]; ν_{max} (Nujol) 1622 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.73 (2H, m, Ph), 7.44 (3H, m, Ph), 4.83 (1H, d, *J* 8 Hz, H5-isoxaz.), 4.34 (1H, s, CH–N), 3.80 (1H, d, *J* 8 Hz, H4-isoxaz.), 3.27 and 3.40 (2H, AB syst., H3), 2.88 (1H, s, CH), 2.16 (3H, s, CH$_3$), 1.74 (2H, m, CH$_2$); δ_C (75 MHz, $CDCl_3$) 168.1, 156.2, 129.9, 129.7, 128.5, 126.4, 84.6, 61.2, 55.6, 49.6, 39.1, 31.8, 21.7.

5.5. Reactions with NBS/AIBN

N-Acetyl derivatives **14a** and **14b** were dissolved in CCl_4 (40 mL/g), using carefully CCl_4 washed glassware. To the suspensions 1 equiv NBS was added at room temperature along with 10% mol of AIBN. The mixtures were refluxed and the reaction status was monitored by TLC until the starting materials have disappeared. To ensure this, an additional half equivalent of NBS could be added. As the conversion of **14a** and **14b** was completed, the solutions were cooled down to ambient and then to lower temperature with an ice-bath to ensure the maximum separation of succinimide. From the filtrate, solvent was removed upon evaporation and the crude residues were submitted to chromatographic separation on

silica gel by eluting initially with $CHCl_3$ and $CHCl_3$/MeOH 9:1 afterwards. The bromo-cycloadducts **15a** and **15b**, the acetals **16a** and **16a′**, and the aldehydes **17a**, **17a′**, and **17b** were collected and characterized.

15a (47%): White solid from benzene/*n*-hexane, mp 212–215 °C; [Found C, 53.7; H, 4.5; N, 8.3. $C_{15}H_{15}N_2O_2Br$ (MW=335.20) requires C, 53.75; H, 4.51; N, 8.36%]; ν_{max} (Nujol) 1706, 1653 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.72 (2H, m, Ph), 7.47 (3H, m, Ph), 5.37 (1H, s, CH–Br), 5.08 (1H, d, *J* 8 Hz, H5-isoxaz.), 4.54 (1H, s, CH–N), 3.97 (1H, d, *J* 8 Hz, H4-isoxaz.), 2.97 (1H, s, CH), 2.63 (1H, m, CH_2), 2.24 (3H, s, CH_3), 1.77 (1H, m, CH_2); δ_C (75 MHz, $CDCl_3$) 168.5, 154.4, 130.5, 129.1, 128.1, 126.3, 84.5, 64.6, 60.3, 59.5, 49.8, 28.2, 22.2.

15b (47%): White solid from benzene/*n*-hexane, mp 195–200 °C; [Found C, 53.8; H, 4.4; N, 8.4. $C_{15}H_{15}N_2O_2Br$ (MW=335.20) requires C, 53.75; H, 4.51; N, 8.36%]; ν_{max} (Nujol) 1700, 1661 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.74 (2H, m, Ph), 7.45 (3H, m, Ph), 5.45 (1H, s, CH–Br), 4.88 (1H, d, *J* 8 Hz, H5-isoxaz.), 4.51 (1H, s, CH–N), 3.97 (1H, d, *J* 8 Hz, H4-isoxaz.), 2.84 (1H, s, CH), 2.50 (1H, d, *J* 11 Hz, CH_2), 2.18 (3H, s, CH_3), 1.73 (1H, d, *J* 11 Hz, CH_2); δ_C (75 MHz, $CDCl_3$) 169.1, 155.8, 130.4, 129.0, 127.7, 126.8, 85.1, 68.0, 62.0, 54.6, 45.7, 29.7, 22.0.

16a (16%): White solid from benzene/*n*-hexane, mp 140–142 °C; [Found C, 65.8; H, 7.5; N, 8.1. $C_{19}H_{26}N_2O_4$ (MW=346.41) requires C, 65.87; H, 7.57; N, 8.09%]; ν_{max} (Nujol) 3260, 1651 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 7.87 (2H, m, Ph), 7.44 (1H, br s, NH), 7.42 (3H, m, Ph), 5.16 (1H, dd, *J* 10, 3 Hz, H5-isoxaz.), 4.68 (1H, d, *J* 5 Hz, O–CH–O), 4.44 (1H, m, CH–N), 4.09 (1H, dd, *J* 10, 3 Hz, H4-isoxaz.), 3.64 and 3.77 (4H, m, CH_2O), 2.57 (1H, m, CH), 2.05 (1H, m, CH_2), 1.91 (3H, s, CH_3), 1.74 (1H, m, CH_2), 1.24 (6H, t, CH_3); δ_C (75 MHz, CD_3COCD_3) 169.4, 158.5, 130.9, 130.6, 129.8, 128.4, 104.5, 89.2, 64.0, 63.5, 60.5, 55.7, 52.2, 33.9, 23.6, 16.1.

16a′ (12%): White solid from benzene/*n*-hexane, mp 110–112 °C; [Found C, 65.9; H, 7.6; N, 8.0. $C_{19}H_{26}N_2O_4$ (MW=346.41) requires C, 65.87; H, 7.57; N, 8.09%]; ν_{max} (Nujol) 3271, 1645 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 8.12 (2H, m, Ph), 7.50 (1H, br s, NH), 7.43 (3H, m, Ph), 5.12 (1H, dd, *J* 9, 6 Hz, H5-isoxaz.), 4.67 (1H, d, *J* 8.5 Hz, O–CH–O), 4.29 (1H, t, *J* 6 Hz, CH–N), 4.23 (1H, d, *J* 9 Hz, H4-isoxaz.), 3.61 (4H, m, CH_2O), 2.83 (1H, m, CH), 1.94 (3H, s, CH_3), 1.76 (1H, m, CH_2), 1.60 (1H, m, CH_2), 1.18 (6H, t, CH_3); δ_C (75 MHz, CD_3COCD_3) 170.6, 157.7, 130.9, 130.6, 129.8, 128.5, 104.5, 87.7, 63.6, 62.5, 60.8, 55.5, 50.4, 33.0, 23.3, 16.2.

17a/a′ (18%): White solid from benzene/ethanol, mp 119–122 °C; [Found C, 66.3; H, 5.8; N, 10.3. $C_{15}H_{16}N_2O_3$ (MW=272.29) requires C, 66.16; H, 5.92; N, 10.29%]; ν_{max} (Nujol) 3297, 1717, 1653 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.97 [10.01] (1H, s, CHO), 8.11 (2H, m, Ph), 7.46 (3H, m, Ph), 6.25 (1H, m, NH), 5.62 [5.65] (1H, d, *J* 8 Hz, H5-isoxaz.), 5.51 [5.54] (1H, s, CH–N), 4.49 [4.54] (1H, s, CH), 4.36 [4.42] (1H, d, *J* 8 Hz, H4-isoxaz.), 2.01 [2.06] (3H, s, CH_3), 1.89 [1.93] and 2.22 (2H, m, CH_2); δ_C (75 MHz, $CDCl_3$) 170.4, 156.4, 130.3, 128.8, 127.7, 127.5, 84.9 [84.5], 77.1 [77.3], 60.4 [62.0], 57.8 [60.2], 54.8 [55.7], 28.6 [30.0], 22.8 [23.3].

17b (20%): White solid from benzene/*n*-hexane, mp 162–164 °C; [Found C, 66.1; H, 5.9; N, 10.2. $C_{15}H_{16}N_2O_3$ (MW=272.29) requires C, 66.16; H, 5.92; N, 10.29%]; ν_{max} (Nujol) 3320, 1716 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 9.79 (1H, s, CHO), 7.69 (2H, m, Ph), 7.45 (3H, m, Ph), 7.07 (1H, br s, NH), 5.09 (1H, dd, *J* 9, 3 Hz, H5-isoxaz.), 4.73 (1H, dd, *J* 9, 3 Hz, H4-isoxaz.), 4.24 (1H, m, CH–N), 3.07 (1H, m, CH), 2.29 (2H, m, CH_2), 1.85 (3H, s CH_3); δ_C (75 MHz, CD_3COCD_3) 202.5, 170.6, 159.3, 131.2, 130.1, 129.5, 128.1, 91.7, 58.8, 56.8, 51.7, 32.0, 23.3.

5.6. Conversion of the bromo-cycloadducts 15a and 15b into the aldehydes 17a and 17b

To a solution in absolute EtOH of the bromo-cycloadducts **15a** and **15b**, an excess of $NaHCO_3$ was added and the mixtures were refluxed for several days, monitoring the evolution of the reactions by TLC until complete consumption of the starting materials. After filtration of the solid, the solvent was evaporated. The residues constituted a mixture of the acetals **16** and/or the aldehydes **17a** and **17b**. Complete transformation of the acetals into the aldehydes was secured by hydrolysis with AcOH/H_2O 3:7 at room temperature for 48 h. At the end of the reaction, the solution volumes were doubled with water and the pH adjusted at 7.5 with NaOH 20%. The water phases were extracted with DCM three times. The organic phases were dried over anhydrous Na_2SO_4 and evaporation of the solvent afforded the crude aldehydes **17**. From **15a**, the aldehydes **17a** and **17a′** were obtained as an epimeric mixture, not separated but submitted to the reduction step, while from **15b** the aldehyde **17b** was isolated and found identical to previously prepared sample.

5.7. Reduction of the aldehydes 17a and 17b

To a solution in MeOH of the aldehydes **17a** and **17b**, 2 equiv of $NaBH_4$ were added under stirring at room temperature. The reactions were carried on until complete consumption of the starting materials, monitoring by TLC. The reactions were subsequently quenched with water and saturated with salt. The water phases were extracted with DCM three times. The organic phases were dried over anhydrous Na_2SO_4 and evaporation of the solvent afforded the crude alcohols **18a** and **18b** in quantitative yields. Purification of the products was secured by column chromatography: from isomer **18a** the two stereoisomers **18a** and **18a′** were easily separated on silica gel by eluting with $CHCl_3$ initially and $CHCl_3$/MeOH 9:1 afterwards.

18a (100%): White solid from acetone, mp 160–161 °C; [Found C, 65.6; H, 6.6; N, 10.2. $C_{15}H_{18}N_2O_3$ (MW=274.31) requires C, 65.67; H, 6.61; N, 10.21%]; ν_{max} (Nujol) 3535, 3297, 1653 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 7.89 (2H, m, Ph), 7.70 (1H, b, NH), 7.43 (3H, m, Ph), 5.13 (1H, dd, *J* 9.6, 4 Hz, H5-isoxaz.), 4.42 (1H, m, CH–N), 4.36 (1H, t, *J* 5 Hz, CH), 4.11 (1H, dd, *J* 9.6, 4 Hz, H4-isoxaz.), 3.73 and 3.84 (2H, m, CH_2–O), 2.38 (1H, m, OH), 2.13 (1H, m, CH_2), 1.89 (3H, s, CH_3), 1.62 (1H, m, CH_2); δ_C (75 MHz, CD_3COCD_3) 170.5, 155.9, 134.9, 130.8, 129.8, 128.4, 90.5, 64.2, 60.8, 55.7, 50.8, 36.0, 23.6.

18a′ (100%): White solid from acetone, mp 152–154 °C; [Found C, 65.6; H, 6.6; N, 10.1. $C_{15}H_{18}N_2O_3$ (MW=274.31) requires C, 65.67; H, 6.61; N, 10.21%]; ν_{max} (Nujol) 3316, 1676 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 8.11 (2H, m, Ph), 7.52 (1H, b, NH), 7.42 (3H, m, Ph), 5.20 (1H, dd, *J* 9, 5 Hz, H5-isoxaz.), 4.29 (2H, m, CH–N and CH), 4.27 (1H, d, *J* 9 Hz, H4-isoxaz.), 3.71 and 3.86 (2H, m, OH and CH_2–O), 2.73 (1H, m, CH), 1.94 (3H, s, CH_3), 1.47 (2H, dd, *J* 13, 6 Hz, CH_2); δ_C (75 MHz, CD_3COCD_3) 170.7, 157.7, 130.9, 130.6, 129.8, 128.5, 87.6, 62.0, 60.9, 55.7, 49.4, 33.7, 23.3.

18b (100%): White solid from acetone, mp 142–145 °C; [Found C, 65.7; H, 6.7; N, 10.3. $C_{15}H_{18}N_2O_3$ (MW=274.31) requires C, 65.67; H, 6.61; N, 10.21%]; ν_{max} (Nujol) 3378, 3318, 1653 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 7.81 (2H, m, Ph), 7.50 (1H, b, NH), 7.45 (3H, m, Ph), 4.99 (1H, dd, *J* 9, 4 Hz, H5-isoxaz.), 4.53 (1H, t, *J* 5 Hz, OH), 4.39 (1H, m, CH–N), 4.18 (1H, dd, *J* 9, 4 Hz, H4-isoxaz.), 3.74 and 3.83 (2H, m, CH_2–O), 2.37 (1H, m, CH), 2.14 (2H, s, CH_2), 1.87 (3H, m, CH_3); δ_C (75 MHz, CD_3COCD_3) 169.6, 160.4, 130.9, 130.0, 129.8, 128.3, 93.8, 65.7, 58.0, 54.8, 47.9, 35.0, 23.5.

5.8. Hydrolysis of the alcohols 18a and 18b

To a solution in MeOH of the alcohols **18a**, **18a′**, and **18b**, an equivalent volume of HCl 3 M was added and the solutions were heated overnight under a nitrogen atmosphere. The solution volumes were doubled with water and the pH adjusted to 8 with $NaHCO_3$. The solutions were then saturated with salt and extracted with DCM three times. The organic phases were dried over anhydrous Na_2SO_4 and evaporation of the solvent afforded quantitatively the crude aminols **19a**, **19a′**, and **19b**, which were purified by crystallization.

19a (100%): White solid from benzene/ethanol, mp 116–118 °C; [Found C, 67.2; H, 6.9; N, 12.1. $C_{13}H_{16}N_2O_2$ (MW=232.27) requires C, 67.22; H, 6.94; N, 12.06%]; ν_{max} (Nujol) 3334, 3266, 3176, 1604 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 7.67 (2H, m, Ph), 7.44 (3H, m, Ph), 5.21 (1H, dd, *J* 9, 2 Hz, H5-isoxaz.), 5.16 (2H, b, NH_2), 4.02 (2H, m, CH–N and H4-isoxaz.), 3.70 (2H, AB syst., CH_2–O), 2.52 (1H, m, CH), 2.10 (2H, m, CH_2); δ_C (75 MHz, CD_3COCD_3) 158.8, 131.0, 130.8, 129.9, 128.2, 91.7, 66.2, 64.7, 62.2, 51.2, 37.8.

19a′ (100%): White solid from benzene/ethanol, mp 153–158 °C; [Found C, 67.3; H, 7.0; N, 12.1. $C_{13}H_{16}N_2O_2$ (MW=232.27) requires C, 67.22; H, 6.94; N, 12.06%]; ν_{max} (Nujol) 3329, 3257, 3158, 1591 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 7.66 (2H, m, Ph), 7.45 (3H, m, Ph), 5.28 (1H, dd, *J* 9, 5 Hz, H5-isoxaz.), 4.03 (1H, d, *J* 9 Hz, H4-isoxaz.), 3.97 (1H, s, CH–N), 3.67 and 3.87 (2H, m, CH_2–O), 2.84 (1H, m, CH), 1.58 (2H, m, CH_2); δ_C (75 MHz, CD_3COCD_3) 158.6, 131.9, 130.1, 129.6, 128.0, 89.1, 65.4, 62.2, 62.1, 49.6, 36.3.

19b (100%): White solid from benzene/ethanol, mp 130–131 °C; [Found C, 67.1; H, 6.8; N, 12.0. $C_{13}H_{16}N_2O_2$ (MW=232.27) requires C, 67.22; H, 6.94; N, 12.06%]; ν_{max} (Nujol) 3370, 3291, 3176, 1594 cm^{-1}; δ_H (300 MHz, CD_3COCD_3) 7.83 (2H, m, Ph), 7.47 (3H, m, Ph), 5.26 (2H, b, NH_2), 4.79 (1H, d, *J* 9 Hz, H5-isoxaz.), 4.26 (1H, dd, *J* 9, 2 Hz, H4-isoxaz.), 4.13 (1H, d, *J* 6 Hz, CH–N), 3.70 (2H, AB syst., CH_2–O), 2.48 (1H, m, CH), 2.11 (2H, m, CH_2) 1.51 (1H, d, *J* 13 Hz, CH_2); δ_C (75 MHz, CD_3COCD_3) 160.5, 130.9, 130.8, 130.0, 128.2, 95.0, 68.0, 66.2, 55.2, 49.1, 37.5.

5.9. X-ray crystallography

Unit-cell dimensions for compound **10b** were obtained by least-squares fit of 2θ values for 25 reflections, using an Enraf–Nonius CAD4 diffractometer with graphite-monochromated Mo Kα radiation at the Centro Grandi Strumenti (CGS) dell'Università, Pavia, Italy.

A summary of crystal data, data collection, and structure refinement for compounds **10b** is presented in Table 3. Table 4 reports the bond lengths and angles while Table 5 the torsion angles.

An approximate absolute scale factor and a mean thermal parameters of 3.31 Å was determined by Wilson's method.[29] The structure was solved by direct method and the E-map correctly revealed the non-hydrogen atoms in the molecules and refined anisotropically in subsequent three-cycle least-squares. The positions of the hydrogen atoms were located from a difference Fourier synthesis, and refined isotropically in the subsequent least-squares refinement. The program SHELXL[30] was used to solve the structure. The ORTEP[31] program was used for molecular graphics.

CCDC 291,467 contains the supplementary crystallographic data. These data can be obtained free of charge via the internet web site at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB21EZ, UK, or deposit@ccdc.cam.ac.uk).

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References and notes

1. (a) Mizuno, Y. *The Organic Chemistry of Nucleic Acids*; Kadansha: Tokyo, 1986; (b) Ueda, T. *Chemistry of Nucleosides and Nucleotides*; Townsend, Ed.; Plenum: New York, NY, 1988; Vol. 1, Chapter 1; (c) Srivasta, P. C.; Robins, R. K.; Meyer, R. B., Jr. *Chemistry of Nucleosides and Nucleotides*; Townsend, Ed.; Plenum: New York, NY, 1988; Vol. 1, Chapter 2; (d) Revenkar, G. R.; Robins, R. K. *Chemistry of Nucleosides and Nucleotides*; Townsend, Ed.; Plenum: New York, NY, 1988; Vol. 2, Chapter 4.
2. (a) Merino, P. *Curr. Med. Chem.: Anti-Infect. Agents* **2002**, *1*, 389–411; (b) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229–9272; (c) Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, S. R.; Earl, R. A.; Guedj, R. *Tetrahedron* **1994**, *50*, 10611–10670.
3. (a) Kitade, Y.; Kojima, H.; Zulfiqur, F.; Kim, H. S.; Wataya, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3963–3965; (b) Ramesh,

N. G.; Klunder, A. J. H.; Zwanenburg, B. *J. Org. Chem.* **1999**, *64*, 3635–3641; (c) Wu, J.; Schneller, S. W.; Seley, K. L.; DeClerq, E. *Heterocycles* **1998**, *47*, 757–763; (d) Siddiqi, S. M.; Raissian, M.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; DeClerq, E. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 663–666; (e) Da Silva, A. D.; Coimbra, E. S.; Fourrey, J. L.; Machado, A. S.; Robert-Gero, M. *Tetrahedron Lett.* **1993**, *34*, 6745–6748; (f) Koga, M.; Schneller, S. W. *Tetrahedron Lett.* **1990**, *31*, 5861–5864.

4. Quadrelli, P.; Scrocchi, R.; Caramella, P.; Rescifina, A.; Piperno, A. *Tetrahedron* **2004**, *60*, 3643–3651.
5. (a) Kirby, G. W. *Chem. Soc. Rev.* **1977**, *6*, 1–24; (b) Vogt, P. F.; Miller, M. J. *Tetrahedron* **1998**, *54*, 1317–1348; (c) Boger, L.; Weinreb, S. M. *Hetero Diels–Alder Methodology in Organic Synthesis*; Academic: San Diego, CA, 1987.
6. (a) Quadrelli, P.; Gamba Invernizzi, A.; Caramella, P. *Tetrahedron Lett.* **1996**, *37*, 1909–1996; (b) Quadrelli, P.; Mella, M.; Gamba Invernizzi, A.; Caramella, P. *Tetrahedron* **1999**, *55*, 10497–10510.
7. (a) Quadrelli, P.; Mella, M.; Paganoni, P.; Caramella, P. *Eur. J. Org. Chem.* **2000**, 2613–2620; (b) Quadrelli, P.; Fassardi, V.; Cardarelli, A.; Caramella, P. *Eur. J. Org. Chem.* **2002**, 2058–2065.
8. Borthwick, A. D.; Biggadike, K. *Tetrahedron* **1992**, *48*, 571–623.
9. (a) Archelos, A.; Morin, C. *Tetrahedron Lett.* **1984**, *25*, 1277–1278; (b) Daluge, S.; Vince, R. *J. Org. Chem.* **1978**, *43*, 2311–2320; (c) Jagt, J. C.; van Leusen, A. M. *J. Org. Chem.* **1974**, *39*, 564–566; (d) Ishikura, M.; Katagiri, N. *Recent Research Developments in Organic Chemistry* **2004**, *8*, 197–205; (e) Ishikura, M.; Katagiri, N. *Trends Heterocycl. Chem.* **2003**, *9*, 83–90; (f) Ruecroft, G.; Taylor, S. *Chem. Br.* **2002**, *38*, 46–49; (g) Griffiths, G. J.; Previdoli, F. E. *J. Org. Chem.* **1993**, *58*, 6129–6131.
10. For an example of pricing see: Aldrich Handbook, ed. 2005–2006, p 249.
11. (a) Grieco, P. A.; Bahsas, A. *J. Org. Chem.* **1987**, *52*, 5746–5749; (b) Larsen, S. D.; Grieco, P. A. *J. Am. Chem. Soc.* **1985**, *107*, 1768–1769; (c) Rodriguez-Borges, J. E.; Garcia-Mera, X.; Fernandez, F.; Lopes, V. H. C.; Magalhaes, A. L.; Cordeiro, M. N. D. S. *Tetrahedron* **2005**, *61*, 10951–10957; (d) Grieco, P. A.; Parker, D. T.; Fobare, W. F.; Ruckle, R. *J. Am. Chem. Soc.* **1987**, *109*, 5859–5861; (e) Grieco, P. A.; Larsen, S. D.; Fobare, W. F. *Tetrahedron Lett.* **1986**, *27*, 1975–1978; (f) Alonso, D. A.; Bertilsson, S. K.; Johnsson, S. Y.; Nordin, S. J. M.; Södergren, M. J.; Andersson, P. G. *J. Org. Chem.* **1999**, *64*, 2276–2280; (g) Bailey, P. D.; Wilson, R. D.; Brown, G. R. *Tetrahedron Lett.* **1989**, *30*, 6781–6784; (h) Waldman, H. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 274–275; (i) Abraham, H.; Stella, L. *Tetrahedron* **1992**, *48*, 9707–9718; (j) Stella, L.; Abraham, H. *Tetrahedron Lett.* **1990**, *31*, 2603–2606.
12. Grieco, P. A.; Larsen, S. D. *Org. Synth.* **1990**, *68*, 206–209.
13. Caramella, P.; Grünager, P. *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, NY, 1984; Vol. 1, pp 291–392.
14. Fliege, W.; Huisgen, R. *Justus Liebigs Ann. Chem.* **1973**, 2038–2047.
15. (a) Yu, L.; Li, J.; Ramirez, J.; Chen, D.; Weng, P. G. *J. Org. Chem.* **1997**, *62*, 903–907; (b) Pinho, P.; Andersson, P. G. *Chem. Commun.* **1999**, 597–598.
16. (a) Huisgen, R.; Ooms, P. H. J.; Mingin, M.; Allinger, N. L. *J. Am. Chem. Soc.* **1980**, *102*, 3951–3953; (b) Rondan, N. G.; Paddon-Row, M. N.; Caramella, P.; Houk, K. N. *J. Am. Chem. Soc.* **1981**, *103*, 2436–2438; (c) Caramella, P.; Rondan, N. G.; Paddon-Row, M. N.; Houk, K. N. *J. Am. Chem. Soc.* **1981**, *103*, 2438–2440; (d) Houk, K. N.; Rondan, N. G.; Brown, F. K.; Jorgensen, W. L.; Madura, J. D.; Spellmeyer, D. C. *J. Am. Chem. Soc.* **1983**, *105*, 5980–5988.
17. Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; Wiley: New York, NY, 1994; pp 627–628.
18. (a) Bonin, M.; Romero, J. R.; Grierson, D. S.; Huson, H. P. *J. Org. Chem.* **1984**, *49*, 2392–2400; (b) Ferrer, M.; Sanchez-Baeza, F.; Messeguer, A. *Tetrahedron* **1997**, *53*, 15877–15888; (c) Bergstad, K.; Bäckvall, J. E. *J. Org. Chem.* **1998**, *63*, 6650–6655; (d) Choudary, B. M.; Bharathi, B.; Venkat Reddy, C.; Lakshmi Kantam, M.; Raghavan, K. V. *Chem. Commun.* **2001**, 1736; (e) Buston, J. E. H.; Coldham, I.; Mulholland, K. R. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2327.
19. (a) Grierson, D. S.; Husson, H. P. *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 6, pp 909–947; (b) Michelot, R. *Bull. Soc. Chim. Fr.* **1969**, 4377–4385.
20. (a) Lebrun, B.; Braekman, J. C.; Daloze, D. *Magn. Reson. Chem.* **1999**, *37*, 60–64 and references therein; (b) Parham, W. E.; Sloan, K. B.; Ratnammal Reddy, K.; Olson, P. E. *J. Org. Chem.* **1973**, *38*, 927–931.
21. Any attempt to perform direct oxidation of C3 with CrO_3-3,5-dimethylpyrazole as suggested by literature for similar compounds failed and the starting *N*-acetyl derivatives were collected unchanged after application of various reaction conditions. For some examples see: (a) Blay, G.; Cardona, L.; Garcia, B.; Garcia, C. L.; Pedro, J. R. *Tetrahedron Lett.* **1997**, *38*, 8257–8260; (b) Corey, E. J.; Fleet, G. W. J. *Tetrahedron Lett.* **1973**, 4499–4501; (c) Salmond, W. G.; Barta, M. A.; Havens, J. L. *J. Org. Chem.* **1978**, *43*, 2057–2059.
22. Doering, W. V. E.; Levitz, M.; Sayigh, A.; Sprecher, M.; Whelan, W. P. *J. Am. Chem. Soc.* **1953**, *75*, 1008.
23. Zhu, X.-F.; Nydegger, F.; Gossauer, A. *Helv. Chim. Acta* **2004**, *87*, 2245–2265.
24. (a) Gershtein, N. A.; Shostakoskii, M. F. *Zh. Obshch. Khim.* **1948**, *18*, 451–458; (b) Shostakoskii, M. F.; Gershtein, N. A. *Zh. Obshch. Khim.* **1946**, *16*, 937–951; (c) Schultz, Forrest S. *Book of Abstracts*, 218th ACS National Meeting, New Orleans, LA, Aug 22–26, 1999; (d) Shimizu, H.; Saito, N.; Matsuzawa, K.; Aizawa, S. Jpn. Kokai Tokkyo Koho JP 07069955, 1995.
25. Domenicano, A.; Vaciago, A.; Coulson, C. A. *Acta Crystallogr., Sect. B* **1975**, *31*, 221–234.
26. Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354–1358.
27. Cahn, R. S.; Ingold, C. K.; Prelog, V. *Experientia* **1956**, *12*, 81–94.
28. Grundmann, C.; Grünanger, P. *The Nitrile Oxide*; Springer: Heidelberg, 1971.
29. Wilson, A. J. C. *Nature* **1942**, *150*, 151–152.
30. Sheldrick, G. M. *SHELXL-93. Program for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1993.
31. Johnson, C. K. *ORTEP, Report ORNL-3793*; Oak Ridge National Laboratory: Oak Ridge, TN, 1965.

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Regioselectivity in the 1,3-dipolar cycloaddition of adamantylidenefulvene and its modification by inclusion in cyclodextrins' solutions

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Abstract—The 1,3-dipolar cycloaddition of adamantylidenefulvene (**1**) with 2 equiv of nitrile oxides **2a–d** gave 1/1 cycloadducts, **3a–d** and **4a–d**, as the major products, and four other 1/2 minor cycloadducts **5–8a**,**b**. The ratios of 1/1 cycloadducts **3a–d** to **4a–d** in THF solution were about 1/1 in the four different nitrile oxides **2a–d** studied and microwave was found to accelerate the reactions and enhance their yields. It is noteworthy that the regioselectivity of **3a**/**4a** was enhanced to 71/29 in β-cyclodextrin (β-CD) aqueous solution compared to that of 40/60 in the absence of β-CD. The regioselectivity of **3b**/**4b** was further enhanced to 99/1 when 4-*tert*-butylphenyl hydroximinoyl chloride (**9b**) was complexed with β-CD and then proceeded to react with **1**; this is in sharp contrast with that of 33/67 in the absence of β-CD. The binding constant of **1**·β-CD in acetone-d_6/D_2O (1/1) was determined to be 188±9 M^{-1} by ^1H NMR titration experiments. The binding mode of **1**·β-CD was further determined by ROESY experiment. Furthermore, molecular dynamic simulations were carried out to provide information of the complexation modes of **1**·β-CD, **3a**·β-CD, **4a**·β-CD, **9a**·β-CD, and **9b**·β-CD. It was found that both steric and electrostatic effects play important roles in determining the regio- and stereochemistry of 1,3-dipolar cycloaddition of **1**. Finally, β-CD is shown to serve as a chiral shift reagent to differentiate the enantiomers of **4a** in ^1H NMR.

1. Introduction

Cycloaddition reactions on fulvenes have attracted much attention because there are many possible reaction pathways involved in them and their reaction products are usually versatile and complicated.[1] Compounds containing isoxazolines or isoxazoles have also received considerable attention[2] because they are excellent precursors in transforming to a variety of bifunctional compounds[3] and they show diverse biological activity.[4] 1,3-Dipolar cycloaddition of fulvenes with benzonitrile oxide (**2a**) was first reported by Grünanger and co-workers in 1952,[5a] and recently elaborated by Nair and co-workers using 6-(2-phenylethenyl)fulvene.[5c] In their studies, at least 2–5 products that contained 1/1 and 1/2 cycloadducts were reported.[5] Even though the reactions of fulvenes with nitrile oxides lead to complex isoxazolines products, they provide us an opportunity to fine tune or control the reaction products.

Cyclodextrins (CDs) can be described as a truncated cone with the narrow rim bearing the primary hydroxy groups and the secondary hydroxy groups as the wider rim, and they possess hydrophobic cavities that enable them to include a variety of organic compounds in aqueous solution[6,7] (Chart 1). Because of their inclusion ability, CDs have become one of the most commonly used host systems and have shown great potential in areas such as drug delivery[6,8] and chromatographic separations.[6,9] Furthermore, CDs have been found to enhance reaction rates and control product

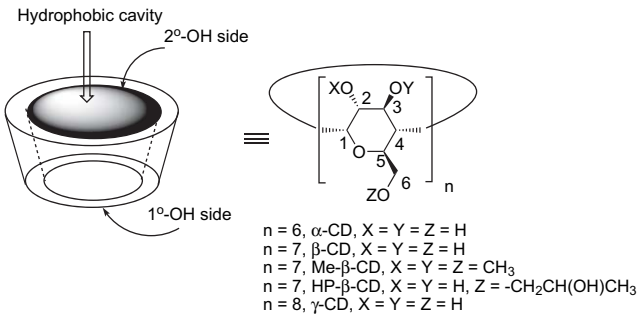

Chart 1.

Keywords: Regioselectivity; Fulvene; Steric effect; Inclusion complex; 1,3-Dipolar cycloaddition; Chiral shift reagent; Molecular reactor.
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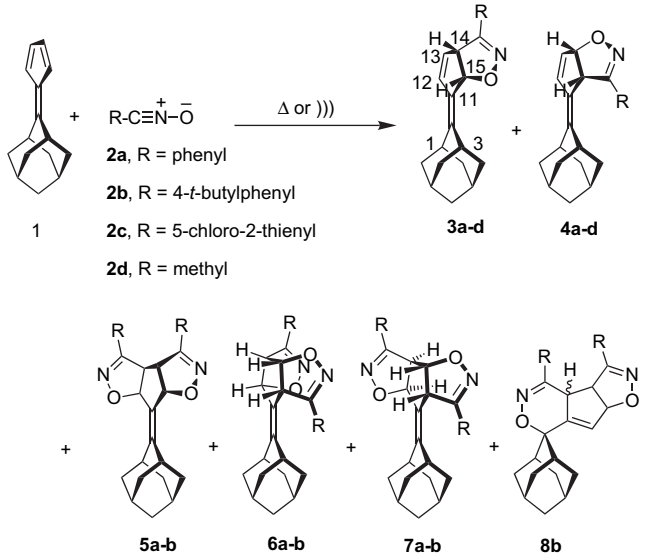
distribution in various reactions including nucleophilic substitution,[6,7,10] electrophilic substitution,[6,11] Diels–Alder reactions,[6,12] and [2+2] photochemical cycloadditions.[6,13]

Rama Rao and co-workers have exploited the use of both CDs and baker's yeast together to enhance the enantioselectivity and regioselectivity of the 1,3-dipolar cycloadditions in several molecular systems;[14] however, Simpson and co-workers later reported that baker's yeast was not required to achieve the high selectivities.[15a] Moreover, Easton and co-workers have elegantly demonstrated that the regioselectivity can be *dramatically reversed* by a dipolarophile (e.g., terminal alkyne) tethered β-CD in 1,3-dipolar cycloaddition.[15] As advocated by Easton and co-workers[3b,c,9b,15] and our continuous interests in using CDs as molecular reactors,[7e,12f,g,13a,c] we report here our studies of the 1,3-dipolar cycloaddition of adamantylidenefulvene (**1**) with nitrile oxides **2a–d** and the application of CDs to modulate the regio- and stereochemistry of the reaction, and indeed, a highly regioselective product distribution is achieved (vide infra).

2. Results and discussion

Adamantylidenefulvene (**1**) was prepared using a literature procedure.[16] The 1,3-dipolar cycloaddition of **1** with 2 equiv of nitrile oxides **2a–d** (R = phenyl, 4-*tert*-butylphenyl, 5-chloro-2-thienyl, and methyl) prepared in situ in THF solution gave a mixture of 2–6 products in total yields of 40–60%. The reaction took 24 h under reflux condition but was completed within 20 min under microwave irradiation and concurrently with higher yields (ca. 50–80%). Column chromatography on silica gel with *n*-hexane and ethyl acetate (20/1) as eluent afforded two major 1/1 cycloadducts **3** and **4** and 2–4 minor 1/2 cycloadducts **5–8** (Scheme 1). The ratios and yields of the major products **3** and **4** are shown in Table 1 and the minor 1/2 cycloadducts are: **5a** (8%), **6a** (6%), and **7a** (6%) when reacting with **2a**; **5b** (15%), **6b** (9%), **7b** (12%), and **8b** when reacting with **2b** (total yields, 50–80%).



Scheme 1.

Table 1. Ratios (**3/4**) of the 1/1 cycloadducts from 1,3-dipolar cycloaddition of **1** with nitrile oxides **2a–d** in THF under thermal condition or microwave irradiation

Entry	Nitrile oxide	**3/4** ratio[a] (yield, %)	
		Thermal reaction[b]	Microwave irradiation[c]
1	**2a**, R = phenyl	60/40 (41%)	66/34 (59%)
2	**2b**, R = 4-*tert*-butylphenyl	51/49 (60%)	64/36 (51%)
3	**2c**, R = 5-chloro-2-thienyl	50/50 (40%)	60/40 (50%)
4	**2d**, R = methyl	49/51 (45%)	45/55 (60%)

[a] Product ratios were determined by ^{1}H NMR at 300 and 500 MHz; error limit ±5%.
[b] The reaction was refluxed in THF for 24 h.
[c] Microwave irradiation (20 min) was set at 300 W and temperature was controlled below 66 °C.

In principle, a [6+3] cycloaddition product (e.g., **8**) is also possible[1c,5b] in the 1,3-dipolar cycloaddition of **1** with nitrile oxides **2a–d**, however, only a trace of [6+3] product **8b** was observed in ^{1}H NMR spectra of the crude products. The molar ratio between **1** and benzonitrile oxide (**2a**) was varied to look for possible variation in product distribution, however, no obvious change was found despite that products' yield increased slightly at higher concentrations of the nitrile oxide. The reactions of **1** with four different nitrile oxides **2a–d** gave 1/1 cycloadducts **3a–d** and **4a–d** as the major products. In order to rationalize product distribution of the reactions, AM1 calculation was carried out and the HOMO and LUMO energies of **1** and **2a–d** are summarized in Figure S-1. As can be seen, the HOMO energy of **1** is higher than those of the four dipoles, thus, the 1,3-dipolar cycloadditions of **1** with **2a–c** (but not **2d**), are believed to be $LUMO_{(dipole)}$–$HOMO_{(dipolarophile)}$ controlled reactions. For the reaction of **1** with **2d**, both the $LUMO_{(dipole)}$–$HOMO_{(dipolarophile)}$ and $HOMO_{(dipole)}$–$LUMO_{(dipolarophile)}$ interactions are important.

The structures of all cycloadducts were determined by ^{1}H, ^{13}C NMR, H,H-COSY, H,C-COSY, MS, and NOE spectral data. The regio and stereo structural assignments of a 1/1 cycloadduct **3b** is described as follows. Compound **3b** is assigned to be a 1/1 head-to-tail adduct of nitrile oxide on **1** where the C_{14} (one of the aliphatic tertiary carbon next to carbon atoms, δ 58.6) and C_{15} (a tertiary carbon next to one oxygen atom, δ 83.7) of the fused dihydrocyclopentaisoxazolines can be readily assigned from its ^{13}C NMR and DEPT. H_{14} and H_{15} can then be assigned through H,C-COSY from the identified C_{14} and C_{15}. From H,H-COSY, we found that H_{14} not only coupled with H_{15} and H_{13} but also coupled with H_{12}, therefore, NOE experiments were carried out to assist the assignment of H_{12} and H_{13}. When H_{14} was irradiated, substantial NOE was found on H_{15} (8.1%), H_{13} (3.6%), and on the *ortho*-protons of the aryl group (5.6%), whereas no NOE was found on H_{12}. In contrast, when H_{12} was irradiated, intense NOE was found on H_1 (15.1%) and H_{13} (6.4%), however, no NOE was found on H_{14}. Based on these observations, H_{12} and H_{13} can be assigned unambiguously and the results of NOE experiments on **3b** are summarized in Figure 1a. The peaks for C_{12} and C_{13} can subsequently be assigned from H,C-COSY through correlation with H_{12} and H_{13}, respectively. Finally, the structure of **3b** was confirmed by a single crystal

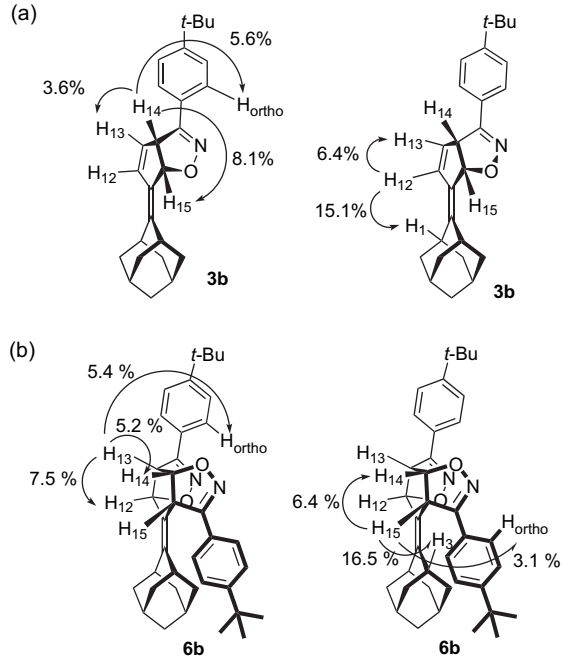


Figure 1. NOE results of (a) compound **3b** and (b) compound **6b**.

X-ray crystallography analysis to be a 1/1 head-to-tail cycloadduct (Fig. 2a).

The detailed regio and stereo structural assignment of a 1/2 cycloadduct **6b** is described in Supplementary data. NOE results (Fig. 1b) and single crystal X-ray crystallography of a 1/2 cycloadduct **6a**, an analogue of **6b**, confirmed the structure to be a *syn* head-to-tail biscycloadduct (Fig. 2b). The characteristic ^{1}H and ^{13}C NMR of all cycloadducts on the fused dihydro- and tetrahydrocyclopentaisoxazolines are summarized in Supplementary data (Tables S-1 and S-2).

2.1. Control of regioselectivity by reacting in CDs

After structural assignments of all products, we then explored the application of CDs on the control of regioselectivity of 1,3-dipolar cycloaddition of **1** with benzonitrile oxides (**2a**,**b**). The regioselectivity (**3a/4a**) of the 1,3-dipolar cycloaddition of **1** with **2a** changed from 60/40 in THF to 40/60 in acetone/water (v/v = 1/1) co-solvent.[17] It is noteworthy that the regioselectivity of **3/4** can be dramatically reversed when the 1,3-dipolar cycloaddition (between **1** and **2**) is executed in the presence of CDs (see Table 2). The regioselectivity reached a maximum value of 83/17 for **3a/4a** but was further enhanced to 99/1 for **3b/4b** when 6 equiv of β-CD versus **1** was added to the reaction mixture (Fig. 3a,b, and S-3). Interestingly, higher values of **3/4** were achieved if inclusion complex of hydroximinoyl chlorides (**9a**,**b**)·β-CD were prepared first (instead of **1**·β-CD), and then proceeded to react with the dipolarophile **1** (filled circles in Fig. 3a,b). The results imply that the hydroximinoyl chlorides **9a**,**b** form stronger complexes with β-CD than **1** does, because electrostatic interactions are involved between them.

There was basically no effect on the product distribution when either α- or γ-CD was used. This is understandable

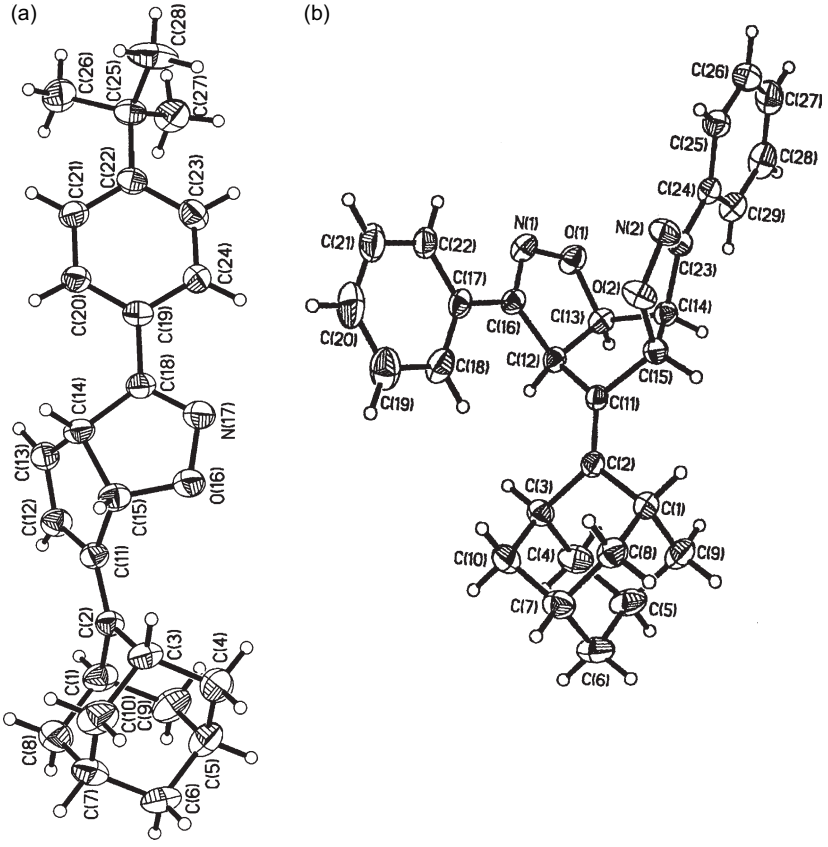


Figure 2. ORTEP structures of (a) compound **3b** and (b) compound **6a**.

Table 2. Ratios of cycloadducts (**3/4**) from 1,3-dipolar cycloaddition of **1** with benzonitrile oxide (**2a**) and 4-*tert*-butylphenyl nitrile oxide (**2b**) in various CD solutions at 310 K[a,b]

Entry	[CDs]/[**1**]	**3a/4a**		**3b/4b**	
		β-CD[c]	β-CD[d]	β-CD[c]	β-CD[d]
1	0	40/60	40/60	33/67	33/67
2	1.0	43/57	43/57	37/63	39/61
3	3.0	58/42	64/36	49/51	48/52
4	5.0	68/32	78/22	57/43	77/21
5	6.0	71/29	83/17	80/20	99/1

[a] Solvent system was acetone/H_2O (v/v = 1/1).
[b] The product ratio was determined by ^1H NMR integration; error limit is ±5%.
[c] Complex of **1**·β-CD was prepared first, followed by adding phenyl hydroximinoyl chloride **9a** or **9b** into the solution.
[d] Complex (**9a** or **9b**)·β-CD was prepared first, followed by adding **1** into the solution.

since the cavity of α-CD is known to bind adamantane moiety only shallowly; and the cavity of γ-CD is too large for a snug fit of the adamantane moiety. Permethylation on all hydroxyl groups of β-CD (i.e., Me–β-CD) or perhydroxypropylation on all primary alcohols of the β-CD (i.e., HP–β-CD), highly enhanced their water solubility, however, not much change on the regioselectivity was found in either cases. Furthermore, it is noteworthy that product yields of the reaction were in the range of 40–45% when [β-CD] was below 3 mM, but they decreased to 20–30% when [β-CD] was above 6 mM. In other words, our results show that a higher regioselectivity was accompanied with a lower reaction yield at high concentrations of β-CD. These results suggest that β-CD plays as a 'steric shield' instead of a 'promoter' in the 1,3-dipolar reactions studied here.

Evidences for complexation of **1** with β-CD came from ^1H NMR spectra, which showed that H_3 ($\Delta\delta=-0.03$ ppm) and H_5 ($\Delta\delta=-0.04$ ppm) of β-CD, oriented toward the interior of the CD cavity, were considerably upfield shifted in the presence of **1**. By contrast, H_1, H_2, H_4, and H_6 all located on the exterior wall of β-CD, either showed little downfield shifts or were unaffected (Fig. 4). These observations are consistent with the notion that a complex is formed between β-CD and **1**, and they most likely have 1/1 stoichiometric ratios, similar to those of adamantane derivatives found in several X-ray crystallography data.[18] The binding constant for complexation of **1** with β-CD was determined to be 188±9 M^{-1} by Benesi–Hilderbrand plot,[19] where the reciprocal chemical shift differences of guest **1** are plotted with the reciprocal concentration of β-CD (See Fig. S-2).

The regioselective results of 1,3-dipolar cycloadditions of **1** in β-CD can be explained by complexes **A** or **B**, where the hydrophilic nitrile oxide can only attack the fulvene from sterically less hindered sites. Complexes **C** and **D** are less likely because the cavity of β-CD is too small to accommodate both fulvene **1** and nitrile oxide **2a** concomitantly (Chart 2). Had complexes **C** and **D** been the favored complexes, one would have observed predominant formation of **4a**; however, **3a** became the major product when high equivalent of β-CD versus **1** was used. Furthermore,

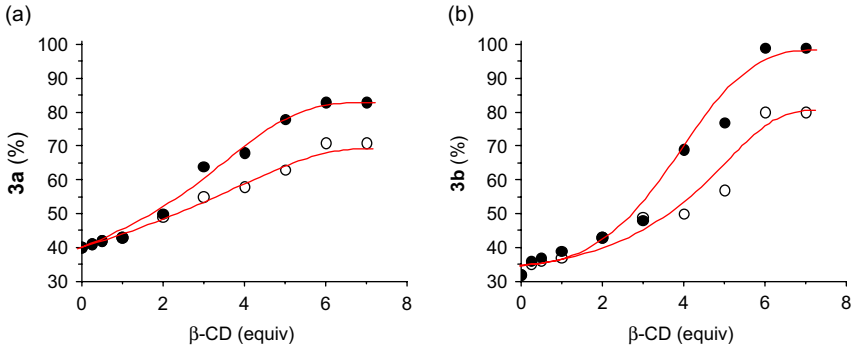


Figure 3. The percentage of (a) **3a** over **4a** and (b) **3b** over **4b** in the 1,3-dipolar cycloadditions of **1** (5 mM) with **2a** and **2b** (10 mM) in aqueous solution as a function of [β-CD]. Where ● denotes data obtained when inclusion complex of hydroximinoyl chloride (**9a** or **9b**)·β-CD was prepared first and proceeded to react with **1**, and ○ denotes data obtained when inclusion complex of **1**·β-CD was prepared first then proceeded to react with corresponding dipoles **2a** or **2b**.

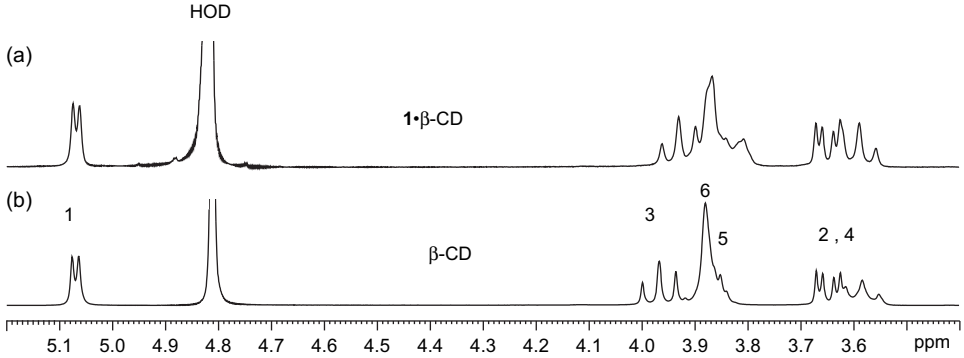


Figure 4. ^1H NMR spectra of (a) **1**·β-CD where [**1**]=[β-CD]=2 mM in D_2O, and (b) [β-CD]=5 mM in D_2O at 300 K.

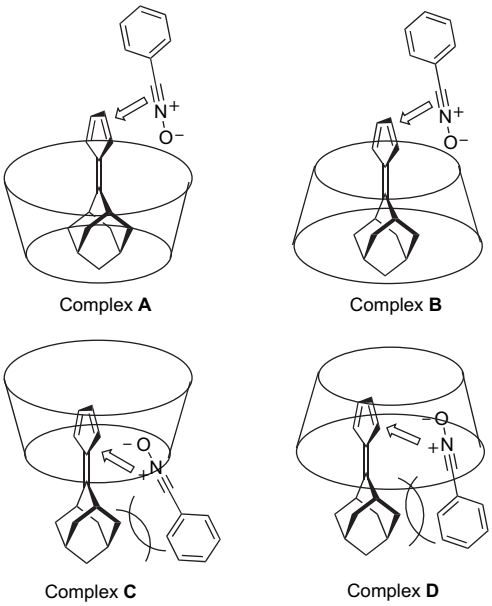


Chart 2.

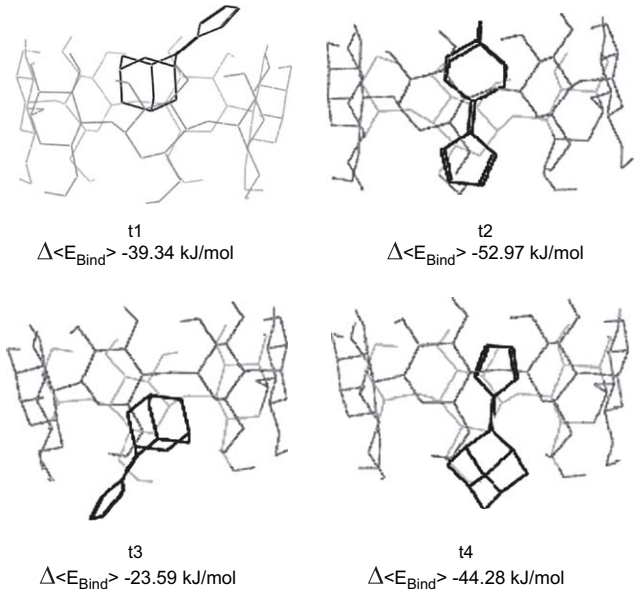


Figure 5. The calculated $\Delta\langle E_{\text{Bind}}\rangle$ of **1**·β-CD from four trajectories (t1–t4), each starting from a different binding configuration (see Supplementary data). For each trajectory, the lowest energy structure obtained from optimization of sampled structures during 5000 ps MD is shown. Hydrogen atoms are omitted for clarity, and all structures are shown with the wider secondary hydroxyl rim of β-CD on the top.

cycloadducts **3a** and **4a** were proven to be stable under the reaction conditions; therefore, they are formed in kinetically controlled processes. ROESY experiment (Fig. S-4)[20d] was carried out on **1**·β-CD (acetone-d_6/D_2O = 1/1), which suggests that complex **B** is the favored binding mode; namely, the reactive fulvene group is pointing toward the primary side of β-CD.

Previously, we have shown that molecular dynamic (MD) simulations[22–24] with a multi-trajectory approach are useful in explaining the product preference in a β-CD mediated Diels–Alder reaction;[12g] each trajectory starts with distinctly different host·guest geometry. The same approach was used here to examine the complexes of **1**·β-CD, **3a**·β-CD, **4a**·β-CD, **3a**-**TS**·β-CD, **4a**-**TS**·β-CD (TS stands for transition state structure, which was located quantum mechanically),[25] **9a**·β-CD, and **9b**·β-CD. MD simulations of **1**·β-CD showed trajectory t2, with the reactive fulvene pointing toward the primary side of β-CD, to be the statistically most stable binding mode and this is in agreement with the observed spectroscopic data (Figs. 5 and S-4). The calculated results of the products and their transition state structures with β-CD showed that on average both **3a** and **3a**-**TS** bound more tightly within β-CD than **4a** and **4a**-**TS** (see Supplementary data). For example, for **3a**-**TS**·β-CD the calculated $\Delta\langle E_{\text{Bind}}\rangle$ from different trajectories ranged from −38.03 to −70.62 kJ mol^{-1}, which are more stable than **4a**-**TS**·β-CD with $\Delta\langle E_{\text{Bind}}\rangle$ in the range of −26.41 to −60.11 kJ mol^{-1} (Tables S-6 and S-7). These results support the notion that the CD cavity provides steric control stabilizing the formation of the transition state structure **3a**-**TS**, giving **3a** as the major product.

It is noteworthy that the **3a**/**4a** product ratio reversed from 60/40 in THF to 40/60 in acetone/water (1/1). This may imply that when reacted in acetone/water (1/1) the transition state leading to **3a** is relatively destabilized compared to that leading to **4a**. Higher regioselectivity was achieved in the 1,3-dipolar cycloaddition of **1** when phenyl hydroximinoyl chloride (**9a**) was complexed with β-CD first and then proceeded to react with **1**. MD simulations of **1**·β-CD showed that, with the presence of adamantane moiety, the stability of **1**·β-CD is very dependent on how the guest molecule is bound in the CD cavity; the calculated binding energy, $\Delta\langle E_{\text{Bind}}\rangle$, of four different trajectories t1–t4 are −39.34, −52.97, −23.59, and −44.28 kJ mol^{-1}, respectively. It can be seen in Figure 5 that t2 and t4 enjoy stronger binding energy at the expense of blocking the ene group from reaction. For **9a**·β-CD, $\Delta\langle E_{\text{Bind}}\rangle$ is less trajectory dependent with a phenyl core (−47.01 and −51.00 kJ mol^{-1} for hydroximinoyl chloride pointing to the top and bottom rims of CD, respectively). In each trajectory, the hydroximinoyl chloride part moves in and out of β-CD rather frequently. The calculated results indicate that while **1**·β-CD might have binding modes with stronger binding energies, the modes effective for 1,3-dipolar cycloaddition are in fact having weaker binding energies than those of **9a**·β-CD. This explains the smaller regioselectivity when **1**·β-CD was prepared first.

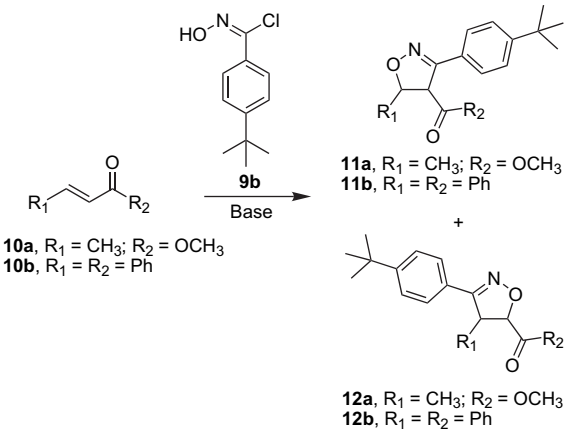
Easton and co-workers reported that 1,3-dipolar cycloaddition of a dipolarophile tethered β-CD with 4-*tert*-butylphenyl nitrile oxide (**2b**) led to an excellent regioselective product.[15a–c] Indeed, **3b** became the exclusive product and almost no **4b** was found when 4-*tert*-butylphenyl hydroximinoyl chlorides (**9b**) formed a complex with β-CD first and then proceeded to react with fulvene **1**. It would be desirable to obtain binding constants of the aryl hydroximinoyl chlorides **9a** and **9b** (precursors for nitrile oxides **2a** and **2b**) with β-CD, however, we were not successful in both cases. The two aryl hydroximinoyl chlorides **9a** and **9b** were too unstable to be detected in D_2O even in the presence of β-CD, and they tend to form diphenyl- and di-*tert*-butylphenyl furoxanes (dimers of the nitrile oxides) instead of the intended cycloaddition with **1**. The latter fact explains why the yields

of 1,3-dipolar cycloaddition of **2a** and **2b** in water are usually below 50%.

MD simulations of **9b**·β-CD showed that it has a higher binding affinity than **9a**·β-CD ($\Delta\Delta\langle E_{\text{Bind}}\rangle = -6.21$ and -4.74 kJ mol^{-1} with the hydroximinoyl chloride pointing to the top and bottom rims of CD, respectively) (Tables S-4 and S-5). Thus the *tert*-butyl group helps to anchor **9b** in the CD cavity and sterically blocking reactant **1** from approaching in the *syn* position relative to **9b**, preventing the formation of product **4b**.

It is possible though not necessary that a reaction in the chiral CD cavity may lead to induced optical activity in the product.[20a–c] This possibility was investigated for the 1,3-dipolar cycloaddition of benzonitrile oxide (**2a**) with **1** in D_2O. In the presence of β-CD, the ^{1}H NMR spectrum of the product **4a** showed two well resolved doublets around δ 6.8 for methine proton H_{12}; without β-CD, only a doublet is showed around δ 7.2 (Fig. S-5). Any enantiomeric excess can thus be determined from the area ratios of the respective isoxazolines; however, the enantiomers are present in 1/1 ratio within experimental error. Unfortunately, spectrum of **3a**·β-CD was not obtained due to its poor solubility in D_2O.

In order to exploit the high regioselectivity of **9b**·β-CD in 1,3-dipolar cycloaddition, we tested its reaction with other dipolarophiles such as methyl *trans*-crotonate **10a** and *trans*-chalcone **10b** (Scheme 2). The product ratio of **11a/12a** was found to be enhanced from 65/35 in THF[21] to that of 86/14 in acetone/water (1/1). The product ratio of **11b/12b** enhanced from 36/64 in THF[21] to that of >99/1 in acetone/water (1/1). Unfortunately, no further improvement in regioselectivity was found when the above reactions were carried out in acetone/water (1/1) in the presence of β-CD (**11a/12a**, 82/18 and **11b/12b**, >99/1, respectively). The results suggest that solvent polarity alone has a strong influence on the regioselectivity of 1,3-dipolar cycloadditions of **10a**,**b** with **9b**.



Scheme 2.

3. Conclusion

The 1,3-dipolar cycloaddition of adamantylidenefulvene **1** with aryl and alkyl nitrile oxides **2a–d** under thermal and microwave reactions gave 1/1 cycloadducts, **3a–d** and **4a–d**, as the major products, and four other 1/2 cycloadducts **5–8a–d** as minor products. All the isoxazolines adducts **3a–7a**, **3b–7b**, **3c**, **4c**, **3d**, and **4d** were isolated and fully characterized by ^{1}H, ^{13}C NMR, MS, NOE, H,H-COSY, and H,C-COSY spectral data. Furthermore, the regioselectivity of the 1,3-dipolar cycloaddition of **1** with nitrile oxides **2a** or **2b** was highly enhanced by inclusion in β-CD solution. MD simulation results support that the *CD cavity provides steric control stabilizing the formation of the transition state structure* **3a**-**TS** (or **3b**-**TS**), giving **3a** (or **3b**) as the major product. The results further expand the scope of using CDs as a molecular reactor in a regio- and stereo-selective fashion. Finally, β-CD was found to be a useful chiral shift reagent for the differentiation of enantiomers of **4a**.

4. Experimental

4.1. General

^{1}H NMR spectra were measured on a 300, 500, and 600 MHz spectrometer. Natural abundance ^{13}C NMR spectra were measured using pulse Fourier transform, on a 300 MHz NMR spectrometer operating at 75.4 MHz. Broad-band decoupling, DEPT, NOE, H,H-COSY, and H,C-COSY were carried out to simplify the spectra and aid peak identification. Chemical shifts are given in parts per million (ppm) and coupling constant J in hertz (Hz) for both nuclei, with the solvent (usually $CDCl_3$) peak as an internal standard. The reference peak for ^{1}H is at δ 7.25 of $CHCl_3$, and for ^{13}C it is the central peak at δ 77. All reported yields here were from an average of three runs and were based on uncovered starting materials. The microwave reactions were carried out in a CEM MARS-5 magnetron with temperature controller.

4.2. Computation details

Stochastic molecular dynamics simulations were carried out using MacroModel V 8.0[22] with an all-atom amber* forcefield[23] in a continuum GB/SA model[24] for water. Default amber* charges were used except for **3a**-**TS** and **4a**-**TS**, which were obtained from CHELPG electrostatic potential fitting using Gaussian 03 at the HF/6-31G* level.[25] Geometric constraint to the optimized HF geometries for **3a**-**TS** and **4a**-**TS** was also applied. Constant dielectric treatment was used to estimate electrostatic interactions. A 200 ps equilibration step with 1 fs time step was followed by a 5000 ps MD run with 1 fs time step at 300 K. Structures were sampled at regular interval of 1 ps during the simulations. The sampled structures were then minimized using PR conjugated gradient method to obtain the lowest energy structure in each simulation.

4.3. General procedures for the reaction of 1 with 2a–c

To a well-stirred solution of **1** (100.0 mg, 0.50 mmol) with hydroximinoyl chloride (**9a**: 155.4 mg, 1.00 mmol; **9b**: 211.3 mg, 1.00 mmol; **9c**: 196.6 mg, 1.00 mmol) in THF (or benzene) (15 mL) was added triethylamine (15 drops) and refluxed for 24 h. After cooled to rt, the solution was washed with water (10 mL) and the water layer was

extracted three times with methylene chloride (10 mL×3). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel column by elution with *n*-hexane/ethyl acetate (20/1) to give **3a**–**8a** 50%; **3b**–**8b** 60%; **3c**–**4c** 40%.

4.3.1. 6-Adamantan-2-ylidene-3-phenyl-3*a*,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 3a. Colorless solid; mp 154–155 °C; δ_H 1.80–2.12 (m, 12H), 2.90 (br s, 1H), 3.01 (br s, 1H), 4.74 (ddd, *J*=8.8, 2.4, 2.3 Hz, 1H), 5.81 (d, *J*=8.8 Hz, 1H), 5.97 (dd, *J*=5.8, 2.4 Hz, 1H), 6.48 (dd, *J*=5.8, 2.3 Hz, 1H), 7.39–7.45 (m, 3H), 7.74–7.80 (m, 2H); δ_C 28.1 (2×CH), 35.3 (CH), 35.5 (CH), 37.1 (CH_2), 39.0 (CH_2), 39.1 (CH_2), 39.2 (CH_2), 39.7 (CH_2), 58.5 (CH), 84.0 (CH), 126.8 (CH), 128.3 (CH), 128.7 (CH), 129.7 (CH), 131.1 (CH), 132.2 (Cq), 149.0 (Cq), 156.3 (Cq); MS (EI, *m*/*z*) 317 (M^+, 83), 300 (43), 198 (100); HRMS *m*/*z* calcd for $C_{22}H_{23}NO$: 317.1780; found: 317.1776. Anal. Calcd for $C_{22}H_{23}NO$: C, 83.24; H, 7.30; N, 4.41; found: C, 82.85; H, 7.37; N, 4.37.

4.3.2. 4-Adamantan-2-ylidene-3-phenyl-3*a*,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 4a. Colorless solid; mp 126–128 °C; δ_H 0.40–0.46 (m, 1H), 1.30–1.37 (m, 1H), 1.46–1.55 (m, 1H), 1.64–1.93 (m, 9H), 2.51 (br s, 1H), 2.82 (br s, 1H), 4.74 (d, *J*=8.2 Hz, 1H), 5.86 (dd, *J*=8.2, 2.1, 0.8 Hz, 1H), 5.94 (dd, *J*=5.8, 2.1 Hz, 1H), 6.63 (dd, *J*=5.8, 0.8 Hz, 1H), 7.34–7.43 (m, 5H); δ_C 27.7 (CH), 28.0 (CH), 35.2 (CH), 35.9 (CH), 36.7 (CH_2), 37.6 (CH_2), 38.5 (CH_2), 39.4 (CH_2), 39.5 (CH_2), 54.8 (CH), 88.8 (CH), 127.5 (Cq), 128.2 (CH), 128.3 (CH), 129.1 (CH), 130.5 (Cq), 130.6 (CH), 132.8 (CH), 144.9 (Cq), 159.0 (Cq); MS (EI, *m*/*z*) 317 (M^+, 43), 300 (52), 198 (100); HRMS *m*/*z* calcd for $C_{22}H_{23}NO$: 317.1780; found: 317.1776.

4.3.3. 7-Adamantan-2-ylidene-3,4-diphenyl-3*a*,3*b*,6*a*,7*a*-tetrahydro-3*aH*-cyclopenta-[2,1-*d*:3,4-*d*′]diisoxazole 5a. Colorless solid; mp 230–231 °C; δ_H 1.87–2.04 (m, 12H), 3.03 (br s, 2H), 4.07 (d, *J*=7.6 Hz, 1H), 5.79 (d, *J*=7.6 Hz, 2H), 7.20–7.27 (m, 4H), 7.35–7.41 (m, 6H); δ_C 27.9 (CH), 36.1 (CH), 36.9 (CH_2), 38.9 (CH_2), 39.5 (CH_2), 56.6 (CH), 87.2 (CH_2), 126.2 (Cq), 127.8 (CH), 128.2 (Cq), 128.7 (CH), 130.1 (CH), 158.9 (2×Cq); MS (EI, *m*/*z*) 436 (M^+, 68), 317 (100), 198 (56), 119 (46), 91 (35), 77 (36); HRMS *m*/*z* calcd for $C_{29}H_{28}N_2O_2$: 436.2152; found: 436.2153. Anal. Calcd for $C_{29}H_{28}N_2O_2$: C, 79.79; H, 6.47; N, 6.42; found: C, 79.88; H, 6.67; N, 6.13.

4.3.4. (3*aR*,3*bR*,6*aS*,7*aR*)-7-Adamantan-2-ylidene-3,6-diphenyl-3*a*,3*b*,6*a*,7*a*-tetrahydro-3*aH*-cyclopenta[1,2-*d*:3,4-*d*′]diisoxazole 6a. Colorless solid; mp 237–238 °C; δ_H 0.98–1.03 (m, 1H), 1.58–1.97 (m, 11H), 2.68 (br s, 1H), 3.01 (br s, 1H), 4.17 (dd, *J*=8.7, 8.7 Hz, 1H), 4.96 (d, *J*=8.7 Hz, 1H), 5.57 (dd, *J*=8.7, 8.7 Hz, 1H), 5.71 (d, *J*=8.7 Hz, 1H), 7.30–7.53 (m, 8H), 7.71–7.75 (m, 2H); δ_C 27.5 (CH), 27.9 (CH), 35.3 (CH), 35.7 (CH), 36.6 (CH_2), 37.8 (CH_2), 38.8 (CH_2), 39.3 (CH_2), 39.5 (CH_2), 57.5 (CH), 58.0 (CH), 85.7 (CH), 86.2 (CH), 121.4 (Cq), 127.2 (CH), 128.2 (CH), 128.4 (CH), 128.5 (CH), 129.3 (Cq), 129.4 (CH), 129.7 (CH), 130.2 (Cq), 153.0 (Cq), 158.7 (Cq), 158.9 (Cq); MS (EI, *m*/*z*) 436 (M^+, 100), 408 (32), 317 (44), 289 (40), 198 (34), 104 (40), 91 (33), 77 (58); HRMS *m*/*z* calcd for $C_{29}H_{28}N_2O_2$: 436.2152; found: 436.2158.

X-ray crystal data for compound **6a**: $C_{29}H_{28}N_2O_2$, *M*=436.53, monoclinic, *a*=11.3842(2) Å, *b*=17.894(3) Å, *c*=11.099(2) Å, *α*=90°, *β*=97.291(1)°, *γ*=90°, *V*=2085.6(3) Å^3, *V*=2242.5(7) Å^3, space group $P2_1/c$, *Z*=4, calculated density 1.293 Mg m^{-3}, crystal dimensions (mm^3): 0.60×0.50×0.40, *T*=293(2) K, *λ* (Mo K*α*)=0.71073 Å, *μ*=0.081 mm^{-1}, 3958 reflections collected, 3958 independent (R_{int}=0.0000), 299 parameter refined on F^2, R_1=0.0422, $wR2[F^2]$=0.1198 (all data), GOF on F^2 1.099, $\Delta\rho_{max}$=0.162 eÅ^{-3}. Crystallographic data for the structure have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 606881. 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: data_request@ccdc.cam.ac.uk].

4.3.5. 6-Adamantan-2-ylidene-3-(4-*tert*-butyl-phenyl)-6,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 3b. Colorless solid; mp 172–173 °C; δ_H 1.32 (s, 9H), 1.78–2.07 (m, 12H), 2.90 (br s, 1H), 3.01 (br s, 1H), 4.72 (ddd, *J*=8.8, 2.3, 2.3 Hz, 1H), 5.79 (d, *J*=8.8 Hz, 1H), 5.97 (dd, *J*=5.8, 2.3 Hz, 1H), 6.47 (dd, *J*=5.8, 2.3 Hz, 1H), 7.42 (d, *J*=8.7 Hz, 2H), 7.68 (d, *J*=8.7 Hz, 2H); δ_C 28.2 (2×CH), 31.2 (CH_3), 34.8 (Cq), 35.3 (CH), 35.5 (CH), 37.1 (CH_2), 39.0 (CH_2), 39.1 (CH_2), 39.2 (CH_2), 39.7 (CH_2), 58.6 (CH), 83.7 (CH), 125.7 (CH), 126.6 (CH), 126.6 (Cq), 128.5 (CH), 131.0 (CH), 132.3 (Cq), 148.9 (Cq), 153.0 (Cq), 156.1 (Cq); MS (EI, *m*/*z*) 373 (M^+, 47), 356 (39), 225 (14), 198 (100), 161 (23), 91 (14); HRMS *m*/*z* calcd for $C_{26}H_{31}NO$: 373.2406; found: 373.2408.

X-ray crystal data for compound **3b**: $C_{26}H_{31}NO$, *M*=373.52, monoclinic, *a*=16.5261(13) Å, *b*=10.5956(9) Å, *c*=11.9249(9) Å, *α*=90°, *β*=92.796(2)°, *γ*=90°, *V*=2085.6(3) Å^3, space group $P2_1/c$, *Z*=4, calculated density 1.19 Mg m^{-3}, crystal dimensions (mm^3): 0.45×0.30×0.13, *T*=295(2) K, *λ* (Mo K*α*)=0.71073 Å, *μ*=0.071 mm^{-1}, 16867 reflections collected, 3670 independent (R_{int}=0.0506), 272 parameter refined on F^2, R_1=0.0862, $wR2[F^2]$=0.2483 (all data), GOF on F^2 1.049, $\Delta\rho_{max}$=0.383 eÅ^{-3}. Crystallographic data for the structure have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 606882. 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: data_request@ccdc.cam.ac.uk].

4.3.6. 4-Adamantan-2-ylidene-3-(4-*tert*-butyl-phenyl)-4,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 4b. Colorless solid; mp 145–146 °C; δ_H 0.26–0.35 (m, 1H), 1.21–1.29 (m, 1H), 1.29 (s, 9H), 1.44–1.53 (m, 1H), 1.60–1.90 (m, 9H), 2.47 (br s, 1H), 2.81 (br s, 1H), 4.71 (d, *J*=8.2 Hz, 1H), 5.4 (ddd, *J*=8.2, 2.0, 0.7 Hz, 1H), 5.93 (dd, *J*=5.8, 2.0 Hz, 1H), 6.62 (dd, *J*=5.8, 0.7 Hz, 1H), 7.27 (d, *J*=8.4 Hz, 2H), 7.35 (d, *J*=8.4 Hz, 2H); δ_C 27.7 (CH), 28.0 (CH), 31.2 (CH_3), 34.7 (Cq), 35.2 (CH), 35.8 (CH), 36.8 (CH_2), 37.4 (CH_2), 38.5 (CH_2), 39.4 (CH_2), 39.5 (CH_2), 55.0 (CH), 88.7 (CH), 125.1 (CH), 127.5 (Cq), 127.6 (Cq), 128.0 (CH), 130.6 (CH), 132.7 (CH), 144.9 (Cq), 152.2 (Cq), 159.0 (Cq); MS (EI, *m*/*z*) 373 (M^+, 87), 356 (39), 225 (17), 198 (100), 161 (18), 91

(9); HRMS *m/z* calcd for $C_{26}H_{31}NO$: 373.2406; found: 373.2404.

4.3.7. 7-Adamantan-2-ylidene-3,4-bis-(4-*tert*-butyl-phenyl)-3*b*,6*a*,7,7*a*-tetrahydro-3*aH*-cyclopenta[2,1-*d*:3,4-*d*′]-diisoxazole 5b. Colorless solid; mp 218–219 °C; δ_H 1.32 (s, 18H), 1.83–2.08 (m, 12H), 3.01 (br s, 2H), 4.06 (d, *J*=7.7 Hz, 2H), 5.75 (d, *J*=7.7 Hz, 2H), 7.26 (d, *J*=8.5 Hz, 4H), 7.33 (d, *J*=8.5 Hz, 4H); δ_C 27.9 (CH), 31.2 (CH_3), 34.8 (Cq), 36.0 (CH), 36.9 (CH_2), 38.8 (CH_2), 39.5 (CH_2), 56.4 (CH), 87.2 (CH), 125.3 (Cq), 125.5 (CH), 126.2 (Cq), 127.6 (CH), 153.4 (Cq), 156.6 (Cq), 158.9 (Cq); MS (EI, *m/z*) 548 (M^+, 6), 373 (33), 356 (39), 225 (17), 198 (100), 91 (9); HRMS *m/z* calcd for $C_{37}H_{44}N_2O_2$: 548.3403; found: 548.3394.

4.3.8. (3*aR*,3*bR*,6*aS*,7*aR*)-7-Adamantan-2-ylidene-3,6-bis-(4-*tert*-butyl-phenyl)-3*b*,6*a*,7,7*a*-tetrahydro-3*aH*-cyclopenta[1,2-*d*:3,4-*d*′]diisoxazole 6b. Colorless solid; mp 238–239 °C; δ_H 0.93–1.02 (m, 1H), 1.28 (s, 9H), 1.32 (s, 9H), 1.56–1.96 (m, 11H), 2.68 (br s, 1H), 3.02 (br s, 1H), 4.13 (dd, *J*=8.6, 8.6 Hz, 1H), 4.93 (d, *J*=8.6 Hz, 1H), 5.55 (dd, *J*=8.6, 8.6 Hz, 1H), 5.68 (d, *J*=8.6 Hz, 1H), 7.34 (d, *J*=6.8 Hz, 2H), 7.41 (d, *J*=6.8 Hz, 2H), 7.44 (d, *J*=6.8 Hz, 2H), 7.66 (d, *J*=6.8 Hz, 2H); δ_C 27.6 (CH), 28.0 (CH), 31.2 (CH_3), 34.7 (Cq), 34.8 (Cq), 35.2 (CH), 35.7 (CH), 36.6 (CH_2), 37.6 (CH_2), 38.7 (CH_2), 39.4 (CH_2), 39.5 (CH_2), 57.6 (CH), 58.1 (CH), 85.6 (CH), 86.1 (CH), 121.7 (Cq), 125.1 (CH), 125.5 (CH), 126.4 (Cq), 126.9 (CH), 127.4 (Cq), 128.0 (CH), 152.4 (Cq), 152.7 (Cq), 152.8 (Cq), 158.5 (Cq), 158.6 (Cq); MS (EI, *m/z*) 548 (M^+, 100), 520 (91), 373 (49), 345 (64), 259 (60), 214 (41), 198 (61), 144 (28), 91 (19); HRMS *m/z* calcd for $C_{37}H_{44}N_2O_2$: 548.3403; found: 548.3402.

4.3.9. (3*aS*,3*bS*,6*aR*,7*aR*)-7-Adamantan-2-ylidene-3,4-bis-(4-*tert*-butyl-phenyl)-3*b*,6*a*,7,7*a*-tetrahydro-3*aH*-cyclopenta[2,1-*d*:3,4-*d*′]diisoxazole 7b. Colorless solid; mp 245–246 °C; δ_H 0.68–0.80 (m, 1H), 1.31 (s, 9H), 1.34 (s, 9H), 1.35–1.47 (m, 1H), 1.56–2.03 (m, 10H), 2.48 (br s, 1H), 3.00 (br s, 1H), 4.18 (dd, *J*=10.6, 4.2 Hz, 1H), 4.83 (d, *J*=8.6 Hz, 1H), 5.16 (dd, *J*=8.6, 4.2 Hz, 1H), 5.69 (d, *J*=10.6 Hz, 1H), 7.39 (br s, 4H), 7.47 (d, *J*=8.4 Hz, 2H), 7.82 (d, *J*=8.4 Hz, 2H); δ_C 27.4 (CH), 27.9 (CH), 31.1 (CH_3), 34.8 (Cq), 34.9 (Cq), 36.2 (CH), 36.3 (CH), 36.7 (CH_2), 37.3 (CH_2), 38.2 (CH_2), 39.6 (CH_2), 39.7 (CH_2), 58.1 (CH), 60.8 (CH), 85.2 (CH), 88.0 (CH), 123.6 (Cq), 125.3 (CH), 125.5 (Cq), 125.9 (CH), 126.2 (Cq), 127.0 (CH), 128.0 (CH), 152.9 (Cq), 153.5 (Cq), 153.9 (Cq), 157.0 (Cq), 159.4 (Cq); MS (EI, *m/z*) 548 (M^+, 65), 520 (39), 373 (62), 356 (42), 345 (36), 259 (30), 214 (35), 198 (100), 144 (28), 91 (14); HRMS *m/z* calcd for $C_{37}H_{44}N_2O_2$: 548.3403; found: 548.3399.

4.3.10. Compound 8b. It is a mixture of at least two stereoisomers, and the ratio of the two isomers is ca. 3/1; colorless solid; mp>320 °C (decomp.); δ_H 1.29 (s, 18H), 1.30 (s, 18H), 1.74–1.94 (m, 10H), 1.97–2.06 (m, 2H), 2.08–2.23 (m, 4H), 2.31–2.40 (m, 4H), 2.41–2.53 (m, 4H), 2.70–2.79 (m, 2H), 3.02 (d, *J*=9.0 Hz, 1H), 3.08 (d, *J*=9.0 Hz, 1H), 4.42 (dd, *J*=9.5, 9.5 Hz, 1H), 4.43 (dd, *J*=9.5, 9.5 Hz, 1H), 6.09 (d, *J*=9.5 Hz, 2H), 7.38 (d, *J*=8.4 Hz, 4H), 7.39 (d, *J*=8.4 Hz, 4H), 7.47 (d, *J*=8.4 Hz, 4H), 7.56 (d, *J*=8.4 Hz, 4H); MS (EI, *m/z*) 548 (M^+, 50), 373 (74), 356 (34), 198 (62), 161 (50), 97 (42), 91 (17), 85 (60), 57 (100); HRMS *m/z* calcd for $C_{37}H_{44}N_2O_2$: 548.3403; found: 548.3406.

4.3.11. 6-Adamantan-2-ylidene-3-(5-chloro-thiophen-2-yl)-6,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 3c. Light yellow solid; mp 144–145 °C; δ_H 1.77–2.10 (m, 12H), 2.89 (br s, 1H), 2.97 (br s, 1H), 4.61 (ddd, *J*=8.9, 2.3, 2.3 Hz, 1H), 5.80 (d, *J*=8.9 Hz, 1H), 5.93 (dd, *J*=5.7, 2.3 Hz, 1H), 6.49 (dd, *J*=5.7, 2.3 Hz, 1H), 6.88 (d, *J*=3.9 Hz, 1H), 7.05 (d, *J*=3.9 Hz, 1H); δ_C 28.1 (2×CH), 35.3 (CH), 35.6 (CH), 37.0 (CH_2), 38.9 (CH_2), 39.1 (2×CH_2), 39.7 (CH_2), 39.1 (CH_2), 58.7 (CH), 84.5 (CH), 126.3 (CH), 126.7 (CH), 127.6 (CH), 131.1 (Cq), 131.6 (CH), 131.8 (Cq), 132.8 (Cq), 149.7 (Cq), 151.9 (Cq); MS (EI, *m/z*) 359 (M^++2, 34), 357 (M^+, 87), 340 (53), 327 (86), 198 (100), 129 (28), 91 (32); HRMS *m/z* calcd for $C_{20}H_{20}{}^{35}ClNOS$: 357.0954; found: 357.0952.

4.3.12. 4-Adamantan-2-ylidene-3-(5-chloro-thiophen-2-yl)-4,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 4c. Light yellow oil; δ_H 1.43–1.52 (m, 1H), 1.67–2.04 (m, 11H), 2.81 (br s, 1H), 2.88 (br s, 1H), 4.60 (d, *J*=7.9 Hz, 1H), 5.84 (ddd, *J*=7.9, 1.9, 0.8 Hz, 1H), 5.90 (dd, *J*=5.8, 1.9 Hz, 1H), 6.65 (dd, *J*=5.8, 0.8 Hz, 1H), 6.83 (d, *J*=3.9 Hz, 1H), 6.96 (d, *J*=3.9 Hz, 1H); δ_C 27.8 (CH), 28.1 (CH), 35.3 (CH), 36.1 (CH), 36.8 (CH_2), 37.8 (CH_2), 38.1 (CH_2), 39.7 (CH_2), 40.1 (CH_2), 53.6 (CH), 89.7 (CH), 126.2 (CH), 127.3 (CH), 127.5 (Cq), 130.1 (Cq), 130.3 (CH), 132.0 (Cq), 133.1 (CH), 145.1 (Cq), 153.0 (Cq); MS (EI, *m/z*) 357 (M^+, 51), 340 (100), 325 (68), 198 (97), 91 (26), 71 (29), 57 (36); HRMS *m/z* calcd for $C_{20}H_{20}{}^{35}ClNOS$: 357.0954; found: 357.0951.

4.4. General procedures for the reaction of 1 with 2d

To a well-stirred solution of **1** (68.0 mg, 0.34 mmol), nitroethane (50.0 mg, 0.69 mmol), and phenyl isocyanate (163.2 mg, 1.14 mmol) in THF (or benzene) (10 mL) was added triethylamine (10 drops) and then refluxed for 24 h. After cooled to rt, to the solution was added 1–2 mL of water and stirring was continued for 30 min in an ice bath; the solution was filtered to remove urea and dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel column by eluting with *n*-hexane/ethyl acetate (20/1) to give **3d** and **4d** in a combined yield of 45%.

4.4.1. 6-Adamantan-2-ylidene-3-methyl-6,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 3d. Light yellow oil; δ_H 1.73–2.05 (m, 12H), 1.98 (s, 3H), 2.87 (br s, 1H), 2.94 (br s, 1H), 4.15 (ddd, *J*=8.9, 2.4, 2.2 Hz, 1H), 5.61 (d, *J*=8.9 Hz, 1H), 5.86 (dd, *J*=5.8, 2.4 Hz, 1H), 6.64 (dd, *J*=5.8, 2.2 Hz, 1H); δ_C 12.4 (CH_3), 28.1 (2×CH), 35.2 (CH), 35.4 (CH), 37.0 (CH_2), 38.9 (CH_2), 39.1 (CH_2), 39.2 (CH_2), 39.6 (CH_2), 62.0 (CH), 82.4 (CH), 127.4 (CH), 131.2 (CH), 132.6 (Cq), 148.7 (Cq), 154.9 (Cq); MS (EI, *m/z*) 255 (M^+, 33), 238 (12), 214 (18), 198 (100), 129 (14), 91 (18); HRMS *m/z* calcd for $C_{17}H_{21}NO$: 255.1623; found: 255.1627.

4.4.2. 4-Adamantan-2-ylidene-3-methyl-4,6*a*-dihydro-3*aH*-cyclopenta[*d*]isoxazole 4d. Light yellow oil; δ_H

1.69–2.07 (m, 12H), 1.92 (s, 3H), 2.75 (br s, 1H), 2.90 (br s, 1H), 4.20 (dd, J=8.2, 1.0 Hz, 1H), 5.66 (ddd, J=8.2, 2.0, 1.0 Hz, 1H), 5.87 (dd, J=5.9, 2.0 Hz, 1H), 6.54 (dd, J=5.9, 1.0 Hz, 1H); δ_C 12.5 (CH_3), 28.0 (CH), 28.1 (CH), 35.1 (CH), 35.8 (CH), 36.9 (CH_2), 38.6 (CH_2), 39.3 (CH_2), 39.5 (CH_2), 39.6 (CH_2), 54.9 (CH), 87.6 (CH), 128.2 (Cq), 131.4 (CH), 132.2 (CH), 144.0 (Cq), 155.9 (Cq); MS (EI, *m/z*) 255 (M^+, 17), 238 (9), 214 (14), 198 (100), 155 (10), 128 (9), 115 (9), 91 (10); HRMS *m/z* calcd for $C_{17}H_{21}NO$: 255.1623; found: 255.1619.

The ratio of cycloadducts and their isolated yields are summarized in Table 1. Compounds **3**–**8** were eluted (*n*-hexane/ethyl acetate = 6/1) in the following sequence (R_f): **3a** (0.38), **4a** (0.43), **5a** (0.48), **6a** (0.69); **3b** (0.69), **4b** (0.50), **5b** (0.53), **6b** (0.36), **7b** (0.57), **8b** (0.50); **3c** (0.26), **4c** (0.35); **3d** (0.65) and **4d** (0.53). Although, compound **7a** was not isolated its existence can be assured by comparing its ^{1}H NMR spectrum with that of **7b**.

4.5. Synthesis of compound 11a,b and 12a,b

The synthetic procedures of **11a**,**b** and **12a**,**b** are similar to those of compounds **3**–**8**. The amount of reagent used in the reaction was as follows: compound **10a** (200 mg, 2.00 mmol), **9b** (365 mg, 3.00 mmol), Et_3N (320 mg, 3.17 mmol), THF (10 mL). The combined yield of compound **11a** and **12a** was 70% and the isolated yield of compounds **11b** and **12b** was 85%.

4.5.1. 3-(4-*tert*-Butyl-phenyl)-5-methyl-4,5-dihydro-isoxazole-4-carboxylic acid methyl ester 11a. Colorless oil; δ_H 1.31 (s, 9H), 1.43 (d, J=6.4 Hz, 3H), 3.71 (s, 3H), 4.06 (d, J=5.8 Hz, 1H), 5.01–5.10 (m, 1H), 7.40 (d, J=8.6 Hz, 2H), 7.62 (d, J=8.6 Hz, 2H); δ_C 20.8 (CH_3), 31.1 (CH_3), 34.8 (Cq), 52.9 (CH_3), 60.1 (CH), 82.1 (CH), 125.7 (CH), 125.9 (Cq), 126.5 (CH), 153.5 (Cq), 153.6 (Cq), 170.0 (Cq); MS (EI, *m/z*) 275 (M^+, 24), 260 (100), 160 (13), 116 (8), 91 (5); HRMS *m/z* calcd for $C_{16}H_{21}NO_3$: 275.1521; found: 275.1520.

4.5.2. 3-(4-*tert*-Butyl-phenyl)-4-methyl-4,5-dihydro-isoxazole-5-carboxylic acid methyl ester 12a. Colorless oil; δ_H 1.32 (s, 9H), 1.42 (d, J=7.2 Hz, 3H), 3.78 (s, 3H), 3.92–4.02 (m, 1H), 4.77 (d, J=3.9 Hz, 1H), 7.42 (d, J=8.7 Hz, 2H), 7.62 (d, J=8.7 Hz, 2H); δ_C 18.2 (CH_3), 31.1 (CH_3), 34.9 (Cq), 47.1 (CH), 52.7 (CH_3), 84.8 (CH), 124.7 (Cq), 125.8 (CH), 127.1 (CH), 153.9 (Cq), 160.3 (Cq), 171.1 (Cq); MS (EI, *m/z*) 275 (M^+, 32), 260 (65), 216 (100), 188 (19), 91 (8); HRMS *m/z* calcd for $C_{16}H_{21}NO_3$: 275.1521; found: 275.1529.

4.5.3. [3-(4-*tert*-Butyl-phenyl)-5-phenyl-4,5-dihydro-isoxazol-4-yl]-phenyl-methanone 11b. Colorless oil; δ_H 1.27 (s, 9H), 5.36 (d, J=6.6 Hz, 1H), 5.74 (d, J=6.6 Hz, 1H), 7.31–7.42 (m, 7H), 7.48–7.55 (m, 4H), 7.62–7.69 (m, 1H), 7.90–7.95 (m, 2H); δ_C 31.1 (CH_3), 34.8 (Cq), 65.0 (CH), 87.6 (CH), 125.6 (Cq), 125.8 (CH), 125.9 (CH), 126.8 (CH), 128.9 (CH), 129.0 (CH), 129.1 (CH), 129.2 (CH), 134.3 (CH), 135.2 (Cq), 139.8 (Cq), 153.6 (Cq), 155.2 (Cq), 195.4 (Cq); MS (EI, *m/z*) 383 (M^+, 2), 105 (100), 77 (19); HRMS *m/z* calcd for $C_{26}H_{25}NO_2$: 383.1885; found: 383.1888.

4.5.4. [3-(4-*tert*-Butyl-phenyl)-4-phenyl-4,5-dihydro-isoxazol-5-yl]-phenyl-methanone 12b. Colorless oil; δ_H 1.26 (s, 9H), 5.41 (d, J=4.6 Hz, 1H), 5.65 (d, J=4.6 Hz, 1H), 7.25–7.64 (m, 12H), 8.03–8.08 (m, 2H); δ_C 31.1 (CH_3), 34.8 (Cq), 55.3 (CH), 90.0 (CH), 125.2 (Cq), 125.6 (CH), 127.4 (CH), 127.9 (CH), 128.0 (CH), 128.7 (CH), 129.4 (CH), 129.6 (CH), 133.9 (CH), 134.4 (Cq), 138.5 (Cq), 153.5 (Cq), 158.4 (Cq), 193.6 (Cq); MS (EI, *m/z*) 383 (M^+, 4), 278 (100), 207 (37), 105 (90), 91 (39), 77 (40); HRMS *m/z* calcd for $C_{26}H_{25}NO_2$: 383.1885; found: 383.1885.

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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.2006.05.021.

References and notes

1. (a) Neuenschwander, M. *Chemistry of Double Bonded Functional Groups*; Patai, S., Ed.; Wiley: Chichester, UK, 1989; Vol. 2, p 1131; (b) Zhang, X.; Khan, S. I.; Foote, C. S. *J. Org. Chem.* **1995**, *60*, 4102–4107; (c) Hong, B.-C.; Chen, F.-L.; Chen, S.-H.; Liao, J.-H.; Lee, G.-H. *Org. Lett.* **2005**, *7*, 557–560 and references cited therein.
2. (a) Grünanger, P. *The Nitrile Oxide*; Springer: New York, NY, 1971; (b) *1,3-Dipolar Cycloadditions*; Padwa, A., Ed.; Wiley: New York, NY, 1984; (c) Jäger, V.; Colinas, P. A. *The Chemistry of Heterocyclic Compounds*; Wiley: New York, NY, 2002; Vol. 59, pp 361–472; (d) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. *J. Am. Chem. Soc.* **2005**, *127*, 210–216.
3. (a) Curran, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 5826–5833; (b) Easton, C. J.; Hughes, C. M. M.; Savage, G. P.; Simpson, G. W. *Adv. Heterocycl. Chem.* **1994**, *60*, 261–327; (c) Easton, C. J.; Hughes, C. M.; Kirby, K. D.; Savage, G. P.; Simpson, G. W.; Tiekink, E. R. T. *J. Chem. Soc., Chem. Commun.* **1994**, 2035; (d) Bode, J. W.; Carreira, E. M. *Org. Lett.* **2001**, *3*, 1587–1590; (e) Tranmer, G. K.; Tam, W. *Org. Lett.* **2002**, *4*, 4101–4104.
4. (a) Miguel del Corral, J. M.; Gordaliza, M.; Castro, M. A. *Tetrahedron* **1997**, *53*, 6555–6564; (b) Lee, J. Y.; Chung, Y. J.; Bae, Y. S.; Ryu, S. H.; Kim, B. H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 359–365; (c) Quadrelli, P.; Scrocchi, R.; Caramella, P.; Rescifina, A.; Piperno, A. *Tetrahedron* **2004**, *60*, 3643–3651.
5. (a) Quilico, A.; Grünanger, P.; Mazzini, R. *Gazz. Chim. Ital.* **1952**, *82*, 349–361; (b) Caramella, P.; Frattini, P.; Grünanger, P. *Tetrahedron Lett.* **1971**, *41*, 3817–3820; (c) Nair, V.; Nandakumar, M. V.; Maliakal, D.; Mathen, J. S.; Rath, N. P. *Tetrahedron* **2000**, *56*, 8001–8005.
6. (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: New York, NY, 1978; (b) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344–362; (c) Wenz, G.

Angew. Chem., Int. Ed. Engl. **1994**, *33*, 803–822; (d) Szejtli, J.; Osa, T. *Cyclodextrins in Comprehensive Supramolecular Chemistry*; Lehn, J.-M., Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds.; Pergamon: New York, NY, 1996; Vol. 3; (e) D'Souza, V. T., Lipkowitz, K. B., Eds.; *Chem. Rev.* **1998**, *98*, 1741–2076; (f) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011.

7. (a) Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325–1357; (b) Lincoln, S. F.; Easton, C. J. *Structure Diversity and Functional Versatility of Polysaccharides*; Dumitriu, S., Ed.; Marcel Dekker: New York, NY, 1998; p 473; (c) Lock, J. S.; May, B. L.; Clements, P.; Loncoln, S. F.; Easton, C. J. *Org. Biomol. Chem.* **2004**, *2*, 337–344; (d) Mieusset, J.-L.; Krois, D.; Pacar, M.; Brecker, L.; Giester, G.; Brinker, U. H. *Org. Lett.* **2004**, *6*, 1967–1970; (e) Chu, J.-H.; Li, W.-S.; Chao, I.; Chung, W.-S. *Tetrahedron* **2004**, *60*, 9493–9501; (f) Kano, K.; Nishiyabu, R.; Dio, R. *J. Org. Chem.* **2005**, *70*, 3667–3673.
8. (a) Brown, S. E.; Coates, J. H.; Easton, C. J.; Lincoln, S. F.; Luo, Y.; Stephens, A. K. W. *Aust. J. Chem.* **1991**, *44*, 855–862; (b) Badawy, S. I. F.; Marshall, A. L.; Ghorab, M. M.; Adeyeye, C. M. *Drug Dev. Ind. Pharm.* **1996**, *22*, 959–966.
9. (a) Li, S.; Purdy, W. C. *Chem. Rev.* **1992**, *92*, 1457–1470; (b) Easton, C. J.; Lincoln, S. F. *Chem. Soc. Rev.* **1996**, 163–170; (c) Ahuja, S. *Chiral Separations by Chromatography*; American Chemical Society: Washington, DC, 2000.
10. (a) Breslow, R.; Campbell, P. *J. Am. Chem. Soc.* **1969**, *91*, 3085; (b) Komiyama, M.; Hirai, H. *J. Am. Chem. Soc.* **1984**, *106*, 174–178; (c) de Rossi, R. H.; Barra, M.; de Vargas, E. B. *J. Org. Chem.* **1986**, *51*, 2157–2162; (d) Luzhkov, V.; Åqvist, J. *J. Am. Chem. Soc.* **1998**, *120*, 6131–6137.
11. (a) Rideout, D. C.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *102*, 7816–7817; (b) Ye, H.; Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1991**, *32*, 5231–5234; (c) Dumanski, P. G.; Easton, C. J.; Lincoln, S. F.; Simpson, J. S. *Aust. J. Chem.* **2003**, *56*, 1107–1111.
12. (a) Sternbach, D. D.; Rossana, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 5853–5854; (b) Schneider, H.-J.; Sangnwan, N. K. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 896–897; (c) Breslow, R.; Guo, T. *J. Am. Chem. Soc.* **1988**, *110*, 5613–5617; (d) Sangwan, N. K.; Schneider, H. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1223–1227; (e) Wernick, D. L.; Yazbek, A.; Levy, J. *J. Chem. Soc., Chem. Commun.* **1990**, 956–957; (f) Chung, W.-S.; Wang, J.-Y. *J. Chem. Soc., Chem. Commun.* **1995**, 971–972; (g) Li, W.-S.; Chung, W.-S.; Chao, I. *Chem.—Eur. J.* **2003**, *9*, 951–962.
13. (a) Chung, W.-S.; Turro, N. J.; Silver, J.; le Noble, W. J. *J. Am. Chem. Soc.* **1990**, *112*, 1202–1205; (b) Moorthey, J. N.; Venkatesan, K.; Weiss, R. G. *J. Org. Chem.* **1992**, *57*, 3292–3297; (c) Chung, W.-S.; Wang, N.-J.; Liu, Y.-D.; Leu, Y.-J.; Chiang, M.-Y. *J. Chem. Soc., Perkin Trans. 2* **1995**, 307–313; (d) Herrmann, W.; Wehrle, S.; Wenz, G. *Chem. Commun.* **1997**, 1709–1710; (e) Inokuma, S.; Kimura, K.; Funaki, T.; Nishimura, J. *J. Incl. Phenom. Macro. Chem.* **2001**, *39*, 35–40.
14. (a) Rama Rao, K.; Bhanumathi, N.; Srinivasan, T. N.; Sattur, P. B. *Tetrahedron Lett.* **1990**, *31*, 899–902; (b) Rama Rao, K.; Bhanumathi, N.; Sattur, P. B. *Tetrahedron Lett.* **1990**, *31*, 3201–3204; (c) Rama Rao, K. *Pure Appl. Chem.* **1992**, *64*, 1141–1145.
15. (a) Easton, C. J.; Hughes, C. M. M.; Tiekink, E. R. T.; Savage, G. P.; Simpson, G. W. *Tetrahedron Lett.* **1995**, *36*, 629–632; (b) Meyer, A. G.; Easton, C. J.; Lincoln, S. F.; Simpson, G. W. *Chem. Commun.* **1997**, 1517–1518; (c) Meyer, A. G.; Easton, C. J.; Lincoln, S. F.; Simpson, G. W. *J. Org. Chem.* **1998**, *63*, 9069–9075.
16. (a) Olah, G. A.; Prakash, G. K. S.; Liang, G. *J. Org. Chem.* **1977**, *42*, 661–666; (b) Alper, H.; Laycock, D. E. *Synthesis* **1980**, 799.
17. Due to poor solubility of adamantylidenefulvene (**1**) in water, co-solvent of acetone/water (1/1) was used.
18. (a) Hamilton, J. A.; Steinrauf, L. K.; vanEtten, R. L. *Acta Crystallogr.* **1968**, *B24*, 1560–1562; (b) Crugler, M.; Eckle, E.; Stezowski, J. *J. Chem. Soc., Chem. Commun.* **1981**, 1291–1292.
19. (a) Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 362–386 and references cited therein; (b) Connors, K. A. *Binding Constants, The Measurement of Molecular Complex Stability*; Wiley: New York, NY, 1987; (c) Benesi, H. A.; Hilderbrand. *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707.
20. (a) For an excellent review on 'chiral discrimination by modified cyclodextrins' see Ref. 9b and Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755–1785; (b) Smith, K. J.; Wilcox, J. D.; Mirick, G. E.; Wacker, L. S.; Ryan, N. S.; Vensel, D. A.; Readling, R.; Domush, H. L.; Amonoo, E. P.; Shariff, S. S.; Wenzel, T. J. *Chirality* **2003**, *15*, S150–S158; (c) Berthod, A.; Li, W.; Armstrong, D. W. *Anal. Chem.* **1992**, *64*, 873–879; (d) For the use of ROESY in structural identification on inclusion complex see: Ref. 7f.
21. For related studies, see: (a) Christl, M.; Huisgen, R. *Chem. Ber.* **1973**, *106*, 3345–3367; (b) Bianchi, G.; De Micheli, C.; Gandolfi, R.; Grünanger, P.; Finzi, P. V.; de Pava, O. V. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1148–1155.
22. Mohamandi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Liption, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 400–467.
23. (a) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Chio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765–784; (b) Weiner, S. J.; Kollman, P. A.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230–252.
24. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
25. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Peterson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Ciosloeski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03, Revision A.1*; Gaussian: Pittsburg, PA, 2003.

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Photochemical behavior of the drug atorvastatin in water

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Abstract—Atorvastatin undergoes a self-sensitized photooxygenation by sunlight in water. The main photoproducts, isolated by chromatographic techniques, have been identified by spectroscopic means. They present a lactam ring arising from an oxidation of pyrrole ring and an alkyl/aryl shift. A mechanism involving singlet oxygen addition and an epoxide intermediate is suggested.

1. Introduction

Atorvastatin calcium is one of the most prescribed drugs in the US and in Europe.[1] It is a synthetic lipid-lowering agent and is widely used in the prevention of cardiovascular events. It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Moreover, recently it has been observed that atorvastatin, like other statin drugs, can be efficient against Alzheimer's disease.[2] Less than 5% of a dose of atorvastatin is recovered in urine following oral administration. The presence of atorvastatin in sewage effluents and surface waters has been observed in concentration at μg/l levels.[3] The presence of pharmaceuticals in surface and ground waters is an increasingly relevant issue in environmental chemistry.[4] These substances enter into the aquatic environment, and thus they are potential pollutants for the aquatic ecosystem, possibly with adverse effects on aquatic organisms. The occurrence of pharmaceuticals in surface waters has been extensively reviewed, but data on the fate of these xenobiotics in the environment are still limited. These chemicals can be transformed through abiotic processes (hydrolysis, photolysis) into different products, and many searches are now addressed to the identification of these transformation products.[5–8]

In this context, we decided to study the photo-induced transformation processes of atorvastatin in water. Particular attention has been focused on the isolation and characterization of its main photoproducts as well as to the elucidation of the possible mechanistic pathways that lead to the observed products.

2. Results and discussion

Preliminary experiments showed that the drug was recovered unchanged by keeping it in the dark in aqueous solution (at different pHs) even after 30 days.

The photochemical behavior of atorvastatin in water was then studied under different conditions. First of all a solution of atorvastatin (10 μM) was exposed to natural sunlight. The ^{1}H NMR analysis of the irradiation mixture showed the presence of the drug and several products. In order to get amounts of photoproducts suitable for spectroscopic analyses, a series of irradiation experiments were performed on a preparative scale. A dispersion of the drug (80 mg/l) was irradiated by a solar simulator and the irradiation was monitored by RP-TLC analysis. After 14 days, the irradiation mixture was analyzed by NMR spectroscopy revealing that atorvastatin was completely transformed. The same photoproducts as in sunlight irradiation were observed by NMR and RP-TLC analyses. The main photoproducts were then separated by chromatographic techniques employing several stationary and mobile phases. Repeated column/TLC chromatographies and preparative HPLC of the mixture were necessary to isolate compounds **2–5** (Fig. 1).[9] Careful NMR analysis of the fractions containing **5** showed the presence of very close double signals, which were attributed to two diasteromers. The latter could be separated as methyl esters after performing a methylation reaction with CH_2N_2.[10]

The structures for all compounds were elucidated by NMR techniques (COSY, TOCSY, HSQC, HMBC, NOESY) and MALDI-MS experiments.

Keywords: Atorvastatin; Pyrroles; Pyrrol-2(3*H*)-ones; Photooxygenation; 1,2-Migration.
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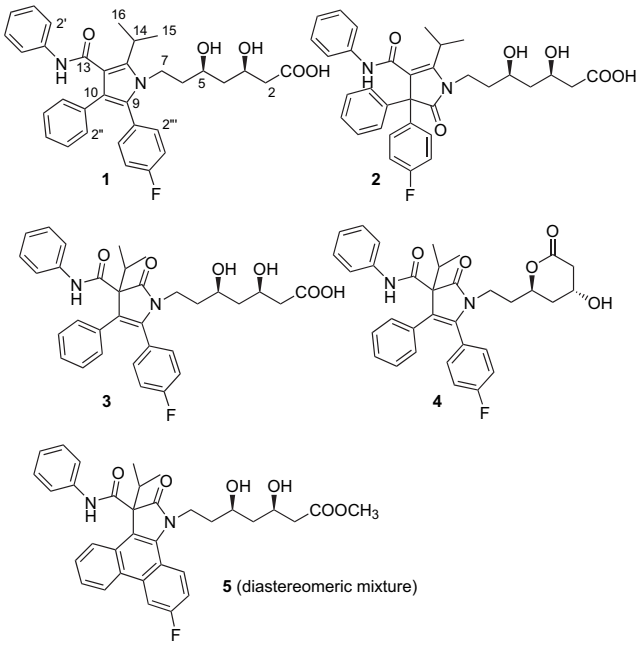


Figure 1. Structure of atorvastatin and photoproducts **2**–**5**.

Compound **2** showed a molecular peak at *m/z* 575 $[M+H]^+$ in the MALDI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{33}H_{34}FN_2O_6$. The UV spectrum revealed a band at 203 nm. In the ^1H NMR spectrum, 14 aromatic protons were present in the 7.00–7.40 ppm range; furthermore three methine at δ 4.17, 3.76, and 3.27, eight methylene protons at δ 3.76, 2.40, 1.85, and 1.66, and two methyls at δ 1.45 were in the aliphatic region. The ^{13}C NMR spectrum showed 27 carbon signals. The DEPT spectrum showed two methyls, four methylenes, and eleven methines. A close inspection of the ^1H and ^{13}C NMR spectra of **2** by DEPT and HSQC experiments and comparison with the spectral data for atorvastatin **1** revealed the presence of the following functionalities: three carbonyl groups, one quaternary sp^3-carbon (C-10), three aliphatic methines (C-3, C-5, and C-14), the first two bearing oxygen, four aliphatic methylene carbons (C-2, C-4, C-6, and C-7), two methyls (C-15 and C-16), two quaternary sp^2-carbons (C-11 and C-12), two monosubstituted aromatic rings (C-1′–C-6′, C-1″–C-6″), and one disubstituted aromatic ring (C-1‴–C-6‴). The connection of these functional groups was determined on the basis of ^1H–^1H COSY and HMBC correlations. Long-range correlations from the H-7 protons at δ 3.76 to the carbonyl carbon (δ 181.4) and C-12 (δ 153.4), the H-14 proton at δ 3.27 to the C-11 (δ 119.3) and C-12 quaternary carbons, the latter also correlated with H-14/H-15 methyls in the HMBC spectrum indicating the presence of 1-*H*-pyrrol-2(3*H*)-one. The correlation from H-2″/H-6″ and H-2‴/H-6‴ protons (δ 7.30 and 7.34) to the C-10 (δ 66.6) indicated the linkage of two phenyl groups at the sp^3 quaternary carbon. These correlations were consistent with structure **2**.

Compound **3** showed a molecular peak at *m/z* 575 $[M+H]^+$ in the ESI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{33}H_{34}FN_2O_6$. The UV spectrum revealed a band at 204 nm. The ^{13}C NMR spectrum showed 27 carbon signals. The DEPT spectrum showed two methyls, four methylenes, and eleven methines. A close inspection of the ^1H and ^{13}C NMR spectra of **3** by DEPT and HSQC experiments and comparison with the spectral data for the atorvastatin **1** revealed the presence of the following functionalities: three carbonyl groups, one quaternary sp^3-carbon (C-11), three aliphatic methines (C-3, C-5, and C-14), two of them bearing oxygen, four aliphatic methylene carbons (C-2, C-4, C-6, and C-7), two methyls (C-15 and C-16), two quaternary sp^2-carbons (C-9 and C-10), two monosubstituted aromatic rings (C-1′–C-6′, C-1″–C-6″), and one disubstituted aromatic ring (C-1‴–C-6‴). The connection of these functional groups was determined on the basis of ^1H–^1H COSY and HMBC correlations. Long-range correlations from the H-7 protons at δ 3.79 and 3.56 to the carbonyl carbon (δ 178.2) and C-9 (δ 140.4), the H-14 proton at δ 2.67 to C-10 (120.0), C-11 (δ 69.9), C-12, and C-13; C-11 also correlated with H-14/H-15 methyls in the HMBC spectrum indicating the presence of 1-*H*-pyrrol-2(3*H*)-one. These correlations were consistent with the structure **3** as depicted.

According to the δ lactone structure, a molecular ion at *m/z* 579 $[M+Na]^+$ in the ESI-MS was present for compound **4**. The ^1H–^1H COSY experiment showed a correlation series beginning with signal of a methylene at δ 3.79 and 3.56 assigned to H-7 to methylene at δ 1.85, which in turn was coupled with methine at δ 4.52. This latter was correlated to methylene at δ 1.77, which in turn was coupled with methine at δ 4.16 correlated with methylene at δ 2.59 and 2.42. Two doublets were attributed to two methyls at δ 1.16 and 0.95 correlated to methine at δ 2.78. The ^{13}C NMR spectrum of **4** showed 27 carbon signals due to two methyls, four methylenes, eleven methines, and ten quaternary carbons. An HMQC experiment allowed to assign the protons to the corresponding carbons. In the HMBC spectrum, H-7 protons were correlated with C-5, C-9, and C-12, while the H-14 proton was correlated with the C-10, C-11, C-12, C-13, C-15, and C-16. The multiplet at δ 4.52 (H-5) was correlated with C-1 and C-3, while H-3 was correlated with C-1, C-2, and C-5, thus completely dating the structure of **4**.

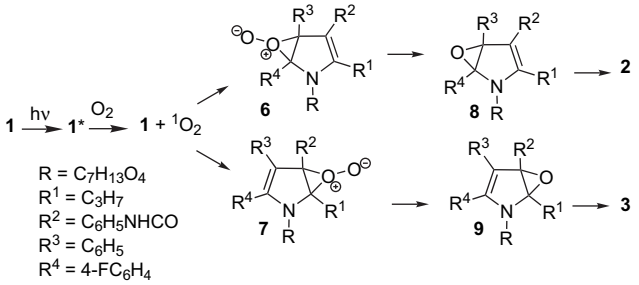
Compound **5** was a ca. 1:1 mixture of diasteromers, which were separated and characterized as methyl esters. One isomer of **5** showed a molecular peak at *m/z* 587 $[M+H]^+$ in the ESI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{34}H_{35}FN_2O_6$. The ^1H NMR spectrum showed the presence of one aromatic ring with three coupled protons, which were also coupled with fluorine atom, and resulted as two double doublet at δ 8.51 (H-6‴), 8.45 (H-3‴) and a double double doublet at δ 7.45. Furthermore, four protons of a 1,2 disubstituted aromatic ring at δ 8.76 (H-6″), 8.60 (H-3″), as doublets and at δ 7.69 (H-5″) and 7.60 (H-4″) as triplets and a phenyl group (H-2′–H-6′) were present in the aromatic region. The aliphatic region of ^1H NMR spectrum presented the usual C7 chain, the isopropyl group, and a methyl. In the ^{13}C NMR spectrum, 30 carbon signals were present, and the DEPT experiment evidenced three methyls, four methylenes, and thirteen methines. The ^1H and ^{13}C resonances were assigned by combination of COSY, DEPT, HMQC, and HMBC experiments. In particular, the HMBC spectrum showed cross-peaks of both the H-7 and H-14 with carbonyl (C-12), the first was correlated with C-5 and C-9, both the H-14 and the

methyls (H-15 and H-16) with the C-11, and the first with C-10 and C-13 carbons. These data indicated the presence of 1-*H*-pyrrol-2(3*H*)-one. In the HMBC spectrum, correlations between H-6‴ and C-9, C4‴, and C3‴ carbons, H-2″ and C-10, C-2″, and C-4″ carbons, and H-3″ and C-2‴ and C-5″ indicate a phenathrene unit.

The other isomer of **5** had the molecular formula $C_{34}H_{35}FN_2O_6$ as deduced from the molecular peak at *m*/*z* 587 $[M+H]^+$ in the ESI-MS spectrum. The general features of the NMR spectra closely resembled those of its isomer, except for the shift of the methyl signals and 6″ methine.

As shown in Figure 1, all photoproducts characterized arise from an oxidation of pyrrole ring and an alkyl/aryl shift with formation of a lactam ring. In addition, compounds **4** and **5** derive from **3** via lactonization and cyclization, respectively. To get information on the possible pathways leading to compounds **2**–**5**, irradiation experiments were run under different conditions, and to shorten the reaction times UV-lamp (Pyrex filter) was used as light source. With this lamp, the drug was converted to compounds **2**–**5** in 8 h (~70%). When the irradiation was carried out under argon atmosphere, the drug was recovered unreacted, confirming that O_2 was directly involved in the phototransformation. Degradation slowed down in the presence of a radical inhibitor as 3-*tert*-butyl-4-anisole (BHA), while it was completely inhibited by sodium azide (NaN_3), a well-known quencher of singlet oxygen. Moreover, when the photooxygenation was carried out in the presence of Rose Bengal, a typical singlet oxygen sensitizer, the reaction was complete already after 8 h. NMR analysis and TLC showed that atorvastatin was transformed in compounds **2**–**5** as main photoproducts.

These data suggest that singlet oxygen may be involved in the photodegradation of the drug and, hence, a Type II (singlet oxygen-mediated) photooxygenation may occur.[11] It is well known that pyrroles are good substrates for singlet oxygen. They often give a mixture of products derived both from 2,5- and 2,3-oxygen addition as well as from hydroperoxides or zwitterionic intermediates, depending on the reaction conditions and on the substituents, and the question of primary adducts is still an open point.[12] Anyhow, hydroxylactams have sometime been found as photooxygenation products from α- or α,α′-unsubstituted pyrroles.[12] So, on the basis of experimental results and literature data a plausible mechanistic interpretation is reported in Scheme 1. The first step should be an energy transfer from the excited drug to oxygen producing singlet oxygen. Then, a 2,3-oxygen addition to the pyrrole moiety probably occurs to give



Scheme 1. Suggested pathways for compounds **2** and **3**.

perepoxides **6** or **7**, which evolve to epoxides **8** or **9**.[13] Migration of the aryl or alkyl moiety gives pyrrolones **2** or **3**, respectively.[14]

Epoxides **8** and **9** were not detected in our experiments, probably due to their easy rearrangement to the corresponding carbonyl compounds.[15] In order to prove the involvement of these intermediates, an oxidation reaction of atorvastatin was carried out by using dimethyldioxirane (DMD). This compound is a well-known epoxidizing agent for diverse unsaturated substrates from alkenes to aromatic compounds, and it reacts with *N*-acylindoles to give the corresponding epoxides that open to form 2-indolinones as rearranged products.[16] The reaction of **1** with DMD was carried out in acetone and after 4 h the reaction mixture showed the presence of compounds **2** and **3**.

Compounds **4** and **5** seem to arise from further modification of compound **3**. Lactonization of a six-membered ring of the dihydroxy heptanoic acid side chain affords compound **4**, while phenantrene **5** is formed through a well-known[17] process in the photochemistry of stilbene-like compounds. In particular, it would be formed by photochemical electrocyclization followed by oxidation, under aerobic conditions, of the dihydrophenantrene intermediate.

3. Conclusions

In conclusion, atorvastatin has been found to be sensitive to sunlight under aerobic conditions and the main photoproducts have been isolated and fully characterized by spectral means (NMR and MS). This behavior agrees with the observation reported in two recent works that atorvastatin is phototransformed with a quantum yield for direct photolysis of 4.5×10^{-3}.[9]

Our data evidenced that in the presence of light and oxygen, the drug is able to act as a self-sensitizer and generate singlet oxygen,[18] and this observation appears of particular interest in the field of photosensitization phenomena by drugs.[19]

4. Experimental

4.1. Chemicals

Atorvastatin calcium was obtained from KEMPROTEC Limited. This and all the other products were used without further treatment: Rose Bengal (RB, Aldrich), NaN_3 (Carlo Erba). Solutions and suspensions of atorvastatin were prepared using Milli Q water. All other solvents were of HPLC grade.

4.2. General procedures

HPLC experiments were carried out on an Agilent 1100 HPLC system equipped with an UV detector, the column used was a RP-18 column (Luna Prep C-18, 10 μm, 250×10 mm). Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for [^{1}H] and 125 MHz for [^{13}C] on a Fourier Transform NMR Varian 500 Unity Inova spectrometer and at 400 MHz for [^{1}H] and 100 MHz for [^{13}C] on

a Bruker AC 400 spectrometer. The carbon multiplicity was evidenced by DEPT experiments. The proton couplings were evidenced by ^{1}H–^{1}H COSY experiments. The heteronuclear chemical shift correlations were determined by HMQC and HMBC pulse sequences. ^{1}H–^{1}H proximities through space within a molecule were determined by NOESY. Matrix assisted laser desorption ionization (MALDI) mass spectra were recorded using a Voyager-DE MALDI-TOF mass spectrometer. Electrospray mass spectra were recorded using a WATERS Z-Q mass spectrometer equipped with an electrospray ionization (ESI) probe operating in positive or negative ion mode. The scan range was 80–2000 *m/z*. UV–vis spectra were recorded in MeOH on a Perkin–Elmer Lambda 7 spectrophotometer. IR spectra were recorded in CH_2Cl_2 on a Nicolet 5700 FT-IR spectrometer. Reverse phase liquid chromatography was performed over Lichroprep RP-18 resin (Merck). Analytical TLC was performed on precoated Merck aluminum sheet (DC-Alufolien Kielselgel 60 F_{254}, 0.2 mm) or RP-18 F_{254} plates with 0.2 mm film thickness. The spots were visualized by UV light or by spraying with H_2SO_4/AcOH/H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Prep. TLC was performed on a Merck Kiesegel 60 F_{254} plates, with 0.5 or 1 mm film thickness.

4.3. Irradiation conditions

Irradiation experiments were performed with a 150 W solar simulator Oriel equipped with a Xenon lamp (spectral output 200–2400 nm) on a suspension of atorvastatin calcium (40 mg) in Milli Q water (500 ml), or solution 10 μM, in a Pyrex beaker for 14 days at room temperature.

An analogous preparation of atorvastatin calcium was exposed to sunlight for 14 days (on September in Naples) in an open Pyrex flask.

In an attempt to gain some mechanistic information, other irradiation experiments were performed using a photoreactor equipped with a 500 W high-pressure mercury lamp (through a Pyrex filter).

In a typical procedure, a suspension of atorvastatin calcium (10 mg in 20 ml of water) in the presence of a sensitizer (molar ratio atorvastatin:sens = 10:1 or as otherwise indicated) in an open Pyrex tube was irradiated at room temperature under stirring at a distance of 15 cm from the lamp.

NaN_3 and the pharmaceutical was used in a 1:3 molar ratio. In the experiment carried out under argon atmosphere, the suspension was saturated with the gas for 30 min before irradiation and then kept closed.

4.4. Photoproduct isolation

The suspension after 14 days irradiation by solar simulator was dried under vacuum, and the acetone-soluble reaction mixture (40 mg) was separated by silica-gel TLC eluting with the organic phase of the mixture AcOEt/EtOH/H_2O (3:1:5) to give five fractions.

Fractions 2 and 4 were pure photoproduct **2** (11 mg) and **3** (4 mg), while fractions 1, 3, and 5 were mixtures of several products.

Fraction 3 (8 mg) was chromatographed on silica-gel TLC eluting with CH_2Cl_2/MeOH (95:5) to afford photoproduct **4** (4 mg) and the diastereomeric mixture of **5** in small quantities.

In order to obtain greater quantities of these products, a suspension of atorvastatin was kept at sunlight exposure (40 mg) for 14 days. The irradiation mixture was dried under vacuum and chromatographed on a RP-18 resin open column eluting with CH_3CN/H_2O (1:1) to afford four fractions (A, B, C, and D). Fraction D was a crude mixture of photoproducts **5** (8 mg). To achieve the separation of the diasteroisomeric compounds, the mixture was subjected to methylation with excess of diazomethane in ether solution. Methylation was quantitative and the resulting mixture was separated on a RP-18 HPLC column eluting with CH_3CN/H_2O (1:1).

4.5. Reaction with dimethyldioxirane

A solution of DMD (~0.5–0.12 M) in acetone was obtained according to literature procedure.[20] To a solution of atorvastatin calcium (5 mg) in acetone (10 ml), 5 ml of the DMD solution (~0.5–0.12 M) in acetone were added under stirring. The reaction mixture was dried under vacuum and analyzed by ^{1}H NMR spectroscopy. NMR analysis evidenced the formation of photoproducts **3** and **4**.

4.6. Spectral data

4.6.1. Atorvastatin (1). UV λ_{max} (CH_3OH) nm: 206 (log ε 7.0); ν_{max} ($CHCl_3$) 2964, 1723, 1658, 1607 cm^{-1}; ESI-MS *m/z* (%): 597 (12), 581 (25), 559 (85), 415 (100); δ_H (500 MHz, CD_3OD) 7.55–6.98 (14H), 4.18 (2H, m, H-7a and H-3), 3.93 (1H, m, H-7b), 3.67 (1H, m, H-5), 3.40 (1H, m, H-14), 2.36 (1H, dd, *J* 10.2, 4.8 Hz, H-2a), 2.26 (1H, dd, *J* 10.2, 2.4 Hz, H-2), 1.70 (2H, m, H-6), 1.60 (1H, m, H-4), 1.50 (6H, d, *J* 7.2 Hz, H-15, H-16), 1.41 (1H, m, H-4); δ_C (125 MHz, CD_3OD) 181.5, 170.0, 164.5, 140.5, 139.8, 137.5, 135.5, 131.5, 130.6, 130.1, 129.4, 128.3, 126.6, 124.0, 122.0, 119.8, 117.2, 117.0, 69.6, 45.5, 44.7, 42.5, 42.0, 28.1, 23.3.

4.6.2. Compound 2. Colorless oil; UV λ_{max} (CH_3OH) nm: 203 (log ε 11.9); ν_{max} ($CHCl_3$) 3040, 2920, 2854, 1712, 1602, 1097 cm^{-1}; ESI-MS *m/z* (%): 613 (15), 597 (20), 575 (100). Anal. calcd for $C_{33}H_{35}FN_2O_6$: C, 68.97, H, 6.14, F, 3.31, N, 4.87. Found: C, 76.09, H, 6.05, F, 3.28, N, 4.84; δ_H (500 MHz, CD_3OD) 7.40–7.00 (14H), 4.17 (1H, m, H-3), 3.76 (3H, m, H-5, H-7), 3.27 (1H, m, H-14), 2.40 (2H, m, H-2), 1.85 (2H, m, H-6), 1.66 (2H, m, H-4), 1.45 (6H, d, *J* 6.5 Hz, H-15, H-16); δ_C (125 MHz, CD_3OD) 181.4 (C-9), 176.8 (C-1), 166.2 (C-13), 164.0 (C-4‴), 153.4 (C-12), 141.3 (C-1‴), 139.3 (C-1′), 132.4 (C-1″), 130.7, 130.2, 129.5, 126.2, 122.1, 119.3 (C-11), 116.7, 116.6, 68.7 (C-5), 68.3 (C-3), 66.6 (C-10), 45.0 (C-4), 43.9 (C-2), 39.6 (C-7), 37.7 (C-6), 28.5 (C-14), 21.4, 21.2 (C-15, C-16).

4.6.3. Compound 3. Amorphous white powder; UV λ_{max} (CH_3OH) nm: 204 (log ε 12.5); ν_{max} ($CHCl_3$) 2957, 2924, 2855, 1714, 1712, 1600, 1364, 1088 cm^{-1}; ESI-MS *m/z* (%): 613 (10), 597 (25), 575 (100). Anal. calcd for $C_{33}H_{35}FN_2O_6$: C, 68.97, H, 6.14, F, 3.31, N, 4.87. Found:

C, 76.10, H, 6.00, F, 3.29, N, 4.89; δ_H (500 MHz, CD_3OD) 7.53 (2H, d, *J* 8.0 Hz), 7.36 (4H, m), 7.12 (8H, m), 4.02 (1H, m, H-3), 3.79 (1H, m, H-7a), 3.72 (1H, m, H-5), 3.56 (1H, m, H-7b), 2.67 (1H, m, H-14), 2.38 (2H, m, H-2), 1.48–1.68 (4H, m, H-4, H-6), 1.18 (3H, d, *J* 6.8 Hz, H-15), 0.92 (3H, d, *J* 6.8 Hz, H-16); δ_C (125 MHz, CD_3OD) 178.2 (C-12), 175.8 (C-1), 168.6 (C-13), 164.0 (C-4‴), 140.4 (C-9), 135.3, 134.0, 132.0, 131.9, 130.3, 129.6, 128.8, 128.2, 126.4, 123.4, 120.0, 117.4, 117.2, 69.9 (C-11),69.6 (C-5), 68.6 (C-3), 45.0 (C-4), 44.1 (C-2), 39.9 (C-7), 36.2 (C-6), 33.5 (C-14), 18.8 (C-15), 17.9 (C-16).

4.6.4. Compound 4. Amorphous white powder; UV λ_{max} (CH_3OH) nm: 202 (log ε 0.42). ν_{max} ($CHCl_3$) 2925, 2854, 1730, 1700, 1605, 1428, 1236, 1157, 1074 cm^{-1}; MALDI-MS *m/z* (%): 579 (30), 556 (100). Anal. calcd for $C_{33}H_{35}FN_2O_6$: C, 71.21, H, 5.98, F, 3.41, N, 5.03. Found: C, 71.18, H, 6.00, F, 3.39, N, 5.00; δ_H (500 MHz, CD_3OD) 7.50–7.24 (6H, m), 7.16 (8H, m), 4.52 (1H, m, H-5), 4.16 (1H, m, H-3), 3.79 (1H, m, H-7a), 3.56 (1H, m, H-7b), 2.78 (1H, m, H-14), 2.59 (1H, dd, *J* 3.9, 17.6 Hz, H-2a), 2.42 (1H, dd, *J* 17.6 Hz, H-2a), 1.85 (2H, m, H-6), 1.77 (2H, m, H-4), 1.16 (3H, d, *J* 7.0 Hz, H-15), 0.95 (3H, d, *J* 7.0 Hz, H-16); δ_C (125 MHz, CD_3OD) 178.2 (C-12, C-13), 172.5 (C-1), 162.5 (C-4‴), 143.7 (C-9), 138.0, 135.1, 134.5, 134.1, 132.2, 130.3, 130.1, 129.6, 128.8, 126.6, 123.8, 122.2 (C-10), 117.4, 117.2, 76.1 (C-5), 63.6 (C-3), 39.4 (C-2, C-7), 35.6 (C-6), 33.1 (C-14), 18.8 (C-15), 17.6 (C-16).

4.6.5. Compound 5. Colorless oil (one diastereomer); UV λ_{max} (CH_3OH) nm: 204, 238, 332; ν_{max} ($CHCl_3$) 3481, 3334, 3010, 2932, 1717, 1697, 1601, 1450, 1314, 1163 cm^{-1}; ESI-MS *m/z* (%): 609 (44), 587 (100). Anal. calcd for $C_{33}H_{35}FN_2O_6$: C, 69.61, H, 6.01, F, 3.24, N, 4.78. Found: C, 69.58, H, 5.98, F, 3.25, N, 4.75; δ_H (500 MHz, CD_3OD) 8.76 (1H, d, *J* 9.0 Hz, H-6″), 8.60 (1H, d, *J* 9.0 Hz, H-3″), 8.51 (1H, dd, *J* 5.5, 9.5 Hz, H-6‴), 8.45 (1H, dd, *J* 2.0, 11.5 Hz, H-3‴), 7.69 (1H, t, *J* 7.0 Hz, H-5″), 7.60 (1H, t, *J* 7.0 Hz, H-4″), 7.49 (2H, d, *J* 8.0 Hz, H-2′, H-6′), 7.45 (1H, ddd, *J* 2.0, 9.5, 11.0 Hz, H-5‴), 7.29 (2H, t, *J* 8.0 Hz, H-3′, H-5′), 7.09 (1H, t, *J* 8.0 Hz, H-4′), 4.67 (1H, m, H-7a), 4.45 (1H, m, H-7b), 4.20 (1H, m, H-3), 4.02 (1H, m, H-5), 3.42 (1H, m, H-14), 2.44 (2H, m, H-2), 2.15 (1H, m, H-6a), 2.05 (1H, m, H-6b), 1.74 (2H, m, H-4), 1.35 (3H, d, *J* 6.5 Hz, H-15), 0.66 (3H, d, *J* 6.5 Hz, H-16); δ_C (125 MHz, CD_3OD) 179.2 (C-12), 173.0 (C-1), 165.5 (C-13), 162.0 (C-4‴), 137.6 (C-1′), 137.0 (C-9), 134.7 (C-2‴, C-1″), 129.1 (C-3′, C-5′), 129.0 (C-3″), 128.0 (C-2′, C-6′), 128.6 (C-5″), 127.1 (C-6″), 125.5 (C-6‴), 125.2 (C-4″), 124.9 (C-4′), 123.4 (C-3″), 120.7 (C-2′, C-6′), 120.0 (C-10), 118.2 (C-1‴), 116.1 (C-5‴), 109.5 (C-3‴), 69.3 (C-5), 69.0 (C-3), 66.4 (C-11), 42.0 (C-4), 41.4 (C-2), 40.0 (C-7), 37.2 (C-6), 35.6 (C-14), 18.2 (C-16), 17.7 (C-15). The other diastereomer. Colorless oil; UV λ_{max} (CH_3OH) nm: 204, 238, 332; ν_{max} ($CHCl_3$) 3481, 3334, 3010, 2932, 1717, 1697, 1601, 1450, 1314, 1163 cm^{-1}; ESI-MS *m/z* (%): 609 (44), 587 (100). Anal. calcd for $C_{33}H_{35}FN_2O_6$: C, 69.61, H, 6.01, F, 3.24, N, 4.78. Found: C, 69.58, H, 5.98, F, 3.25, N, 4.75; δ_H (500 MHz, CD_3OD) 8.68 (1H, d, *J* 8.0 Hz, H-6″), 8.58 (1H, d, *J* 8.0 Hz, H-3″), 8.49 (1H, dd, *J* 5.5, 9.5 Hz, H-6‴), 8.46 (1H, dd, *J* 2.0, 11.0 Hz, H-3‴), 7.67 (1H, t, *J* 7.5 Hz, H-5″), 7.59 (1H, t, *J* 7.5 Hz, H-4″), 7.45 (2H, d, *J* 8.5 Hz, H-2′, H-6′), 7.45 (1H, obscured, H-5‴), 7.25 (2H, t, *J* 8.5 Hz, H-3′, H-5′), 7.07 (1H, t, *J* 8.0 Hz, H-4′), 4.65 (1H, m, H-7a), 4.44 (1H, m, H-7b), 4.32 (1H, m, H-3), 4.18 (1H, m, H-5), 3.42 (1H, m, H-14), 2.48 (2H, m, H-2), 2.08 (2H, m, H-6), 1.68 (2H, m, H-4), 1.38 (3H, d, *J* 7.0 Hz, H-15), 0.63 (3H, d, *J* 7.0 Hz, H-16); δ_C (125 MHz, CD_3OD) 177.8 (C-12), 173.6 (C-1), 166.2 (C-13), 162.3 (C-4‴), 137.6 (C-1′), 137.0 (C-9), 134.5 (C-2‴, C-1″), 129.0 (C-3′, C-5′), 128.8 (C-3″), 128.1 (C-2′, C-6′, C-5″), 126.5(C-6″, C-6‴), 125.4 (C-4′, C-4″), 124.8 (C-3″), 123.4 (C-2′, C-6′), 119.3 (C-10), 118.2 (C-1‴), 116.1 (C-5‴), 109.5 (C-3‴), 70.5 (C-5), 69.1 (C-3), 52.1 (C-11), 42.4 (C-4), 41.5 (C-2), 40.9 (C-7), 37.6 (C-6), 35.0 (C-14), 18.4 (C-16), 17.5 (C-15).

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References and notes

1. Class, S. *Chem. Eng. News* **2005**, *83, December 5*, 15–32.
2. Rovner, S. L. *Chem. Eng. News* **2005**, *83, February 21*, 38–45.
3. Ternes, T. A. *Water Res.* **1998**, *32*, 3245–3260.
4. Kummerer, K. *Pharmaceuticals in the Environment*; Springer: Heidelberg, 2004; pp 3–11.
5. DellaGreca, M.; Fiorentino, A.; Iesce, M. R.; Isidori, M.; Nardelli, A.; Previtera, L.; Temussi, F. *Environ. Toxicol. Chem.* **2003**, *22*, 534–539.
6. DellaGreca, M.; Brigante, M.; Isidori, M.; Nardelli, A.; Previtera, L.; Rubino, M.; Temussi, F. *Environ. Chem. Lett.* **2004**, *1*, 237–241.
7. DellaGreca, M.; Fiorentino, A.; Isidori, M.; Lavorgna, M.; Previtera, L.; Rubino, M.; Temussi, F. *Chemosphere* **2004**, *54*, 629–637.
8. Cermola, M.; DellaGreca, M.; Iesce, M. R.; Previtera, L.; Rubino, M.; Temussi, F.; Brigante, M. *Environ. Chem. Lett.* **2005**, *3*, 43–47.
9. In previous papers, Lam, M. W.; Mabury, S. A. *Aquat. Sci.* **2005**, *67*, 177–188 , on the photolysis of atorvastatin, two photoproducts were detected by LC–MS and suggested to derive one from *N*-chain elimination and the other from an oxygenation of *N*-aryl moiety. The latter has also been detected as metabolite in biological fluids (Black, A. E.; Hayes, R. N.; Roth, B. D.; Woo, P.; Woolf, T. F. *Drug Metab. Dispos.* **1999**, *27*, 916–923). Under our experimental conditions, we were not able to detect these photoproducts.
10. We were not be able to distinguish diastereomers for the other products **2**–**4**, despite the presence of a new stereogenic center in these molecules, likely due to a very close similarity of spectral data.
11. The slight decrease of degradation rate in the presence of the radical inhibitors indicates that radical species may also be involved, although to a lesser extent.
12. Iesce, M. R.; Cermola, F.; Temussi, F. *Curr. Org. Chem.* **2005**, *9*, 109–139.

13. Wasserman, H. H.; Frechette, R.; Rotello, V. M. *Tetrahedron Lett.* **1991**, *32*, 7571–7574.
14. In photooxygenation reactions of heterocycles, products have been sometime isolated, which correspond stoichiometrically to two molecules of substrates and one molecule of oxygen, and this occurs particularly in the presence of water or starting from aryl-substituted furans: Gorman, A. A.; Rodgers, M. A. J. *Chem. Soc. Rev.* **1981**, *10*, 205–231.
15. For rearrangements of labile epoxides to carbonyl compounds, see for example: (a) Adam, W.; Hadjiarapoglou, L.; Wang, X. *Tetrahedron Lett.* **1991**, *32*, 1295–1298; (b) Baylon, C.; Hanna, I. *Tetrahedron Lett.* **1995**, *36*, 6475–6478; (c) Katritzky, A. R.; Xie, L.; Serdyuk, L. *J. Org. Chem.* **1996**, *61*, 7564–7570; Cermola, F.; Iesce, M. R. *J. Org. Chem.* **2002**, *67*, 4937–4944.
16. Adam, W.; Ahrweiler, M.; Peters, K.; Schmiedeskamp, B. *J. Org. Chem.* **1994**, *59*, 2733–2739; Xiaojun, Z.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 8867–8868.
17. Gilbert, A. *Organic Photochemistry and Photobiology*, 2nd ed.; Horspool, W., Lenci, F., Eds.; CRC: Boca Raton, FL, 2004; Chapter 33, pp 1–11.
18. The capability of producing singlet oxygen has been noted in diverse pyrrole-containing molecules: Lin, Y. Y.; Li, G. B.; Zhang, Y. H.; Leung, H. K. *Photochem. Photobiol.* **1997**, *65*, 82–84; Nigorikawa, K. JP 2,002,274,813, 2002; *Chem. Abstr. 137*, 270353.
19. Quintero, B.; Miranda, M. A. *Ars. Pharmaceutica* **2000**, *41*, 27–46.
20. Singh, M.; Murray, R. W. *J. Org. Chem.* **1992**, *57*, 4263–4270.

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Synthesis of the novel conjugated ω,ω′-diaryl/heteroaryl hexatriene system with the central double bond in a heteroaromatic ring: photochemical transformations of 2,3-divinylfuran derivatives

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Abstract—New β,β′-aryl/heteroaryl 2,3-divinylfuran derivatives (**9a–d**) in which a hexatriene system is a part of heteroaromatic ring have been synthesized and their photochemical properties were investigated. The primary process observed was the isomerization to *trans*,*trans*-isomers **9a–d** followed by photochemical rearrangement of the furan ring giving the phototransposition products (**I–IV**). Stilbenes (**20**, **21**) and phenanthrenes (**22**, **25**, and **26**), formed as secondary products from the competitive intermolecular cycloadditions, were also observed.

1. Introduction

In our first paper about a photochemical approach to heteropolycyclic compounds from hexatriene systems in which the central double bond is placed in a benzene ring (**1**), we described the furan derivative of *o*-divinylbenzene (**2a**) and its transformation into the fused bicyclo[3.2.1]octadiene structure **3** (Fig. 1).[1]

The investigation was extended to many modified furan,[2–9] pyrrole,[10–16] and sydnone[17,18] analogues (**1a**,**b**; **2b–e**), where their photochemical behavior was examined. We have observed that the photochemistry of furan derivatives of *o*-divinylbenzene[1,2,4,6,7,9] results in a [2+2] cycloaddition and formation of intramolecular cycloadducts **3** or **4** (Fig. 1), obtained via 1,6- or 1,4-biradical ring closure, respectively (Scheme 1). In the case of β-(3-substituted-2-furyl)-*o*-divinylbenzenes (**2b–d**),[7] the 1,4-biradical ring closure leading to benzobicyclo[2.1.1]hexene derivatives (**4**) was preferred due to steric reasons. The same benzobicyclo-[2.1.1]hexene structure was obtained on irradiation of β-aryl *o*-divinylbenzenes (**1c**).[19–31]

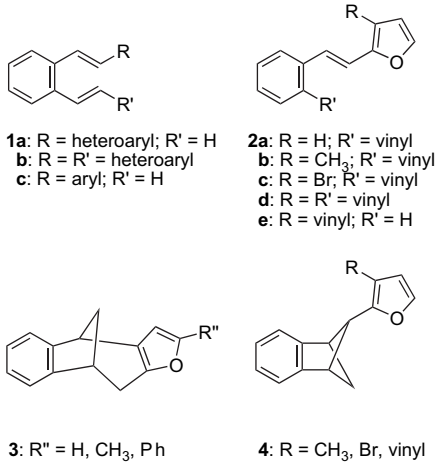

Figure 1.

On irradiation of compound **2d** (unsaturated system with the vinyl group on benzene and on the furan ring) the benzobicyclo[2.1.1]hexene structure **5** was isolated.[7] The furobicyclo[2.1.1]hexene derivative **6** (Fig. 2), formed as a result of the vinyl-furan group participating in a cycloaddition, was not isolated. If the cycloadduct **6** was formed, further $[\pi^2+\sigma^2]$ cycloaddition[19] and formation of tricyclic structure **7** via the excited state of the styryl group would be expected. The tricyclic structure **8** was not obtained due to the nonabsorption of the vinyl-furan derivative **5** under the experimental

Keywords: Oxygen heterocycles; Pericyclic reaction; Photochemistry; Rearrangement; Synthesis.
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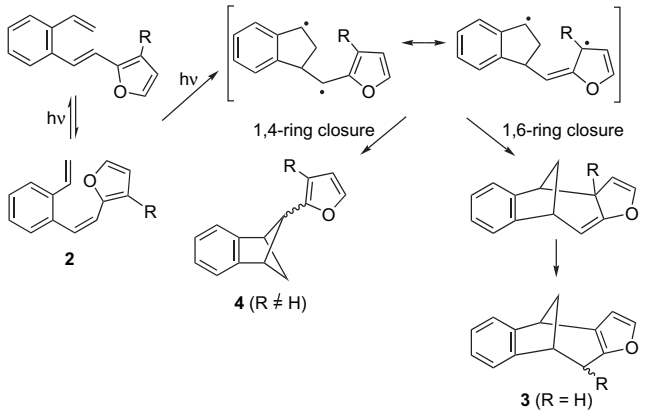


Scheme 1.

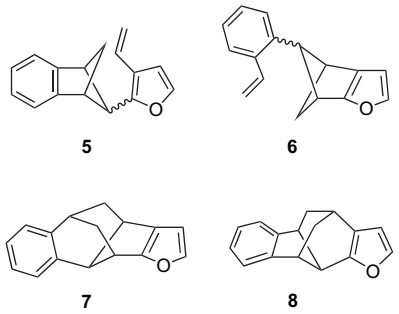


Figure 2.

conditions. On irradiation of **2e** (hexatriene system in which the central double bond is a part of only a furan ring) mostly high-molecular-weight material was obtained.[7]

In order to get a deeper understanding of the photochemical behavior of the hexatriene system with the central double bond in a heteroaromatic ring, we describe herein for the first time the synthesis and photochemistry of β,β′-aryl/heteroaryl 2,3-divinylfurans **9** (Fig. 3), compounds with carefully chosen aryl/heteroaryl substituents were used. There are examples of β,β′-disubstituted 2,3-divinylfurans[32–42] in the literature but to the best of our knowledge, there are no examples with aryl/heteroaryl substituents.

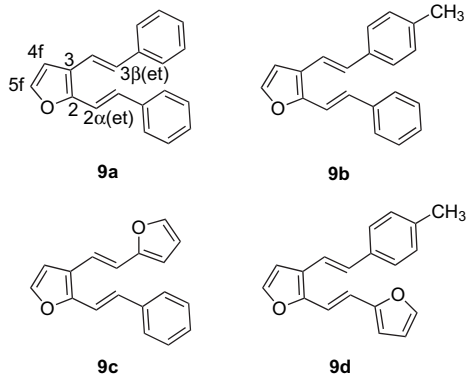


Figure 3.

2. Results and discussion

Introduction of the second aromatic moiety to the β-position of the vinyl group of **2e** to give compounds **9a–d** could result in intramolecular complexation[9] and formation of the bicyclic structures (Scheme 2). In such a way the access to a variety of differently functionalized heteropolycyclic compounds could be enabled.

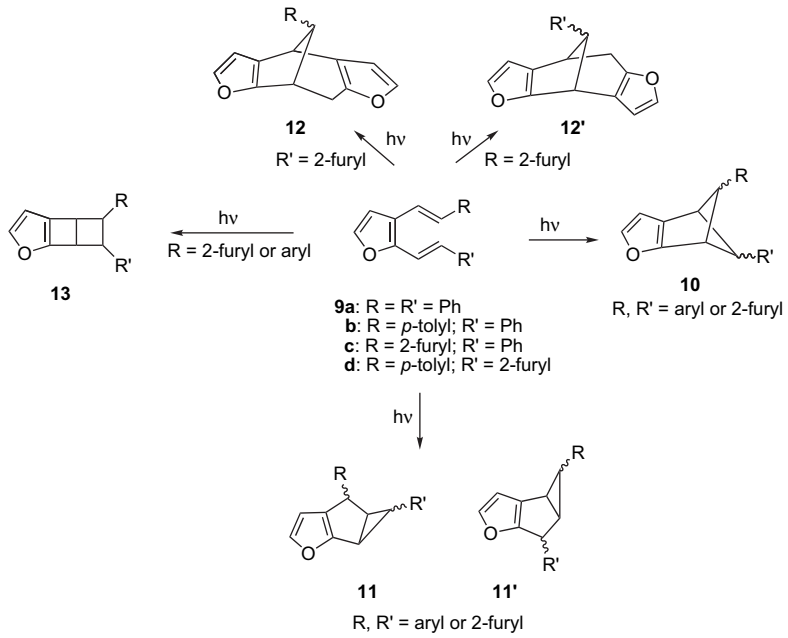
If we consider the 2,3-divinylfurans **9** as heteroaromatic analogues of disubstituted *o*-divinylbenzenes (**1b**),[9] we might expect the formation of several furobicyclo structures (**10–13**) on irradiation of **9**. The furobicyclo[2.1.1]hexene (**10**) and furobicyclo[3.1.0]hexene (**11**, **11′**) derivatives might be formed by initial [2+2] cycloaddition via 1,4-biradicals **14** and/or **14′** (Fig. 4), followed by ring closure.

In the formation of products **10**, the aromatic character of the furan ring is pronounced while in the case of **11/11′**, through the participation of two of the furan π electrons and vinylcyclopropane–cyclopentene rearrangement,[43,44] the diene character of the furan ring is prevailing. If one of the β-substituents is a furan ring (**9c**,**d**), then 1,6-biradical ring closure[1,2,4,6,7,9] may occur leading to furobicyclo-[3.2.1]octadiene structures (**12**, **12′**) by the mechanism described therein. If the head-to-head [2+2] cycloaddition took place, the furobicyclo[2.2.0]hexene **13** would be formed (Scheme 2).

Novel β,β′-aryl/heteroaryl-substituted 2,3-divinylfurans **9a–d**, were prepared by a series of reactions according to Scheme 3. The corresponding *cis*- and *trans*-aryl/heteroaryl-2-vinylfurans **16** are prepared by a Wittig reaction from aldehydes **15** in moderate-to-good yields (47–82%). To reduce the number of isomers of the final products **9a–d**, the isomers **16a–c** were separated by column chromatography on silica gel. The *trans*-**16b** and *trans*-**16c** were transformed to *trans*-**17a** and *trans*-**17b**, respectively, by formylation with *n*-butyl lithium and DMF (~50% yield). The *trans*-**16a** was converted to **9e**,**f** (Scheme 3) in three steps giving a mixture of *cis*,*trans*- and *trans*,*trans*-isomers. After column chromatography, the *trans*,*trans*-**9e** and *trans*,*trans*-**9f** were debrominated to *trans*,*trans*-**9b** and *trans*,*trans*-**9c**, respectively. By using a Wittig reaction with formyl derivatives *trans*-**17a**,**b**, the corresponding triphenylphosphonium salts, **9a–d** were prepared as mixtures of *cis*,*trans*- and *trans*,*trans*- isomers (65–95%). All isolated compounds were identified and characterized spectroscopically. The ratios of the isomers were determined from the NMR spectra and by GC–MS measurements.

Diaryl-substituted 2,3-divinylfurans **9a**,**b** (Fig. 3) have very similar patterns in their respective ^{1}H NMR. Generally, all *cis*,*trans*- and *trans*,*trans*-**9** derivatives show ethylenic doublets at 6.6–7.1 ppm. Within this region for all *trans*,*trans*-**9** derivatives considerable shifts of one of the ethylenic doublets to higher fields are observed compared to the other three ethylenic protons. The 4f proton of the furan ring A of all *trans*,*trans*-**9** is found at 6.6–6.7 ppm while the 4f proton of the furan ring B (**9c**,**d**) is shifted by 0.2 ppm to the higher field and is well recognizable.

Compounds **9a–d** are the starting materials for irradiation experiments and show strong absorption maxima at



Scheme 2. Hypothetic photochemical products of intramolecular [2+2] cycloaddition of ethylenic bonds.

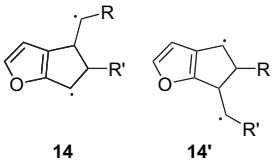
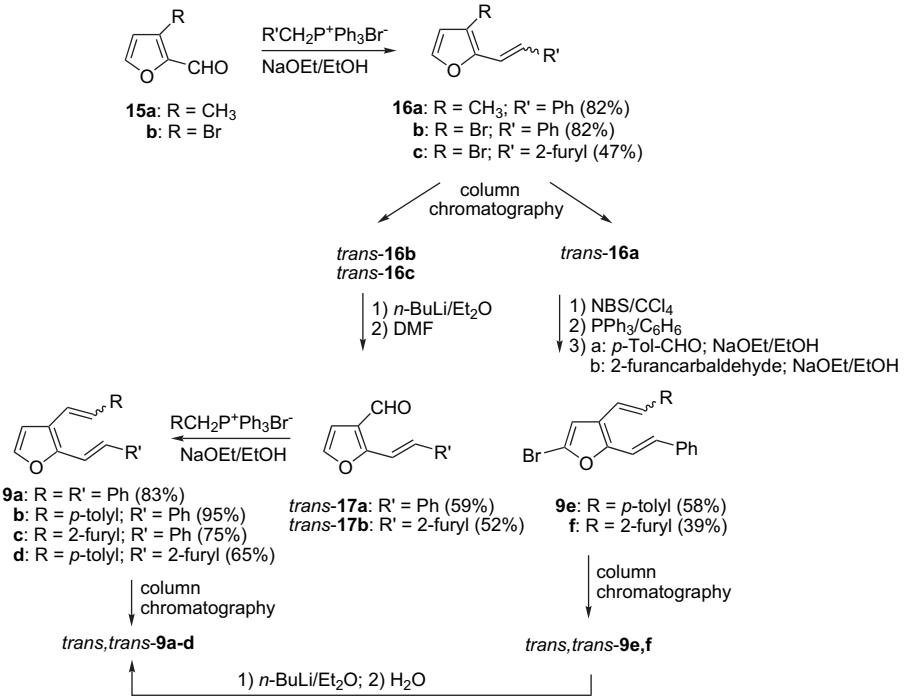


Figure 4. Possible 1,4-biradicals.

350–360 nm with similar molar absorption coefficients. The furan derivatives *trans*,*trans*-**9c** and *trans*,*trans*-**9d** demonstrate bathochromic shifts of 5 and 6 nm, respectively, in comparison to the corresponding aryl analogues (*trans*, *trans*-**9a**, *trans*,*trans*-**9b**) due to the conjugation from the oxygen lone pair through the furan ring. The ethanolic solution of *cis*,*trans*-**9a** (UV concentration) was irradiated and the process was followed by UV measurements (Fig. 5). After 15 s, the shift of the absorption maximum to the longer wavelength (359 nm) with concomitant formation of another maximum at 275 nm was observed. The new maxima (275 and 359 nm) correspond to the *trans*,*trans*-**9** isomer. These maxima decrease on further irradiation as it is comparable with the results of the pure *trans*,*trans*-**9a** irradiations (Fig. 6).



Scheme 3.

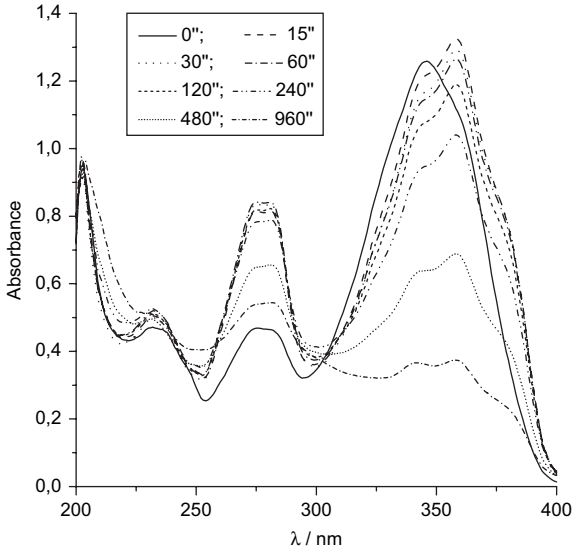


Figure 5.

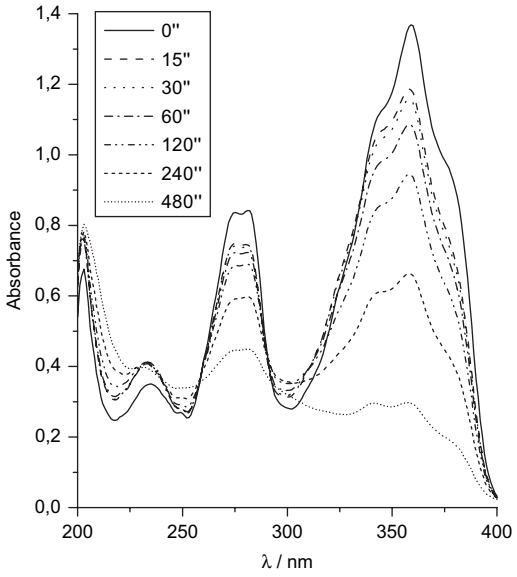


Figure 6.

Compounds **9a–d** with concentrations of 10^{-3} M were irradiated in benzene solutions under anaerobic conditions in Rayonet reactor at 350 nm. The reaction course of all these substituted divinylfurans **9** was followed by GC–MS measurements and the results were similar. In the first 15 min of irradiation, only the isomerization of *cis*,*trans*- to *trans*,*trans*-isomers **9** was detected (Fig. 7; **9b** presented).

On further irradiation for 30 min (Fig. 7), the new signals appeared having similar retention times with molecular ions identical to the starting compounds. The ratios of the sums of these new signals (see Section 4) in the experiments with **9a–d** to the signals of the corresponding starting compounds *trans*,*trans*-**9** were ca. 1:4. After 1 h of irradiation, the ratio of the signals remained the same (Fig. 8) and did not change on further irradiation. After 2 h of irradiation, besides already described signals, the signals, which correspond to stilbenes and styrylfurans (**20**, **21**; Scheme 4)

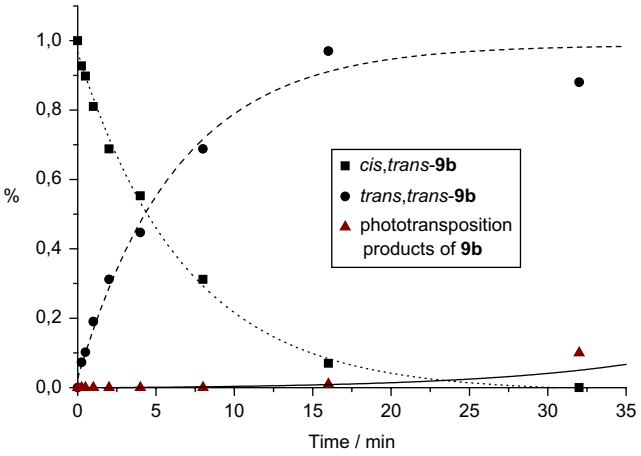


Figure 7.

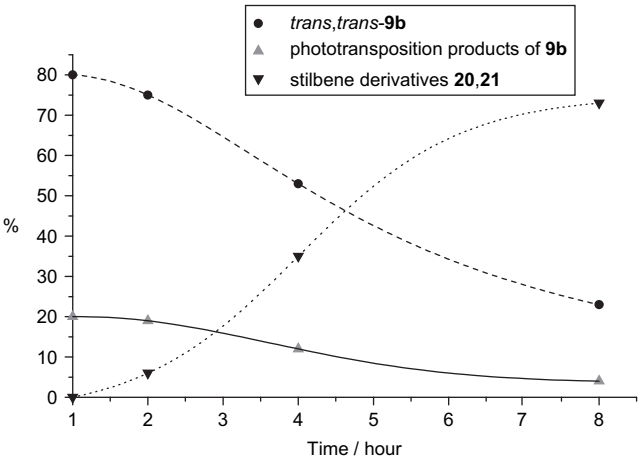
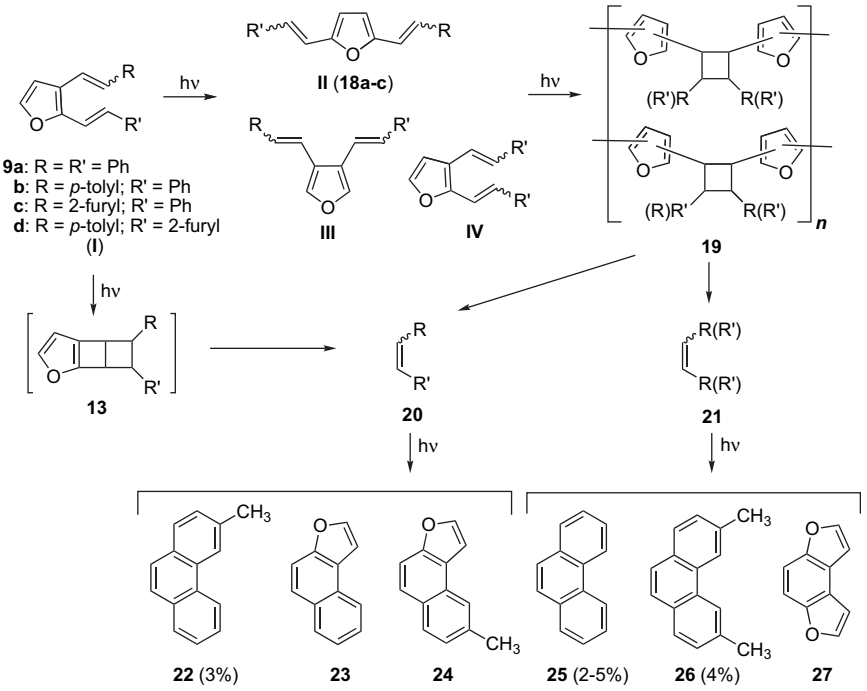


Figure 8.

appeared and become more intensive on further irradiation. The ^{1}H NMR spectra of all photomixtures showed neither signals for the aliphatic protons of the furobicyclic structures (**10–13**) nor cyclobutane dimeric structures as in the case of irradiation experiments of β,β′-disubstituted *o*-divinylbenzenes.[9] In a lower magnetic field, the signals of new aromatic protons at 7.5–8.0 ppm have been observed along with new ethylenic protons at 6.5–7.0 ppm, having characteristic coupling constants for *cis*- and *trans*-isomers. In the case of the methyl derivatives (**9b** and **9d**) several new methyl signals appear.

When the irradiation of **9a–d** was performed at higher concentrations (~10^{-1} M), the appearance of stilbenes and styrylfurans (**20**, **21**; Scheme 4) was observed by GC–MS already after 30 min. On further irradiation, the signals of **20** and **21** increase faster than at lower concentrations and on prolonged irradiation time, regardless of concentration, they are the only detectable compounds besides phenanthrenes (**22**, **25**, and **26**) and a high amount of tarry material. The naphthofurans **23** and **24** and furobenzofuran **27** are not detected in the reaction mixture, although their corresponding precursors **20** and **21** are found, and this is not a surprising fact. Electrocyclization reactions of styrylfurans[4] are not efficient reactions and compete with the formation of high-molecular-weight products.



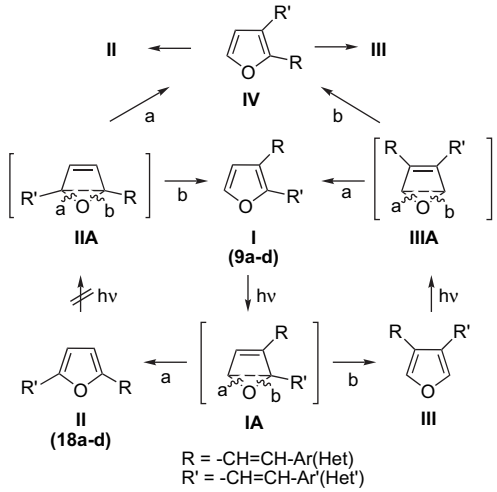
Scheme 4.

From the NMR spectra of the photomixtures, combined with the GC data, one can conclude that the signals observed in GC–MS correspond to the phototransposition products, the constitutional isomers **I–IV** of distyrylfurans **9a–d** (Scheme 4).

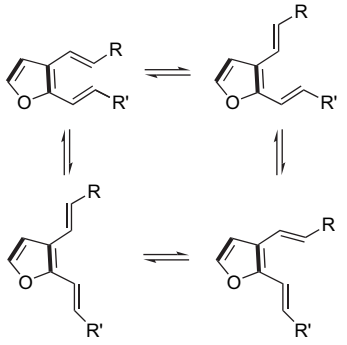
To confirm this assumption one of these constitutional isomers, the substituted 2,5-divinylfuran **II** (**18a–c**; Scheme 4) was prepared by independent synthesis (Section 4). The retention times of synthesized **18a–c**, as mixtures of configurational isomers (*cis*,*cis*-, *cis*,*trans*-, and *trans*,*trans*-**18**), were compared to the signals of the photomixtures of irradiation experiments of **9a–c**. Only the signal for *trans*,*trans*-2,5-divinylfuran derivative **II** (**18a–c**) coincides to one of the signals of the irradiation mixture of **I** (**9a–c**). After addition of *trans*,*trans*-2,5-divinylfuran **II** to the photomixture the coincident signal increases. It is not unexpected that *cis*,*cis*- or *cis*,*trans*-isomers **II** have not been found in the photomixture because they undergo fast cis–trans isomerization and that is confirmed by independent irradiation of **II**. The remaining GC-signals in the photomixture of **9**, having similar retention times and the same molecular ions, could correspond to the structures **III** and/or **IV**.[45]

The formation of phototransposition furan derivatives (**I–IV**) can be explained by formal [2+2] cycloaddition of the π systems in the furan ring and rearrangements of the obtained epoxy-furan derivatives (**IA–IIIA**; Scheme 5).[46–48] The anticipated dienic character of the furan ring might be a reason for their formation and mutual rearrangement. This process of cycloaddition is prevailing due to the more convenient and rigid conformation of furan double bonds compared to ethylenic π system (Scheme 6).

The existence of compounds **20** on irradiation of **9** can be explained by thermal cleavage of the intra- (**13**) or



Scheme 5. Schematic representation of the possible phototransposition products.



Scheme 6. Possible conformations of *trans*,*trans*-**9** derivatives.

intermolecular cycloadducts (**19**), formed by intra- or intermolecular [2+2] cycloaddition of the ethylenic bonds (Scheme 4). Since in the irradiation mixture of unsymmetrically substituted 2,3-divinylfuran derivatives **9b–d** besides **20** (Scheme 4) the symmetrically substituted stilbene derivatives **21** were found; it is concluded that the stilbene derivatives were derived from the intermolecular cycloadduct **19**, although the formation of **20** and the intramolecular process via unstable **13** cannot be excluded. Moreover, on irradiation of 2,5-divinylfuran derivatives **18a–c** no phototransposition products were detected showing that **II** (**18a–c**) does not undergo an intramolecular furan rearrangement process to the constitutional isomers (Scheme 5). Instead, they polymerize by intermolecular cycloaddition reactions of ethylenic bonds (**19**). Even though no evidence of a cyclobutane structure was found in the ^{1}H NMR spectra, we are sure of its formation due to the detection of all combination of ethene derivatives **20** and **21** in GC–MS measurements. Only some of their corresponding phenanthrenes (**22**, **25**, and **26**) are the isolated products from the photoreaction mixture after prolonged irradiation and complete conversion of the starting compounds **9**. The phenanthrene derivatives are formed by electrocyclization reaction of stilbenes via oxidation of dihydrophenanthrenes, as a result of imperfect anaerobic reaction conditions.[49]

3. Conclusion

The conjugated hexatriene systems with a central double bond placed in a furan ring (**9a–d**) have been studied. They behave completely different from the hexatriene system with the central double bond incorporated in benzene ring (**1**) under the same reaction conditions. Compared to diheteroaryl systems (**1b**) in which the intramolecular cycloaddition and cis–trans isomerization were the competitive processes it is obvious that in this system the primary process is cis–trans isomerization to *trans*,*trans*-isomers. Because of dienic character of the furan ring and prevailing conformation of the furan double bonds, the excitation of the hexatriene causes the [2+2] cycloaddition within the furan ring followed by rearrangement, rather than the [2+2] cycloaddition of ethylenic bonds. The photochemical process within the furan ring is competitive with the intermolecular cycloaddition processes and leads to the formation of phototransposition (**I–IV**) and high-molecular-weight products, respectively. The phototransposition process is confirmed by independent synthesis of one of the representatives (**18a–c**).

4. Experimental

4.1. General

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 Spectrometer at 300 or 600 MHz and 75 or 150 MHz, respectively. All NMR spectra were measured in $CDCl_3$ using tetramethylsilane as reference. The assignment of the signals is based on 2D CH-correlation and 2D HH-COSY, LRCOSY, and NOESY experiments. UV spectra were measured on a Varian Cary 50 UV–vis Spectrophotometer. IR spectra were recorded on Perkin–Elmer Spectrum One. Mass spectra were obtained on a GC–MS (Varian CP-3800 Gas Chromatograph–Varian Saturn 2200) equipped with FactorFour Capillary Column VF-5ms. Irradiations were performed in a Pyrex vessel in benzene solutions in a Rayonet reactor equipped with RPR 3500 Å lamps. All irradiation experiments were carried out in deoxygenated solutions by bubbling a stream of argon prior to irradiation. Melting points were obtained using an Original Kofler Mikroheitztisch apparatus (Reichert, Wien) and are uncorrected. Elemental analyses were carried out on Perkin–Elmer, Series II, CHNS Analyzer 2400. Silica gel (Merck 0.063–0.2 mm) was used for chromatographic purifications. Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates. Solvents were purified by distillation. Boiling range of petroleum ether, used for chromatographic separation, was 40–70 °C.

Furan-2-carbaldehyde was obtained from a commercial source. Benzyltriphenylphosphonium bromide, *p*-methylbenzyltriphenylphosphonium bromide, and 2-furylmethyltriphenylphosphonium bromide were synthesized from the corresponding bromides[50] and triphenylphosphine in benzene solution.

3-Methyl-2-furancarbaldehyde (**15a**)[51] was prepared by oxidation of 3-methyl-2-furfurylalcohol[52] that was obtained from 3-methyl-2-furoate[53] with $LiAlH_4$. 3-Bromo-2-furancarbaldehyde (**15b**) was prepared from 2-furancarboxylic acid according to a described procedure.[54] Compound **17a** was prepared as described in the literature.[7]

4.2. Preparation of 16a and 16c

Starting compounds **16a** and **16c** were prepared from benzyltriphenylphosphonium bromide and the corresponding aldehydes, 3-methyl-2-furancarbaldehyde (**15a**) and 3-bromo-2-furancarbaldehyde (**15b**), respectively. Starting compound 3-bromo-2-(2-phenylethenyl)furan (**16b**) was obtained according to the described procedure.[7]

A solution of sodium ethoxide (0.12 g, 5.2 mmol in 10 mL ethanol) was added dropwise to a stirred solution of benzyltriphenylphosphonium bromide (1.69 g, 4.0 mmol) and 3-methyl-2-furancarbaldehyde (**15a**) (4.0 mmol) in absolute ethanol (100 mL). Stirring was continued under a stream of nitrogen for 1 day at room temperature. After removal of the solvent, the residue was worked up with water and benzene. The benzene extracts were dried with $MgSO_4$ and concentrated. The crude reaction mixture was purified and the isomers of **16a** were separated by repeated column chromatography on silica gel using petroleum ether/diethyl ether (0–3%) mixture as eluent. The first fractions yielded *trans*-isomer and the last fractions yielded *cis*-isomer. Starting compound **16c** was prepared also by Wittig reaction from 2-furylmethyltriphenylphosphonium bromide and 3-bromo-2-furancarbaldehyde (**15b**). Characterization data of the new compounds **16a** and **16c** are given below.

4.2.1. 3-Methyl-2-(2-phenylethenyl)furan (16a). Yield 82.0%; according to ^{1}H NMR spectroscopy, a mixture of 45% *cis* and 55% *trans* isomers was obtained.

trans-**16a**: R_f 0.61 (petroleum ether); colorless crystals; mp 50–51 °C; UV (EtOH) λ_{max} (log ε) 340 (4.37, sh), 322

(4.50), 310 (4.43, sh) nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.47 (d, J=7.8 Hz, 2H), 7.33 (m, 3H), 7.22 (t, J=7.2 Hz, 1H), 6.97 (d, J=16.5 Hz, H-et, 1H), 6.89 (d, J=16.5 Hz, H-et, 1H), 6.27 (s, H-4f, 1H), 2.12 (s, CH_3, 3H); ^{13}C NMR ($CDCl_3$) δ_C 148.70 (s), 141.20 (d), 137.33 (s), 128.52 (2d), 127.11 (d), 126.03 (2d), 125.38 (d), 118.60 (s), 114.57 (d), 114.08 (d), 10.00 (q); MS (EI) *m*/*z* (%) 184 (M^+, 100), 141 (10), 115 (5); HRMS (EI) Calcd for $C_{13}H_{12}O$: 184.088266. Found: 184.093546.

cis-**16a**: R_f 0.55 (petroleum ether); colorless oil; UV (EtOH) λ_{max} (log ε) 325 (3.83, sh), 317 (4.16), 311 (4.18) nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.43 (d, J=7.5 Hz, 1H), 7.23–7.35 (m, 4H-ar), 7.21 (d, J=1.8 Hz, 1H, H-5f), 6.44 (d, J=12.3 Hz, 1H, H-et), 6.28 (d, J=12.3 Hz, 1H, H-et), 6.20 (d, J=1.8 Hz, 1H, H-4f), 1.98 ppm (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ_C 147.76 (s), 140.88 (d), 137.31 (s), 128.81 (2d), 127.68 (2d), 126.91 (2d), 120.08 (s), 115.36 (d), 113.45 (d), 10.37 (q); MS (EI) *m*/*z* (%) 184 (M^+, 100), 141 (25), 115 (10); Anal. Calcd for $C_{13}H_{12}O$: C, 84.75; H, 6.57. Found: C, 84.68; H, 6.67.

4.2.2. 3-Bromo-2-(2-furylethenyl)furan (16c). Yield 47.0%; according to ^{1}H NMR spectroscopy, a mixture of 34% *cis* and 66% *trans* isomers was obtained.

trans-**16c**: R_f 0.61 (petroleum ether); colorless crystals; mp 45–46 °C; UV (EtOH) λ_{max} (log ε) 337 (4.27), 319 (4.46), 312 (4.40, sh) nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.46 (m, 1H, H-1), 7.35 (d, J=1.8 Hz, 1H, H-5), 6.89 (AB_q, J=16.1 Hz, 2H, H-et), 6.49 (d, J=1.8 Hz, 1H, H-4), 6.45 (m, 1H, H-2), 6.40 (d, J=3.2 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ_C 152.71 (s), 149.81 (s), 142.56 (d), 142.02 (d), 116.36 (d), 115.20 (d), 111.98 (d), 111.87 (d), 109.71 (d), 99.14 (s); MS (EI) *m*/*z* (%) 238/240 (M^+, 100), 159 (10), 131 (70).

cis-**16c**: R_f 0.52 (petroleum ether); colorless oil; UV (EtOH) λ_{max} (log ε) 321 (3.80, sh), 315 (4.09), 311 (4.14) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.46 (d, J=1.9 Hz, 1H, H-5), 7.43 (m, 1H, H-1), 7.10 (d, J=3.4 Hz, 1H, H-3), 6.52 (d, J=1.9 Hz, 1H, H-4), 6.46 (m, 1H, H-2), 6.37 (d, J=13.4 Hz, 1H, H-et), 6.19 (d, J=13.4 Hz, 1H, H-et); ^{13}C NMR ($CDCl_3$) δ_C 151.58 (s), 148.77 (s), 141.94 (d), 141.51 (d), 115.75 (d), 114.44 (d), 111.43 (d), 110.74 (d), 109.52 (d), 100.92 (s); MS (EI) *m*/*z* (%) 238/240 (M^+, 75), 159 (10), 131 (100); Anal. Calcd for $C_{10}H_7BrO_2$: C, 82.42; H, 5.38. Found: C, 82.20; H, 5.45.

4.3. Preparation of 17b

To a stirred solution of *trans*-**16c** (3.0 mmol) in anhydrous diethyl ether (40 mL) cooled to −70 °C, *n*-butyl lithium (3.3 mmol, 1.6 M hexane solution) was added under a stream of nitrogen over 30 min. After additional stirring for 30 min, anhydrous *N*,*N*-dimethylformamide (6.6 mmol, 0.48 mL) was added. After 1 h at that temperature, the mixture was allowed gradually to warm up to 0 °C, over 3 h. Dilute hydrochloric acid (1.5 mL, 7 mol/L) was added and the layers were separated. After extraction, the combined organic phase was dried over $MgSO_4$. The reaction mixture was purified and separated by column chromatography on silica gel using petroleum ether/diethyl ether (0–10%) as the eluent. After the separation of the starting compound from the first fractions, the last fractions yielded 2-*trans*-(2-furylethenyl)-furan-3-carbaldehyde (**17b**) in 52% yield. Compound **17b** was confirmed by GC–MS measurements before the next reaction step, the preparation of **9d**.

4.4. Preparation of 9a–d

Starting compounds **9a–c** were prepared by Wittig reaction from the 2-*trans*-(2-phenylethenyl)furan-3-carbaldehyde (*trans*-**17a**) and corresponding triphenylphosphonium salts, benzyltriphenylphosphonium bromide, *p*-methylbenzyltriphenylphosphonium bromide, and 2-furylmethyltriphenylphosphonium bromide, respectively. To a stirred solution of the corresponding triphenylphosphonium salts (1.9 mmol) and the 2-*trans*-(2-phenylethenyl)furan-3-carbaldehyde (*trans*-**17a**) (0.350 g, 1.8 mmol) in absolute ethanol (50 mL), a solution of sodium ethoxide (0.067 g, 2.9 mmol in 10 mL ethanol) was added dropwise. Stirring was continued under a stream of nitrogen for 1 day at room temperature. After removal of the solvent, water was added to the residue and extracted with benzene. The benzene extracts were dried and concentrated. The crude reaction mixture was purified and the isomers of products **9a–c** were separated by repeated column chromatography on silica gel using petroleum ether and petroleum ether/diethyl ether (0–5%) mixture as eluent. Starting compound **9d** was prepared similarly by Wittig reaction from the 2-*trans*-[2-(2-furyl)ethenyl]furan-3-carbaldehyde (*trans*-**17b**) and *p*-tolyltriphenylphosphonium bromide. The first fractions yielded *cis*,*trans*-isomer and the last fractions yielded *trans*,*trans* isomers. Characterization data of the new compounds **9a–d** are given below.

4.4.1. 2-(2-Phenylethenyl)-3-(2-phenylethenyl)furan (9a). Yield 83.2%; according to ^{1}H NMR spectroscopy, a mixture of 29% *cis*,*trans*- and 71% *trans*,*trans* isomers was obtained.

cis,*trans*-**9a**: R_f 0.40 (petroleum ether); yellow-green crystals; mp 85–86 °C; UV (EtOH) λ_{max} (log ε) 346 (4.46), 279 (4.02), 235 (4.02) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.44 (d, J=7.2 Hz, 2H, H-ar), 7.28–7.36 (m, 6H), 7.21–7.26 (m, 2H), 7.20 (d, J=1.8 Hz, 1H, H-5f), 7.05 (d, J=16.2 Hz, 1H, H(et)-2β), 6.97 (d, J=16.2 Hz, 1H, H(et)-2α), 6.62 (d, J=12.0 Hz, 1H, H(et)-3β), 6.53 (d, J=12.0 Hz, 1H, H(et)-3α), 6.11 (d, J=1.8 Hz, 1H, H-4f); ^{13}C NMR ($CDCl_3$) δ_C 150.65 (s, C-2), 141.37 (d, C-5_f), 137.40 (s), 136.99 (s), 130.08 (d, C-3β), 128.77 (2d), 128.56 (2d), 128.07 (2d), 127.54 (d), 127.43 (d), 127.13 (d), 126.32 (2d), 119.99 (s, C-3), 119.46 (d, C-3α), 114.37 (d, C-2α), 111.60 (d, C-4f); IR (evaporated film from $CHCl_3$) 2985, 2908, 2853, 1580, 1500, 965, 750 cm^{-1}; MS (EI) *m*/*z* (%) 272 (M^+, 100), 243 (7), 115 (6).

trans,*trans*-**9a**: R_f 0.38 (petroleum ether); yellow crystals; mp 165–166 °C; UV (EtOH) λ_{max} (log ε) 376 (4.36, sh), 359 (4.51), 343 (4.41, sh), 279 (4.29), 236 (3.91) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.50–7.53 (m, 4H), 7.35–7.39 (m, 5H), 7.24–7.28 (m, 2H), 7.13 (d, J=16.2 Hz, 1H, H(et)-3α), 7.12 (d, J=16.2 Hz, 1H, H(et)-2α), 7.09 (d, J=16.2 Hz, 1H, H(et)-2β), 6.84 (d, J=16.2 Hz, 1H, H(et)-3β), 6.68 (d, J=1.8 Hz, 1H, H-4f); ^{13}C NMR ($CDCl_3$) δ_C 150.00 (s, C-2), 142.40 (d, C-5f), 137.27 (s), 136.96 (s),

128.80 (d, C-3β), 128.60 (2d), 128.57 (2d), 128.35 (d), 128.21 (d), 127.59 (d), 127.40 (d), 127.36 (d), 126.33 (2d), 126.12 (2d), 121.99 (s, C-3), 117.78 (d, C-3α), 113.69 (d, C-2α), 108.69 (d, C-4f); IR (evaporated film from $CHCl_3$) 2973, 2915, 2849, 1583, 1500, 1433, 950, 750, 683 cm^{-1}; MS (EI) *m/z* (%) 272 (M^+, 100), 243 (8), 115 (5); Anal. Calcd for $C_{20}H_{16}O$: C, 88.20; H, 5.92. Found: C, 88.53; H, 5.65.

4.4.2. 2-(2-Phenylethenyl)-3-[2-(4-methylphenyl)ethenyl]furan (9b). Yield 95.1%; according to ^1H NMR spectroscopy, a mixture of 35% *cis*,*trans*- and 65% *trans*,*trans*-isomers was obtained.

cis,*trans*-**9b**: R_f 0.43 (petroleum ether); yellow-green crystals; mp 124 °C; UV (EtOH) λ_{max} (log ε) 346 (4.48), 284 (4.04), 235 (4.04) nm; ^1H NMR (600 MHz, $CDCl_3$) δ_H 7.49 (d, *J*=7.8 Hz, 2H), 7.30–7.36 (m, 5H), 7.14 (d, *J*=12.6 Hz, 1H), 7.13 (d, *J*=7.8 Hz, 2H), 7.08 (d, *J*=16.2 Hz, 1H), 6.89 (d, *J*=12.6 Hz, 1H), 6.73 (d, *J*=16.2 Hz, 1H), 6.37 (s, 1H, H-4f), 2.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ_C 152.31 (s), 141.23 (d), 137.65 (s), 136.34 (s), 133.29 (s), 129.02 (2d), 128.39 (d), 128.26 (2d), 128.06 (d), 127.45 (d), 126.08 (2d), 126.00 (2d), 123.10 (s, C-3), 113.90 (d), 112.89 (d), 112.58 (d), 20.80 (q); IR (evaporated film from $CHCl_3$) 3000, 2915, 2849, 1583, 1500, 950, 750 cm^{-1}; MS (EI) *m/z* (%) 286 (M^+, 100).

trans,*trans*-**9b**: R_f 0.39 (petroleum ether); yellow crystals; mp 134 °C; UV (EtOH) λ_{max} (log ε) 378 (4.38, sh), 360 (4.51), 344 (4.41, sh), 284 (4.36), 278 (4.34, sh), 235 (3.99) nm; ^1H NMR (600 MHz, $CDCl_3$) δ_H 7.53 (d, *J*=7.8 Hz, 2H), 7.41 (d, *J*=7.8 Hz, 2H), 7.37 (m, 4H), 7.18 (d, *J*=7.8 Hz, 2H), 7.13 (d, *J*=16.0 Hz, 1H), 7.10 (d, *J*=16.0 Hz, 1H), 7.09 (d, *J*=16.0 Hz, 1H), 6.83 (d, *J*=16.0 Hz, 1H), 6.69 (d, *J*=1.6 Hz, 1H, H-4f), 2.37 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ_C 149.41 (s), 141.98 (d), 136.91 (s), 136.69 (s), 134.16 (s), 128.92 (2d), 128.47 (d), 128.22 (2d), 127.15 (d), 126.84 (d), 125.94 (2d), 125.67 (2d), 122.00 (s), 116.45 (d), 113.45 (d), 108.35 (d), 20.74 (q); IR (evaporated film from $CHCl_3$) 3000, 2907, 2849, 1583, 1500, 1432, 965, 750 cm^{-1}; MS (EI) *m/z* (%) 286 (M^+, 100); Anal. Calcd for $C_{21}H_{18}O$: C, 88.08; H, 6.34. Found: C, 88.35; H, 6.19.

4.5. Photochemical isomerization of the mixture of isomers of 9c,d into *trans*,*trans*-9c,d

A mixture of *cis*,*trans*- and *trans*,*trans*-isomers of **9c**,**d** (~1:1) in benzene (7.7 mM) was purged with argon for 15 min and irradiated at 350 nm in a Rayonet reactor in a Pyrex tube for 30 min. The photochemical isomerization from *cis*,*trans*-isomers of **9c**,**d** to *trans*,*trans*-isomers of **9c**,**d** was followed by GC–MS measurements with time. After 15 min, the reaction mixture contained 91% of the *trans*,*trans*-isomers of **9c**,**d**. The solvent was removed in vacuum and the oily residue chromatographed on silica gel column using petroleum ether to get pure *trans*,*trans*-**9c**,**d** in the last fractions.

4.5.1. 2-(2-Phenylethenyl)-3-(2-furylethenyl)furan (9c). Yield 75.3%; according to ^1H NMR spectroscopy, a mixture of 27% *cis*,*trans*- and 73% *trans*,*trans*-isomers was obtained.

trans,*trans*-**9c**: R_f 0.43 (petroleum ether); yellow crystals; mp 110 °C; UV (EtOH) λ_{max} (log ε) 383 (4.38, sh), 364 (4.49), 346 (4.40, sh), 292 (4.34), 280 (4.30, sh) nm; ^1H NMR (600 MHz, $CDCl_3$) δ_H 7.53 (d, *J*=7.6 Hz, 2H), 7.41 (d, *J*~1 Hz, 1H), 7.36 (m, 2H), 7.26 (t, *J*=7.6 Hz, 2H), 7.11 (d, *J*=16.1 Hz, 1H), 7.07 (d, *J*=16.1 Hz, 1H), 7.05 (d, *J*=15.9 Hz, 1H), 6.62 (d, *J*=15.9 Hz, 1H), 6.61 (d, *J*~1 Hz, 1H), 6.43 (m, 1H), 6.32 (d, *J*=3.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ_C 152.83 (s), 149.76 (s), 142.06 (d), 141.53 (d), 136.62 (s), 128.22 (2d), 127.20 (d), 127.04 (d), 125.96 (2d), 121.41 (s), 116.19 (d), 116.03 (d), 113.33 (d), 111.20 (d), 108.03 (d), 107.68 (d); IR (evaporated film from $CHCl_3$) 2915, 2824, 1608, 1500, 1440, 965, 750 cm^{-1}; MS (EI) *m/z* (%) 262 (M^+, 100), 233 (10), 115 (7); Anal. Calcd for $C_{18}H_{14}O_2$: C, 82.42; H, 5.38. Found: C, 82.73; H, 5.09.

4.5.2. 2-(2-Furylethenyl)-3-[2-(4-methylphenyl)ethenyl]furan (9d). Yield 64.8%.

trans,*trans*-**9d**: R_f 0.40 (petroleum ether); yellow crystals; mp 117 °C; UV (EtOH) λ_{max} (log ε) 385 (4.41), 366 (4.50), 347 (4.36, sh), 289 (4.32), 280 (4.28, sh), 249 (4.03), 241 (4.06) nm; ^1H NMR (600 MHz, $CDCl_3$) δ_H 7.41 (d, *J*=7.9 Hz, 2H), 7.39 (d, *J*~1 Hz, 2H), 7.34 (d, *J*=1.3 Hz, 1H), 7.16 (d, *J*=7.9 Hz, 2H), 7.07 (d, *J*=16.1 Hz, 1H), 7.03 (d, *J*=15.8 Hz, 1H), 6.84 (d, *J*=15.8 Hz, 1H), 6.80 (d, *J*=16.1 Hz, 1H), 6.66 (d, *J*=1.3 Hz, 1H), 6.43 (m, 1H), 6.35 (d, *J*=3.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ_C 152.72 (s), 149.23 (s), 141.96 (d), 141.73 (d), 136.86 (s), 134.17 (s), 128.90 (2d), 128.36 (d), 125.68 (2d), 121.96 (s), 116.44 (d), 114.32 (d), 111.81 (d), 111.40 (d), 108.68 (d), 108.36 (d), 20.73 (q); IR (evaporated film from $CHCl_3$) 3049, 2923, 2851, 1596, 1495, 1446, 961, 741 cm^{-1}; MS (EI) *m/z* (%) 276 (M^+, 100), 219 (2), 115 (2); Anal. Calcd for $C_{19}H_{16}O_2$: C, 82.58; H, 5.84. Found: C, 82.28; H, 5.49.

4.6. Preparation of 9e and 9f

To a solution of *trans*-3-methyl-2-(2-phenylethenyl)furan (*trans*-**16a**) (0.197 g, 1.0 mmol) in CCl_4 (20 mL) were added freshly crystallized *N*-bromosuccinimide (NBS) (0.430 g, 2.2 mmol) and a few mg (5–6 mg) of AIBN. The mixture was heated under reflux until the NBS was consumed (usually 6–7 h). The reaction mixture was cooled to room temperature and filtered to remove the succinimide and concentrated under reduced pressure to give a brown oil of the corresponding *trans*-dibromide. The oil was dissolved in benzene (10 mL) and triphenylphosphine (0.317 g, 1.1 mmol) was added and the solution was stirred overnight. The precipitated phosphonium salt was filtered and subjected to a Wittig reaction with the corresponding aldehydes, *p*-tolylaldehyde (0.144 g, 1.0 mmol) and furan-2-carbaldehyde (0.096 g, 1.0 mmol) in a similar manner as described above for **9a–d**. After column chromatography on silica gel with petroleum ether/diethyl ether (0–3%) mixture as eluent, a 57.8% yield of **9e** and 39% yield of **9f** were obtained for three reaction steps. In the first fractions *cis*,*trans*-isomers **9e**,**f** were isolated and in the last fractions *trans*,*trans*-isomers **9e**,**f** were isolated.

4.6.1. 5-Bromo-2-(2-phenylethenyl)-3-[2-(4-methylphenyl)ethenyl]furan (9e). Yield 57.8%; according to ^1H NMR spectroscopy, a mixture of 38% *cis*,*trans*-isomers and 62% *trans*,*trans*-isomers were obtained.

cis,*trans*-**9e**: Too small a quantity to be analyzed completely; R_f 0.62 (petroleum ether/diethyl ether 19:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.38 (d, J=7.9 Hz, 2H), 7.27–7.36 (m, 3H), 7.17 (d, J=7.9 Hz, 2H), 7.08 (d, J=7.9 Hz, 2H), 6.98 (d, J=15.9 Hz, 1H, H-et), 6.82 (d, J=15.9 Hz, 1H, H-et), 6.56 (d, J=12.0 Hz, 1H, H-et), 6.34 (d, J=12.0 Hz, 1H, H-et), 6.04 (s, 1H), 2.30 (s, 3H, CH_3); MS (EI) *m*/*z* (%) 364/366 (M^+, 100), 285 (20), 257 (30), 115 (20).

trans,*trans*-**9e**: R_f 0.57 (petroleum ether/diethyl ether 19:1); yellow-green crystals; mp 98–100 °C; UV (EtOH) λ_{max} (log ε) 380 (4.19, sh), 364 (4.33), 347 (4.24, sh), 286 (4.23), 237 (3.85) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.47 (d, J=7.9 Hz, 2H), 7.33 (m, 4H), 7.22 (t, J=7.9 Hz, 1H), 7.13 (d, J=7.9 Hz, 2H), 7.02 (d, J=16.0 Hz, 1H, H-et), 6.96 (d, J=16.0 Hz, 1H, H-et), 6.94 (d, J=16.0 Hz, 1H, H-et), 6.72 (d, J=16.0 Hz, 1H, H-et), 6.56 (s, 1H), 2.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ_C 151.61 (s), 137.81 (s), 136.85 (s), 134.21 (s), 129.86 (d), 129.49 (2d), 128.78 (2d), 127.90 (d), 127.71 (d), 126.51 (2d), 126.29 (2d), 124.37 (s), 123.38 (s), 115.77 (d), 112.81 (d), 110.54 (d), 21.31 (q); IR (evaporated film from $CHCl_3$) 2932, 2873, 1600, 1500, 1456, 956, 940, 750 cm^{-1}; MS (EI) *m*/*z* (%) 364/366 (M^+, 100), 285 (15), 257 (30), 115 (15); Anal. Calcd for $C_{21}H_{17}BrO$: C, 69.05; H, 4.69. Found: C, 69.43; H, 4.33.

4.7. Photochemical isomerization of the mixture of isomers of 9f into *trans*,*trans*-9f

A mixture of *cis*,*trans*- and *trans*,*trans*-isomers of **9f** (~2:3) in benzene (9.5 mM) was purged with argon for 15 min and irradiated at 350 nm in a Rayonet reactor in a Pyrex tube. The photochemical isomerization from *cis*,*trans*-isomers of **9c**,**d** to *trans*,*trans*-isomers of **9f** was followed by GC–MS measurements with time. After 15 min, the reaction mixture contained 95% of the *trans*,*trans*-isomers of **9f**. The solvent was removed in vacuum and the oily residue was chromatographed on silica gel column using petroleum ether to get pure *trans*,*trans*-**9f** from the last fractions.

4.7.1. 5-Bromo-2-(2-phenylethenyl)-3-[2-(2-furyl)ethenyl]furan (*trans*,*trans*-9f). R_f 0.59 (petroleum ether/diethyl ether 19:1); yellow crystals; mp 95 °C; UV (EtOH) λ_{max} (log ε) 385 (4.38), 367 (4.47), 351 (4.36, sh), 298 (4.38), 288 (4.36), 239 (3.80) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.50 (dd, J=7.5, 1.6 Hz, 2H), 7.41 (d, J=1.5 Hz, 1H), 7.35 (dt, J=7.5, 1.6 Hz, 2H), 7.25 (m, 1H), 7.07 (d, J=16.0 Hz, 1H), 6.98 (d, J=16.0 Hz, 1H), 6.96 (d, J=16.2 Hz, 1H), 6.56 (d, J=16.2 Hz, 1H), 6.53 (s, 1H), 6.43 (dd, J=3.3, 1.5 Hz, 1H), 6.33 (d, J=3.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ_C 148.15 (s), 147.18 (s), 137.57 (d), 132.03 (s), 124.02 (2d), 123.18 (d), 123.14 (d), 121.79 (2d), 119.19 (s), 118.77 (s), 112.66 (d), 110.51 (d), 107.94 (d), 107.07 (d), 105.46 (d), 104.14 (d); IR (evaporated film from $CHCl_3$) 3044, 2922, 2840, 1595, 1508, 1494, 949, 928, 771 cm^{-1}; MS (EI) *m*/*z* (%) 340/342 (M^+, 100), 261 (5), 233 (5); Anal. Calcd for $C_{18}H_{13}BrO_2$: C, 63.36; H, 3.84. Found: C, 63.73; H, 3.53.

4.8. Preparation of 9b,c from 9e,f

To a stirred solution of *trans*,*trans*-**9e** or *trans*,*trans*-**9f** (0.9 mmol), respectively, in anhydrous diethyl ether (40 mL) cooled to −70 °C, *n*-butyl lithium (1.0 mmol, 1.6 M hexane solution) was added under a stream of nitrogen over 30 min. After additional stirring for 30 min, the mixture was allowed to gradually warm up to room temperature, over 4 h. To a stirred reaction mixture, water (1.0 mmol, 0.02 mL) was added and the solution was stirred overnight. Dilute hydrochloric acid (0.3 mL, 7 mol/L) was added and the layers were separated. After extraction, the combined organic phases were dried over $MgSO_4$. The reaction mixture was purified and separated by column chromatography on silica gel using petroleum ether/diethyl ether (0–2%) as eluent. After the separation of the starting compound from the first fractions, the last fractions yielded 2-(2-phenylethenyl)-3-[2-(4-methylphenyl)ethenyl]furan (*trans*,*trans*-**9b**) in a 71.3% yield or 2-(2-phenylethenyl)-3-[2-(2-furyl)ethenyl)ethenyl]-furan (*trans*,*trans*-**9c**) in a 65.5% yield.

4.9. Preparation of 18a–c

Starting compounds **18a–c** were prepared in three steps by a Wittig reaction and Vilsmeier formylation. In the first step, 2-styrylfuran was prepared by Wittig reaction from benzyltriphenylphosphonium bromide and freshly distilled furan-2-carbaldehyde. To a stirred solution of benzyltriphenylphosphonium bromide (8.29 g, 20.0 mmol) and furan-2-carbaldehyde (1.70 g, 18.0 mmol) in absolute ethanol (100 mL), a solution of sodium ethoxide (0.615 g, 27.0 mmol in 10 mL ethanol) was added dropwise. Stirring was continued under a stream of nitrogen for 1 day at room temperature. After removal of the solvent, the residue was worked up with water and benzene. The benzene extracts were dried and concentrated. The crude reaction mixture was purified and the pure mixture of isomers of 2-styrylfuran (81.8%) was separated by column chromatography on silica gel using petroleum ether as eluent. Vilsmeier formylation was carried out from 2-styrylfuran (1.26 g, 7.4 mmol) dissolved in dimethylformamide (1.71 mL, 22.0 mmol). After being stirred at ~12 °C for 15 min, phosphorus oxychloride (1.14 g, 7.4 mmol) was added and the reaction mixture was allowed gradually to warm up to room temperature and stirred for 4 days. The reaction mixture was decomposed by the continuous addition (with cooling) of 15% sodium hydroxide solution and the product was worked up with diethyl ether. The diethyl ether extracts were washed with water. After removal of the solvent, the crude reaction mixture of 2-formyl-5-styrylfuran (as a mixture of *cis*- and *trans*-isomer) was used in a Wittig reaction to prepare 2,5-distyrylfuran derivatives **18a–c**. To a stirred solution of formyl derivative (0.285 g, 1.4 mmol) and the phosphonium salts (1.6 mmol), benzyltriphenylphosphonium bromide, *p*-methylbenzyltriphenylphosphonium bromide, or 2-furylmethyltriphenylphosphonium bromide, respectively, in absolute ethanol (100 mL) a solution of sodium ethoxide (0.05 g, 2.2 mmol in 10 mL ethanol) was added dropwise. Stirring was continued under a stream of nitrogen for 1 day at room temperature. After removal of the solvent, the residue was worked up with water and benzene. The benzene extracts were dried and concentrated. The crude reaction mixture was purified and the mixture of four isomers of products **18a–c** were isolated by column chromatography on silica gel using petroleum ether/diethyl ether (0–3%) mixture as eluent. After the photochemical isomerization of the mixture of four isomers of **18a–c** (see the procedure for isolating *trans*,*trans*-**9f**) into *trans*,*trans*-isomers of **18a–c**. The

solvent was removed in vacuum and the oily residue was chromatographed on silica gel column using petroleum ether to isolate pure *trans*,*trans*-**18a–c**. Characterization data of the new compounds *trans*,*trans*-**18b** and *trans*,*trans*-**18c** are given below.[55]

4.9.1. 2-(2-Phenylethenyl)-5-[2-(4-methylphenyl)ethenyl]furan (*trans*,*trans*-18b). Yield 58.7%; R_f 0.63 (petroleum ether/CH_2Cl_2 9:1); yellow-green crystals; mp 91–92 °C; UV (EtOH) λ_{max} (log ε) 386 (4.50), 281 (4.33), 276 (4.34) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.49 (d, J=7.6 Hz, 2H), 7.39 (d, J=8.0 Hz, 2H), 7.34 (t, J=7.6 Hz, 2H), 7.24 (t, J=7.6 Hz, 2H), 7.16 (d, J=8.0 Hz, 1H), 7.11 (d, J=16.2 Hz, 1H), 7.10 (d, J=16.1 Hz, 1H), 6.87 (d, J=16.2 Hz, 1H), 6.83 (d, J=16.1 Hz, 1H), 6.37 (d, J=3.4 Hz, 1H), 6.34 (d, J=3.4 Hz, 1H), 2.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ_C 152.75 (s), 152.37 (s), 137.10 (s), 136.67 (s), 133.84 (s), 128.98 (2d), 128.24 (2d), 127.07 (d), 126.89 (d), 126.64 (d), 125.88 (2d), 125.84 (2d), 115.80 (d), 114.88 (d), 110.79 (d), 110.33 (d), 20.80 (q); IR (evaporated film from $CHCl_3$) 2981, 2925, 1592, 1507, 1442, 1017, 956, 782, 750 cm^{-1}; MS (EI) *m*/*z* (%) 286 (M$^+$, 100); Anal. Calcd for $C_{21}H_{18}O$: C, 88.08; H, 6.34. Found: C, 88.27; H, 6.18.

4.9.2. 2-(2-Furylethenyl)-5-(2-phenylethenyl)furan (*trans*,*trans*-18c). Yield 64.2%; R_f 0.61 (petroleum ether/CH_2Cl_2 9:1); yellow crystals; mp 87 °C; UV (EtOH) λ_{max} (log ε) 383 (4.52), 283 (4.35), 274 (4.34) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.44 (d, J=7.6 Hz, 2H), 7.35 (d, J=1.4 Hz, 1H), 7.31 (t, J=7.6 Hz, 2H), 7.21 (m, 1H), 7.06 (d, J=16.1 Hz, 1H), 6.88 (d, J=16.1 Hz, 1H), 6.82 (d, J=16.1 Hz, 1H), 6.75 (d, J=16.1 Hz, 1H), 6.39 (m, 1H), 6.33 (d, J=3.4 Hz, 2H), 6.31 (d, J=3.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ_C 152.65 (s), 152.49 (s), 152.26 (s), 141.75 (d), 136.60 (s), 128.22 (d), 127.10 (2d), 126.78 (d), 125.89 (d), 125.86 (d), 115.69 (d), 114.53 (d), 114.08 (d), 111.36 (d), 110.97 (d), 110.90 (d), 108.49 (d); IR (evaporated film from $CHCl_3$) 2932, 2874, 1508, 956, 815, 782 cm^{-1}; MS (EI) *m*/*z* (%) 262 (M$^+$, 100); Anal. Calcd for $C_{18}H_{14}O_2$: C, 82.42; H, 5.38. Found: C, 82.29; H, 5.53.

4.10. Irradiation experiments

A mixture of *cis*,*trans*- and *trans*,*trans*-isomers of **9a–d** in benzene (3.3 mM) was purged with argon for 30 min and irradiated at 350 nm in a Rayonet reactor in a Pyrex tube. The reaction course was followed by GC–MS. The GC–MS analysis of the photomixtures was performed on a Varian CP-3800 Gas Chromatograph–Varian Saturn 2200 equipped with FactorFour Capillary Column VF-5ms, 30 m×0.25 mm ID; GC operating conditions for all experiments: column temperature programed from 110 to 300 °C (3 min isothermal) at a rate of 33 °C/min; carrier gas: helium; flow rate: 1 mL/min; injector temperature: 300 °C; volume injected: 5 μL.

According to chromatograms, the following data for irradiation of 2,3-divinylfuran derivatives **9a–d** were obtained [irrad. time/h, t_R./min, M$^+$, %, compd; 2-[2-(2-furyl)ethenyl]furan (M$^+$ 160), 2-styrylfuran (M$^+$ 170), stilbene (M$^+$ 180), 2-[2-(4-methylphenyl)ethenyl]furan (M$^+$ 184), 4-methylstilbene (M$^+$ 194), 4,4′-dimethylstilbene (M$^+$ 208)]:

Compound **9a**: 0 h: 7.53′, M$^+$ 272, 23%, *cis*,*trans*-**9a**; 8.38′, M$^+$ 272, 77%, *trans*,*trans*-**9a**; after 1 h: 7.02′, M$^+$ 272, 5%, **III/IV**; 7.97′, M$^+$ 272, 4%, **III/IV**; 8.12′, M$^+$ 272, 4%, **III/IV**; 8.38′, M$^+$ 272, 80%, *trans*,*trans*-**9a**; 8.88′, M$^+$ 272, 7%, *trans*,*trans*-**18a**; after 2 h: 4.69′, M$^+$ 180, 2%; 5.39′, M$^+$ 180, 1%; 7.02′, M$^+$ 272, 4%, **III/IV**; 7.97′, M$^+$ 272, 4%, **III/IV**; 8.12′, M$^+$ 272, 4%, **III/IV**; 8.38′, M$^+$ 272, 77%, *trans*,*trans*-**9a**; 8.88′, M$^+$ 272, 8%, *trans*,*trans*-**18a**; after 4 h: 4.69′, M$^+$ 180, 21%; 5.39′, M$^+$ 180, 6%; 7.02′, M$^+$ 272, 3%, **III/IV**; 7.97′, M$^+$ 272, 3%, **III/IV**; 8.12′, M$^+$ 272, 4%, **III/IV**; 8.38′, M$^+$ 272, 58%, *trans*,*trans*-**9a**; 8.88′, M$^+$ 272, 5%, *trans*,*trans*-**18a**; after 8 h: 4.69′, M$^+$ 180, 44%; 4.75′, M$^+$ 178, 5%, **25**; 5.39′, M$^+$ 180, 27%; 7.02′, M$^+$ 272, 1%, **III/IV**; 7.97′, M$^+$ 272, 1%, **III/IV**; 8.12′, M$^+$ 272, 1%, **III/IV**; 8.38′, M$^+$ 272, 19%, *trans*,*trans*-**9a**; 8.88′, M$^+$ 272, 2%, *trans*,*trans*-**18a**.

Compound **9b**: 0 h: 7.86′, M$^+$ 286, 33%, *cis*,*trans*-**9b**; 8.95′, M$^+$ 286, 67%, *trans*,*trans*-**9b**; after 1 h: 7.38′, M$^+$ 286, 4%, **III/IV**; 7.84′, M$^+$ 286, 5%, **III/IV**; 8.74′, M$^+$ 286, 4%, **III/IV**; 8.95′, M$^+$ 286, 80%, *trans*,*trans*-**9b**; 9.27′, M$^+$ 286, 7%, *trans*,*trans*-**18b**; after 2 h: 4.55′, M$^+$ 208, 2%; 4.69′, M$^+$ 180, 3%; 5.39′, M$^+$ 180, 1%; 7.38′, M$^+$ 286, 3%, **III/IV**; 7.84′, M$^+$ 286, 4%, **III/IV**; 8.74′, M$^+$ 286, 6%, **III/IV**; 8.95′, M$^+$ 286, 75%, *trans*,*trans*-**9b**; 9.27′, M$^+$ 286, 6%, *trans*,*trans*-**18b**; after 4 h: 4.55′, M$^+$ 208, 8%; 4.69′, M$^+$ 180, 13%; 5.11′, M$^+$ 194, 10%; 5.38′, M$^+$ 194, 1%; 5.39′, M$^+$ 180, 3%; 7.38′, M$^+$ 286, 2%, **III/IV**; 7.84′, M$^+$ 286, 3%, **III/IV**; 8.74′, M$^+$ 286, 3%, **III/IV**; 8.95′, M$^+$ 286, 53%, *trans*,*trans*-**9b**; 9.27′, M$^+$ 286, 4%, *trans*,*trans*-**18b**; after 8 h: 4.55′, M$^+$ 208, 17%; 4.69′, M$^+$ 180, 31%; 5.11′, M$^+$ 194, 12%; 5.31′, M$^+$ 192, 3%, **22**; 5.38′, M$^+$ 194, 3%; 5.39′, M$^+$ 180, 7%; 7.84′, M$^+$ 286, 1%, **III/IV**; 8.74′, M$^+$ 286, 1%, **III/IV**; 8.95′, M$^+$ 286, 23%, *trans*,*trans*-**9b**; 9.27′, M$^+$ 286, 2%, *trans*,*trans*-**18b**.

Compound **9c**: 0 h: 6.56′, M$^+$ 262, 41%, *cis*,*trans*-**9c**; 7.45′, M$^+$ 262, 59%, *trans*,*trans*-**9c**; after 1 h: 6.33′, M$^+$ 262, 4%, **III/IV**; 6.92′, M$^+$ 262, 4%, **III/IV**; 7.17′, M$^+$ 262, 3%, **III/IV**; 7.45′, M$^+$ 262, 82%, *trans*,*trans*-**9c**; 8.03′, M$^+$ 262, 7%, *trans*,*trans*-**18c**; after 2 h: 3.65′, M$^+$ 160, 1%; 4.08′, M$^+$ 170, 3%; 4.60′, M$^+$ 170, 1%; 4.69′, M$^+$ 180, 2%; 6.33′, M$^+$ 262, 4%, **III/IV**; 6.92′, M$^+$ 262, 3%, **III/IV**; 7.17′, M$^+$ 262, 2%, **III/IV**; 7.45′, M$^+$ 262, 78%, *trans*,*trans*-**9c**; 8.03′, M$^+$ 262, 6%, *trans*,*trans*-**18c**; after 4 h: 3.65′, M$^+$ 160, 6%; 4.08′, M$^+$ 170, 17%; 4.60′, M$^+$ 170, 6%; 4.69′, M$^+$ 180, 4%; 6.33′, M$^+$ 262, 2%, **III/IV**; 6.92′, M$^+$ 262, 3%, **III/IV**; 7.17′, M$^+$ 262, 1%, **III/IV**; 7.45′, M$^+$ 262, 57%, *trans*,*trans*-**9c**; 8.03′, M$^+$ 262, 4%, *trans*,*trans*-**18c**; after 8 h: 3.65′, M$^+$ 160, 13%; 4.08′, M$^+$ 170, 31%; 4.60′, M$^+$ 170, 19%; 4.69′, M$^+$ 180, 11%; 4.75′, M$^+$ 178, 2%, **25**; 6.33′, M$^+$ 262, 1%, **III/IV**; 6.92′, M$^+$ 262, 1%, **III/IV**; 7.45′, M$^+$ 262, 21%, *trans*,*trans*-**9c**; 8.03′, M$^+$ 262, 1%, *trans*,*trans*-**18c**.

Compound **9d**: 0 h: 7.10′, M$^+$ 276, 17%, *cis*,*trans*-**9d**; 7.88′, M$^+$ 276, 83%, *trans*,*trans*-**9d**; after 1 h: 6.85′, M$^+$ 276, 5%, **II/III/IV**; 7.29′, M$^+$ 276, 3%, **II/III/IV**; 7.48′, M$^+$ 276, 5%, **II/III/IV**; 7.88′, M$^+$ 276, 81%, *trans*,*trans*-**9d**; 8.41′, M$^+$ 276, 6%, **II/III/IV**; after 2 h: 3.65′, M$^+$ 160, 1%; 4.33′, M$^+$ 184, 2%; 4.55′, M$^+$ 208, 4%; 6.85′, M$^+$ 276, 5%, **II/III/IV**; 7.29′, M$^+$ 276, 4%, **II/III/IV**; 7.48′, M$^+$ 276, 5%, **II/III/IV**; 7.88′, M$^+$ 276, 75%, *trans*,*trans*-**9d**; 8.41′, M$^+$ 276, 4%, **II/III/IV**; after 4 h: 3.65′, M$^+$ 160, 2%; 4.33′, M$^+$ 184, 6%; 4.55′, M$^+$ 208, 24%; 6.85′, M$^+$ 276, 3%, **II/III/IV**; 7.29′, M$^+$ 276, 3%,

II/III/IV; 7.48′, M^+ 276, 3%, **II/III/IV**; 7.88′, M^+ 276, 55%, *trans*,*trans*-**9d**; 8.41′, M^+ 276, 4%, **II/III/IV**; after 8 h: 3.65′, M^+ 160, 8%; 4.33′, M^+ 184, 17%; 4.50′, M^+ 206, 4%, **26**; 4.55′, M^+ 208, 40%; 6.85′, M^+ 276, 1%, **II/III/IV**; 7.29′, M^+ 276, 1%, **II/III/IV**; 7.88′, M^+ 276, 27%, *trans*,*trans*-**9d**; 8.41′, M^+ 276, 2%, **II/III/IV**.

After complete conversion (8–10 h) of the starting material (**9a**–**d**) the solvent was removed in vacuum and the oily residue was chromatographed on a silica gel column using petroleum ether/diethyl ether (2–50%). The only obtained products (besides high amount of tarry material) were small quantities of stilbene derivatives (**20**, **21**) and their oxidation products phenanthrenes (**22**, **25**, and **26**), whose ratio varied depending on experimental efficiency of accomplishing anaerobic reaction conditions. In the representative example after complete conversion of the starting compound, from irradiation of 50 mg of **9a**, 20 mg of **20/25** was isolated.

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References and notes

1. Šindler-Kulyk, M.; Špoljarić, L.; Marinić, Ž. *Heterocycles* **1989**, *29*, 679–682.
2. Šindler-Kulyk, M.; Kragol, G.; Piantanida, I.; Tomšić, S.; Vujković Cvijin, I.; Marinić, Ž.; Metelko, B. *Croat. Chem. Acta* **1996**, *69*, 1593–1602.
3. Vujković Cvijin, I.; Marinić, Ž.; Šindler-Kulyk, M. *Spectrosc. Lett.* **1998**, *31*, 989–1000.
4. Šindler-Kulyk, M.; Škorić, I.; Tomšić, S.; Marinić, Ž.; Mrvoš-Sermek, D. *Heterocycles* **1999**, *51*, 1355–1369.
5. Škorić, I.; Marinić, Ž.; Šindler-Kulyk, M. *Heterocycles* **2000**, *53*, 55–68.
6. Škorić, I.; Basarić, N.; Marinić, Ž.; Šindler-Kulyk, M. *Heterocycles* **2001**, *55*, 1889–1896.
7. Škorić, I.; Hutinec, A.; Marinić, Ž.; Šindler-Kulyk, M. *ARKIVOC* **2003**, 87–97.
8. Škorić, I.; Marinić, Ž.; Šindler-Kulyk, M. *Croat. Chem. Acta* **2004**, *77*, 161–166.
9. Škorić, I.; Basarić, N.; Marinić, Ž.; Višnjevac, A.; Kojić-Prodić, B.; Šindler-Kulyk, M. *Chem.—Eur. J.* **2005**, *11*, 543–551.
10. Šindler-Kulyk, M.; Tomšić, S.; Marinić, Ž.; Metelko, B. *Recl. Trav. Chim. Pays-Bas* **1995**, *114*, 476–479.
11. Basarić, N.; Tomšić, S.; Marinić, Ž.; Šindler-Kulyk, M. *Tetrahedron* **2000**, *56*, 1587–1593.
12. Basarić, N.; Marinić, Ž.; Šindler-Kulyk, M. *Tetrahedron Lett.* **2001**, *42*, 3641–3643.
13. Basarić, N.; Marinić, Ž.; Višnjevac, A.; Kojić-Prodić, B.; Griesbeck, A. G.; Šindler-Kulyk, M. *Photochem. Photobiol. Sci.* **2002**, *1*, 1017–1023.
14. Rademacher, P.; Basarić, N.; Kowski, K.; Šindler-Kulyk, M. *Eur. J. Org. Chem.* **2002**, 551–556.
15. Basarić, N.; Marinić, Ž.; Šindler-Kulyk, M. *J. Org. Chem.* **2003**, *68*, 7524–7527.
16. Basarić, N.; Marinić, Ž.; Šindler-Kulyk, M. *Tetrahedron Lett.* **2003**, *44*, 7337–7340.
17. Butković, K.; Basarić, N.; Lovreković, K.; Marinić, Ž.; Višnjevac, A.; Kojić-Prodić, B.; Šindler-Kulyk, M. *Tetrahedron Lett.* **2004**, *45*, 9057–9060.
18. Butković, K.; Marinić, Ž.; Šindler-Kulyk, M. *Magn. Reson. Chem.* **2004**, *42*, 1053–1055.
19. Šindler-Kulyk, M.; Laarhoven, W. H. *J. Am. Chem. Soc.* **1976**, *98*, 1052–1053.
20. Šindler-Kulyk, M.; Laarhoven, W. H. *J. Am. Chem. Soc.* **1978**, *100*, 3819–3830.
21. Šindler-Kulyk, M.; Laarhoven, W. H. *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 187–191.
22. Šindler-Kulyk, M.; Laarhoven, W. H. *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 452–459.
23. Op den Brouw, P. M.; Laarhoven, W. H. *J. Org. Chem.* **1982**, *47*, 1546–1555.
24. Op den Brouw, P. M.; Laarhoven, W. H. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 58–67.
25. Op den Brouw, P. M.; Laarhoven, W. H. *J. Chem. Soc., Perkin Trans. 2* **1982**, 795–799.
26. Op den Brouw, P. M.; Laarhoven, W. H. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1015–1020.
27. Op den Brouw, P. M.; de Zeeuw, P.; Laarhoven, W. H. *J. Photochem.* **1984**, *27*, 327–341.
28. Laarhoven, W. H. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 241–254.
29. Laarhoven, W. H. *Pure Appl. Chem.* **1984**, *56*, 1225–1240.
30. Laarhoven, W. H.; Cuppen, Th. J. H. M. *J. Photochem.* **1986**, *32*, 105–118.
31. Tol, A. J. W.; Laarhoven, W. H. *J. Org. Chem.* **1986**, *51*, 1663–1668.
32. Song, Z. Z.; Wong, H. N. C. *J. Chin. Chem. Soc.* **1995**, *42*, 673–679.
33. Song, Z. Z.; Wong, H. N. C.; Yang, Y. *Pure Appl. Chem.* **1996**, *68*, 723–726.
34. Karminski-Zamola, G.; Dogan, J.; Bajić, M.; Blažević, J.; Malešević, M. *Heterocycles* **1994**, *38*, 759–767.
35. Pennanen, S. I. *J. Heterocycl. Chem.* **1977**, *14*, 745–748.
36. Yokoyama, Y.; Takashima, M.; Higaki, Ch.; Shidori, K.; Moriguchi, Sh.; Ando, Ch.; Murakami, Y. *Heterocycles* **1993**, *36*, 1739–1742.
37. Jones, R. R.; Brown, J. M.; Sondheimer, F. *Tetrahedron Lett.* **1975**, *47*, 4183–4186.
38. Shibuya, H.; Yamamoto, Y.; Miura, I.; Kitagawa, I. *Heterocycles* **1982**, *17*, 215–218.
39. Vuligonda, V.; Garst, M. E.; Chandraratna, A. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 589–594.
40. Hiroyasu, T.; Harumasa, Y.; Masayuki, M.; Tadashi, S. Patent No. JP 03,135,570, 1991, 10 pages, Patent written in Japanese.
41. Buckle, D.R. Eur. Pat. Appl. Patent No. EP 194,093, 1986, 58 pages.
42. Shiro, I.; Takamasa, I.; Mutsumi, K.; Hidemi, I. Patent No. JP 09,059,207, 1997, 19 pages, Patent written in Japanese.
43. For a review of hexatriene photochemistry see: Fonken, G. J. *Organic Photochemistry*; Chapman, O. L., Ed.; Marcel Dekker: New York, NY, 1967; Vol. 1, p 222.
44. Dauben, W. G.; Kellog, M. S.; Seeman, J. I.; Vietmeyer, N. D.; Wendschuh, P. H. *Pure Appl. Chem.* **1973**, *33*, 197.
45. No attempt was made to synthesize the other constitutional isomers.
46. Padwa, A. *Rearrangement in Ground and Excited States*; de Mayo, P., Ed.; Academic: New York, NY, 1980; Vol. 3.
47. D'Auria, M. *Heterocycles* **1999**, *50*, 1115–1136.

48. D'Auria, M. *Advances in Heterocyclic Chemistry: Photochemical Isomerization of Pentaatomic Heterocycles*; Katritzky, A. R., Ed.; Academic: San Diego, CA, 2001; pp 41–88.
49. Mallory, F. B.; Wood, C. S.; Gordon, J. T. *J. Am. Chem. Soc.* **1964**, *86*, 3094–3100.
50. Zanetti, J. E. *J. Am. Chem. Soc.* **1927**, *49*, 1065–1067.
51. Cook, M. J.; Forbes, E. J. *Tetrahedron* **1968**, *24*, 4501–4508.
52. Pelter, A.; Siugaram, B. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1383–1386.
53. Burness, D. M. In *Org. Syntheses Coll. Vol. 4*, Rabjohn, N., Ed.; 1963; pp 628–630 and 649–652.
54. Gronovitz, S.; Michael, V. *Ark. Kemi* **1970**, *32*, 283–294.
55. The data for 2,5-di(2-phenylethenyl)furan (**18a**) can be found in the literature.[56,57]
56. Seus, E. J. *J. Heterocycl. Chem.* **1965**, *2*, 318–320.
57. Van Der Looy, J. F. A.; Thys, G. J. H.; Dieltiens, P. E. M.; Schrijver, D. D.; Alsenoy, Ch. V.; Geise, H. J. *Tetrahedron* **1997**, *53*, 15069–15084.

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Convergent synthesis of the common FGHI-ring part of ciguatoxins

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Abstract—Convergent synthesis of the common FGHI-ring part (**54**) of ciguatoxins was achieved via the following key steps: (i) the Nozaki–Hiyama–Kishi reaction connecting the F-ring part (**6**) with the I-ring part (**7**); (ii) regio- and stereoselective epoxidation; (iii) the 6-*exo*-epoxide opening reaction forming simultaneously the H-ring and the quaternary asymmetric center at C30; (iv) inversion of the C29 stereocenter by a two-step oxidation/reduction process, where the successful inversion depended on proper management of the steric environment of the substrate; and (v) final reductive cyclization constructing the G-ring.

1. Introduction

Ciguatoxins (CTXs, Fig. 1)[1] are the principal toxins responsible for ciguatera,[2] a form of sea food poisoning. More than 25,000 people suffer annually from this poisoning in the Pacific and Indian Oceans as well as the Caribbean Sea.[2b] In 1977, Yasumoto and co-workers identified an epiphytic dinoflagellate, *Gambierdiscus toxicus*, as a causative organism.[3] The dinoflagellate-produced toxins are first transferred to herbivorous fish and accumulated most in carnivorous fish through the marine food chain, thus causing human intoxication. The symptoms of ciguatera are characterized by gastrointestinal and neurological disturbances. Since these disturbances often last for months or years, ciguatera has resulted in serious social problems. Thus, CTXs are now studied by many researchers from a variety of viewpoints in order to prevent and treat ciguatera intoxication.[4]

CTXs have been isolated from both poisonous fish and dinoflagellate *G. toxicus* with great effort over several years and despite the extremely low content of CTXs in these organisms. Ciguatoxin (CTX1B, **1**) was first isolated from the moray eel, *Gymnothorax javanicus*, by Scheuer and co-workers in 1967, and characterized to be a polyether compound in 1980.[5] Determination of the relative structure of **1** was achieved by Yasumoto and co-workers in 1989 with only 0.35 mg of **1** isolated from 4000 kg of *G. javanicus*. The absolute structure of **1** was determined by collaboration of Yasumoto et al. in 1997.[6] CTX3C (**2**) was isolated from cultured *G. toxicus* by Yasumoto and co-workers in 1993.[7]

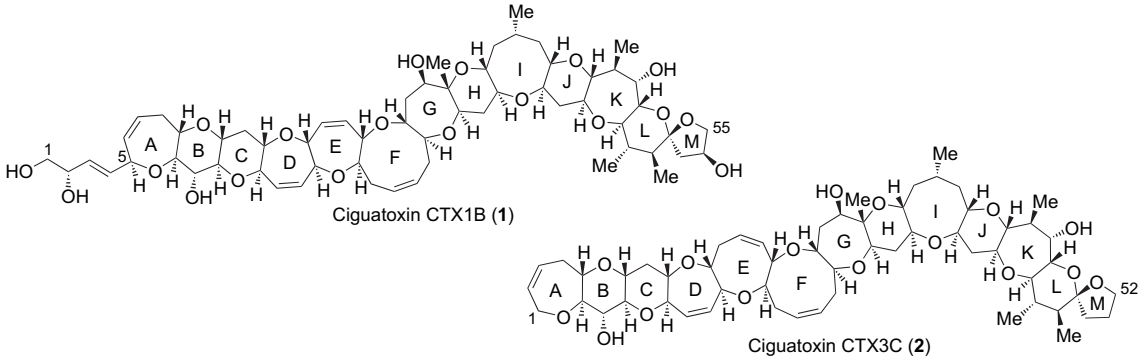

Figure 1. Representative ciguatoxin congeners.

Keywords: Ciguatoxin; *trans*-Fused tetracyclic ether; Reductive etherification; The Nozaki–Hiyama–Kishi reaction; 6-*exo* Hydroxy epoxide opening.
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They elucidated the structure of **2** with only 0.70 mg of **2** isolated from 1100 l of the culture. So far, more than 20 CTXs have been isolated and structurally identified.

Pharmacological studies have disclosed that the potent neurotoxicity of CTXs arises from the activation of voltage-sensitive sodium channels (VSSCs) in neuron cells by the strong binding to site-5 on the channel, and CTXs share the binding site on VSSC with brevetoxins.[8] However, further progress in these studies has been prevented by the insufficient amounts of CTXs from natural sources. Therefore, synthetic supply of CTXs on a practical scale is desired for the advancement of the above studies as well as the development of therapies for ciguatera and methods for screening of ciguateric fish.

Structural features of CTXs, such as the stereochemical complexity, huge molecular size, and ladder-shaped polyether skeleton possessing five- to nine-membered cyclic ethers, provide remarkable synthetic challenges. Therefore, CTXs have been studied extensively by numerous chemists in the synthetic viewpoint.[9,10] To date, many convergent synthetic strategies[11,12] toward the total synthesis of CTXs have been reported.[9,10]

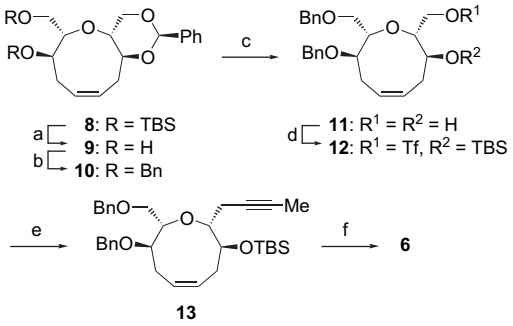
In the course of our program toward the total synthesis of CTXs,[13] we have established a method for the convergent construction of a *trans*-fused X/6/7/X cyclic ether system based on the coupling reaction of an acyl anion equivalent with an aldehyde followed by reductive cyclization reactions.[13g,i,k,14] So far, we reported the synthesis of the ABCDE- and IJKLM-ring parts of **2** by the method[13m,q] as well as by a new procedure for the addition of the F-ring to the E-ring part of **1**, which would also be available for the CTX3C (**2**) synthesis.[13n,o] Accordingly, the remaining issue is development of a synthetic method for the middle (GH-ring) part of **2** from the left (ABCDEF-ring) and the right (IJKLM-ring) segments. Here, a convergent synthesis of the common FGHI-ring part of CTXs from F- and I-ring segments is described.[13r]

2. Synthetic plan for the FGHI-ring part

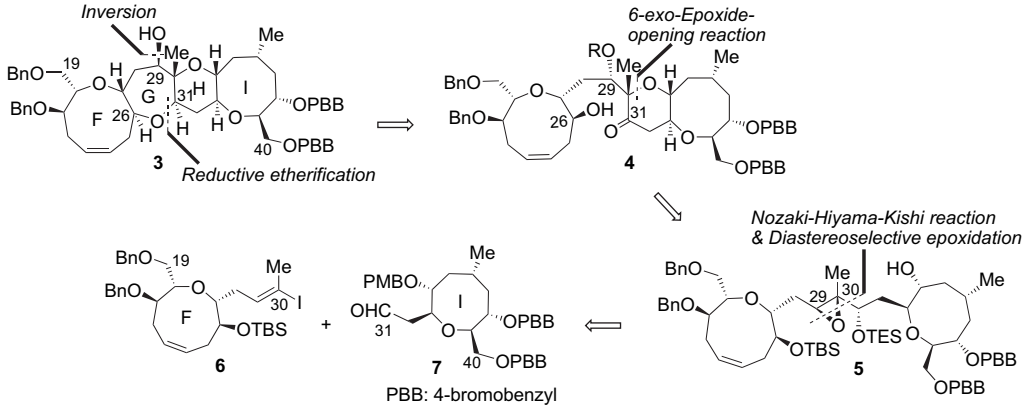
Our synthetic plan for the FGHI-ring part **3** from the F- and I-ring segments (**6** and **7**, respectively) is outlined in Scheme 1. A main issue of the synthesis of **3** was stereocontrolled construction of three contiguous asymmetric centers from C29 to C31 including a quaternary asymmetric center (C30) at the junction between the G- and H-rings.[15] We intended to solve the issue based on the following scheme: (i) The G-ring of **3** was envisioned to be constructed from hydroxy ketone **4** by reductive etherification, which would generate the O26–C31 bond and the C31 stereocenter,[16,17] and inversion of the C29 stereochemistry; (ii) In order to construct the H-ring and the quaternary center at C30 concurrently, the 6-*exo*-epoxide-opening reaction of **5**, whose product was intended to be oxidized to **4**, was planned; (iii) The epoxide **5** would be synthesized from *E*-iodoolefin **6** and aldehyde **7** via the Nozaki–Hiyama–Kishi (NHK) reaction[18] followed by regio- and diastereoselective epoxidation; (iv) Both **6** and **7** would be prepared from our previously reported medium-ring ethers.[10j,p] Although the use of the *cis*-epoxide (C29-*epi*-**5**) corresponding to **5** might be straightforward and excludes the C29-inversion step, the *cis*-epoxide could not be prepared so far because of the difficulty in the synthesis of a *Z*-iodoolefin corresponding to *E*-iodoolefin **6**.[19] Therefore, we decided to adopt the above synthetic plan that employed the NHK reaction with an *E*-iodoolefin at the first stage and C29 inversion at the final stage.

3. Preparation of the F- and I-ring segments

The F-ring segment **6** was synthesized from known **8**[13j] (Scheme 2). Removal of the TBS groups of **8** (98%) followed



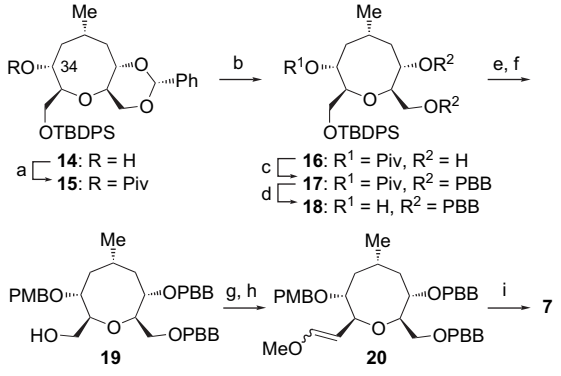
Scheme 2. Reagents and conditions: (a) TBAF, THF, 25 °C, 1 h, 98%; (b) BnBr, NaH, TBAI, THF, 25 °C, 23 h, 98%; (c) THF–3 M HCl (1:1), 21 h, 98%; (d) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C, 15 min, then TBSOTf, 0 °C, 1 h, 95%; (e) propyne, BuLi, THF, −78 °C, 10 min, then **12**, −78→24 °C, 3 h, 99%; (f) Cp_2ZrCl_2, DIBAL, THF, 55 °C, 30 min, then I_2, 0 °C, 15 min, 86%.



Scheme 1. Synthetic plan for the FGHI-ring part (**3**).

by protection with BnBr (98%) provided **10**, which was hydrolyzed to give **11** (98%). The diol **11** was converted to triflate **12** by a one-pot selective triflate formation/TBS-protection process (95%).[20] The subsequent reaction with 1-propynyllithium afforded **13** (99%),[21] which was treated first with a zirconium reagent, prepared from Cp_2ZrCl_2 and DIBAL,[22] and then with I_2 to produce **6** regioselectively (86%).

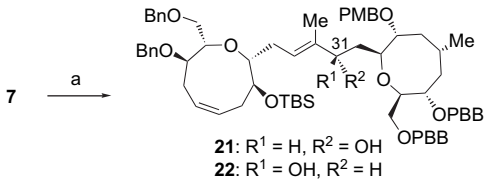
Preparation of the I-ring segment **7** from known **14**[13j,p] is illustrated in Scheme 3. Although direct PMB-protection of the hydroxy group at C34 of **14** was possible, the resulting compound resisted the removal of the benzylidene acetal without detachment of the PMB group. Therefore, the alcohol **14** was first transformed into pivalate **15** (100%), which was converted to PMB ether **19** (overall 83%) by a five-step process [(i) removal of the benzylidene acetal with $Zn(OTf)_2$ and ethanedithiol,[23] (ii) protection of the resulting diol with *p*-bromobenzyl (PBB) bromide, (iii) detachment of the Piv group, (iv) PMB-protection of the resulting alcohol, (v) removal of the TBDPS group]. Oxidation of **19** with Dess–Martin periodinane (DMPI)[24] followed by Wittig reaction afforded **20** (79%), which was hydrolyzed in the presence of $Hg(OAc)_2$ to produce **7** in good yield (99%).[25]



Scheme 3. Reagents and conditions: (a) PivCl, pyridine, 26 °C, 14 h, 100%; (b) $Zn(OTf)_2$, $HS(CH_2)_2SH$, $NaHCO_3$, CH_2Cl_2, 0 °C, 4 h then 25 °C, 1 h, 100%; (c) PBBBr, NaH, TBAI, THF, 25 °C, 14 h, 100%; (d) DIBAL, CH_2Cl_2, −78 °C, 1.5 h, 88%; (e) PMBBr, NaH, TBAI, THF, 26 °C, 21 h; (f) TBAF, THF, 0 °C, 1.5 h, 94% from **18**; (g) DMPI, CH_2Cl_2, 0→23 °C, 50 min; (h) $Ph_3P^+CH_2OMeCl^-$, NHMDS, 0 °C, 30 min, then aldehyde, −78→25 °C, 17 h, 79%; (i) $Hg(OAc)_2$, THF–H_2O (10:1), 23 °C, 1 h, then TBAI, 1.5 h, 99%.

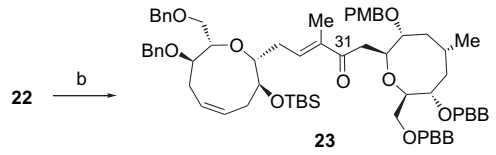
4. Construction of the H-ring

Connection of **6** and **7** is depicted in Scheme 4. According to the Nozaki–Hiyama–Kishi procedure,[18] the segments **6** and **7** were treated with $CrCl_2$ in the presence of $NiCl_2$ (0.5 wt % of $CrCl_2$) in DMSO, and the reaction smoothly proceeded to give **21** (45% from **7**) and its C31-epimer **22** (40% from **7**) in good yield.[26]



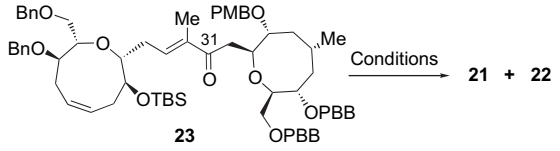
Scheme 4. Reagents and conditions: (a) **6**, $CrCl_2$, $NiCl_2$, DMSO, 25 °C, 25 h, **21**: 45% from **7**, **22**: 40% from **7**.

Since the diastereoselective epoxidation in the next step required *S* configuration at C31 of **21**, inversion of the *R* configuration at C31 of **22** was then examined. Although direct inversion by an S_N2 reaction, such as the Mitsunobu reaction, was unsuccessful, a stepwise oxidation/selective reduction process was found to be effective for the inversion after several examinations (vide infra). Initial oxidation of **22** with DMPI[24] readily afforded α,β-unsaturated ketone **23** in quantitative yield (Scheme 5). Next, stereoselective reduction of **23** was investigated under several conditions (Table 1). Aluminum reducing agents (DIBAL, DIBAL/BuLi,[27] and Red-Al®) exhibited low stereoselectivities (entries 1–3). However, these results suggested that a bulkier reagent would provide better selectivity. Then, boron-reducing agents were examined. In order to avoid conjugate reduction, $NaBH_4$ was first used under the Luche conditions.[28] Although the Luche reduction of **23** at −40 to 0 °C gave low selectivity (**21**:**22**=2:1), the reduction at −78 °C showed enhanced selectivity (4:1) (entries 4 and 5). It was notable that the selectivity of $NaBH_4$ at −78 °C was higher than those of aluminum reductants in spite of the small size of $NaBH_4$. Therefore, bulky L-Selectride® was used instead of $NaBH_4$ under Luche's conditions at −78 °C. As a result, the selectivity increased to 6:1 (entry 6). On the other hand, the reduction with L-Selectride® in the absence of $CeCl_3$ displayed the highest selectivity (>13:1) without side products by conjugate reduction (entry 7). Contrary to the above suggestion, lithium trisiamylborohydride (LS-Selectride®),[29] a bulkier reagent than L-Selectride®, gave only moderate selectivity independently of the presence of $CeCl_3$ (entries 8 and 9).



Scheme 5. Reagents and conditions: (a) DMPI, $NaHCO_3$, CH_2Cl_2, 25 °C, 2.5 h, 100%.

Table 1. Reduction of **23** with several reducing agents

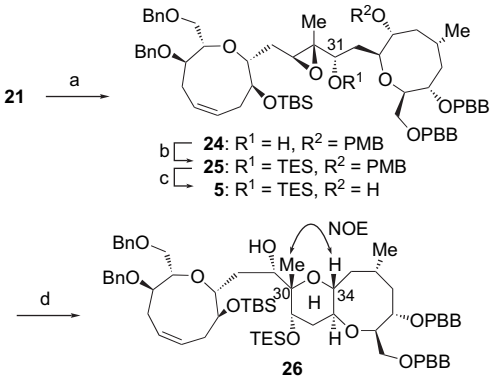


Entry	Conditions	**21**:**22**[a]	Yield (%)[b]
1	DIBAL, CH_2Cl_2, −78 °C, 0.5 h	1:1	~100
2	DIBAL, BuLi, THF, −78 °C, 1 h	2:1	~100
3	Red-Al®, THF, −78→−40 °C, 8 h	3:1	92
4	$CeCl_3\cdot 7H_2O$, $NaBH_4$, MeOH, −40→0 °C, 1.5 h	2:1	75
5	$CeCl_3\cdot 7H_2O$, $NaBH_4$, MeOH, −78 °C, 1.5 h	4:1	35
6	$CeCl_3$, L-Selectride®, THF, −78 °C, 2 h	6:1	~100
7	L-Selectride®, THF, −78 °C, 2 h	>13:1	~100
8	$CeCl_3$, LS-Selectride®, THF, −78→−40 °C, 26 h	6:1	67
9	LS-Selectride®, THF, −40 °C, 18 h	5:1	~100

[a] Determined by ^1H NMR analysis.
[b] Combined yield.

The low reactivity of LS-Selectride® toward **23**, suggested by the fact that LS-Selectride® needed higher temperature to consume the substrate (**23**) than L-Selectride®, was probably due to its excessive bulkiness and might be attributable to the moderate selectivity. Thus, the inversion of the stereochemistry at C31 was efficiently achieved by a two-step Dess–Martin oxidation/L-Selectride® reduction process.

Construction of the H-ring is illustrated in Scheme 6. The $VO(acac)_2$-catalyzed epoxidation of **21** with TBHP exclusively afforded **24** (91%).[30] Protection of the hydroxy group at C31 of **24** by the TES group followed by removal of the PMB group of **25** with DDQ produced **5** in good yield (overall 97%). The hydroxy epoxide **5** was smoothly cyclized with catalytic CSA into **26** (80%). The stereochemistry at C30 of **26** was confirmed by the presence of NOE between H34 and the protons of the methyl group at C30. Thus, the F–HI-ring part **26** was efficiently constructed from **6** and **7** in total seven steps, including the C31-inversion step, in 58% overall yield.

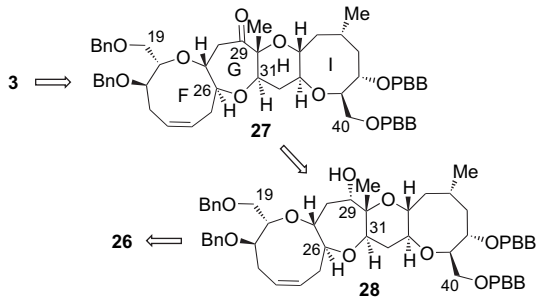


Scheme 6. Reagents and conditions: (a) $VO(acac)_2$, TBHP, toluene, 0 °C, 2 h, 91%; (b) TESOTf, 2,6-lutidine, CH_2Cl_2, 25 °C, 10 min, 100%; (c) DDQ, CH_2Cl_2–pH 7 buffer (10:1), 0 °C, 1 h, 97%; (d) CSA, CH_2Cl_2, 0 °C, 25 min, 80%.

5. Construction of the G-ring

5.1. First-generation approach to the construction of the FGHI-ring part

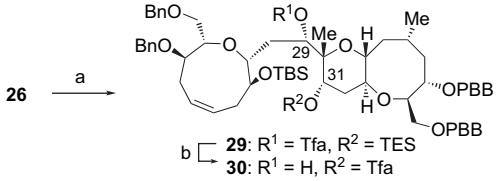
At first, a plan for the construction of the G-ring part including inversion of the stereochemistry at C29 at the final stage, shown in Scheme 7, was examined. The target compound **3** was envisioned to be constructed from ketone **27**, which would be prepared from **28** corresponding to the



Scheme 7. First-generation plan for the construction of the FGHI-ring part (**3**) from **26**.

29-*epi*-FGHI-ring part, by diastereoselective reduction. The reduction of **27** was expected to give **3** with high diastereoselectivity because the methyl group adjacent to the ketone would sterically hinder the approach of a reductant to the ketone from the same side of the methyl group. Accordingly, the construction of the 29-*epi*-FGHI-ring part **28** from **26** was first investigated.

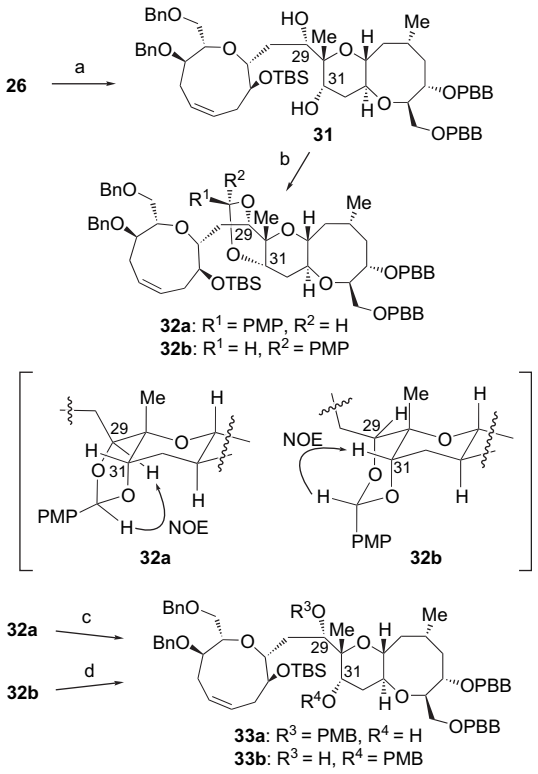
Protection of the hydroxy group at C29 of the F–HI-ring part **26** and the selective deprotection of the TES group at O31 were first examined in order to perform the oxidation at C31 in the later step (Scheme 8). Although the hydroxy group at C29 showed extremely low reactivity to AcCl, Ac_2O, or MsCl, which might be attributable to the steric hindrance of the TES and the C31-methyl groups as well as the F-ring part, the protection with highly reactive trifluoroacetic anhydride successfully afforded Tfa ester **29** in good yield. Then the selective removal of the TES group under mild conditions (THF–H_2O–TFA) was examined. However, the reaction proceeded very slowly with migration of the Tfa group associated with the detachment of the TES group to produce alcohol **30** having a Tfa group at O31 exclusively (63% after three cycles) without the desired O29-protected alcohol.



Scheme 8. Reagents and conditions: (a) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, 0 °C, 1 h, 100%; (b) THF–H_2O–TFA (4:1:0.1), 2 d, 63% after three cycles.

Although selective deprotection of the O31-TES group could not be achieved, this result suggested that the two hydroxy groups at C29 and C31 were in close proximity. Therefore, we next designed a stepwise route for the protection of the C29-hydroxy group via a cyclic acetal, which would be facilely prepared from a 29,31-diol derivative (**31**) of **26** due to close proximity of these two hydroxy groups at C29 and C31 (Scheme 9).

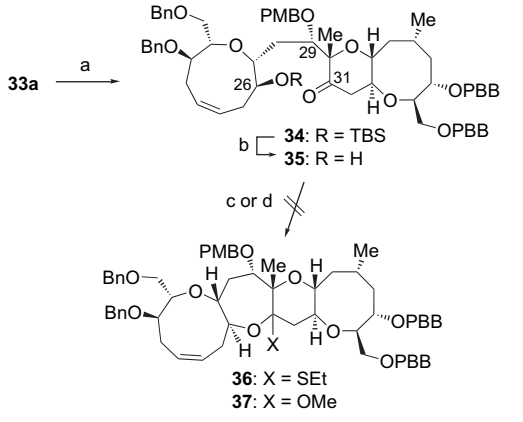
The diol **31** was readily obtained from **26** by selective deprotection of the TES group under mild acidic conditions (Scheme 9).[31] Treatment of **31** with *p*-anisaldehyde under acidic conditions gave a 1:1 mixture of **32a** and **32b** in excellent yield. After the acetals were separated by HPLC, the stereochemistry of **32a** and **32b** was determined from NOE experiments on the basis of *S* configuration at C31 as follows: for **32a**, the presence of NOE between the acetal proton and H29 as well as absence of NOEs between the acetal proton and H31 and between H29 and H31 established the *S* configuration at C29 and *R* at the acetal carbon; for **32b**, the presence of NOE between the acetal proton and H31 as well as the absence of NOEs between the acetal proton and H29 and between H31 and H29 confirmed the *S* configuration at C29 and *S* at the acetal carbon. The reductive cleavage reactions of acetals **32a** and **32b** with DIBAL gave different results. While the cleavage of **32a** showed relatively high selectivity (**33a**:**33b**=5:1), that of **32b** gave opposite but excellent selectivity (**33b**:**33a**>20:1). Although



Scheme 9. Reagents and conditions: (a) PPTS, MeOH–CH_2Cl_2 (4:1), 24 °C, 40 min, 100%; (b) *p*-anisaldehyde, PPTS, benzene, reflux, 3 h, 100% (**32a**:**32b**=1:1); (c) DIBAL, CH_2Cl_2, −30 °C, 1.5 h, 100% (**33a**:**33b**=5:1); (d) DIBAL, CH_2Cl_2, −20 °C, 2 h, 100% (**33b**:**33a**>20:1).

the reason for the regioselectivity of the reductive cleavage with DIBAL cannot be clarified at present, it is suggested that the stereochemistry of the acetal carbon would affect the regioselectivity of the acetal fission. Thus, O29-protected compound **33a** could be obtained though the overall yield from **26** was moderate.

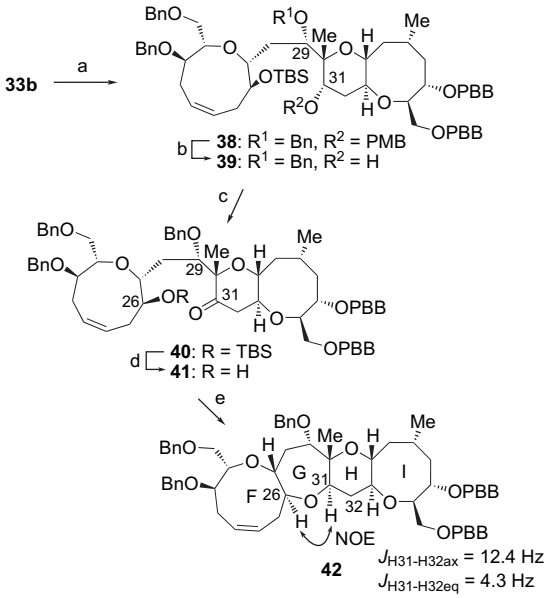
Next, the construction of the G-ring from **33a** via a two-step cyclization/reductive etherification reaction of ketone **35** was examined (Scheme 10). The cyclization precursor **35** was readily synthesized in two steps. Oxidation of **33a** with DMPI[24] followed by deprotection of the TBS group



Scheme 10. Reagents and conditions: (a) DMPI, CH_2Cl_2, 24 °C, 2 h, 70%; (b) HF·Py, THF, 24 °C, 2 d, 63%; (c) $Zn(OTf)_2$, EtSH, $NaHCO_3$, CH_2Cl_2, 25 °C, 1 h; (d) $HC(OMe)_3$, PTS, MeOH, 25 °C, 4 d.

with HF·Py afforded **35** in overall 44% yield. Then, cyclization of **35** into a cyclic *S*,*O*-acetal or a cyclic acetal was attempted. When the ketone **35** was treated with ethanethiol in the presence of $Zn(OTf)_2$,[32] the desired cyclic *S*,*O*-acetal was not produced, and decomposition of **35** due to detachment of the PMB group followed by a retro aldol reaction took place. On the other hand, treatment of **35** with trimethyl orthoformate and catalytic PTS[33] only resulted in recovery of the starting material **35**. These results showed that an acid-labile group, such as PMB, was inappropriate for the protection at O29 during the G-ring formation under acidic conditions. Therefore, an alternative protective group at O29 was then investigated.

Cyclization of the G-ring after protection of O29 as a benzyl ether was performed as shown in Scheme 11. The F–HI-ring part **33b** possessing a PMB group at O31 was used as a starting material. Protection of **33b** with BnBr, which required long reaction time (5 d) for the complete consumption of **33b**, gave **38** in 71% yield. The PMB group of **38** was smoothly deprotected with DDQ to provide **39** (85%). Oxidation of **39** with DMPI[24] followed by deprotection of the TBS group afforded hydroxy ketone **41** in overall 75% yield. The reductive cyclization of **41** with excess Et_3SiH in the presence of TMSOTf furnished the 29-*epi*-FGHI-ring part **42** stereoselectively.[16] The G-ring closure and the desired stereochemistry of C31 in **42** were proved by the presence of NOE between H26 and H31 as well as the large $J_{H31–H32ax}$ (12.4 Hz).



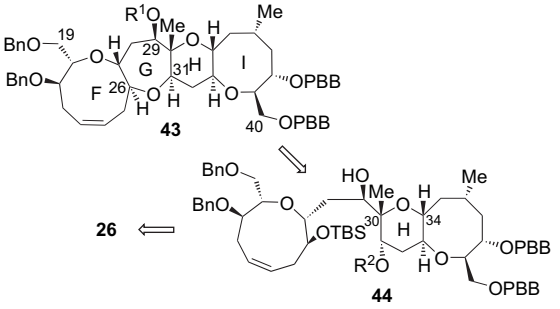
Scheme 11. Reagents and conditions: (a) BnBr, NaH, TBAI, THF, 22 °C, 5 d, 71%; (b) DDQ, CH_2Cl_2–pH 7 buffer (10:1), 0 °C, 35 min, 85%; (c) DMPI, $NaHCO_3$, CH_2Cl_2, 25 °C, 30 min; (d) THF–H_2O–TFA (10:10:1), 25 °C, 2 d, 75% from **39**; (e) TMSOTf, Et_3SiH–CH_2Cl_2 (1:10), 0 °C, 30 min, 70%.

Thus, the 29-*epi*-FGHI-ring part **42** was assembled from **26** in total eight steps in 15% overall yield. Although the synthesis of a key compound (**42**) for the synthesis of the FGHI-ring part **3** succeeded, it is still difficult to supply a reasonable amount of **42** due to some problems, for example, difficulty in the separation of acetals **32a** and **32b**, unusable **33a**, and low reactivity of **33b** in the protection step. On the

other hand, the synthesis of **3** from **42** would require four more steps involving detachment of all Bn groups of **42**, selective protection of the 1,3-diol part, oxidation of the hydroxy group at C29, and reduction of the resulting ketone to **3**. Accordingly, in order to overcome the above difficulties, an alternative synthesis of the FGHI-ring part from **26**, where the C29 configuration was inverted prior to the G-ring cyclization, was designed as described in the next section.

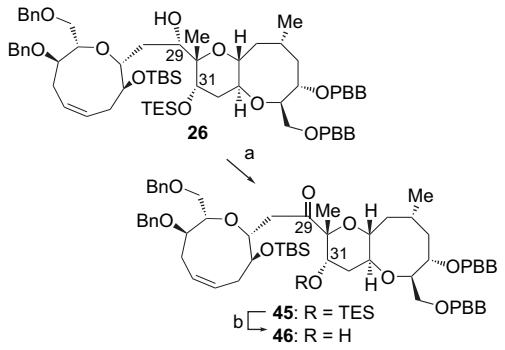
5.2. Second-generation approach to the construction of the FGHI-ring part

Next, a second plan for the synthesis of the FGHI-ring part including inversion of the stereochemistry at C29 in advance of the G-ring formation was investigated. In the plan, outlined in Scheme 12, O29-protected FGHI-ring part **43** was selected as a target compound and envisaged to be synthesized from **26** via the route including (i) inversion of the stereochemistry at C29 providing **44** and (ii) formation of the G ring from **44** through reductive etherification. Success of the plane relied on the C29-inversion step.



Scheme 12. Second-generation plan for the construction of the FGHI-ring part (**43**) from **26**.

At first, inversion of the stereochemistry at C29 of **26** was examined. Since the hydroxy group at C29 of **26** showed seriously low reactivity to electrophiles including several protective groups and MsCl due to steric hindrance around the hydroxy group, as mentioned in the previous section, the inversion at C29 of **26** by an S_N2 reaction was obviously difficult. Therefore, we took an oxidation/reduction process as a reasonable method for inversion of the stereochemistry at C29. Although the alcohol **26** resisted several oxidation reactions (DMPI,[24] SO_3·Py, TPAP, and PCC) owing to the above steric hindrance, Swern oxidation[34] of **26** at higher temperature (−45 °C) for prolonged reaction time (1 h) was able to give the ketone **45** along with recovered **26**. In order to consume the substrate **26**, when the mixture of **45** and **26** was subjected to the Swern oxidation again, the ketone **45** was obtained in 61% yield without recovery of **26** (Scheme 13). However, the reduction of the ketone **45** to C29-*epi*-**26** was not achieved. While treatment of **45** with $NaBH_4$ or $LiAlH_4$ gave only decomposed compounds due to the detachment of the TES and/or TBS groups, DIBAL reduction of **45** regenerated exclusively the original alcohol **26**. Accordingly, we next examined the reduction of **46**, obtained selectively by treatment of **45** with HF·Py (71%), under several conditions as shown in Table 2.

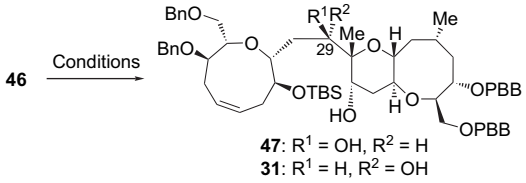


Scheme 13. Reagents and conditions: (a) $(COCl)_2$, DMSO, CH_2Cl_2, −45 °C, 1 h, then Et_3N, 0 °C, 20 min, 61% after two cycles; (b) HF·Py, THF–pyridine (2:1), 25 °C, 6 d, 71%.

Reduction with Red-Al® resulted in exclusive formation of **31** and low yield due to decomposition of the substrate and the product (entry 1, Table 2). Combined use of DIBAL and BuLi at −78 °C also selectively gave **31** along with recovered **46** (entry 2).[27] While reduction with $LiBH_4$ in THF afforded only **31** (entry 3), the reduction in MeOH produced **47** as a mixture with **31** (entry 4). The presence of MeOH in the reaction with $NaBH_4$ and KBH_4 also effectively provided **47** though the selectivity was low (entries 5–8). While use of $Me_4N(AcO)_3BH$[35] afforded only **31** (entry 9), reduction with $NaBH_4$ in the presence of Et_2BOMe[36] gave a similar result as in entries 5–6 (entry 10). Among these experiments, the reduction with $NaBH_4$ in MeOH at 0 °C gave the best result (**47**:**31**=2:1). Although L-Selectride® and Super-Hydride® were also examined, they were not reacted with the ketone in THF at −20 °C. The stereochemistry of the newly generated asymmetric center at C29 in **47** was elucidated at the later stage of the synthesis.

These results suggested that decrease of steric hindrance due to unprotection of C31–OH contributed to increasing reactivity of the ketone. The result from the reaction with $Me_4N(AcO)_3BH$ also suggested that coordination or complexation of the unprotected hydroxyl group at C31 with

Table 2. Reduction of **46** with several reducing agents

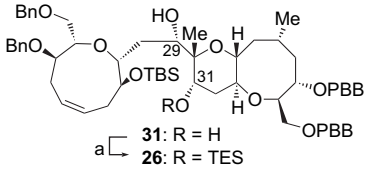


Entry	Conditions	**47**:**31**[a]	Yield (%)[b]
1	Red-Al®, THF, −20 °C, 3 h	0:1	50
2	DIBAL, BuLi, THF, −78 °C, 1.5 h	0:1	ND[c]
3	$LiBH_4$, THF, −20 °C, 40 min	0:1	~100
4	$LiBH_4$, MeOH, −20 °C, 22 h	0.3:1	~100
5	$NaBH_4$, MeOH, −20 °C, 1 h	0.8:1	~100
6	$NaBH_4$, MeOH, 0 °C, 15 min	2:1	~100
7	KBH_4, MeOH, 25 °C, 21 h	1.3:1	ND[c]
8	KBH_4, THF–MeOH (1:1), 0 °C, 22 h	1.5:1	~100
9	$Me_4N(AcO)_3BH$, AcOH, MeCN, 40 °C, 2 d	0:1	~100
10	Et_2BOMe, $NaBH_4$ THF–MeOH (5:1), 0 °C, 15 h	1:1	ND[c]

[a] Determined by ^1H NMR analysis.
[b] Combined yield.
[c] Not determined.

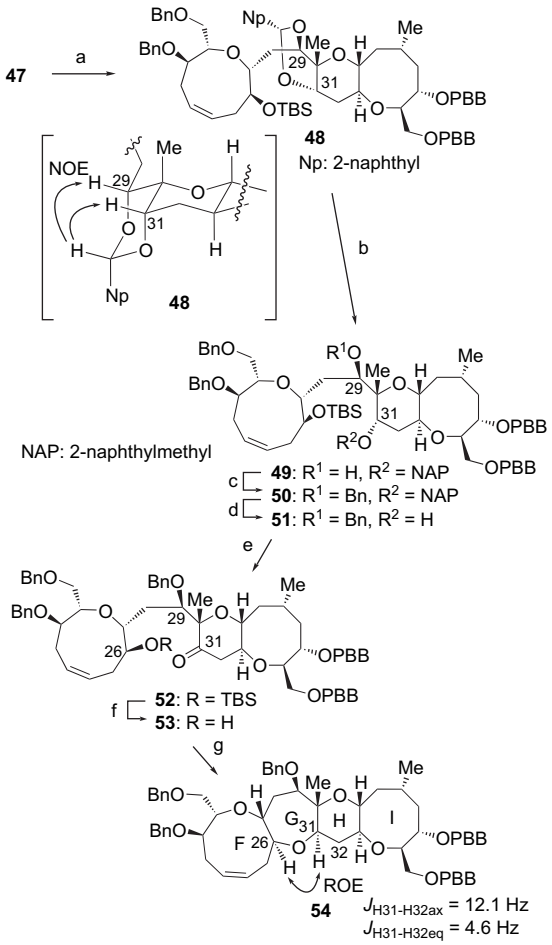
the reducing agent did not participate the production of **47**. Since the reason for the solvent effect of MeOH producing **47** was unclear, we could only speculate the role of MeOH as follows: (i) alteration of the conformation of **46** by hydrogen bonding between the ketone and MeOH, and/or (ii) prohibition of the coordination or complexation of the reducing agent with C31–OH, which would increase external attack of the reagent to the ketone producing **47**.

Thus, the inversion of the stereochemistry at C29 was achieved by the reduction of the β-hydroxy ketone **46** with $NaBH_4$ in MeOH at 0 °C though the selectivity of the reduction was unsatisfactory. Hence, transformation of the undesired diol **31** into the desired **47** was examined. As a result, the hydroxy group at C31 of **31** was simply and selectively protected with TESOTf to provide **26** in excellent yield (Scheme 14), thereby establishing the recycle route from **31** to **47** via **26**.



Scheme 14. Reagents and conditions: (a) TESOTf, 2,6-lutidine, CH_2Cl_2, −40 °C, 1 h, 95%.

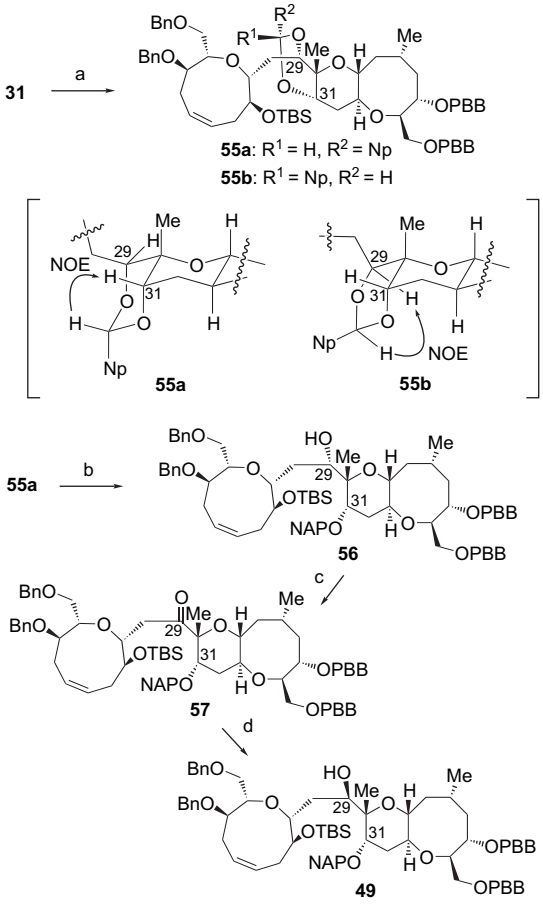
Next, construction of the G-ring from **47** was investigated (Scheme 15). At first, selective protection of the hydroxy group at C29 of the diol **47** via a cyclic acetal was performed. Treatment of **47** with 2-naphthaldehyde dimethyl acetal under acidic conditions gave 2-(2-naphthyl)-1,3-dioxane **48** as the sole product (89%). Stereochemistry of the acetal **48** including the C29-stereocenter, whose formation is described in the above section, was determined by NOE experiment on the basis of *S* configuration at C31. The presence of NOEs between the acetal proton and H31 and between the acetal proton and H29 confirmed the *R* configuration at C29 as well as the *S* configuration at the acetal carbon. Reduction of **48** with DIBAL exclusively afforded **49** possessing the NAP group[35] at O31 in good yield. Protection of the resultant hydroxy group at C29 of **49** with BnBr followed by detachment of the NAP group at O31 provided **51** (overall 91%). Thus, the selective Bn-protection at O29 of **47** was accomplished by the four-step process. Next, G-ring formation via reductive cyclization was executed. The alcohol **51** was oxidized with DMPI,[24] and the resulting **52** was desilylated to give hydroxy ketone **53** quantitatively. The reductive cyclization of **53** with excess Et_3SiH in the presence of TMSOTf at 0 °C produced the FGHI-ring part **54** stereoselectively (78%).[16] The stereochemistry of **54** was confirmed by the presence of ROE between H26 and H31 as well as the large $J_{H31-H32ax}$ (12.1 Hz). Thus, the FGHI-ring part **54** was successfully constructed from the F–HI-ring part **26** in 18% overall yield in 10 steps.



Scheme 15. Reagents and conditions: (a) $NpCH(OMe)_2$, PPTS, benzene, reflux, 1.5 h, 89%; (b) DIBAL, CH_2Cl_2, 10 °C, 3 h, 100%; (c) BnBr, NaH, TBAI, THF, 25 °C, 8 h, 100%; (d) DDQ, CH_2Cl_2–pH 7 buffer (10:1), 0 °C, 20 min, 91%; (e) DMPI, $NaHCO_3$, CH_2Cl_2, 25 °C, 25 min; (f) HF·Py, THF, 25 °C, 2 d, 100% from **51**; (g) TMSOTf, Et_3SiH–CH_2Cl_2 (1:10), 0 °C, 30 min, 78%.

5.2.1. Refinement of the second-generation approach. While construction of the FGHI-ring part was achieved, the yield of β-hydroxy ketone **46** from the F–HI-ring part **26** was low (overall 43%) and the reduction of **46** gave low stereoselectivity (~2:1). Therefore, an improved route for the conversion of the F–HI-ring part **26** to the alcohol **49** was investigated (Scheme 16).

The diol **31** prepared from **26** was treated with 2-naphthaldehyde dimethyl acetal in the presence of PPTS[31] to give a cyclic acetal **55a** as a major product (89%) along with the minor diastereomer **55b** (11%). The stereochemistry of **55a** and **55b** was determined by similar NOE experiments based on the *S* configuration at C31 as described in Section 5.1. The presence of NOE between the acetal proton and H31 as well as the absence of NOEs between the acetal proton and H31 and between H29 and H31 in **55a** confirmed its stereochemistry. The stereochemistry of **55b** was also verified by the presence of NOE between the acetal proton and H29 as well as the absence of NOEs between the acetal proton and H31 and between H29 and H31. The reductive cleavage of the major Np-acetal **55a** with DIBAL selectively provided **56** (93%),[37] where the regioselectivity agreed with the case of **32b** (Section 5.1). Oxidation of **56** with DMPI smoothly afforded the ketone **57** in good yield (90%). Prior to the reduction of **57**, preliminary examinations using ketone **58** (Fig. 2), derived from **33b**, were performed. When the ketone **58** was treated with $NaBH_4$ in MeOH, the starting ketone was only recovered due to



Scheme 16. Reagents and conditions: (a) $NpCH(OMe)_2$, PPTS, benzene, reflux, 2 h, **55a**: 89%, **55b**: 11%; (b) DIBAL, CH_2Cl_2, 0→10 °C, 2 h, 93%; (c) DMPI, $NaHCO_3$, CH_2Cl_2, 25 °C, 8 h, 90%; (d) $NaBH_4$, $CeCl_3\cdot 7H_2O$, THF–H_2O (3:1), 25 °C, 8 d, 96% (**49**:**56**>5:1).

insolubility of the ketone to MeOH. The use of a THF–H_2O (5:1) mixed solvent system in the reduction, where the ketone was soluble, produced alcohols **33b** and 29-*epi*-**33b** as a 1:2 mixture (preliminary data). Therefore, the reduction of **57** with $NaBH_4$ was performed in THF–H_2O (3:1). Although the mixed solvent system gave alcohols **49** and **56**, significant decomposition was observed. After several experiments, the reduction of **57** with $NaBH_4$ in the presence of $CeCl_3\cdot 7H_2O$[28] in THF–H_2O (3:1) was found to proceed cleanly. Although long reaction time (8 d) was required in order to consume the ketone **57**, the reduction showed high yield (96%) and selectivity (**49**:**56**>5:1). Thus, the improved route from the F–HI-ring part **26** to alcohol **49** (overall 59% yield in five steps; previous route: 26% yield in five steps) based on selective protection of O31 and stereoselective reduction of the C29-carbonyl group was developed.

Although it includes preliminary results, we also disclose herein an assessment of the first-generation approach by

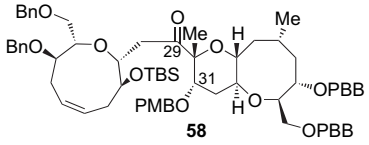
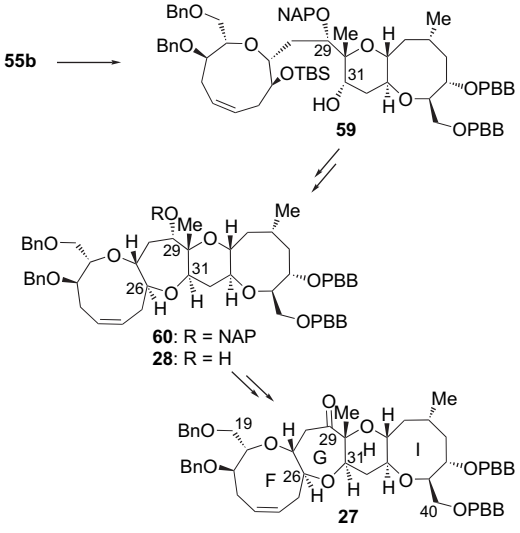


Figure 2.

an alternative method. In the first-generation approach, we expected that the reduction of ketone **27** would produce **3** stereoselectively, but we could not prove the idea (Section 5.1). Since the above-mentioned acetal **55b**, obtained as a minor product, could be converted to 29-*epi*-FGHI-ring part **28** via **59** (Scheme 17), we examined the initial idea with **28**. Reductive cleavage of **55b** with DIBAL exclusively gave **59** possessing an NAP-group at O29, which showed similar stereoselectivity as that of **32b**. Transformation of **59** to a tetracyclic **60**, O29-NAP ether of **28**, was readily performed by the same procedure as that of **33b** to **42**. Deprotection of the NAP-group of **60** with DDQ followed by Dess–Martin oxidation of the resulting hydroxy group afforded the ketone **27**. Contrary to our expectation, reduction of the ketone **27** with $NaBH_4$, $LiAlH_4$, or L-Selectride® provided **28** as a major product. Thus, we found that the first generation-approach was unsuccessful, and the failure suggested that the C29-stereocenter should be constructed at the early stage of the synthesis.



Scheme 17. Synthesis of ketone **27** from **55b**.

6. Conclusion

The aim of this study was the development of an efficient method for the construction of the GH-ring part of ciguatoxins in a convergent manner, which was envisaged to be performed at the final stage of the total synthesis of ciguatoxins. Accordingly, we extensively explored a synthetic route for the common FGHI-ring part (**54**) of ciguatoxins from the F- and I-ring segments. As a result, the convergent synthesis of **54** was achieved via the following key steps: (i) the Nozaki–Hiyama–Kishi reaction connecting the F-ring (**6**) with the I-ring (**7**); (ii) regio- and stereoselective epoxidation; (iii) the 6-*exo*-epoxide opening reaction forming simultaneously the H-ring and the quaternary asymmetric center at C30; (iv) inversion of the C29 stereocenter by a two-step oxidation/reduction process, where the successful inversion depended on proper management of the steric environment of the substrate; and (v) final reductive cyclization constructing the G-ring. Thus, the FGHI-ring part was efficiently synthesized through a novel route in 17 steps in 24% overall yield from **6** and **7**. Further studies toward the

total synthesis of the ciguatoxins are now under way in this laboratory.

7. Experimental

7.1. General methods

All reactions involving air- or moisture-sensitive reagents were carried out under an argon atmosphere in oven-dried glasswares capped with septa, and sensitive liquids and solutions were transferred by using syringe- or cannula-techniques, unless otherwise stated. All commercially available reagents were used without further purification with the following exceptions. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl under argon. Dichloromethane (DCM), dimethylsulfoxide (DMSO), and benzene (PhH) were distilled from CaH_2 prior to use. Normal reagent-grade solvents were used for flash chromatography and extraction. Special reagent-grade solvents were used for high-pressure liquid chromatography (HPLC). All reactions were monitored by thin-layer chromatography (TLC) with precoated silica gel (SiO_2) plates (Merck, silica gel 60 F_{254}). Plates were visualized by ultraviolet light and by treatment with acidic anisaldehyde or phosphomolybdic acid stain followed by heating. SiO_2 (YMC, SIL-60-400/230W) was utilized for flash chromatography. HPLC was run with a JASCO Intelligent HPLC Pump PU-986, equipped with a JASCO Intelligent UV–vis Detector UV-975 and a YMC-Pack SIL-06 (250×10 or 20 mm ID) HPLC column. Melting points were measured on Yanagimoto micro-melting apparatus without calibration. Optical rotations were recorded on a JASCO P-1020 digital polarimeter. Infrared (IR) spectra were measured on a JEOL JIR-WINSPEC100 infrared spectrometer. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-AL300 (^1H at 300 MHz, ^{13}C at 75 MHz), JNM-α-400 (^1H at 400 MHz, ^{13}C at 100 MHz), JNM-α-500 (^{13}C at 125 MHz) or JNM-ECA600 (^1H at 600 MHz, ^{13}C at 150 MHz) NMR spectrometers. ^1H NMR spectra are reported as chemical shifts (δ) in parts per million (ppm) based on tetramethylsilane (0.00 ppm), C_6HD_5 (7.15 ppm) or $CHD_2C(=O)CD_3$ (2.04 ppm). Splitting patterns were designated as 's, d, t, q, m, and br' indicating 'singlet, doublet, triplet, quartet, multiplet, and broad', respectively. Coupling constants (J) are reported in Hertz (Hz). ^{13}C NMR spectra are reported as chemical shifts (δ) in ppm based on $^{13}CDCl_3$ (77.0 ppm) or $^{13}C^{12}C_5D_6$ (128.0 ppm). High-resolution mass spectra (HRMS) were measured on a JEOL JMS-600H mass spectrometer under electron impact ionization (EI) condition and a JEOL JMS-SX102A mass spectrometer under field desorption ionization (FD) condition.

7.1.1. (1*R*,3*S*,4*R*,6*Z*,9*S*)-3-Hydroxymethyl-11-phenyl-2,10,12-trioxabicyclo[7.4.0]tridecan-6-en-4-ol (9). To a solution of **8** (348 mg, 0.649 mmol) in THF (6.0 ml) was added TBAF (1.95 ml, 1.0 M in THF, 1.95 mmol) at 25 °C and the mixture was stirred for 1 h. After that, saturated aqueous NH_4Cl (6 ml) was added and the aqueous layer was extracted with AcOEt (3×30 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=1 to AcOEt) to give **9** (196 mg, 98%). **9**: a colorless solid; mp 154.0–157.0 °C; $[\alpha]_D^{23}$ +29.3 (*c* 0.10, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.49–7.31 (5H, m), 5.88–5.77 (2H, m), 5.43 (1H, s), 4.32 (1H, dd, *J*=3.9, 9.8 Hz), 3.87 (1H, dt, *J*=8.6, 4.4 Hz), 3.73–3.65 (3H, m), 3.63 (1H, dt, *J*=3.9, 9.8 Hz), 3.57 (1H, t, *J*=9.8 Hz), 3.38 (1H, dt, *J*=8.8, 4.4 Hz), 2.71–2.54 (4H, m); IR (KBr), ν (cm^{-1}) 3435, 3091, 2925, 2858, 1455, 1412, 1294, 1219, 1114, 1075, 1015, 963, 948, 916, 883, 759, 700; HR-EIMS, calcd for $C_{17}H_{22}O_5$ $[M]^+$: 306.1467, found: 306.1446.

7.1.2. (1*R*,3*S*,4*R*,6*Z*,9*S*)-4-Benzyloxy-3-benzyloxymethyl-11-phenyl-2,10,12-trioxabicyclo[7.4.0]tridecan-6-ene (10). To a solution of **9** (103 mg, 0.337 mmol) in THF (4.0 ml) were added NaH (33.7 mg, 0.841 mmol) and TBAI (12.4 mg, 0.0337 mmol) at 0 °C and the mixture was stirred for 10 min. Then, benzyl bromide (100 μl, 0.841 mmol) was added at 0 °C, the reaction mixture was warmed to 25 °C, and stirred for 4.5 h. After that, extra NaH (33.7 mg, 0.841 mmol) was added, and the stirring was continued for further 18 h. Saturated aqueous NH_4Cl (4 ml) was added and the aqueous layer was extracted with Et_2O (3×20 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=15 to 5) to give **10** (61.0 mg, 98%). **10**: a colorless solid; mp 110.0–113.0 °C; $[\alpha]_D^{25}$ −12.8 (*c* 0.64, $CHCl_3$); ^1H NMR (300 MHz, C_6D_6), δ (ppm) 7.40–7.00 (10H, m), 5.86–5.70 (2H, m), 5.32 (1H, s), 4.71 (1H, br d, *J*=6.4 Hz), 4.32 (1H, d, *J*=11.7 Hz), 4.31 (1H, d, *J*=12.3 Hz), 4.25 (1H, d, *J*=12.3 Hz), 4.00 (1H, d, *J*=11.7 Hz), 3.57–3.46 (4H, m), 3.48 (1H, br d, *J*=9.7 Hz), 3.31 (1H, dd, *J*=7.5, 9.7 Hz), 3.29–3.21 (1H, m), 2.85–2.78 (1H, m), 2.51–2.44 (2H, m), 2.31–2.25 (1H, m); IR (KBr), ν (cm^{-1}) 3030, 2924, 2856, 1496, 1453, 1393, 1366, 1294, 1213, 1153, 1103, 1027, 976, 747, 697; HR-EIMS, calcd for $C_{31}H_{34}O_5$ $[M]^+$: 486.2406, found: 486.2420.

7.1.3. (2*R*,3*S*,5*Z*,8*R*,9*S*)-8-Benzyloxy-9-benzyloxymethyl-2-hydroxymethyl-2,3,4,7,8,9-hexahydrooxonin-3-ol (11). To a solution of **10** (664 mg, 1.36 mmol) in THF (15.0 ml) was added 3 M HCl (15.0 ml) at 25 °C and the mixture was stirred for 21 h. Then, H_2O (15 ml) was added and the aqueous layer was extracted with AcOEt (4×60 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=5 to AcOEt) to give **11** (529 mg, 98%). **11**: a colorless oil; $[\alpha]_D^{25}$ −68.7 (*c* 1.04, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.37–7.20 (10H, m), 5.87–5.73 (2H, m, H5, 6), 4.62 (1H, d, *J*=11.5 Hz), 4.52 (1H, d, *J*=12.1 Hz), 4.46 (1H, d, *J*=12.1 Hz), 4.28 (1H, d, *J*=11.5 Hz), 3.87–3.80 (2H, m, H3, 10), 3.68 (1H, dd, *J*=5.0, 11.3 Hz, H10), 3.60–3.57 (1H, m, H1), 3.51–3.41 (4H, m, H1, 2, 8, OH), 3.25 (1H, ddd, *J*=3.9, 5.0, 8.8 Hz, H9), 2.81 (1H, ddd, *J*=3.7, 9.9, 13.6 Hz, H4), 2.67–2.60 (1H, m, H7), 2.41–2.36 (1H, m, H7), 2.15 (1H, ddd, *J*=3.7, 4.8, 13.6 Hz, H4); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 137.9 (C), 137.2 (C), 128.5 (CH×2), 128.4 (CH×2), 128.1 (CH×3), 127.9 (CH), 127.8 (CH×2), 127.7 (CH), 127.2 (CH), 87.3 (CH), 84.0 (CH), 78.9 (CH), 73.3 (CH_2), 72.0 (CH_2), 71.2 (CH_2),

70.3 (CH), 63.9 (CH_2), 32.3 (CH_2), 26.9 (CH_2); IR (film), ν (cm^{-1}) 3407, 3063, 3027, 2919, 2860, 1496, 1453, 1367, 1310, 1260, 1207, 1098, 772, 698, 695; HR-EIMS, calcd for $C_{24}H_{30}O_5$ $[M]^+$: 398.2093, found: 398.2112.

7.1.4. (2*R*,3*S*,5*Z*,8*R*,9*S*)-[8-Benzyloxy-9-benzyloxymethyl-3-(*tert*-butyldimethylsilyloxy)-2,3,4,7,8,9-hexahydrooxonin-2-yl]methyl trifluoromethanesulfonate (12). To a solution of **11** (11.4 mg, 28.6 μmol) in DCM (0.70 ml) were added 2,6-lutidine (20.0 μl, 172 μmol) and trifluoromethansulfonic anhydride (5.0 μl, 29.7 μmol) at −78 °C and the mixture was stirred for 15 min. Then, TBSOTf (10.0 μl, 43.5 μmol) was added and the reaction mixture was allowed to warm to 0 °C and stirred for 1 h. After that, H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30) to give **12** (17.5 mg, 95%). **12**: a pale yellow oil; $[\alpha]_D^{19}$ −36.5 (*c* 0.705, $CHCl_3$); ^1H NMR (300 MHz, C_6D_6), δ (ppm) 7.30–7.28 (2H, m), 7.20–7.07 (8H, m), 5.89–5.76 (2H, m), 5.43 (1H, dd, *J*=1.5, 10.1 Hz), 4.54 (1H, d, *J*=12.1 Hz), 4.50 (1H, dd, *J*=1.5, 10.1 Hz), 4.34 (1H, d, *J*=12.1 Hz), 4.30 (1H, d, *J*=12.1 Hz), 4.09 (1H, dt, *J*=3.3, 8.8 Hz), 4.00 (1H, d, *J*=12.1 Hz), 3.64–3.61 (1H, m), 3.50–3.40 (2H, m), 3.27 (1H, dt, *J*=8.4, 3.1 Hz), 3.22 (1H, dt, *J*=8.8, 1.5 Hz), 2.72 (1H, ddd, *J*=3.3, 10.1, 13.8 Hz), 2.56 (1H, ddd, *J*=3.1, 10.1, 13.6 Hz), 2.34 (1H, dt, *J*=13.6, 3.1 Hz), 2.02–1.95 (1H, m), 0.89 (9H, s), 0.03 (3H, s), 0.007 (3H, s); ^{13}C NMR (75 MHz, C_6D_6), δ (ppm) 138.7 (C), 138.5 (C), 128.8 (CH), 128.6 (CH×2), 128.5 (CH×2), 127.4 (CH), 86.4 (CH), 84.5 (CH), 79.0 (CH), 77.1 (CH_2), 73.3 (CH_2), 73.1 (CH_2), 71.3 (CH_2), 69.9 (CH), 32.0 (CH_2), 26.9 (CH_2), 25.8 (CH_3×3), 17.9 (C), −4.3 (CH_3), −5.3 (CH_3) (The signals of seven carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3091, 3035, 2929, 2858, 1496, 1479, 1472, 1454, 1412, 1362, 1336, 1317, 1295, 1245, 1210, 1146, 1102, 1028, 998, 937, 836, 777, 749, 698; HR-EIMS, calcd for $C_{27}H_{34}O_7F_3SiS$ $[M-{}^tBu]^+$: 587.1746, found: 587.1745.

7.1.5. (2*S*,3*R*,5*Z*,8*S*,9*R*)-3-Benzyloxy-2-benzyloxymethyl-8-(*tert*-butyldimethylsilyloxy)-9-(but-2′-ynyl)-2,3,4,7,8,9-hexahydrooxonin (13). To a solution of liquid propyne (excess) in THF (1.0 ml) was added BuLi (0.60 ml, 1.56 M in hexane, 0.936 mmol) at −78 °C and the mixture was stirred for 10 min. Then, a solution of **12** (123 mg, 0.191 mmol) in THF (2.0 ml) was added and the reaction mixture was allowed to warm to 24 °C and stirred for 3 h. After that, H_2O (3 ml) was added and the aqueous layer was extracted with Et_2O (3×15 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=20) to give **13** (101 mg, 99%). **13**: a colorless oil; $[\alpha]_D^{26}$ −78.4 (*c* 1.08, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.38–7.23 (10H, m), 5.79 (1H, dt, *J*=4.5, 10.7 Hz), 5.76 (1H, dt, *J*=4.5, 10.7 Hz), 4.62 (1H, d, *J*=11.6 Hz), 4.54 (2H, s), 4.33 (1H, d, *J*=11.6 Hz), 4.07 (1H, dt, *J*=8.6, 3.3 Hz), 3.74 (1H, dt, *J*=8.6, 3.3 Hz), 3.65 (1H, dd, *J*=2.4, 10.3 Hz), 3.51 (1H, dd, *J*=5.0, 10.3 Hz), 3.33 (1H, ddd, *J*=2.4, 5.0, 8.6 Hz), 3.19 (1H, dt, *J*=8.6, 3.3 Hz), 2.83 (1H, ddd, *J*=3.3, 10.7, 13.5 Hz), 2.79–2.66 (2H, m), 2.39 (1H, ddq, *J*=3.3, 16.9, 2.6 Hz), 2.31 (1H, ddd, *J*=3.3, 4.5, 13.6 Hz), 2.05 (1H, ddd, *J*=3.3, 4.5, 13.5 Hz), 1.79 (3H, t, *J*=2.6 Hz), 0.88 (9H, s), 0.11 (3H, s), 0.094 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.4 (C), 138.3 (C), 128.24 (CH×2), 128.19 (CH×2), 128.1 (CH), 127.8 (CH×2), 127.7 (CH×2), 127.5 (CH×2), 127.3 (CH), 85.0 (CH), 84.5 (CH), 78.9 (CH), 77.2 (C), 76.2 (C), 73.3 (CH_2), 71.9 (CH), 71.8 (CH_2), 71.3 (CH_2), 32.1 (CH_2), 26.8 (CH_2), 25.7 (CH_3×3), 22.3 (CH_2), 17.8 (C), 3.8 (CH_3), −4.5 (CH_3), −5.0 (CH_3); IR (film), ν (cm^{-1}) 3064, 3026, 2956, 2926, 2855, 1471, 1453, 1360, 1309, 1258, 1196, 1099, 1064, 1027, 836, 809, 776, 735, 697; HR-EIMS, calcd for $C_{33}H_{46}O_4Si$ $[M]^+$: 534.3165, found: 534.3209.

7.1.6. (2*S*,3*R*,5*Z*,8*S*,9*R*,2′*E*)-3-Benzyloxy-2-benzyloxymethyl-8-(*tert*-butyldimethylsilyloxy)-9-(3′-iodo-but-2′-enyl)-2,3,4,7,8,9-hexahydrooxonin (6). To a suspension of Cp_2ZrCl_2 (1.47 g, 4.93 mmol) in degassed THF (4.0 ml) were added DIBAL (5.0 ml, 0.94 M in hexane, 4.70 mmol) and a solution of **13** (548 mg, 1.02 mmol) in degassed THF (5.0 ml) at 25 °C. The mixture was heated to 55 °C and stirred for 30 min in the dark. After the mixture was cooled to 0 °C, I_2 (783 mg, 3.08 mmol) in THF (2.0 ml) was added and the reaction mixture was stirred for 15 min. Then, saturated aqueous Na_2SO_3 (5 ml) and saturated aqueous potassium sodium tartrate (10 ml) was added. The mixture was diluted with Et_2O and stirred at 25 °C for 12 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×50 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=75 to 60) to give **6** (584 mg, 86%). **6**: a colorless oil; $[\alpha]_D^{21}$ −81.2 (*c* 0.46, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.33–7.21 (10H, m), 6.30 (1H, tq, *J*=6.6, 1.5 Hz), 5.81–5.70 (1H, m), 4.60 (1H, d, *J*=11.4 Hz), 4.53 (1H, d, *J*=12.0 Hz), 4.43 (1H, d, *J*=12.0 Hz), 4.29 (1H, d, *J*=11.4 Hz), 3.75 (1H, dt, *J*=8.3, 3.4 Hz), 3.71 (1H, dt, *J*=8.6, 3.4 Hz), 3.56 (1H, dd, *J*=2.6, 10.1 Hz), 3.47 (1H, dd, *J*=4.2, 10.1 Hz), 3.32 (1H, ddd, *J*=2.6, 4.2, 8.6 Hz), 3.24 (1H, dt, *J*=8.3, 4.2 Hz), 2.79 (1H, ddd, *J*=3.4, 9.9, 13.4 Hz), 2.66 (1H, ddd, *J*=3.4, 9.9, 13.6 Hz), 2.49 (1H, dddq, *J*=4.2, 6.6, 16.0, 1.7 Hz), 2.36–2.26 (5H, m), 2.05 (1H, dt, *J*=13.4, 3.4 Hz), 0.88 (9H, s), 0.090 (3H, s), 0.023 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.3 (C), 138.2 (C), 137.7 (CH), 128.3 (CH×4), 128.0 (CH×3), 127.8 (CH×2), 127.55 (CH), 127.51 (CH), 127.4 (CH), 94.4 (C), 85.4 (CH), 84.0 (CH), 78.3 (CH), 73.4 (CH), 73.3 (CH_2), 71.3 (CH_2), 71.0 (CH_2), 33.5 (CH_2), 32.0 (CH_2), 28.0 (CH_3), 26.9 (CH_2), 25.8 (CH_3×3), 17.9 (C), −4.1 (CH_3), −4.9 (CH_3); IR (film), ν (cm^{-1}) 3063, 3026, 1496, 1471, 1453, 1388, 1360, 1336, 1297, 1256, 1195, 1099, 1027, 939, 923, 835, 811, 775, 734, 697; HR-EIMS, calcd for $C_{33}H_{47}IO_4Si$ $[M]^+$: 662.2288, found: 662.2258.

7.1.7. (1′*R*,3′*S*,4′*R*,6′*R*,8′*S*)-3′-(*tert*-Butyldiphenylsilyloxymethyl)-6′-methyl-10′-phenyl-2′,9′,11′-trioxabicyclo[6.4.0]-dodecan-4′-yl 2,2-dimethylpropanoate (15). To a solution of **14** (48.6 mg, 87.0 μmol) in pyridine (1.0 ml) was added pivaloyl chloride (40.0 μl, 325 μmol) at 0 °C. The reaction

mixture was allowed to warm to 26 °C and stirred for 14 h. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=20 to 15) to give **15** (54.9 mg, ~100%). **15**: a colorless oil; $[\alpha]_D^{26}$ −13.3 (*c* 0.755, $CHCl_3$); ^{1}H NMR (300 MHz, C_6D_6), δ (ppm) 7.81–7.76 (4H, m), 7.70–7.67 (2H, m), 7.28–7.11 (9H, m), 5.37 (1H, s), 4.95 (1H, dt, *J*=2.8, 9.5 Hz), 4.51 (1H, dd, *J*=4.2, 10.6 Hz), 3.68–3.56 (2H, m), 3.51–3.41 (3H, m), 1.94–1.78 (3H, m), 1.73–1.65 (1H, m), 1.55 (1H, ddd, *J*=6.4, 9.5, 14.7 Hz), 1.18 (9H, s), 1.02 (9H, s), 0.91 (3H, d, *J*=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 176.9 (C), 137.9 (C), 135.6 (CH×2), 135.5 (CH×2), 133.25 (C), 133.21 (C), 129.71 (CH), 129.67 (CH), 128.9 (CH), 128.3 (CH×2), 127.7 (CH×4), 126.2 (CH×2), 100.9 (CH), 86.9 (CH), 80.7 (CH), 78.5 (CH), 72.5 (CH), 70.0 (CH_2), 65.3 (CH_2), 44.6 (CH_2), 41.9 (CH_2), 38.5 (C), 27.8 (CH), 27.0 (CH_3), 26.9 (CH_3×3), 26.8 (CH_3×3), 19.1 (C); IR (film), ν (cm^{-1}) 3071, 3047, 2931, 1959, 1889, 1728, 1590, 1456, 1428, 1396, 1276, 1216, 1138, 975, 912, 823, 754, 703, 615; HR-EIMS calcd for $C_{34}H_{41}O_6Si$ [M−^{t}Bu]$^+$: 573.2627, found: 573.2672.

7.1.8. (2′*S*,3′*R*,5′*R*,7′*S*,8′*R*)-2′-(*tert*-Butyldiphenylsilyloxymethyl)-7′-hydroxy-8′-hydroxymethyl-5′-methyl-oxocan-3′-yl 2,2-dimethylpropionate (16). To a suspension of **15** (1.95 g, 3.09 mmol), 1,2-ethanedithiol (2.60 ml, 31.0 mmol), and $NaHCO_3$ (2.65 g, 3.15 mmol) in DCM (30 ml) was added $Zn(OTf)_2$ (1.13 g, 3.11 mmol) at 0 °C and the mixture was stirred for 4 h. After that, the mixture was warmed to 25 °C and stirred for 1 h. Saturated aqueous $NaHCO_3$ (30 ml) was added and the aqueous layer was extracted with Et_2O (2×150 ml) and AcOEt (150 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=10 to 1) to give **16** (1.68 g, ~100%). **16**: a colorless oil; $[\alpha]_D^{26}$ −1.86 (*c* 0.850, $CHCl_3$); ^{1}H NMR (300 MHz, C_6D_6), δ (ppm) 7.80–7.76 (4H, m), 7.25–7.17 (6H, m), 4.66 (1H, dt, *J*=2.8, 9.9 Hz), 4.19 (1H, ddd, *J*=3.3, 9.5, 12.8 Hz), 4.02 (1H, dd, *J*=2.9, 9.5 Hz), 3.94–3.83 (2H, m), 3.76 (1H, dd, *J*=2.8, 10.6 Hz), 3.70 (1H, dd, *J*=8.1, 10.6 Hz), 3.60 (1H, ddd, *J*=3.3, 7.9, 9.0 Hz), 3.46–3.37 (1H, m), 1.85–1.76 (1H, m), 1.70 (1H, dt, *J*=14.3, 2.8 Hz), 1.62–1.55 (1H, m), 1.46 (1H, m), 1.42–1.31 (2H, m), 1.22 (9H, s), 0.93 (9H, s), 0.86 (3H, d, *J*=7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 177.0 (C), 135.6 (CH×2), 135.5 (CH×2), 132.50 (C), 132.47 (C), 129.9 (CH×2), 127.8 (CH×4), 87.5 (CH), 85.9 (CH), 72.7 (CH), 72.0 (CH), 66.7 (CH_2), 65.5 (CH_2), 48.1 (CH_2), 42.5 (CH_2), 38.4 (C), 27.4 (CH), 27.3 (CH_3), 26.8 (CH_3×3), 26.7 (CH_3×3), 19.0 (C); IR (film), ν (cm^{-1}) 3447, 3072, 2932, 2859, 1728, 1473, 1461, 1428, 1395, 1363, 1282, 1154, 1113, 1034, 823, 740, 701; HR-EIMS, calcd for $C_{27}H_{37}O_6Si$ [M−^{t}Bu]$^+$: 485.2359, found: 485.2359.

7.1.9. (2′*S*,3′*R*,5′*R*,7′*S*,8′*R*)-7′-(4-Bromobenzyloxy)-8′-(4-bromobenzyloxymethyl)-2′-(*tert*-butyldiphenylsilyloxymethyl)-5′-methyl-oxocan-3′-yl 2,2-dimethylpropionate (17). To a suspension of **16** (7.0 mg, 12.9 μmol) and TBAI (8.5 mg, 23.0 μmol) in THF (0.50 ml) was added NaH (6.2 mg, 155 μmol) at 0 °C and the mixture was stirred for 10 min. Then, to the mixture was added *p*-bromobenzyl bromide (33.0 mg, 132 μmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 16 h. Saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=20 to 15) to give **17** (11.4 mg, ~100%). **17**: a colorless oil; $[\alpha]_D^{25}$ +14.6 (*c* 1.08, $CHCl_3$); ^{1}H NMR (300 MHz, C_6D_6), δ (ppm) 7.87–7.76 (4H, m), 7.31–7.15 (10H, m), 6.88–6.81 (4H, m), 5.25 (1H, dt, *J*=3.2, 9.4 Hz), 4.22 (1H, d, *J*=12.0 Hz), 4.20 (1H, d, *J*=12.1 Hz), 4.10 (1H, d, *J*=12.1 Hz), 3.99 (1H, d, *J*=12.0 Hz), 3.97–3.92 (1H, m), 3.85–3.80 (3H, m), 3.76 (1H, dd, *J*=2.8, 9.7 Hz), 3.59 (1H, dd, *J*=5.5, 9.7 Hz), 3.48 (1H, dt, *J*=3.2, 8.4 Hz), 2.12–2.09 (1H, m), 1.96–1.84 (2H, m), 1.76 (1H, dt, *J*=14.2, 9.4 Hz), 1.59 (1H, dt, *J*=8.4, 14.5 Hz), 1.18 (9H, s), 1.07 (9H, s), 0.94 (3H, d, *J*=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 176.9 (C), 140.2 (C×2), 137.7 (CH×2), 137.5 (CH×2), 133.5 (C), 133.3 (C), 131.4 (CH×2), 131.3 (CH×2), 129.6 (CH×2), 129.4 (CH×2), 129.3 (CH×2), 127.7 (CH×2), 127.6 (CH×2), 121.4 (C), 121.3 (C), 86.0 (CH), 85.6 (CH), 78.3 (CH), 72.6 (CH_2), 72.0 (CH), 71.2 (CH_2), 70.7 (CH_2), 65.9 (CH_2), 41.9 (CH_2), 41.1 (CH_2), 38.5 (C), 27.7 (CH), 26.93 (CH_3), 26.88 (CH_3×3), 26.85 (CH_3×3), 19.2 (C); IR (film), ν (cm^{-1}) 3071, 2930, 2858, 1897, 1726, 1591, 1487, 1461, 1428, 1396, 1361, 1281, 1113, 1012, 823, 804, 739, 702; HR-FDMS, calcd for $C_{45}H_{56}^{79}Br_2O_6Si$ [M+H]$^+$: 879.2286, found: 879.2316.

7.1.10. (2*S*,3*R*,5*S*,7*S*,8*R*)-7-(4-Bromobenzyloxy)-8-(4-bromobenzyloxymethyl)-2-(*tert*-butyldiphenylsilyloxymethyl)-5-methyloxocan-3-ol (18). To a solution of **17** (11.4 mg, 12.9 μmol) in DCM (0.60 ml) was added DIBAL (0.14 ml, 0.95 M in hexane, 133 μmol) at −78 °C and the mixture was stirred for 2 h. After that, MeOH (0.10 ml) and saturated aqueous potassium sodium tartrate (1 ml) were added. The mixture was diluted with Et_2O (5 ml) and stirred at 25 °C for 3 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=10 to 3) to give **18** (9.0 mg, 88%). **18**: a colorless oil; $[\alpha]_D^{26}$ +48.1 (*c* 1.26, $CHCl_3$); ^{1}H NMR (300 MHz, C_6D_6), δ (ppm) 7.81–7.74 (4H, m), 7.29–7.16 (10H, m), 6.82 (2H, d, *J*=8.3 Hz), 6.76 (2H, d, *J*=8.4 Hz), 4.18 (1H, d, *J*=11.9 Hz), 4.11 (1H, dd, *J*=5.2, 10.3 Hz), 4.09 (1H, d, *J*=12.4 Hz), 4.02 (1H, dd, *J*=5.9, 10.3 Hz), 3.99 (1H, d, *J*=12.4 Hz), 3.91 (1H, d, *J*=11.9 Hz,), 3.85 (1H, dt, *J*=9.0, 2.6 Hz), 3.68–3.61 (2H, m), 3.58 (1H, dd, *J*=2.6, 9.8 Hz), 3.35 (1H, dd, *J*=6.4, 9.8 Hz), 3.30 (1H, dt, *J*=3.3, 9.1 Hz), 2.69 (1H, d, *J*=2.6 Hz), 1.94–1.73 (4H, m), 1.54 (1H, ddd, *J*=7.9, 9.1, 14.3 Hz), 1.13 (9H, s), 0.98 (3H, d, *J*=7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 137.2 (C), 137.1 (C), 135.52 (CH×2), 135.46 (CH×2), 132.5 (C), 132.4 (C), 131.4 (CH×2), 131.3 (CH×2), 129.93 (CH), 129.91 (CH), 129.2 (CH×2), 129.1 (CH×2), 127.8 (CH×4), 121.4 (C),

121.2 (C), 85.4 (CH), 85.1 (CH), 78.6 (CH), 74.8 (CH), 72.3 (CH_2), 71.5 (CH_2), 70.5 (CH_2), 67.5 (CH_2), 46.3 (CH_2), 42.0 (CH_2), 27.5 (CH), 27.4 (CH_3), 26.7 ($CH_3\times3$), 19.0 (C); IR (film), ν (cm^{-1}) 3481, 3071, 2928, 2858, 1591, 1487, 1471, 1428, 1391, 1361, 1113, 1070, 1012, 823, 802, 740, 701; HR-FDMS, calcd for $C_{40}H_{49}^{79}Br_2O_5Si$ $[M+H]^+$: 795.1711, found: 795.1718.

7.1.11. (2*S*,3*R*,5*S*,7*S*,8*R*)-[7-(4-Bromobenzyloxy)-8-(4-bromobenzyloxymethyl)-3-(4-methoxybenzyloxy)-5-methyloxocan-2-yl]methanol (19). To a suspension of **18** (85.4 mg, 0.107 mmol) and TBAI (17.8 mg, 0.0482 mmol) in THF (0.70 ml) was added NaH (43.4 mg, 1.09 mmol) at 0 °C and the mixture was stirred for 10 min. Then, to the mixture was added a solution of *p*-methoxybenzyl bromide (110 mg, 0.547 mmol) in THF (0.30 ml) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 21 h. Saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/AcOEt=10) to give a crude product (100 mg), and it was used in the next reaction without further purification. To a solution of the above crude product in THF (1.0 ml) was added TBAF (0.50 ml, 1.0 M in THF, 0.50 mmol) at 25 °C and the mixture was stirred for 2 h. The solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=5 to 1) to give **19** (68.3 mg, 94% from **18**). **19**: a colorless oil; $[\alpha]_D^{26}$ +12.2 (*c* 1.02, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.49–7.42 (4H, m), 7.21–7.17 (4H, m), 7.05 (2H, d, *J*=8.4 Hz), 6.85 (2H, d, *J*=8.6 Hz), 4.52–4.48 (4H, m), 4.27 (1H, d, *J*=11.0 Hz), 4.21 (1H, d, *J*=11.6 Hz), 4.09–4.03 (1H, m), 3.95–3.88 (1H, m), 3.80 (3H, s), 3.76–3.72 (2H, m), 3.67 (1H, dt, *J*=3.1, 9.0 Hz), 3.43 (1H, dd, *J*=9.0, 11.1 Hz), 3.36 (1H, t, *J*=8.8 Hz), 3.22 (1H, dt, *J*=3.1, 9.5 Hz), 3.14 (1H, dt, *J*=3.3, 10.5 Hz), 2.03–1.98 (1H, m), 1.93–1.88 (1H, m), 1.79–1.55 (3H, m), 1.07 (3H, d, *J*=7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 159.2 (C), 136.9 (C), 136.2 (C), 131.6 (CH×2), 131.5 (CH×2), 130.1 (C), 129.6 (CH×2), 129.4 (CH×2), 129.3 (CH×2), 121.9 (C), 121.6 (C), 113.8 (CH×2), 87.6 (CH), 85.3 (CH), 79.5 (CH), 78.7 (CH), 72.7 (CH_2), 72.6 (CH_2), 71.1 (CH_2), 70.2 (CH_2), 65.6 (CH_2), 55.2 (CH_3), 42.7 (CH_2), 41.8 (CH_2), 27.39 (CH), 27.38 (CH_3); IR (film), ν (cm^{-1}) 3447, 3048, 2927, 1612, 1592, 1513, 1487, 1463, 1428, 1405, 1362, 1302, 1248, 1173, 1070, 1011, 804, 737, 703; HR-FDMS, calcd for $C_{32}H_{38}^{79}Br_2O_6$ $[M]^+$: 676.1035, found: 676.1042.

7.1.12. (2*R*,3*S*,5*S*,7*R*,8*S*,1′*E*)-3-(4-Bromobenzyloxy)-2-(4-bromobenzyloxymethyl)-7-(4-methoxybenzyloxy)-8-(2′-methoxyvinyl)-5-methyloxocane and (2*R*,3*S*,5*S*,7*R*,8*S*,1′*Z*)-3-(4-bromobenzyloxy)-2-(4-bromobenzyloxymethyl)-7-(4-methoxybenzyloxy)-8-(2′-methoxyvinyl)-5-methyloxocane (20). To a solution of **19** (295 mg, 0.435 mmol) in DCM (4.0 ml) was added DMPI (368 mg, 0.868 mmol) at 0 °C. The reaction mixture was warmed to 23 °C and stirred for 1 h. After the mixture was diluted with Et_2O (10 ml), saturated aqueous Na_2SO_3 (2 ml) was added and the aqueous layer was extracted with Et_2O (2×5 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant crude aldehyde was used in the next reaction without purification. To a solution of $Ph_3P^+CH_2OMeCl^-$ (748 mg, 2.18 mmol) in THF (2.0 ml) was added NHMDS (2.1 ml, 1.0 M in THF, 2.10 mmol) at 0 °C and the mixture was stirred for 30 min at the same temperature before cooling to −78 °C. After that, to the mixture was added a solution of the above crude aldehyde in THF (4.0 ml) at −78 °C and the mixture was stirred for 20 min. The reaction mixture was warmed to 23 °C and stirred for 17 h. Then, brine (6 ml) was added and the aqueous layer was extracted with Et_2O (3×30 ml). The combined organic layers were washed with H_2O, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=15 to 2) to give **20** (241 mg, 79% from **19**, *E*/*Z*=1/1 from ^{1}H NMR). **20**: a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.44–7.39 (4H, m), 7.26–7.16 (4H, m), 7.08 (2H, d, *J*=8.1 Hz), 6.86–6.82 (2H, m), 6.54 (0.5H, d, *J*=12.7 Hz), 5.98 (0.5H, d, *J*=5.5 Hz), 4.82 (0.5H, dd, *J*=7.3, 12.7 Hz), 4.57–4.35 (6H, m), 4.29–4.24 (1H, m), 3.94–3.83 (1.5H, m), 3.79 (3H), 3.74–3.67 (0.5H, m), 3.64–3.41 (5H, m), 3.36–3.24 (1H, m), 1.96–1.61 (5H, m), 1.05 (1.5H, d, *J*=7.2 Hz), 1.02 (1.5H, d, *J*=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 159.1 (C×0.5), 159.0 (C×0.5), 149.5 (CH×0.5), 148.2 (CH×0.5), 137.8 (C×0.5), 137.5 (C×0.5), 137.4 (C×0.5), 137.3 (C×0.5), 131.41 (CH), 131.36 (CH), 131.3 (CH), 131.2 (CH), 130.8 (C×0.5), 130.5 (C×0.5), 129.5 (CH), 129.31 (CH), 129.25 (CH×4), 121.4 (C×0.5), 121.32 (C×0.5), 121.27 (C×0.5), 121.1 (C×0.5), 113.7 (CH), 113.6 (CH), 107.4 (CH×0.5), 103.4 (CH×0.5), 84.5 (CH×0.5), 83.91 (CH×0.5), 83.86 (CH×0.5), 81.5 (CH×0.5), 80.8 (CH×0.5), 79.0 (CH×0.5), 78.50 (CH×0.5), 78.46 (CH×0.5), 72.5 ($CH_2\times0.5$), 72.4 ($CH_2\times0.5$), 71.6 ($CH_2\times0.5$), 71.3 ($CH_2\times0.5$), 71.1 ($CH_2\times0.5$), 70.9 ($CH_2\times0.5$), 70.6 ($CH_2\times0.5$), 70.5 ($CH_2\times0.5$), 59.7 ($CH_3\times0.5$), 55.8 ($CH_3\times0.5$), 55.27 ($CH_3\times0.5$), 55.25 ($CH_3\times0.5$), 42.1 (CH_2), 41.2 (CH_2), 27.9 (CH×0.5), 27.7 (CH×0.5), 27.3 ($CH_3\times0.5$), 27.2 ($CH_3\times0.5$); IR (film), ν (cm^{-1}) 2926, 2862, 1657, 1612, 1586, 1513, 1487, 1462, 1358, 1302, 1248, 1201, 1172, 1088, 1037, 1011, 803; HR-FDMS, calcd for $C_{34}H_{40}^{79}Br_2O_6$ $[M]^+$: 702.1192, found: 702.1213.

7.1.13. (2*S*,3*R*,5*S*,7*S*,8*R*)-[7-(4-Bromobenzyloxy)-8-(4-bromobenzyloxymethyl)-3-(4-methoxybenzyloxy)-5-methyloxocan-2-yl]ethanal (7). To a solution of **20** (21.9 mg, 31.1 μmol) in THF/H_2O (10/1, v/v, 1.1 ml) was added $Hg(OAc)_2$ (47.3 mg, 148 μmol) at 24 °C and the mixture was stirred for 1 h. Then, TBAI (172 mg, 466 μmol) was added at 24 °C. After the mixture was stirred for 2 h, saturated aqueous NH_4Cl (1 ml) was added and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=4 to 3) to give **7** (21.3 mg, 99%). **7**: a colorless oil; $[\alpha]_D^{26}$ +12.7 (*c* 0.803, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm) 9.66 (1H, t, *J*=2.4 Hz), 7.46–7.41 (4H, m), 7.24–7.14 (4H, m), 7.07 (2H, d, *J*=8.3 Hz), 6.86 (2H, d, *J*=8.6 Hz), 4.51 (1H, d, *J*=11.6 Hz), 4.50 (1H, d, *J*=10.9 Hz), 4.44 (1H, d, *J*=12.3 Hz), 4.38 (1H, d, *J*=12.3 Hz), 4.27 (1H, d, *J*=10.9 Hz), 4.24 (1H, d,

J=11.6 Hz), 4.04 (1H, ddd, J=4.7, 7.5, 9.0 Hz), 3.80 (3H, s), 3.78–3.74 (1H, m), 3.59 (1H, dd, J=2.4, 9.7 Hz), 3.39 (1H, dd, J=6.5, 9.7 Hz), 3.38–3.35 (1H, m), 3.23 (1H, dt, J=2.7, 9.0 Hz), 2.77 (1H, ddd, J=2.4, 4.7, 15.8 Hz), 2.54 (1H, ddd, J=2.4, 7.5, 15.8 Hz), 2.01–1.85 (3H, m), 1.77–1.64 (2H, m), 1.09 (3H, d, J=7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 201.2 (CH), 159.3 (C), 137.2 (C×2), 131.44 (CH×2), 131.43 (CH×2), 129.8 (C), 129.7 (CH×2), 129.4 (CH×2), 129.2 (CH×2), 121.5 (C), 121.4 (C), 113.9 (CH×2), 84.6 (CH), 81.5 (CH), 80.8 (CH), 78.6 (CH), 72.6 (CH_2), 71.8 (CH_2), 70.7 (CH_2), 70.6 (CH_2), 55.3 (CH_3), 48.8 (CH_2), 41.0 (CH_2), 40.1 (CH_2), 28.1 (CH), 26.7 (CH_3); IR (film), ν (cm^{-1}) 2923, 2863, 1723, 1612, 1513, 1486, 1456, 1374, 1301, 1248, 1173, 1070, 1011, 804; HR-FDMS, calcd for $C_{33}H_{38}^{79}Br_2O_6$ $[M]^+$: 688.1035, found: 688.1044.

7.1.14. (2*S*,3*E*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,2″*S*,3″*R*,5″*S*,7″*S*,8″*R*)-5-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-[7″-(4-bromobenzyloxy)-8″-(4-bromobenzyloxymethyl)-3″-(4-methoxybenzyloxy)-5″-methyloxocan-2″-yl]-3-methylpent-3-en-2-ol (21) and (2*R*,3*E*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,2″*S*,3″*R*,5″*S*,7″*S*,8″*R*)-5-[8′-benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-[7″-(4-bromobenzyloxy)-8″-(4-bromobenzyloxymethyl)-3″-(4-methoxybenzyloxy)-5″-methyloxocan-2″-yl]-3-methylpent-3-en-2-ol (22). To a suspension of $CrCl_2$ (420 mg, 3.42 mmol) and $NiCl_2$ (2.2 mg, 0.0174 mmol) in degassed DMSO (1.0 ml) was added a solution of **6** (742 mg, 1.12 mmol) and **7** (226 mg, 0.327 mmol) in degassed DMSO (5.0 ml) at 25 °C. The reaction mixture was stirred for 25 h in the dark. Then, saturated aqueous NH_4Cl (6 ml) was added and the aqueous layer was extracted with Et_2O (2×30 ml) and AcOEt (2×30 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=30 to 10) to give **21** (181 mg, 45%) and **22** (160 mg, 40%). **21**: a colorless oil; $[\alpha]_D^{24}$ −40.4 (c 0.250, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 7.43–7.41 (4H, m), 7.31–7.15 (14H, m), 7.04 (2H, d, J=8.1 Hz), 6.84 (2H, d, J=8.6 Hz), 5.78 (1H, dt, J=5.1, 10.1 Hz), 5.74 (1H, dt, J=4.9, 10.1 Hz), 5.62 (1H, t, J=5.7 Hz), 4.60 (1H, d, J=11.5 Hz), 4.50 (2H, d, J=12.9 Hz), 4.49 (1H, d, J=10.7 Hz), 4.46 (1H, d, J=12.9 Hz), 4.42 (1H, d, J=10.9 Hz), 4.39 (1H, d, J=12.9 Hz), 4.33 (1H, d, J=11.7 Hz), 4.30 (1H, d, J=11.7 Hz), 4.20 (1H, d, J=11.5 Hz), 4.41–4.34 (1H, m), 3.91–3.79 (4H, m), 3.77 (3H, s), 3.65–3.58 (3H, m), 3.50 (1H, dd, J=3.3, 10.1 Hz), 3.36–3.26 (5H, m), 2.81–2.76 (1H, m), 2.71–2.65 (1H, m), 2.52–1.48 (1H, m), 2.32–2.28 (2H, m), 2.04–1.93 (3H, m), 1.89–1.71 (4H, m), 1.60 (3H, s), 1.55–1.49 (1H, m), 1.07 (3H, d, J=7.0 Hz), 0.87 (9H, s), 0.06 (3H, s), −0.01 (3H); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 159.1 (C), 138.6 (C), 138.5 (C), 138.4 (C), 137.2 (C), 136.5 (C), 131.5 (CH×2), 131.4 (CH×2), 130.3 (C), 129.8 (CH×2), 129.23 (CH×2), 129.20 (CH×2), 128.3 (CH), 128.23 (CH×2), 128.21 (CH×2), 127.8 (CH×2), 127.7 (CH×2), 127.44 (CH), 127.36 (CH), 127.2 (CH), 121.7 (C), 121.4 (C), 121.2 (CH), 113.7 (CH×2), 86.1 (CH), 83.7 (CH), 82.7 (CH), 82.5 (CH), 81.4 (CH), 78.8 (CH), 78.2 (CH), 73.4 (CH), 73.1 (CH_2), 72.8 (CH), 72.6 (CH_2), 72.2 (CH_2), 71.3 (CH_2), 71.0 (CH_2), 70.6 (CH_2), 70.3 (CH_2), 55.2 (CH_3), 39.8 (CH_2), 38.9 (CH_2), 38.0 (CH_2), 32.0 (CH_2), 30.4 (CH_2), 28.4 (CH), 27.0 (CH_2), 26.2 (CH_3), 25.8 (CH_3×3), 17.9 (C), 12.3 (CH_3), −4.2 (CH_3), −4.9 (CH_3); IR (film), ν (cm^{-1}) 3482, 2924, 2853, 1611, 1512, 1487, 1452, 1361, 1300, 1248, 1172, 1098, 1011, 775, 697; HR-FDMS, calcd for $C_{66}H_{86}^{79}Br_2O_{10}Si$ $[M]^+$: 1224.4357, found: 1224.4386. **22**: a colorless oil; $[\alpha]_D^{25}$ −29.4 (c 0.190, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 7.42–7.39 (4H, m), 7.31–7.16 (14H, m), 7.05 (2H, d, J=8.3 Hz), 6.81 (2H, d, J=8.8 Hz), 5.78 (1H, dt, J=5.3, 10.6 Hz), 5.74 (1H, dt, J=5.0, 10.6 Hz), 5.58 (1H, t, J=6.2 Hz), 4.58 (1H, d, J=11.5 Hz), 4.51 (1H, d, J=11.3 Hz), 4.48 (1H, d, J=11.3 Hz), 4.46 (2H, d, J=11.5 Hz), 4.39 (1H, d, J=11.3 Hz), 4.35 (2H, d, J=11.3 Hz), 4.22 (1H, d, J=11.5 Hz), 4.23–4.21 (1H, m), 4.00–3.97 (1H, m), 3.85 (1H, t, J=11.3 Hz), 3.83 (1H, dt, J=10.1, 3.9 Hz), 3.78–3.74 (2H, m), 3.75 (3H, s), 3.64 (1H, dd, J=2.1, 9.6 Hz), 3.59 (1H, dd, J=2.4, 10.0 Hz), 3.50–3.43 (3H, m), 3.34 (1H, dt, J=8.4, 2.7 Hz), 3.28 (1H, dt, J=8.1, 3.9 Hz), 3.21–3.18 (1H, m), 2.83–2.77 (1H, m), 2.72–2.66 (1H, m), 2.48–2.45 (1H, m), 2.32–2.28 (2H, m), 2.05–2.01 (2H, m), 1.91–1.71 (5H, m), 1.68–1.61 (1H, m), 1.59 (3H, s), 1.06 (3H, d, J=6.8 Hz), 0.87 (9H, s), 0.07 (3H, s), 0.01 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 159.1 (C), 138.5 (C×3), 137.3 (C), 136.9 (C), 131.40 (CH×2), 131.37 (CH×2), 130.2 (C), 129.7 (CH×2), 129.3 (CH×2), 129.2 (CH×2), 128.3 (CH), 128.23 (CH×2), 128.16 (CH×2), 127.8 (CH×2), 127.7 (CH×2), 127.4 (CH), 127.3 (CH), 127.2 (CH), 121.7 (CH), 121.5 (C), 121.4 (C), 113.7 (CH×2), 86.2 (CH), 85.2 (CH), 83.5 (CH), 82.5 (CH), 81.9 (CH), 78.30 (CH), 78.25 (CH), 77.2 (CH), 73.7 (CH), 73.0 (CH_2), 72.6 (CH_2), 71.3 (CH_2), 71.2 (CH_2), 70.9 (CH_2), 70.7 (CH_2), 70.5 (CH_2), 55.2 (CH_3), 40.1 (CH_2), 38.5 (CH_2), 37.6 (CH_2), 31.9 (CH_2), 30.7 (CH_2), 28.3 (CH), 27.0 (CH_2), 26.0 (CH_3), 25.8 (CH_3×3), 17.9 (C), 12.4 (CH_3), −4.2 (CH_3), −4.8 (CH_3); IR (film), ν (cm^{-1}) 3505, 3026, 2926, 2856, 1612, 1513, 1487, 1454, 1381, 1349, 1249, 1172, 1098, 1012, 835, 805, 776, 698; HR-FDMS, calcd for $C_{66}H_{86}^{79}Br_2O_{10}Si$ $[M]^+$: 1224.4357, found: 1224.4386.

7.1.15. (1*R*,1′*R*,2′*E*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*,2‴*S*,3‴*R*,5‴*S*,7‴*S*,8‴*R*)-4′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-[7‴-(4-bromobenzyloxy)-8‴-(4-bromobenzyloxymethyl)-3‴-(4-methoxybenzyloxy)-5‴-methyloxocan-2‴-ylmethyl-2′-methylbut-2′-enyl]-1-methoxy-1-trifluoromethyl-1-phenylacetate {(+)MTPA ester}. To a solution of **22** (2.4 mg, 1.96 μmol) in DCM (0.05 ml) was added triethylamine (16.0 μl, 115 μmol), (+)MTPACl (10.0 μl, 53.4 μmol), and DMAP (3.0 mg, 24.6 μmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 7 h. Then, saturated aqueous $NaHCO_3$ (0.5 ml) was added and the organic layer was extracted with Et_2O (4×3 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=50 to 10) to give (+)MTPA ester of **22** (2.1 mg, 74%). (+)MTPA ester of **22**: a colorless oil; $[\alpha]_D^{26}$ −13.3 (c 0.110, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 7.52–7.49 (2H, m), 7.40–7.34 (7H, m), 7.25–7.16 (14H, m), 7.03 (2H, d, J=8.3 Hz), 6.81

(2H, d, J=8.8 Hz), 5.85 (1H, dd, J=5.9, 9.8 Hz), 5.83 (1H, t, J=6.4 Hz), 5.75 (1H, dt, J=5.1, 10.7 Hz), 5.70 (1H, dt, J=5.1, 10.7 Hz), 4.58 (1H, d, J=11.5 Hz), 4.59 (1H, d, J=11.7 Hz), 4.51 (1H, d, J=11.2 Hz), 4.49 (1H, d, J=12.2 Hz), 4.47 (1H, d, J=11.7 Hz), 4.44 (1H, d, J=11.2 Hz), 4.37 (1H, d, J=11.7 Hz), 4.29 (1H, d, J= 12.2 Hz), 4.23 (1H, d, J=11.2 Hz), 4.22 (1H, d, J=11.7 Hz), 4.16 (1H, d, J=11.2 Hz), 3.79 (1H, dt, J=8.3, 3.4 Hz), 3.75 (3H, s), 3.64 (1H, dt, J=8.1, 3.9 Hz), 3.56–3.53 (2H, m), 3.50 (3H, s), 3.49–3.46 (1H, m), 3.46–3.29 (4H, m), 3.25–3.23 (1H, m), 3.15 (1H, dt, J=2.4, 8.8 Hz), 2.71–2.66 (2H, m), 2.56 (1H, ddd, J=2.9, 10.7, 14.2 Hz), 2.29–2.17 (2H, m), 2.12 (1H, ddd, J=2.9, 9.8, 13.7 Hz), 1.97–1.93 (1H, m), 1.87–1.75 (3H, m), 1.70–1.57 (3H, m), 1.53 (3H, s), 1.05 (3H, d, J=6.8 Hz), 0.84 (9H, s), 0.03 (3H, s), −0.08 (3H, s); IR (film), ν (cm^{-1}) 2925, 2854, 1743, 1612, 1513, 1487, 1453, .1299, 1250, 1169, 1100, 1070, 1012, 836; HR-FDMS, calcd for $C_{76}H_{93}^{79}Br_2F_3O_{12}Si$ $[M]^+$: 1440.4755, found: 1440.4736.

7.1.16. (1*S*,1′*R*,2′*E*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*,2‴*S*,3‴*R*,5‴*S*, 7‴*S*,8‴*R*)-4′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydro-oxonin-2″-yl]-1′-[7‴-(4-bromobenzyloxy)-8‴-(4-bromo-benzyloxymethyl)-3‴-(4-methoxybenzyloxy)-5‴-methyl-oxocan-2‴-ylmethyl-2′-methylbut-2′-enyl]-1-methoxy-1-trifluoromethyl-1-phenylacetate {(−)MTPA ester}. To a solution of **22** (1.2 mg, 0.978 μmol) in DCM (0.05 ml) was added triethylamine (16.0 μl, 115 μmol), (−)MTPACl (10.0 μl, 52.8 μmol), and DMAP (3.0 mg, 24.6 μmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 7 h. Then, saturated aqueous $NaHCO_3$ (0.5 ml) was added and the organic layer was extracted with Et_2O (4×3 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=50 to 10) to give (−)MTPA ester of **22** (1.1 mg, 78%). (−)MTPA ester of **22**: a colorless oil; $[\alpha]_D^{26}$ −22.6 (c 0.055, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) 7.52–7.48 (2H, m), 7.41–7.33 (7H, m), 7.30–7.16 (14H, m), 7.02 (2H, d, J=8.3 Hz), 6.79 (2H, d, J=8.8 Hz), 5.85 (1H, dd, J=5.1, 10.0 Hz), 5.82 (1H, t, J=6.4 Hz), 5.75 (1H, dt, J=4.9, 10.7 Hz), 5.69 (1H, dt, J=4.9, 10.7 Hz), 4.60 (1H, d, J= 12.7 Hz), 4.52–4.46 (4H, m), 4.39–420 (4H, m), 4.15 (1H, d, J=11.7 Hz), 3.82–8.76 (1H, m), 3.73 (3H, s), 3.68 (1H, dd, J=2.0, 9.3 Hz), 3.64–3.60 (2H, m), 3.56 (1H, dd, J= 4.4, 9.3 Hz), 3.51 (3H, s), 3.49–3.29 (6H, m), 3.27–3.23 (2H, m), 2.72–2.65 (2H, m), 2.59–2.52 (1H, m), 2.33–2.18 (3H, m), 1.96–1.87 (4H, m), 1.81–1.64 (3H, m), 1.41 (3H, s), 1.08 (3H, d, J=6.8 Hz), 0.85 (9H, s), 0.04 (3H, s), −0.05 (3H, s); IR (film), ν (cm^{-1}) 2918, 2849, 1744, 1612, 1487, 1462, .1250, 1168, 1100, 836; HR-FDMS, calcd for $C_{76}H_{93}^{79}Br_2F_3O_{12}Si$ $[M]^+$: 1440.4755, found: 1440.4752.

7.1.17. (3*E*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,2″*S*,3″*R*,5″*S*,7″*S*,8″*R*)-5-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldi-methylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-[7″-(4-bromobenzyloxy)-8″-(4-bromobenzyloxy-methyl)-3″-(4-methoxybenzyloxy)-5″-methyloxocan-2″-yl]-3-methylpent-3-en-2-one (23). To a solution of **22** (7.0 mg, 5.70 μmol) in DCM (0.50 ml) were added $NaHCO_3$ (11.1 mg, 132 μmol) and DMPI (9.0 mg, 21.2 μmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 2.5 h. After the mixture was diluted with Et_2O (5 ml), saturated aqueous Na_2SO_3 (1 ml) was added and the aqueous layer was extracted with Et_2O (2×3 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=20) to give **23** (7.0 mg, ∼100%). **23**: a colorless oil; $[\alpha]_D^{19}$ −31.4 (c 0.195, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.42–7.16 (16H, m), 7.10–7.03 (4H, m), 6.85–6.79 (3H, m), 5.83 (2H, m), 4.60 (1H, d, J=11.4 Hz), 4.50 (1H, d, J=11.0 Hz), 4.47 (1H, d, J=11.9 Hz), 4.43 (2H, d, J=12.5 Hz), 4.34–4.24 (5H, m), 4.17 (1H, dt, J=2.9, 8.5 Hz), 3.83–3.77 (2H, m), 3.75 (3H, s), 3.64 (1H, dt, J=8.2, 3.4 Hz), 3.56 (1H, dd, J=3.8, 10.2 Hz), 3.52–3.40 (3H, m), 3.39–3.34 (2H, m), 3.29 (1H, dt, J=2.6, 8.5 Hz), 3.10 (1H, dd, J=2.9, 16.3 Hz), 2.86 (1H, dd, J=8.5, 16.3 Hz), 2.83–2.76 (1H, m), 2.74–2.69 (1H, m), 2.67–2.61 (1H, m), 2.50 (1H, ddd, J=2.8, 7.8, 17.1 Hz), 2.32 (1H, dt, J=13.8, 3.4 Hz), 2.05 (1H, dt, J=13.2, 3.4 Hz), 1.96–1.86 (3H, m), 1.78–1.72 (2H, m), 1.69 (3H, s), 1.06 (3H, d, J=6.8 Hz), 0.86 (9H, s), 0.054 (3H, s), −0.055 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 199.7 (C), 159.1 (C), 139.4 (CH), 138.6 (C), 138.3 (C), 138.1 (C), 137.58 (C), 137.56 (C), 131.3 (CH×4), 131.2 (CH×2), 130.4 (C), 129.3 (CH×2), 129.2 (CH×2), 129.1 (CH×2), 128.28 (CH×2), 128.26 (CH×2), 127.9 (CH×2), 127.7 (CH×2), 127.55 (CH), 127.53 (CH), 127.50 (CH), 121.18 (C), 121.15 (C), 113.7 (CH×2), 85.2 (CH), 84.20 (CH), 84.15 (CH), 82.0 (CH), 80.8 (CH), 78.5 (CH), 78.1 (CH), 73.3 (CH), 73.1 (CH_2), 72.4 (CH_2), 71.7 (CH_2), 71.3 (CH_2), 70.8 (CH_2), 70.5 (CH_2), 70.4 (CH_2), 55.2 (CH_3), 42.6 (CH_2), 40.9 (CH_2), 40.4 (CH_2), 32.1 (CH_2), 31.8 (CH_2), 28.0 (CH), 26.9 (CH_2), 26.8 (CH_3), 25.7 (CH_3×3), 17.9 (C), 11.9 (CH_3), −4.1 (CH_3), −4.9 (CH_3); IR (film), ν (cm^{-1}) 3026, 2925, 2856, 1665, 1612, 1513, 1487, 1462, 1453, 1361, 1301, 1249, 1207, 1172, 1099, 1012, 836, 805, 776, 698; HR-FDMS, calcd for $C_{66}H_{84}^{79}Br_2O_{10}Si$ $[M]^+$: 1222.4200, found: 1222.4165.

7.1.18. Reduction of 23. To a solution of **23** (2.8 mg, 2.29 μmol) in THF (0.80 ml) was added L-Selectride® (0.10 ml, 1.0 M in THF, 0.10 mmol) at −78 °C and the reaction mixture was stirred for 2 h. After that, 5 M NaOH (1 ml) and 30% aqueous H_2O_2 (1 ml) were added. The mixture was diluted with Et_2O (5 ml) and stirred at 25 °C for 15 h. Then, saturated aqueous $Na_2S_2O_3$ (0.5 ml) was added at 0 °C and the mixture was stirred for 30 min. The layers were separated and the aqueous layer was extracted with Et_2O (2×5 ml) and AcOEt (2×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=40 to 10) to give a mixture of **21** and **22** (2.8 mg, ∼100%, **21**:**22**≥13:1 from ^1H NMR).

7.1.19. (2*S*,3*S*,4*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,2″*S*,3″*R*,5″*S*, 7″*S*,8″*R*)-5-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethysilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-[7″-(4-bromobenzyloxy)-8″-(4-bromobenzyloxy-methyl)-3″-(4-methoxybenzyloxy)-5″-methyloxocan-2″-yl]-3-methyl-3,4-epoxypentan-2-ol (24). To a solution of

21 (287 mg, 0.234 mmol) in toluene (2.0 ml) were added $VO(acac)_2$ (5.6 mg, 0.0211 mmol) and TBHP (0.10 ml, 7.2 M in toluene, 0.720 mmol) at 0 °C. The reaction mixture was stirred for 2.5 h at the same temperature. Then, to the reaction mixture was added dimethylsulfide (0.1 ml) and the mixture was stirred for 0.5 h at 24 °C. After the mixture was diluted with Et_2O (10 ml), saturated aqueous $NaHCO_3$ (2 ml) was added and the aqueous layer was extracted with Et_2O (2×10 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=5 to 3) to give **24** (263 mg, 91%). **24**: a colorless oil; $[\alpha]_D^{23}$ −72.6 (*c* 0.370, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) 7.42–7.40 (4H, m), 7.32–7.21 (12H, m), 7.14 (2H, d, *J*=8.3 Hz), 7.04 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.8 Hz), 5.80 (1H, dt, *J*=4.9, 10.7 Hz), 5.76 (1H, dt, *J*=5.1, 10.7 Hz), 4.61 (1H, d, *J*=11.5 Hz), 4.49 (1H, d, *J*=11.6 Hz), 4.48–4.30 (6H, m), 4.29 (1H, d, *J*=11.5 Hz), 4.21 (1H, d, *J*=11.6 Hz), 4.11–4.08 (1H, m), 3.86–3.81 (2H, m), 3.75 (3H, s), 3.66–3.63 (2H, m), 3.59–3.55 (2H, m), 3.45 (1H, dd, *J*=4.8, 10.1 Hz), 3.41–3.33 (5H, m), 3.29–3.24 (2H, m), 2.83 (1H, ddd, *J*=2.8, 10.7, 13.2 Hz), 2.83 (1H, ddd, *J*=3.2, 10.7, 13.7 Hz), 2.34–2.24 (2H, m), 2.07–2.02 (1H, m), 1.93–1.57 (8H, m), 1.17 (3H, s), 1.06 (3H, d, *J*=6.8 Hz), 0.87 (9H, s), 0.12 (3H, s), 0.079 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 159.0 (C), 138.4 (C), 138.2 (C), 137.3 (C), 136.6 (C), 131.4 (CH×2), 131.3 (CH×2), 130.4 (C), 129.6 (CH×2), 129.3 (CH×2), 129.2 (CH×2), 128.3 (CH), 128.22 (CH×2), 128.19 (CH×2), 127.9 (CH×2), 127.7 (CH×2), 127.5 (CH), 127.4 (CH), 127.1 (CH), 121.6 (C), 121.3 (C), 113.6 (CH×2), 85.1 (CH), 84.0 (CH), 82.9 (CH), 82.1 (CH), 81.3 (CH), 78.6 (CH), 78.5 (CH), 73.3 (CH), 73.0 (CH_2), 72.5 (CH_2), 71.7 (CH_2), 71.6 (CH_2), 71.2 (CH_2), 70.8 (CH), 70.6 (CH_2), 70.3 (CH_2), 60.7 (C), 58.3 (CH), 55.1 (CH_3), 39.8 (CH_2), 39.0 (CH_2), 35.9 (CH_2), 32.1 (CH_2), 30.7 (CH_2), 28.3 (CH), 26.9 (CH_2), 26.3 (CH_3), 25.8 (CH_3×3), 17.9 (C), 12.9 (CH_3), −4.4 (CH_3), −4.5 (CH_3); IR (film), ν (cm^{-1}) 3490, 3028, 2931, 2858, 1612, 1514, 1488, 1454, 1360, 1302, 1251, 1211, 1173, 1011, 939, 836, 777, 698; HR-FDMS, calcd for $C_{66}H_{86}^{79}Br_2O_{11}Si$ $[M]^+$: 1240.4306, found: 1243.4263.

7.1.20. (2*S*,3*R*,5*Z*,8*S*,9*R*,2′*S*,3′*S*,4′*S*,2″*S*,3″*R*,5″*S*,7″*S*,8″*R*)-3-Benzyloxy-2-benzyloxymethyl-9-{5′-[7″-(4-bromobenzyloxy)-8″-(4-bromobenzyloxymethyl)-3″-(4-methoxybenzyloxy)-5″-methyloxocan-2″-yl]-3′-methyl-4′-triethylsilyloxy-2′,3′-epoxypentyl}-8-(*tert*-butyldimethylsilyloxy)-2,3,4,7,8,9-hexahydrooxonin (25). To a solution of **24** (4.5 mg, 3.62 μmol) in DCM (0.50 ml) were added 2,6-lutidine (13.0 μl, 112 μmol) and TESOTf (12.0 μl, 53.1 μmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (0.5 ml) was added and the aqueous layer was extracted with Et_2O (4×3 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=15 to 10) to give **25** (4.9 mg, ∼100%). **25**: a colorless oil; $[\alpha]_D^{23}$ −102.3 (*c* 1.07, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ (ppm) 7.43–7.37 (4H, m), 7.31–7.15 (14H, m), 7.05 (2H, d, *J*=8.4 Hz), 6.82 (2H, d, *J*=8.8 Hz), 5.79 (1H, dt, *J*=4.9, 10.3 Hz), 5.72 (1H, dt, *J*=4.9, 10.3 Hz), 4.58 (1H, d, *J*=11.5 Hz), 4.53–4.34 (6H, m), 4.33 (1H, d, *J*=11.0 Hz), 4.25 (1H, d, *J*=11.5 Hz), 4.24 (1H, d, *J*=11.7 Hz), 4.12 (1H, dt, *J*=8.4, 3.2 Hz), 3.87–3.82 (2H, m), 3.76 (3H, s), 3.66 (1H, dd, *J*=2.1, 9.3 Hz), 3.60–3.41 (7H, m), 3.38–3.33 (1H, m), 3.25 (1H, dt, *J*=2.9, 6.8 Hz), 3.11 (1H, dd, *J*=1.1, 9.1 Hz), 2.75 (1H, ddd, *J*=3.2, 10.3, 13.2 Hz), 2.63 (1H, ddd, *J*=3.0, 10.3, 13.6 Hz), 2.35–2.24 (2H, m), 2.05–1.98 (2H, m), 1.89–1.60 (6H, m), 1.52 (1H, ddd, *J*=2.4, 9.1, 14.8 Hz), 1.31 (3H, s), 1.06 (3H, d, *J*=6.8 Hz), 0.93 (9H, t, *J*=7.9 Hz), 0.87 (9H, s), 0.58 (6H, q, *J*=7.9 Hz), 0.11 (3H, s), 0.058 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 158.9 (C), 138.4 (C), 138.3 (C), 137.7 (C), 137.6 (C), 131.35 (CH×2), 131.29 (CH×2), 130.9 (C), 129.4 (CH×2), 129.1 (CH×2), 128.8 (CH×2), 128.3 (CH), 128.23 (CH×2), 128.19 (CH×2), 127.6 (CH×4), 127.43 (CH), 127.37 (CH), 127.0 (CH), 121.3 (C), 121.2 (C), 113.6 (CH×2), 85.4 (CH), 84.2 (CH), 82.6 (CH), 82.3 (CH), 80.7 (CH), 78.6 (CH), 78.4 (CH), 73.4 (CH), 73.1 (CH), 72.7 (CH_2), 72.6 (CH_2), 72.4 (CH_2), 71.4 (CH_2), 71.2 (CH_2), 70.23 (CH_2), 70.15 (CH_2), 60.7 (C), 58.2 (CH), 55.2 (CH_3), 39.6 (CH_2), 38.7 (CH_2), 38.3 (CH_2), 32.1 (CH_2), 30.7 (CH_2), 28.3 (CH), 26.8 (CH_2), 26.2 (CH_3), 25.9 (CH_3×3), 17.9 (C), 13.0 (CH_3), 7.1 (CH_3×3), 5.4 (CH_2×3), −4.4 (CH_3), −4.5 (CH_3); IR (film), ν (cm^{-1}) 3063, 2957, 1612, 1586, 1513, 1487, 1454, 1360, 1301, 1249, 1172, 1108, 938, 836, 745, 697; HR-FDMS, calcd for $C_{72}H_{100}^{79}Br_2O_{11}Si_2$ $[M]^+$: 1354.5171, found: 1354.5188.

7.1.21. (2*S*,3*R*,5*S*,7*S*,8*R*,2′*S*,3′*S*,4′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-2-{5′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-3′-methyl-2′-triethylsilyloxy-3′,4′-epoxypentyl}-8-(4-bromobenzyloxy)-9-(4-bromobenzyloxymethyl)-5-methyloxocan-3-ol (5). To a solution of **25** (267 mg, 0.197 mmol) in DCM–pH 7 buffer (10:1, v/v, 2.0 ml) was added DDQ (50.0 mg, 0.220 mmol) at 0 °C and the mixture was stirred for 50 min. Then, to the mixture was added DDQ (20.8 mg, 0.0916 mmol) at 0 °C and the stirring was continued for further 15 min. Saturated aqueous $NaHCO_3$ (2 ml) was added and the aqueous layer was extracted with Et_2O (4×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=10 to 4) to give **5** (236 mg, 97%). **5**: a colorless oil; $[\alpha]_D^{24}$ −22.3 (*c* 0.615, $CHCl_3$); ^1H NMR (300 MHz, CD_3COCD_3), δ (ppm) 7.53–7.45 (4H, m), 7.35–7.22 (14H, m), 5.76 (1H, dt, *J*=4.5, 10.7 Hz), 5.72 (1H, dt, *J*=4.5, 10.7 Hz), 4.66 (1H, d, *J*=11.9 Hz), 4.61 (1H, d, *J*=11.9 Hz), 4.59–4.47 (4H, m), 4.36 (2H, d, *J*=11.9 Hz), 4.19 (1H, dt, *J*=8.2, 3.3 Hz), 3.94 (1H, dd, *J*=2.2, 9.5 Hz), 3.79 (1H, ddd, *J*=2.2, 5.9, 8.3 Hz), 3.73 (1H, dt, *J*=2.2, 8.7 Hz), 3.70–3.62 (3H, m), 3.57–3.48 (3H, m), 3.46–3.34 (4H, m), 3.22 (1H, dd, *J*=1.1, 9.4 Hz), 2.73 (1H, ddd, *J*=3.3, 10.7, 13.3 Hz), 2.60 (1H, ddd, *J*=3.1, 10.7, 13.3 Hz), 2.48 (1H, ddd, *J*=1.1, 5.0, 14.7 Hz), 2.33 (1H, dt, *J*=13.3, 4.5 Hz), 2.07–1.71 (5H, m), 1.64 (1H, ddd, *J*=2.2, 8.7, 13.9 Hz), 1.50 (1H, ddd, *J*=2.2, 8.7, 14.7 Hz), 1.13 (3H, s), 1.03 (3H, d, *J*=7.0 Hz), 0.96 (9H, t, *J*=7.9 Hz), 0.89 (9H, s), 0.66 (6H, q, *J*=7.9 Hz), 0.16 (3H, s), 0.12 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.31 (C), 138.25 (C), 137.32

(C), 137.29 (C), 131.4 (CH×4), 129.4 (CH×2), 129.2 (CH), 128.2 (CH×4), 128.1 (CH), 127.6 (CH×4), 127.44 (CH), 127.41 (CH), 127.1 (CH), 121.37 (C), 121.35 (C), 85.2 (CH), 85.1 (CH), 84.9 (CH), 84.2 (CH), 78.9 (CH), 78.7 (CH), 75.2 (CH), 74.1 (CH), 73.1 (CH), 72.8 (CH_2), 72.6 (CH_2), 72.4 (CH_2), 72.0 (CH_2), 71.1 (CH_2), 70.4 (CH_2), 60.9 (C), 59.5 (CH), 47.3 (CH_2), 41.2 (CH_2), 41.1 (CH_2), 32.1 (CH_2), 30.5 (CH_2), 28.0 (CH), 27.2 (CH_3), 26.7 (CH_2), 25.8 (CH_3×3), 17.9 (C), 11.9 (CH_3), 6.9 (CH_3×3), 5.0 (CH_2×3), −4.3 (CH_3), −4.5 (CH_3); IR (film), ν (cm^{-1}) 3447, 3063, 3026, 2927, 1593, 1487, 1453, 1360, 1311, 1250, 1210, 1095, 1012, 938, 836, 776, 744, 697; HR-FDMS, calcd for $C_{64}H_{92}^{79}Br_2O_{10}Si_2$ $[M]^+$: 1234.4596, found: 1234.4553.

7.1.22. (1*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,1″*S*,3″*R*,4″*S*,6″*S*,8″*R*,10″*R*,11″*S*)-2-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-{4″-(4-bromobenzyloxy)-3″-(4-bromobenzyloxymethyl)-6″,10″-dimethyl-11″-triethylsilyloxy-2″,9″-dioxabicyclo[6.4.0]dodecan-10″-yl}ethanol (26). To a solution of **5** (233 mg, 0.188 mmol) in DCM (3.0 ml) was added CSA (4.3 mg, 0.0185 mmol) at 0 °C and the mixture was stirred for 25 min. Then, Et_3N (0.1 ml) was added at 0 °C and the mixture was warmed to 25 °C and stirred for 30 min. The solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=7 to 2) to give **26** (186 mg, 80%). **26**: a colorless oil; $[\alpha]_D^{23}$ +12.2 (*c* 0.495, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3COCD_3), δ (ppm) 7.52–7.47 (4H, m), 7.37–7.23 (14H, m), 5.70–5.64 (2H, m), 4.65 (1H, d, *J*=11.4 Hz), 4.63 (1H, d, *J*=12.1 Hz), 4.53–4.49 (3H, m), 4.61 (1H, d, *J*=11.4 Hz), 4.38 (1H, d, *J*=11.4 Hz), 4.34 (1H, d, *J*=11.7 Hz), 4.11–4.07 (1H, m), 4.04–4.01 (1H, m), 3.92 (1H, t, *J*=2.9 Hz), 3.86–3.81 (1H, m), 3.78–3.74 (2H, m), 3.70–3.67 (1H, m), 3.64–3.52 (4H, m), 3.44–3.35 (3H, m), 3.28 (1H, d, *J*=4.0 Hz), 2.84–2.63 (2H, m), 2.35 (1H, dt, *J*=12.8, 4.4 Hz), 2.11–2.09 (2H, m), 1.93–1.78 (5H, m), 1.69–1.67 (2H, m), 1.59–1.49 (1H, m), 1.09 (3H, s), 1.03 (3H, d, *J*=7.0 Hz), 0.93 (9H, t, *J*=8.1 Hz), 0.89 (9H, s), 0.60 (6H, q, *J*=8.1 Hz), 0.11 (3H, s), 0.10 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.5 (C), 138.3 (C), 137.5 (C), 137.3 (C), 131.4 (CH×2), 131.3 (CH×2), 129.31 (CH×2), 129.30 (CH×2), 129.0 (CH), 128.2 (CH×4), 127.6 (CH×4), 127.4 (CH), 127.3 (CH), 126.2 (CH), 121.4 (C), 121.3 (C), 85.4 (CH), 84.5 (CH), 80.9 (CH), 79.1 (CH), 78.6 (CH), 77.6 (C), 77.1 (CH), 76.6 (CH), 73.4 (CH), 73.1 (CH_2), 72.5 (CH_2), 71.7 (CH_2), 71.6 (CH), 71.2 (CH_2), 70.5 (CH_2), 70.1 (CH), 69.4 (CH_2), 45.4 (CH_2), 40.2 (CH_2), 36.1 (CH_2), 34.6 (CH_2), 29.2 (CH_2), 28.3 (CH), 26.8 (CH_3), 26.3 (CH_2), 25.9 (CH_3×3), 18.0 (C), 14.3 (CH_3), 7.0 (CH_3×3), 5.0 (CH_2×3), −4.6 (CH_3), −4.7 (CH_3); IR (film), ν (cm^{-1}) 3502, 3030, 2926, 1594, 1488, 1456, 1362, 1256, 1206, 1099, 1012, 885, 836, 775, 735, 697; HR-FDMS, calcd for $C_{64}H_{92}^{79}Br_2O_{10}Si_2$ $[M]^+$: 1234.4596, found: 1234.4587.

7.1.23. (1*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,1″*S*,3″*R*,4″*S*,6″*S*,8″*R*,10″*R*,11″*S*)-2-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-{4″-(4-bromobenzyloxy)-3″-(4-bromobenzyloxymethyl)-6″,10″-dimethyl-11″-triethylsilyloxy-2″,9″-dioxabicyclo[6.4.0]dodecan-10″-yl}ethyl trifluoroacetate (29). To a solution of **26** (10.8 mg, 8.23 μmol) in pyridine (0.60 ml) was added trifluoroacetic anhydride (20.0 μl, 144 μmol) at 0 °C and the mixture was stirred for 1 h. Then, H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=100 to 40) to give **29** (11.0 mg, ~100%). **29**: a colorless oil; $[\alpha]_D^{25}$ −2.10 (*c* 0.660, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3/C_6D_6$=1:1), δ (ppm) 7.35–7.31 (6H, m), 7.26–7.11 (8H, m), 7.03 (2H, d, *J*=8.1 Hz), 6.90 (2H, d, *J*=8.1 Hz), 5.84–5.76 (2H, m), 5.67 (1H, t, *J*=6.1 Hz), 4.63 (1H, d, *J*=12.4 Hz), 4.56 (1H, d, *J*=12.4 Hz), 4.47 (1H, d, *J*=11.6 Hz), 4.32 (1H, d, *J*=12.6 Hz), 4.31 (1H, d, *J*=11.5 Hz), 4.29 (1H, d, *J*=12.6 Hz), 4.20 (1H, d, *J*=11.6 Hz), 3.99 (1H, d, *J*=11.5 Hz), 3.93 (1H, q, *J*=6.1 Hz), 3.88 (1H, dd, *J*=1.7, 10.0 Hz), 3.85–3.83 (1H, m), 3.80–3.70 (4H, m), 3.56 (1H, dd, *J*=2.0, 9.5 Hz), 3.54–3.48 (2H, m), 3.45 (1H, dt, *J*=2.6, 9.9 Hz), 3.34 (1H, dd, *J*=6.6, 9.5 Hz), 3.24 (1H, dt, *J*=2.1, 9.4 Hz), 2.84–2.79 (1H, m), 2.74–2.69 (1H, m), 2.33 (1H, dt, *J*=14.9, 6.1 Hz), 2.29–2.24 (1H, m), 2.18 (1H, dt, *J*=13.7, 4.1 Hz), 2.09 (1H, dt, *J*=13.7, 4.5 Hz), 1.86–1.78 (5H, m), 1.64–1.50 (2H, m), 1.16 (3H, s), 0.99 (3H, d, *J*=7.1 Hz), 0.93 (9H, t, *J*=7.9 Hz), 0.92 (9H, s), 0.59 (6H, q, *J*=7.9 Hz), 0.084 (3H, s), 0.080 (3H, s); IR (film), ν (cm^{-1}) 3032, 2927, 1792, 1593, 1487, 1456, 1374, 1336, 1217, 1164, 1098, 1012, 960, 836, 775, 735, 697; HR-FDMS, calcd for $C_{66}H_{91}^{79}Br_2F_3O_{11}Si_2$ $[M]^+$: 1330.4419, found: 1330.4448.

7.1.24. (1*S*,3*R*,4*S*,6*S*,8*R*,10*S*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{2′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-hydroxyethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-yl trifluoroacetate (30). To a solution of **29** (11.0 mg, 8.23 μmol) in THF–H_2O (4:1, v/v, 0.75 ml) was added TFA (15.0 μl) at 22 °C and the mixture was stirred for 2 d. Then, the solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30 to 7) to give **30** and recovery of **29**. For this recovered starting material, the above procedure was repeated twice to give **30** (6.3 mg, 63%, after three cycles). **30**: a colorless oil; $[\alpha]_D^{19}$ +31.3 (*c* 0.265, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.37–7.28 (6H, m), 7.20–7.05 (8H, m), 6.92 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.3 Hz), 5.87 (1H, dt, *J*=6.1, 10.1 Hz), 5.80 (1H, dt, *J*=6.1, 10.1 Hz), 4.52 (1H, d, *J*=11.5 Hz), 4.33 (2H, d, *J*=11.7 Hz), 4.24 (1H, d, *J*=11.5 Hz), 4.20–4.16 (2H, m), 4.13 (1H, d, *J*=12.2 Hz), 4.07 (1H, d, *J*=11.7 Hz), 3.98–3.91 (2H, m), 3.86 (1H, d, *J*=12.2 Hz), 3.82–3.80 (1H, m), 3.75 (1H, dd, *J*=1.8, 10.1 Hz), 3.70–3.66 (1H, m), 3.65 (1H, dd, *J*=3.3, 10.1 Hz), 3.61–3.47 (4H, m), 3.32 (1H, dd, *J*=7.1, 9.8 Hz), 3.26 (1H, dt, *J*=2.9, 8.5 Hz), 2.78–2.69 (2H, m), 2.44 (1H, ddd, *J*=3.0, 4.6, 14.9 Hz), 2.54–2.16 (2H, m), 2.09–2.06 (1H, m), 1.93–1.74 (5H, m), 1.64 (1H, dt, *J*=14.1, 9.8 Hz), 1.55–1.47 (1H, m), 1.04 (3H, s), 1.03 (3H, d, *J*=6.6 Hz), 0.96 (9H, s), 0.036 (3H, s), 0.032 (3H, s); IR (film), ν (cm^{-1}) 3476, 3027, 2931, 1784, 1593, 1488, 1454, 1387, 1167, 1098, 939, 879, 837, 754, 698; HR-FDMS, calcd for $C_{60}H_{77}^{79}Br_2F_3O_{11}Si$ $[M]^+$: 1216.3554, found: 1216.3586.

7.1.25. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{2′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-hydroxyethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-ol (31). To a solution of **26** (60.2 mg, 48.7 μmol) in MeOH–DCM (4:1, v/v, 1.0 ml) was added PPTS (11.3 mg, 45.0 μmol) at 24 °C and the mixture was stirred for 50 min. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml) and AcOEt (5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=7 to 3) to give **31** (54.7 mg, ~100%). **31**: a colorless oil; $[\alpha]_D^{19}$ +14.5 (*c* 0.545, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.33–7.26 (5H, m), 7.20–7.05 (9H, m), 7.00 (2H, d, *J*=8.1 Hz), 6.84 (2H, d, *J*=8.3 Hz), 5.96 (1H, dt, *J*=6.0, 10.5 Hz), 5.87 (1H, dt, *J*=5.6, 10.5 Hz), 4.40 (1H, d, *J*=11.8 Hz), 4.35 (1H, d, *J*=11.8 Hz), 4.24 (1H, d, *J*=12.7 Hz), 4.21–4.13 (5H, m), 4.12 (1H, d, *J*=11.8 Hz), 3.97–3.88 (3H, m), 3.87–3.82 (2H, m), 3.69 (1H, dt, *J*=9.0, 3.3 Hz), 3.67–3.65 (1H, m), 3.63–3.56 (3H, m), 3.54 (1H, dd, *J*=2.3, 10.0 Hz), 3.43 (1H, dd, *J*=6.6, 10.0 Hz), 3.34 (1H, dt, *J*=2.7, 8.5 Hz), 3.26–3.20 (1H, m), 2.87–2.75 (2H, m), 2.47 (1H, dt, *J*=13.4, 3.9 Hz), 2.27 (1H, ddd, *J*=3.3, 5.6, 13.8 Hz), 2.19 (1H, dt, *J*=13.2, 6.0 Hz), 2.07–2.03 (1H, m), 2.00–1.79 (5H, m), 1.60 (1H, ddd, *J*=6.1, 8.5, 14.6 Hz), 1.09 (3H, s), 1.05 (3H, d, *J*=7.1 Hz), 0.99 (9H, s), 0.13 (3H, s), 0.086 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.4 (C), 137.9 (C), 137.5 (C), 137.3 (C), 131.41 (CH×2), 131.38 (CH×2), 129.3 (CH×4), 129.1 (CH), 128.33 (CH×2), 128.29 (CH×2), 127.8 (CH×2), 127.7 (CH×2), 127.65 (CH), 127.56 (CH), 126.1 (CH), 121.4 (C), 121.3 (C), 85.23 (CH), 85.15 (CH), 80.3 (CH), 79.8 (CH), 78.8 (CH), 77.2 (C), 76.3 (CH), 76.1 (CH), 74.7 (CH), 73.3 (CH), 73.2 (CH_2), 72.5 (CH_2), 71.7 (CH_2), 71.3 (CH_2), 70.5 (CH_2), 70.3 (CH), 68.5 (CH_2), 45.2 (CH_2), 40.3 (CH_2), 34.8 (CH_2), 33.9 (CH_2), 29.5 (CH_2), 28.3 (CH), 26.9 (CH_3), 26.4 (CH_2), 25.8 (CH_3×3), 18.0 (C), 15.8 (CH_3), −4.6 (CH_3), −4.7 (CH_3); IR (film), ν (cm^{-1}) 3474, 3027, 2926, 2854, 1593, 1487, 1453, 1360, 1255, 1205, 1096, 1011, 939, 836, 804, 775, 751, 697; HR-FDMS, calcd for $C_{58}H_{78}^{79}Br_2O_{10}Si$ $[M]^+$: 1120.3731, found: 1120.3763.

7.1.26. (1*R*,3*R*,4*S*,6*R*,8*S*,10*S*,12*R*,13*S*,15*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*)-4-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-13-(4-bromobenzyloxy)-12-(4-bromobenzyloxymethyl)-6-(4-methoxyphenyl)-3,15-dimethyl-2,5,7,11-tetraoxatricyclo[8.6.0.0^{3,8}]hexadecane (32a) and (1*R*,3*R*,4*S*,6*S*,8*S*,10*S*,12*R*,13*S*,15*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*)-4-[8′-benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-13-(4-bromobenzyloxy)-12-(4-bromobenzyloxymethyl)-6-(4-methoxyphenyl)-3,15-dimethyl-2,5,7,11-tetraoxatricyclo[8.6.0.0^{3,8}]hexadecane (32b). To a solution of **31** (13.2 mg, 11.8 μmol) in benzene (2.0 ml) were added *p*-anisaldehyde (30.0 μl, 247 μmol) and PPTS (3.2 mg, 12.7 μmol). The reaction mixture was heated to 80 °C and stirred for 3 h. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30 to 7) to give **32** (14.6 mg, ~100%, a 1:1 mixture of **32a** and **32b** from ^1H NMR). This mixture of **32a** and **32b** was separated by HPLC (hexane/AcOEt=7) to give **32a** as less-polar eluate and **32b** as polar eluate. **32a**: a colorless oil; $[\alpha]_D^{24}$ +6.71 (*c* 0.235, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.51–7.49 (4H, m), 7.30–7.05 (12H, m), 6.97 (2H, d, *J*=8.3 Hz), 6.90 (2H, d, *J*=8.8 Hz), 6.82 (2H, d, *J*=8.5 Hz), 6.07 (1H, s), 6.04 (1H, dt, *J*=5.1, 10.6 Hz), 5.99 (1H, dt, *J*=5.1, 10.6 Hz), 4.72 (1H, d, *J*=11.8 Hz), 4.54 (1H, dd, *J*=4.1, 8.8 Hz), 4.52 (1H, d, *J*=11.8 Hz), 4.49 (1H, dt, *J*=8.7, 3.2 Hz), 4.45 (1H, d, *J*=11.8 Hz), 4.21 (1H, d, *J*=12.3 Hz), 4.19 (1H, d, *J*=11.8 Hz), 4.18 (1H, d, *J*=11.5 Hz), 4.13 (1H, d, *J*=12.3 Hz), 3.98 (1H, dt, *J*=8.5, 3.2 Hz), 3.93 (1H, t, *J*=4.8 Hz), 3.88 (1H, d, *J*=11.5 Hz), 3.86–3.83 (2H, m), 3.76 (1H, dt, *J*=4.8, 9.0 Hz), 3.64–3.53 (4H, m), 3.43–3.40 (1H, m), 3.38 (1H, dd, *J*=6.6, 9.8 Hz), 3.32 (3H, s), 3.27 (1H, dt, *J*=2.4, 8.7 Hz), 3.07 (1H, ddd, *J*=3.2, 10.6, 13.5 Hz), 2.80 (1H, ddd, *J*=3.2, 10.6, 13.7 Hz), 2.46 (1H, dt, *J*=13.8, 4.8 Hz), 2.31–2.25 (1H, m), 2.17–2.14 (3H, m), 1.98–1.75 (5H, m), 1.56 (1H, ddd, *J*=6.1, 8.7, 14.6 Hz), 1.18 (3H, s), 1.05 (3H, d, *J*=6.6 Hz), 1.04 (9H, s), 0.22 (3H, s), 0.091 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 160.4 (C), 139.52 (C), 139.46 (C), 138.2 (C), 138.0 (C), 132.2 (C), 131.6 (CH×4), 129.4 (CH×2), 129.2 (CH×2), 128.6 (CH×2), 128.4 (CH×2), 127.5 (CH×2), 121.6 (C), 121.5 (C), 113.8 (CH×2), 98.6 (CH), 85.1 (CH), 85.0 (CH), 84.9 (CH), 81.0 (CH), 79.8 (CH), 78.7 (CH), 77.4 (CH), 76.1 (C), 74.8 (CH), 73.4 (CH), 73.3 (CH_2), 72.6 (CH_2), 72.4 (CH_2), 72.0 (CH_2), 71.42 (CH), 71.35 (CH_2), 70.3 (CH_2), 54.8 (CH_3), 45.2 (CH_2), 33.0 (CH_2), 32.7 (CH_2), 31.9 (CH_2), 30.2 (CH_2), 28.9 (CH), 27.5 (CH_2), 27.0 (CH_3), 26.2 (CH_3×3), 18.2 (C), 16.3 (CH_3), −3.9 (CH_3), −4.3 (CH_3) (The signals of 10 carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3063, 3026, 2925, 2853, 1614, 1588, 1513, 1487, 1453, 1360, 1301, 1249, 1213, 1170, 1095, 1011, 939, 833, 804, 776, 734, 697; HR-FDMS, calcd for $C_{66}H_{84}^{79}Br_2O_{11}Si$ $[M]^+$: 1238.4150, found: 1238.4125. **32b**: a colorless oil; $[\alpha]_D^{23}$ −8.95 (*c* 0.200, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.75 (2H, d, *J*=8.8 Hz), 7.35–7.21 (8H, m), 7.18–7.04 (6H, m), 6.98 (2H, d, *J*=8.5 Hz), 6.87 (2H, d, *J*=8.8 Hz), 6.83 (2H, d, *J*=8.5 Hz), 5.96 (1H, dt, *J*=5.4, 10.2 Hz), 5.90 (1H, s), 5.89 (1H, dt, *J*=5.4, 10.2 Hz), 4.50 (1H, dd, *J*=2.8, 11.3 Hz), 4.47 (1H, d, *J*=11.8 Hz), 4.43 (1H, d, *J*=11.7 Hz), 4.42 (1H, d, *J*=11.8 Hz), 4.22–4.14 (5H, m), 3.92–3.87 (1H, m), 3.86 (1H, d, *J*=12.0 Hz), 3.73–3.58 (6H, m), 3.56–3.50 (3H, m), 3.40 (1H, dd, *J*=6.8, 10.0 Hz), 3.31–3.26 (4H, m), 2.81 (1H, ddd, *J*=2.6, 10.2, 13.6 Hz), 2.71 (1H, ddd, *J*=2.3, 10.2, 12.7 Hz), 2.43 (1H, ddd, *J*=2.8, 4.6, 13.9 Hz), 2.31–2.25 (2H, m), 2.09 (1H, dt, *J*=13.6, 5.4 Hz), 2.02–1.90 (4H, m), 1.88–1.81 (2H, m), 1.57–1.49 (1H, m), 1.04 (3H, s), 1.03 (3H, d, *J*=7.1 Hz), 1.00 (9H, s), 0.15 (3H, s), 0.084 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 160.5 (C), 139.3 (C), 139.2 (C), 138.2 (C), 138.1 (C), 132.1 (C), 131.7 (CH×2), 131.6 (CH×2), 129.22 (CH×2), 129.16 (CH×2), 128.54 (CH×2), 128.50 (CH×2), 128.45 (CH×2), 127.5 (CH), 121.5 (C), 121.4 (C), 113.7 (CH×2), 97.0 (CH), 84.8 (CH), 84.1 (CH), 83.8 (CH), 80.3 (CH), 79.7 (CH),

78.8 (CH), 75.3 (CH), 75.0 (CH), 74.8 (CH), 74.3 (C), 74.2 (CH), 73.3 (CH_2), 72.8 (CH_2), 72.7 (CH_2), 72.4 (CH_2), 71.3 (CH_2), 70.2 (CH_2), 54.8 (CH_3), 45.1 (CH_2), 33.9 (CH_2), 32.3 (CH_2), 31.2 (CH_2), 30.2 (CH_2), 28.9 (CH), 27.6 (CH_2), 26.8 (CH_3), 26.2 ($CH_3\times3$), 18.2 (C), 16.5 (CH_3), −4.08 (CH_3), −4.14 (CH_3) (The signals of seven carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3063, 3026, 2927, 2855, 1614, 1589, 1514, 1487, 1453, 1360, 1301, 1249, 1214, 1170, 1098, 1011, 940, 834, 804, 775, 753, 697; HR-FDMS, calcd for $C_{66}H_{84}^{79}Br_2O_{11}Si$ $[M]^+$: 1238.4150, found: 1238.4172.

7.1.27. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{2′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-(4-methoxybenzyloxy)ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-ol (33a) and (1*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,1″*S*,3″*R*,4″*S*,6″*S*,8″*R*,10″*S*,11″*S*)-2-[8′-benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-{4″-(4-bromobenzyloxy)-3″-(4-bromobenzyloxymethy)-11″-(4-methoxybenzyloxy)-6″,10″-dimethyl-2″,9″-dioxabicyclo[6.4.0]dodecan-10″-yl}ethanol (33b).

7.1.27.1. Reaction of 32a. To a solution of **32a** (3.8 mg, 3.06 μmol) in DCM (0.50 ml) was added DIBAL (0.10 ml, 0.94 M in hexane, 94.0 μmol) at −30 °C and the mixture was stirred for 1.5 h. Then, MeOH (0.1 ml) and saturated aqueous potassium sodium tartrate (1 ml) were added. The mixture was diluted with Et_2O (5 ml) and stirred at 25 °C for 2 h. The layers were separated and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=7 to 5) to give **33** (3.8 mg, ∼100%, **33a**:**33b**=5:1 from ^1H NMR).

7.1.27.2. Reaction of 32b. To a solution of **32b** (4.0 mg, 3.22 μmol) in DCM (0.50 ml) was added DIBAL (0.10 ml, 0.94 M in hexane, 94.0 μmol) at −20 °C and the mixture was stirred for 2 h. Then, MeOH (0.1 ml) and saturated aqueous potassium sodium tartrate (1 ml) were added. The mixture was diluted with Et_2O (5 ml) and stirred at 25 °C for 3 h. The layers were separated and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=7 to 4) to give **33** (4.0 mg, ∼100%, **33b**:**33a**>20:1 from ^1H NMR). **33a**: a colorless oil; $[\alpha]_D^{21}$ −3.02 (*c* 0.150, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.40 (2H, d, *J*=8.5 Hz), 7.35 (2H, d, *J*=7.1 Hz), 7.29 (2H, d, *J*=8.3 Hz), 7.25–7.05 (8H, m), 6.98 (2H, d, *J*=8.5 Hz), 6.84 (2H, d, *J*=8.3 Hz), 6.81 (2H, d, *J*=8.5 Hz), 6.07 (1H, dt, *J*=5.1, 10.7 Hz), 5.97 (1H, dt, *J*=5.1, 10.7 Hz), 5.20 (1H, d, *J*=10.4 Hz), 4.73 (1H, d, *J*=10.4 Hz), 4.48 (1H, d, *J*=11.7 Hz), 4.46 (1H, d, *J*=11.8 Hz), 4.41 (1H, dt, *J*=7.8, 3.9 Hz), 4.31 (1H, dd, *J*=2.4, 10.2 Hz), 4.30 (1H, d, *J*=11.8 Hz), 4.26–4.16 (4H, m), 4.10–4.04 (1H, m), 3.95–3.92 (1H, m), 3.91 (1H, d, *J*=12.2 Hz), 3.84 (1H, dt, *J*=9.5, 3.4 Hz), 3.73–3.69 (2H, m), 3.65–3.64 (2H, m), 3.57 (1H, dd, *J*=2.4, 10.0 Hz), 3.55–3.49 (2H, m), 3.45 (1H, dd, *J*=6.3, 10.0 Hz), 3.34 (1H, dt, *J*=2.8, 8.8 Hz), 3.27 (3H, s), 2.96–2.89 (1H, m), 2.88–2.81 (1H, m), 2.47 (1H, dt, *J*=13.4, 4.0 Hz), 2.34 (1H, ddd, *J*=3.4, 5.1, 13.8 Hz), 2.14–1.79 (8H, m), 1.67 (1H, ddd, *J*=5.6, 8.9, 14.4 Hz), 1.07–1.05 (6H, m), 1.01 (9H, s), 0.18 (3H, s), 0.038 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 159.8 (C), 139.3 (C), 139.1 (C), 138.2 (C), 138.1 (C), 131.7 (CH×2), 131.64 (CH×2), 131.60 (C), 130.1 (CH×2), 129.3 (CH×4), 129.2 (CH), 128.9 (CH), 128.54 (CH×2), 128.50 (CH×2), 121.6 (C), 121.5 (C), 114.1 (CH×2), 85.5 (CH), 85.1 (CH), 84.6 (CH), 80.8 (CH), 79.8 (CH×2), 78.5 (C), 78.1 (CH), 75.3 (CH), 74.3 (CH), 73.2 (CH_2), 72.7 (CH_2), 72.4 ($CH_2\times2$), 71.33 (CH_2), 71.30 (CH_2), 70.9 (CH), 70.3 (CH_2), 54.7 (CH_3), 45.9 (CH_2), 36.1 (CH_2), 32.5 (CH_2), 31.9 (CH_2), 30.4 (CH_3), 30.2 (CH_2), 28.9 (CH), 27.5 (CH_2), 26.9 (CH_3), 26.2 ($CH_3\times3$), 18.2 (C), −4.2 (CH_3), −4.3 (CH_3) (The signals of six carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3420, 3063, 3026, 2926, 2856, 1612, 1586, 1514, 1487, 1453, 1360, 1301, 1249, 1213, 1173, 1097, 1012, 939, 834, 804, 776, 750, 697; HR-FDMS, calcd for $C_{66}H_{86}^{79}Br_2O_{11}Si$ $[M]^+$: 1240.4306, found: 1240.4355. **33b**: a colorless oil; $[\alpha]_D^{20}$ +15.6 (*c* 0.150, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.36–7.22 (8H, m), 7.18–7.04 (8H, m), 6.94 (2H, d, *J*=8.5 Hz), 6.83 (2H, d, *J*=8.3 Hz), 6.76 (2H, d, *J*=8.8 Hz), 5.93 (1H, dt, *J*=6.2, 10.4 Hz), 5.85 (1H, dt, *J*=5.7, 10.4 Hz), 4.66–4.63 (1H, m), 4.60 (1H, d, *J*=12.1 Hz), 4.51 (2H, s), 4.38 (1H, d, *J*=11.7 Hz), 4.24 (1H, d, *J*=12.1 Hz), 4.22–4.17 (3H, m), 4.15 (1H, d, *J*=11.7 Hz), 4.06–4.03 (1H, m), 3.95–3.90 (3H, m), 3.87 (1H, d, *J*=11.7 Hz), 3.84–3.79 (2H, m), 3.73–3.68 (3H, m), 3.65 (1H, dt, *J*=2.7, 9.6 Hz), 3.61 (1H, d, *J*=2.7 Hz), 3.59 (1H, dd, *J*=2.3, 9.9 Hz), 3.41 (1H, dd, *J*=7.0, 9.9 Hz), 3.32–3.27 (4H, m, H20), 2.90–2.79 (2H, m), 2.45 (1H, ddd, *J*=3.3, 4.5, 13.8 Hz), 2.26 (1H, ddd, *J*=4.1, 5.7, 13.9 Hz), 2.25–2.17 (2H, m), 2.03–1.74 (6H, m), 1.62 (1H, ddd, *J*=5.5, 8.8, 14.8 Hz), 1.27 (3H, s), 1.08 (3H, d, *J*=6.6 Hz), 0.96 (9H, s), 0.080 (3H, s), 0.054 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 159.6 (C), 139.4 (C), 139.1 (C), 138.2 (C), 138.0 (C), 131.73 (C), 131.66 (CH×2), 131.6 (CH×2), 129.3 (CH×2), 129.2 (CH×4), 128.44 (CH×2), 128.37 (CH×2), 127.54 (CH), 127.46 (CH), 126.8 (CH), 121.6 (C), 121.5 (C), 114.1 (CH×2), 86.0 (CH), 85.4 (CH), 81.3 (CH), 80.0 (CH), 79.9 (CH), 78.1 (C), 77.3 (CH), 76.9 (CH), 76.6 (CH), 74.3 (CH), 73.3 (CH_2), 73.0 (CH_2), 72.5 (CH_2), 72.1 (CH), 71.42 (CH_2), 71.37 (CH_2), 70.3 (CH_2), 69.4 (CH_2), 54.7 (CH_3), 45.5 (CH_2), 35.0 (CH_2), 32.0 (CH_2), 30.2 (CH_2), 29.8 (CH_2), 29.0 (CH), 26.9 (CH_2), 26.7 (CH_3), 26.1 ($CH_3\times3$), 18.2 (C), 15.0 (CH_3), −4.5 (CH_3), −4.6 (CH_3) (The signals of five carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3509, 3067, 3032, 2928, 2859, 1616, 1588, 1514, 1488, 1454, 1406, 1361, 1302, 1250, 1207, 1173, 1099, 1012, 940, 836, 805, 775, 735, 698; HR-FDMS, calcd for $C_{66}H_{86}^{79}Br_2O_{11}Si$ $[M]^+$: 1240.4306, found: 1240.4371.

7.1.28. (1*S*,3*R*,4*S*,6*S*,8*R*,10*S*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{2′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-(4-methoxybenzyloxy)ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-one (34). To a solution

of **33a** (3.0 mg, 2.41 μmol) in DCM (0.80 ml) was added DMPI (9.5 mg, 22.4 μmol) at 24 °C and the reaction mixture was stirred for 2 h. After the mixture was diluted with Et_2O (5 ml), saturated aqueous Na_2SO_3 (1 ml) was added and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=15 to 5) to give **34** (2.1 mg, 70%). **34**: a colorless oil; $[\alpha]_D^{21}$ −14.4 (*c* 0.165, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.42–7.28 (8H, m), 7.25–7.09 (8H, m), 6.94 (2H, d, *J*=8.5 Hz), 6.83–6.80 (4H, m), 6.07 (1H, dt, *J*=5.1, 10.6 Hz), 5.97 (1H, dt, *J*=5.1, 10.6 Hz), 4.91 (2H, s), 4.58 (1H, d, *J*=11.7 Hz), 4.49–4.45 (2H, m), 4.35 (1H, d, *J*=11.7 Hz), 4.34 (1H, dd, *J*=2.3, 11.0 Hz), 4.20–4.12 (4H, m), 3.87 (1H, d, *J*=11.7 Hz), 3.85–3.80 (2H, m), 3.68 (1H, dd, *J*=3.2, 10.2 Hz), 3.62 (1H, dd, *J*=2.0, 10.2 Hz), 3.54 (1H, ddd, *J*=2.1, 6.8, 8.8 Hz), 3.52–3.41 (4H, m), 3.33–3.27 (5H, m), 3.21 (1H, dt, *J*=2.1, 8.8 Hz), 2.94–2.87 (1H, m), 2.83–2.76 (1H, m), 2.62 (1H, dd, *J*=6.1, 16.8 Hz), 2.34–2.27 (2H, m), 2.13–2.08 (1H, m), 2.04–2.00 (1H, m), 1.89 (1H, ddd, *J*=2.3, 7.2, 14.5 Hz), 1.82–1.75 (3H, m), 1.61–1.53 (1H, m), 1.28 (3H, s), 1.05 (9H, s), 0.99 (3H, d, *J*=6.6 Hz), 0.25 (3H, s), 0.088 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 210.8 (C), 159.6 (C), 139.4 (C), 139.3 (C), 137.9 (C), 137.8 (C), 132.0 (C), 131.73 (CH×2), 131.69 (CH×2), 129.8 (CH×2), 129.4 (CH×4), 128.9 (CH), 128.5 (CH), 121.7 (C), 121.6 (C), 114.0 (CH×2), 88.1 (C), 85.5 (CH), 85.4 (CH), 85.0 (CH), 83.1 (CH), 82.4 (CH), 79.7 (CH), 78.1 (CH), 75.1 (CH_2), 74.7 (CH×2), 73.1 (CH_2), 72.6 (CH_2), 72.2 (CH_2), 71.4 (CH_2), 71.3 (CH_2), 70.4 (CH_2), 54.8 (CH_3), 46.8 (CH_2), 44.6 (CH_2), 39.4 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 28.7 (CH), 27.6 (CH_2), 26.8 (CH_3), 26.2 (CH_3×3), 18.7 (CH_3), 18.2 (C), −4.0 (CH_3), −4.2 (CH_3) (The signals of 10 carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3062, 3026, 2925, 2855, 1593, 1716, 1613, 1586, 1513, 1487, 1454, 1360, 1301, 1249, 1213, 1173, 1071, 1012, 939, 834, 804, 777, 750, 698; HR-FDMS, calcd for $C_{66}H_{84}^{79}Br_2O_{11}Si$ $[M]^+$: 1238.4150, found: 1238.4119.

7.1.29. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{2′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-hydroxy-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-(4-methoxybenzyloxy)ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-one (35). To a solution of **34** (2.1 mg, 1.69 μmol) in THF (0.80 ml) was added HF·Py at 0 °C. The reaction mixture was warmed to 24 °C and stirred for 1 d. After the reaction mixture was diluted with Et_2O and cooling to 0 °C, saturated aqueous $NaHCO_3$ (1 ml) was added and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with AcOEt (3×5 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=4 to 2) to give **35** (1.2 mg, 63%). **35** was immediately used for the next reaction. **35**: a colorless oil; ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.38–7.33 (4H, m), 7.32–7.27 (4H, m), 7.21–7.15 (6H, m), 7.11–7.07 (2H, m), 6.92 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.5 Hz), 6.79 (2H, d, *J*=8.8 Hz), 5.97 (1H, dt, *J*=5.7, 10.5 Hz), 5.89 (1H, dt, *J*=5.7, 10.5 Hz), 4.86 (1H, d, *J*=11.0 Hz), 4.81 (1H, d, *J*=11.0 Hz), 4.54 (1H, d, *J*=12.2 Hz), 4.41 (1H, d, *J*=11.7 Hz), 4.40 (1H, d, *J*=12.2 Hz), 4.27 (1H, dd, *J*=2.0, 10.2 Hz, H11), 4.20–4.11 (4H, m), 4.02–3.99 (1H, m), 3.87 (1H, d, *J*=11.7 Hz), 3.80 (1H, dt, *J*=9.1, 6.6 Hz), 3.70 (1H, dt, *J*=7.9, 3.3 Hz), 3.63–3.62 (2H, m), 3.56–3.43 (5H, m), 3.30 (1H, dd, *J*=6.8, 10.2 Hz), 3.27 (3H, s), 3.26–3.20 (2H, m), 2.93 (1H, ddd, *J*=3.9, 10.5, 13.4 Hz), 2.72 (1H, ddd, *J*=3.3, 10.5, 13.1 Hz), 2.59 (1H, dd, *J*=6.6, 16.8 Hz), 2.50 (1H, ddd, *J*=1.1, 10.3, 14.6 Hz), 2.30 (1H, ddd, *J*=3.3, 5.7, 13.1 Hz), 2.14–2.08 (1H, m), 2.02 (1H, ddd, *J*=2.0, 7.4, 14.6 Hz), 1.99–1.95 (1H, m), 1.82–1.72 (3H, m), 1.55 (1H, ddd, *J*=5.6, 9.0, 14.4 Hz), 1.30 (3H, s), 0.97 (3H, d, *J*=6.8 Hz).

7.1.30. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{1′-Benzyloxy-2′-[8″-benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-11-(4-methoxybenzyloxy)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecane (38). To a suspension of **33b** (2.5 mg, 2.01 μmol) and TBAI (5.0 mg, 13.5 μmol) in THF (1.0 ml) was added NaH (15.0 mg, 375 μmol) at 0 °C and the mixture was stirred for 10 min. Then, to the mixture was added benzyl bromide (20.0 μmol, 168 μmol) at 0 °C and the reaction mixture was warmed to 25 °C. During 5 d, NaH was added several times to the reaction mixture with stirring until the reaction was complete. After that, H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=50 to 10) to give **38** (1.9 mg, 71%). **38**: a colorless oil; $[\alpha]_D^{24}$ +8.71 (*c* 0.125, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.41–7.03 (17H, m), 6.94 (2H, d, *J*=8.3 Hz), 6.83 (2H, d, *J*=8.5 Hz), 6.72 (2H, d, *J*=8.8 Hz), 6.00 (1H, dt, *J*=5.6, 10.6 Hz), 5.94 (1H, dt, *J*=5.6, 10.6 Hz), 5.03 (1H, d, *J*=12.0 Hz), 4.79 (1H, d, *J*=12.0 Hz), 4.59–4.58 (1H, m), 4.55 (1H, d, *J*=12.8 Hz), 4.48 (1H, d, *J*=12.8 Hz), 4.44 (1H, d, *J*=11.5 Hz), 4.43 (1H, d, *J*=11.3 Hz), 4.24–4.20 (5H, m), 4.17 (1H, d, *J*=11.3 Hz), 3.93 (1H, dt, *J*=8.9, 2.8 Hz), 3.87 (1H, d, *J*=12.0 Hz), 3.86–3.83 (1H, m), 3.82–3.73 (4H, m), 3.72–3.62 (3H, m), 3.61 (1H, dd, *J*=2.2, 9.9 Hz), 3.40 (1H, dd, *J*=7.4, 9.9 Hz), 3.28 (1H, dt, *J*=2.6, 8.7 Hz), 3.26 (3H, s), 2.98–2.84 (2H, m), 2.53 (1H, dt, *J*=14.4, 3.7 Hz), 2.35–2.28 (2H, m), 2.21–2.12 (1H, m), 2.06–1.78 (6H, m), 1.68–1.56 (1H, m), 1.35 (3H, s), 1.08 (3H, d, *J*=6.8 Hz), 1.00 (9H, s), 0.16 (3H, s), 0.048 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 159.6 (C), 140.6 (C), 139.9 (C), 139.5 (C), 138.1 (C), 138.0 (C), 131.69 (CH×2), 131.65 (CH×2), 131.2 (C), 129.7 (CH×2), 129.4 (CH×2), 129.3 (CH×2), 128.5 (CH), 127.3 (CH), 127.23 (CH), 127.15 (CH), 121.63 (C), 121.60 (C), 114.1 (CH×2), 85.7 (CH), 84.4 (CH), 82.5 (CH), 81.2 (CH), 79.9 (CH), 78.8 (C), 78.4 (CH), 77.3 (CH), 77.0 (CH), 75.7 (CH), 74.4 (CH), 73.3 (CH_2), 73.1 (CH_2), 72.54 (CH_2), 72.46 (CH_2), 71.5 (CH_2), 71.1 (CH_2), 70.4 (CH_2), 70.3 (CH_2), 54.7 (CH_3), 45.6 (CH_2), 39.4 (CH_2), 33.3 (CH_2), 31.4 (CH_2), 30.9 (CH_2), 29.0 (CH), 27.4 (CH_2), 26.7 (CH_3), 26.2

($CH_3\times3$), 18.2 (C), 15.6 (CH_3), −4.1 (CH_3), −4.4 (CH_3) (The signals of 13 carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3063, 3028, 2926, 2855, 1612, 1586, 1513, 1487, 1454, 1367, 1301, 1249, 1206, 1172, 1097, 1012, 939, 835, 804, 775, 733, 697; HR-FDMS, calcd for $C_{73}H_{92}^{79}Br_2O_{11}Si$ $[M]^+$: 1330.4776, found: 1330.4784.

7.1.31. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{1′-Benzyloxy-2′-[8″-benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-ol (39). To a solution of **38** (4.4 mg, 3.30 μmol) in DCM–pH 7 buffer (10:1, v/v, 0.70 ml) was added DDQ (5.0 mg, 22.0 μmol) at 0 °C and the mixture was stirred for 20 min. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30 to 5) to give **39** (3.4 mg, 85%). **39**: a colorless oil; $[\alpha]_D^{22}$ −2.29 (*c* 0.170, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.43 (2H, d, *J*=7.1 Hz), 7.35 (2H, d, *J*=6.8 Hz), 7.31–7.28 (4H, m), 7.24–7.08 (11H, m), 6.98 (2H, d, *J*=8.3 Hz), 6.84 (2H, d, *J*=8.3 Hz), 6.05 (1H, dt, *J*=5.2, 10.9 Hz), 5.95 (1H, dt, *J*=5.2, 10.9 Hz), 5.22 (1H, d, *J*=10.7 Hz), 4.76 (1H, d, *J*=10.7 Hz), 4.47 (1H, d, *J*=11.7 Hz), 4.46 (1H, d, *J*=12.1 Hz), 4.38–4.34 (2H, m), 4.30 (1H, d, *J*=12.1 Hz), 4.25 (1H, d, *J*=12.7 Hz), 4.21 (1H, d, *J*=11.9 Hz), 4.19 (1H, d, *J*=12.7 Hz), 4.17 (1H, d, *J*=11.7 Hz), 4.04–3.98 (1H, m), 3.92–3.89 (2H, m), 3.83 (1H, dt, *J*=9.0, 3.0 Hz), 3.71–3.63 (4H, m), 3.57 (1H, dd, *J*=2.1, 9.9 Hz), 3.54–3.52 (1H, m), 3.50 (1H, dt, *J*=9.0, 3.0 Hz), 3.44 (1H, dd, *J*=6.5, 9.9 Hz), 3.32 (1H, dt, *J*=2.6, 8.9 Hz), 2.88–2.78 (2H, m), 2.43 (1H, dt, *J*=13.4, 4.2 Hz), 2.32 (1H, ddd, *J*=3.0, 5.2, 13.7 Hz), 2.11–1.78 (8H, m), 1.64 (1H, ddd, *J*=6.1, 8.9, 14.6 Hz), 1.06 (3H, s), 1.05 (3H, d, *J*=6.8 Hz), 1.01 (9H, s), 0.17 (3H, s), 0.024 (3H, s); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 139.6 (C), 139.3 (C), 139.1 (C), 138.2 (C), 138.1 (C), 131.7 (CH×2), 131.6 (CH×2), 129.33 (CH×2), 129.32 (CH×2), 128.9 (CH), 128.60 (CH×2), 128.55 (CH×2), 128.5 (CH×2), 128.3 (CH×2), 121.6 (C), 121.5 (C), 85.5 (CH), 85.1 (CH), 84.6 (CH), 80.7 (CH), 79.7 (CH×2), 78.5 (C), 78.0 (CH), 75.3 (CH), 74.2 (CH), 73.2 (CH_2), 72.7 (CH_2), 72.4 ($CH_2\times2$), 71.3 (CH_2), 71.2 (CH_2), 70.9 (CH), 70.3 (CH_2), 45.9 (CH_2), 39.9 (CH_2), 36.1 (CH_2), 32.4 (CH_2), 31.8 (CH_2), 30.4 (CH_3), 28.9 (CH), 27.5 (CH_2), 26.9 (CH_3), 26.2 ($CH_3\times3$), 18.2 (C), −4.3 ($CH_3\times2$) (The signals of eight carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3584, 3433, 3063, 3028, 2923, 2857, 1593, 1487, 1454, 1405, 1359, 1298, 1256, 1207, 1096, 1012, 940, 836, 804, 776, 749, 698; HR-FDMS, calcd for $C_{65}H_{84}^{79}Br_2O_{10}Si$ $[M]^+$: 1210.4200, found: 1210.4218.

7.1.32. (1*S*,3*R*,4*S*,6*S*,8*R*,10*S*,11*S*,1′*S*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-[1′-Benzyloxy-2′-(8″-benzyloxy-9″-benzyloxymethyl-3″-hydroxy-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl)ethyl]-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-one (41). To a solution of **39** (3.4 mg, 2.80 μmol) in DCM (0.70 ml) were added $NaHCO_3$ (5.0 mg, 59.5 μmol) and DMPI (5.0 mg, 11.8 μmol) at 25 °C and the reaction mixture was stirred for 30 min. After the mixture was diluted with Et_2O (1 ml), saturated aqueous Na_2SO_3 (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/AcOEt=5 to 4) to give a crude product (3.4 mg), and it was used in the next reaction without further purification. To a solution of the above crude product in THF–H_2O (1:1, v/v, 0.80 ml) was added TFA (40.0 μl) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 2 d. Then, $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with AcOEt (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=5 to 2) to give **41** (2.3 mg, 75% from **39**). **41**: a colorless oil; $[\alpha]_D^{21}$ −21.3 (*c* 0.115, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.41–7.35 (4H, m), 7.32–7.27 (4H, m), 7.21–7.04 (11H, m), 6.92 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.5 Hz), 5.94 (1H, dt, *J*=5.5, 10.2 Hz), 5.87 (1H, dt, *J*=5.5, 10.2 Hz), 4.90 (1H, d, *J*=11.5 Hz), 4.85 (1H, d, *J*=11.5 Hz), 4.53 (1H, d, *J*=12.1 Hz), 4.40 (1H, d, *J*=12.1 Hz), 4.39 (1H, d, *J*=12.1 Hz), 4.28 (1H, dd, *J*=2.0, 10.4 Hz), 4.18 (1H, d, *J*=11.7 Hz), 4.16 (1H, d, *J*=12.1 Hz), 4.12 (1H, d, *J*=12.1 Hz), 3.98 (1H, dt, *J*=8.8, 3.5 Hz), 3.86 (1H, d, *J*=11.7 Hz), 3.76 (1H, dt, *J*=9.0, 6.6 Hz), 3.69 (1H, dt, *J*=8.7, 3.3 Hz), 3.62 (2H, d, *J*=2.4 Hz), 3.55–3.43 (5H, m), 3.30 (1H, dd, *J*=7.1, 10.2 Hz), 3.23–3.17 (2H, m), 2.87 (1H, ddd, *J*=3.5, 10.2, 13.4 Hz), 2.70 (1H, ddd, *J*=3.3, 10.2, 13.4 Hz), 2.58 (1H, dd, *J*=6.6, 16.8 Hz), 2.50 (1H, ddd, *J*=2.0, 10.4, 14.6 Hz), 2.28 (1H, ddd, *J*=3.3, 5.5, 13.4 Hz), 2.07–1.95 (3H, m), 1.81–1.67 (3H, m), 1.53 (1H, ddd, *J*=6.1, 8.8, 14.9 Hz), 1.28 (3H, s), 0.96 (3H, d, *J*=6.6 Hz); ^{13}C NMR (125 MHz, C_6D_6), δ (ppm) 210.5 (C), 139.5 (C), 139.3 (C), 139.2 (C), 137.9 (C), 137.8 (C), 133.0 (C), 131.74 (CH×2), 131.70 (CH×2), 129.4 (CH×4), 128.6 (CH×2), 128.50 (CH×2), 128.47 (CH×2), 127.6 (CH×2), 121.74 (C), 121.69 (C), 87.8 (C), 85.2 (CH), 84.8 (CH), 84.7 (CH), 82.5 (CH), 82.2 (CH), 79.7 (CH), 78.4 (CH), 75.0 (CH_2), 74.6 (CH), 74.4 (CH), 73.1 (CH_2), 72.6 (CH_2), 72.1 (CH_2), 71.9 (CH_2), 71.3 (CH_2), 70.4 (CH_2), 46.5 (CH_2), 44.4 (CH_2), 39.4 (CH_2), 34.5 (CH_2), 33.1 (CH_2), 28.6 (CH), 27.7 (CH_2), 26.8 (CH_3), 18.5 (CH_3) (The signals of nine carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3465, 3063, 3027, 2924, 2858, 1716, 1592, 1487, 1453, 1405, 1366, 1305, 1256, 1215, 1098, 1027, 1012, 911, 839, 803, 753, 698; HR-FDMS, calcd for $C_{59}H_{68}^{79}Br_2O_{10}$ $[M]^+$: 1094.3179, found: 1094.3176.

7.1.33. (1*R*,3*S*,5*Z*,8*R*,9*S*,11*R*,13*S*,14*S*,16*R*,18*S*,20*S*,21*R*,23*S*)-8,13-Dibenzyloxy-9-benzyloxymethyl-20-(4-bromobenzyloxy)-21-(4-bromobenzyloxymethyl)-14,18-dimethyl-2,10,15,22-tetraoxatetracyclo[12.10.0.0^{3,11}.0^{16,23}]-tetracos-5-ene (42). To a solution of **41** (2.3 mg, 2.10 μmol) in DCM–Et_3SiH (10:1, v/v, 0.70 ml) was added TMSOTf (3.0 μl, 16.6 μmol) at 0 °C and the mixture was stirred for 30 min. Then, saturated aqueous $NaHCO_3$ (1 ml) was added

and the aqueous layer was extracted with Et_2O (5 ml) and AcOEt (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=10 to 4) to give **41** (1.6 mg, 70%). **41**: a colorless oil; $[\alpha]_D^{22}$ −60.8 (*c* 0.080, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm) 7.45–7.41 (4H, m), 7.37–7.18 (17H, m), 7.07 (2H, d, *J*=8.5 Hz), 5.81 (1H, dt, *J*=5.2, 10.7 Hz), 5.71 (1H, dt, *J*=5.2, 10.7 Hz), 4.621 (2H, s), 4.617 (1H, d, *J*=11.3 Hz), 4.525 (1H, d, *J*=12.8 Hz), 4.516 (1H, d, *J*=11.5 Hz), 4.50 (1H, d, *J*=12.1 Hz), 4.465 (1H, d, *J*=12.1 Hz), 4.461 (1H, d, *J*=12.8 Hz), 4.30 (1H, d, *J*=11.3 Hz), 4.23 (1H, d, *J*=11.5 Hz), 4.07–4.03 (1H, m), 3.88 (1H, dd, *J*=4.3, 12.4 Hz), 3.72–3.68 (1H, m), 3.59 (1H, dd, *J*=1.8, 9.8 Hz), 3.56–3.48 (3H, m), 3.45 (1H, dd, *J*=5.7, 9.8 Hz), 3.41–3.29 (4H, m), 3.27–3.21 (2H, m), 2.80–2.73 (1H, m), 2.68–2.61 (1H, m), 2.43 (1H, ddd, *J*=5.5, 9.1, 15.4 Hz), 2.33–2.27 (1H, m), 2.18 (1H, dt, *J*=12.4, 4.3 Hz), 2.12–2.06 (1H, m), 1.98–1.90 (3H, m), 1.80–1.75 (1H, m), 1.71–1.51 (3H, m), 1.06 (3H, d, *J*=7.1 Hz), 1.00 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm) 139.5 (C), 138.4 (C), 138.2 (C), 137.4 (C), 137.3 (C), 131.5 (CH×4), 129.4 (CH×2), 129.3 (CH×2), 128.8 (CH), 128.33 (CH×2), 128.29 (CH×2), 128.1 (CH×4), 127.8 (CH×2), 127.6 (CH×2), 127.5 (CH×2), 127.13 (CH), 127.05 (CH), 121.5 (C), 121.4 (C), 85.8 (CH), 85.2 (CH), 84.8 (CH), 84.2 (CH), 82.5 (CH), 81.6 (CH), 79.7 (C), 79.0 (CH), 77.7 (CH), 73.6 (CH), 73.4 (CH_2), 73.2 (CH_2), 72.7 (CH_2), 72.6 (CH), 71.9 (CH_2), 71.4 (CH_2), 71.1 (CH_2), 70.5 (CH_2), 45.3 (CH_2), 40.6 (CH_2), 37.2 (CH_2), 34.6 (CH_2), 32.1 (CH_2), 28.1 (CH), 27.1 (CH_2, CH_3), 13.7 (CH_3); IR (film), ν (cm^{-1}) 3062, 3027, 2923, 2854, 1593, 1495, 1487, 1454, 1376, 1330, 1315, 1259, 1204, 1096, 1027, 1012, 803, 778, 735, 697; HR-FDMS, calcd for $C_{59}H_{68}^{79}Br_2O_9$ $[M]^+$: 1078.3230, found: 1078.3226.

7.1.34. (2*R*,3*S*,5*Z*,8*R*,9*S*,1′*S*,3′*R*,4′*S*,6′*S*,8′*R*,10′*R*,11′*S*)-[8-Benzyloxy-9-benzyloxymethyl-3-*(tert*-butyldimethylsilyloxy)-2,3,4,7,8,9-hexahydrooxonin-2-yl]methyl 4′-(4-bromobenzyloxy)-3′-(4-bromobenzyloxymethyl)-6′,10′-dimethyl-11′-triethylsilyloxy-2′,9′-dioxabicyclo[6.4.0]dodecan-10′-yl ketone (45). To a solution of oxalyl dichloride (21.0 μl, 241 μmol) in DCM (0.30 ml) was added a solution of DMSO (30.0 μl, 423 μmol) in DCM (0.30 ml) at −78 °C and the mixture was stirred for 10 min. Then, a solution of **26** (32.7 mg, 26.4 μmol) in DCM (0.90 ml) was added at −78 °C and the mixture was warmed to −45 °C and stirred for 1 h. After Et_3N (120 μl, 861 μmol) was added, the reaction mixture was warmed to 0 °C and stirred for 15 min. H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/AcOEt=5) to give a mixture of **26** and **45** (31.6 mg). In order to consume **26** completely, the process was repeated as follows: to a solution of oxalyl dichloride (42.0 μl, 481 μmol) in DCM (0.30 ml) was added a solution of DMSO (60.0 μl, 846 μmol) in DCM (0.40 ml) at −78 °C and the mixture was stirred for 10 min. Then, a solution of the above mixture (31.6 mg) in DCM (0.90 ml) was added at −78 °C and the mixture was warmed to −45 °C and stirred for 1 h. After Et_3N (240 μl, 1.72 mmol) was added, the reaction mixture was warmed to 0 °C and stirred for 20 min. H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=15 to 10) to give **45** (19.8 mg, 61%). **45**: a colorless oil; $[\alpha]_D^{23}$ +27.2 (*c* 0.900, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.35–7.23 (8H, m), 7.20–7.06 (6H, m), 6.95 (2H, d, *J*=8.3 Hz), 6.81 (2H, d, *J*=8.3 Hz), 5.96–5.92 (2H, m), 4.51 (1H, d, *J*=12.2 Hz), 4.48 (1H, d, *J*=11.6 Hz), 4.45 (1H, d, *J*=12.2 Hz), 4.39 (1H, ddd, *J*=3.2, 5.4, 8.3 Hz), 4.30 (2H, d, *J*=11.6 Hz), 4.28 (1H, dt, *J*=9.0, 2.6 Hz), 4.21 (1H, d, *J*=11.7 Hz), 4.20 (1H, s), 4.16 (1H, t, *J*=2.9 Hz), 3.98 (1H, dt, *J*=9.0, 3.9 Hz), 3.96 (1H, dt, *J*=2.2, 5.4 Hz), 3.89–3.83 (2H, m), 3.80 (2H, d, *J*=2.6 Hz), 3.72 (1H, ddd, *J*=2.1, 7.1, 9.0 Hz), 3.61 (1H, dd, *J*=2.1, 9.8 Hz), 3.55 (1H, dt, *J*=3.0, 9.4 Hz), 3.38 (1H, dd, *J*=7.1, 9.8 Hz), 3.37 (1H, dd, *J*=8.3, 18.8 Hz), 3.25 (1H, dt, *J*=2.6, 9.0 Hz), 3.03 (1H, dd, *J*=3.2, 18.8 Hz), 2.97–2.90 (1H, m), 2.83 (1H, ddd, *J*=2.9, 9.0, 13.4 Hz), 2.34 (1H, dt, *J*=13.4, 3.9 Hz), 2.25 (1H, dt, *J*=13.4, 5.4 Hz), 2.22 (1H, dt, *J*=13.7, 2.9 Hz), 2.05–2.02 (1H, m), 1.95–1.87 (3H, m), 1.79 (1H, ddd, *J*=2.9, 11.2, 13.7 Hz), 1.65 (1H, ddd, *J*=5.2, 9.0, 14.5 Hz), 1.13 (3H, s), 1.07 (3H, d, *J*=6.3 Hz), 1.004 (9H, t, *J*=7.9 Hz), 0.997 (9H, s), 0.62 (6H, q, *J*=7.9 Hz), 0.19 (3H, s), 0.099 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 212.2 (C), 138.75 (C), 138.68 (C), 137.4 (C), 137.2 (C), 131.5 (CH×2), 131.4 (CH×2), 129.4 (CH×2), 129.3 (CH×2), 128.2 (CH×4), 127.9 (CH×3), 127.8 (CH×3), 127.7 (CH), 127.4 (CH×2), 121.5 (C), 121.4 (C), 85.9 (CH), 83.4 (C), 82.0 (CH), 80.4 (CH), 80.2 (CH), 79.1 (CH), 77.9 (CH), 73.8 (CH), 73.7 (CH), 73.2 (CH_2), 72.5 (CH_2), 72.1 (CH), 72.0 (CH_2), 71.7 (CH_2), 71.4 (CH_2), 70.6 (CH_2), 70.1 (CH_2), 45.6 (CH_2), 44.4 (CH_2), 40.7 (CH_2), 35.7 (CH_2), 32.0 (CH_2), 28.1 (CH), 27.5 (CH_2), 27.0 (CH_3), 26.0 (CH_3×3), 18.20 (CH_3), 18.16 (C), 6.9 (CH_3×3), 4.7 (CH_2×3), −4.5 (CH_3), −4.6 (CH_3); IR (film), ν (cm^{-1}) 3026, 2926, 1719, 1593, 1487, 1453, 1361, 1337, 1257, 1202, 1098, 1070, 1012, 960, 836, 776, 733, 697; HR-FDMS, calcd for $C_{64}H_{90}^{79}Br_2O_{10}Si_2$ $[M]^+$: 1232.4439, found: 1232.4431.

7.1.35. (2*R*,3*S*,5*Z*,8*R*,9*S*,1′*S*,3′*R*,4′*S*,6′*S*,8′*R*,10′*R*,11′*S*)-[8-Benzyloxy-9-benzyloxymethyl-3-*(tert*-butyldimethylsilyloxy)-2,3,4,7,8,9-hexahydrooxonin-2-yl]methyl 4′-(4-bromobenzyloxy)-3′-(4-bromobenzyloxymethyl)-11′-hydroxy-6′,10′-dimethyl-2′,9′-dioxabicyclo[6.4.0]dodecan-10′-yl ketone (46). To a solution of **45** (19.8 mg, 16.0 μmol) in THF–pyridine (2:1, v/v, 1.35 ml) was added HF·Py (excess) at 25 °C. During 6 d, HF·Py was added several times to the reaction mixture with stirring until the reaction was complete. After the reaction mixture was diluted with Et_2O and cooled to 0 °C, saturated aqueous $NaHCO_3$ (1 ml) was added and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=10 to 3) to give **46**

(13.8 mg, 71%). **46**: a colorless oil; $[\alpha]_D^{23}$ +36.0 (*c* 0.150, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.35–7.28 (5H, m), 7.23–7.05 (9H, m), 6.96 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.5 Hz), 5.99 (1H, dt, *J*=4.9, 10.7 Hz), 5.95 (1H, dt, *J*=4.9, 10.7 Hz), 4.48–4.41 (3H, m), 4.24 (1H, dt, *J*=7.6, 3.4 Hz), 4.22–4.14 (4H, m), 4.10 (1H, ddd, *J*=3.4, 5.4, 8.2 Hz), 3.93 (1H, ddd, *J*=2.4, 4.5, 8.3 Hz), 3.88 (1H, d, *J*=12.0 Hz), 3.85–3.73 (3H, m), 3.67 (1H, dd, *J*=4.5, 10.5 Hz), 3.60 (1H, dd, *J*=2.4, 10.5 Hz), 3.58–3.49 (4H, m), 3.38 (1H, dd, *J*=6.6, 10.0 Hz), 3.27 (1H, dt, *J*=2.4, 9.0 Hz), 3.17 (1H, dd, *J*=5.4, 19.5 Hz), 3.04 (1H, ddd, *J*=3.4, 10.7, 13.2 Hz), 2.77 (1H, ddd, *J*=3.0, 10.7, 13.5 Hz), 2.29 (1H, dt, *J*=13.5, 4.9 Hz), 2.23–2.16 (2H, m), 2.00–1.96 (1H, m), 1.92–1.79 (3H, m), 1.70 (1H, ddd, *J*=2.8, 11.0, 13.7 Hz), 1.58 (1H, ddd, *J*=5.4, 9.0, 14.8 Hz), 1.21 (3H, s), 1.04 (3H, d, *J*=6.8 Hz), 0.97 (9H, s), 0.12 (3H, s), 0.075 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 213.3 (C), 138.5 (C), 138.3 (C), 137.4 (C), 137.3 (C), 131.44 (CH×2), 131.41 (CH×2), 129.3 (CH×4), 128.31 (CH×2), 128.26 (CH×2), 127.94 (CH×2), 127.88 (CH), 127.7 (CH×3), 127.6 (CH), 127.5 (CH), 121.5 (C), 121.4 (C), 85.4 (CH), 83.2 (CH), 81.1 (CH), 79.8 (CH), 78.8 (CH), 78.3 (CH), 77.2 (C), 73.7 (CH), 73.1 (CH), 73.0 (CH_2), 72.5 (CH_2), 71.7 (CH_2), 71.2 (CH_2), 70.8 (CH), 70.6 (CH_2), 70.5 (CH_2), 45.3 (CH_2), 41.9 (CH_2), 40.4 (CH_2), 34.5 (CH_2), 32.0 (CH_2), 28.1 (CH), 27.01 (CH_2), 26.96 (CH_3), 25.8 (CH_3×3), 18.2 (CH_3), 17.9 (C), −4.3 (CH_3), −4.8 (CH_3); IR (film), ν (cm^{-1}) 3463, 3026, 2926, 2854, 1715, 1593, 1487, 1453, 1361, 1257, 1204, 1099, 1069, 1011, 836, 803, 776, 735, 697; HR-FDMS, calcd for $C_{58}H_{76}^{79}Br_2O_{10}Si$ $[M]^+$: 1118.3574, found: 1118.3552.

7.1.36. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*R*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{2′-[8″-Benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]-1′-hydroxyethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-ol (47). To a solution of **46** (2.8 mg, 2.31 μmol) in MeOH (0.70 ml) was added $NaBH_4$ (7.3 mg, 193 μmol) at 0 °C and the reaction mixture was stirred for 15 min. Then, H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=15 to 10) to give a mixture of **47** and **31** (2.8 mg, ~100%, **47**:**31**=2:1 from ^1H NMR). This mixture of **47** and **31** was separated by HPLC (hexane/AcOEt=4) to give **47** (1.6 mg, 61%) as less-polar eluate and **31** (0.7 mg, 27%) as polar eluate. **47**: a colorless oil; $[\alpha]_D^{20}$ +7.67 (*c* 0.225, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.40 (2H, d, *J*=7.3 Hz), 7.31−7.05 (12H, m), 7.00 (2H, d, *J*=8.3 Hz), 6.83 (2H, d, *J*=8.3 Hz), 5.93 (1H, dt, *J*=5.5, 10.4 Hz), 5.87 (1H, dt, *J*=5.5, 10.4 Hz), 4.82−4.79 (1H, m), 4.52 (1H, d, *J*=11.6 Hz), 4.40 (1H, d, *J*=11.7 Hz), 4.30 (1H, d, *J*=11.6 Hz), 4.25 (1H, d, *J*=12.3 Hz), 4.24−4.20 (2H, m), 4.19 (1H, d, *J*=12.3 Hz), 4.13 (1H, d, *J*=11.7 Hz), 4.04 (1H, dt, *J*=4.8, 10.4 Hz), 4.02−3.99 (1H, m), 3.96 (1H, dt, *J*=1.8, 5.5 Hz), 3.92−3.89 (2H, m), 3.84−3.82 (1H, m), 3.77−3.72 (3H, m), 3.68 (1H, ddd, *J*=2.2, 6.7, 9.0 Hz), 3.63−3.59 (2H, m), 3.57 (1H, dd, *J*=2.2, 9.8 Hz), 3.45 (1H, dd, *J*=6.6, 9.8 Hz), 3.33 (1H, dt, *J*=2.4, 9.0 Hz), 2.79−2.67 (2H, m), 2.52 (1H, ddd, *J*=2.9, 4.8, 13.3 Hz), 2.29−2.24 (1H, m), 2.15 (1H, dt, *J*=13.5, 5.5 Hz), 2.04−1.77 (7H, m), 1.61 (1H, ddd, *J*=6.1, 9.0, 15.0 Hz), 1.053 (3H, s), 1.050 (3H, d, *J*=6.6 Hz), 0.96 (9H, s), 0.056 (3H, s), 0.030 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 138.3 (C), 137.8 (C), 137.5 (C), 137.4 (C), 131.41 (CH×2), 131.39 (CH×2), 129.31 (CH×2), 129.27 (CH×2), 128.4 (CH), 128.3 (CH×3), 128.2 (CH×2), 127.8 (CH×2), 127.7 (CH), 127.6 (CH), 126.9 (CH), 121.4 (C), 121.3 (C), 85.3 (CH), 83.9 (CH), 81.5 (CH), 80.4 (CH), 78.8 (CH), 77.2 (CH), 75.8 (C), 74.5 (CH), 74.0 (CH), 73.6 (CH), 73.2 (CH_2), 72.9 (CH), 72.5 (CH_2), 71.6 (CH_2), 71.4 (CH_2), 70.5 (CH_2), 69.1 (CH_2), 45.6 (CH_2), 40.5 (CH_2), 35.7 (CH_2), 32.5 (CH_2), 29.7 (CH_2), 28.3 (CH), 27.0 (CH_3), 26.8 (CH_2), 25.8 (CH_3×3), 18.0 (C), 17.5 (CH_3), −4.2 (CH_3), −4.6 (CH_3); IR (film), ν (cm^{-1}) 3397, 3026, 2961, 2851, 1593, 1487, 1454, 1405, 1360, 1296, 1256, 1204, 1100, 1028, 1012, 947, 836, 804, 776, 751, 698; HR-FDMS, calcd for $C_{58}H_{78}^{79}Br_2O_{10}Si$ $[M]^+$: 1120.3731, found: 1120.3730.

7.1.37. Conversion of 31 to 26. To a solution of **31** (2.1 mg, 1.87 μmol) in DCM (0.50 ml) were added 2,6-lutidine (20 μl, 172 μmol) and TESOTf (3.0 μl, 13.3 μmol) at −40 °C. After the mixture was stirred for 25 min, TESOTf (2.0 μl, 8.8 μmol) was added to the mixture at −40 °C. The mixture was stirred for 35 min. Then, saturated aqueous $NaHCO_3$ (0.5 ml) was added and the aqueous layer was extracted with Et_2O (4×3 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=7) to give **26** (2.2 mg, 95%).

7.1.38. (1*R*,3*R*,4*R*,6*S*,8*S*,10*S*,12*R*,13*S*,15*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*)-4-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-13-(4-bromobenzyloxy)-12-(4-bromobenzyloxymethyl)-3,15-dimethyl-6-(2-naphthyl)-2,5,7,11-tetraoxatricyclo[8.6.0.0^{3,8}]hexadecane (48). To a solution of **47** (4.2 mg, 3.74 μmol) in benzene (1.0 ml) were added 2-naphthaldehyde dimethyl acetal (27.1 mg, 134 μmol) and PPTS (4.3 mg, 17.1 μmol). The reaction mixture was heated to 80 °C and stirred for 1.5 h. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30 to 4) to give **48** (4.2 mg, 89%,). **48**: a colorless oil; $[\alpha]_D^{22}$ +39.4 (*c* 0.160, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 8.28 (1H, s), 7.93 (1H, dd, *J*=1.5, 8.4 Hz), 7.81–7.76 (2H, m), 7.62 (1H, *J*=1.5, 7.7 Hz), 7.28–7.01 (16H, m), 6.94 (2H, d, *J*=8.3 Hz), 6.78 (2H, d, *J*=8.3 Hz), 5.99 (1H, dt, *J*=6.7, 10.5 Hz), 5.89 (1H, dt, *J*=6.0, 10.5 Hz), 5.75 (1H, s), 4.46 (1H, d, *J*=12.0 Hz), 4.28–4.11 (8H, m), 4.08 (1H, dt, *J*=9.1, 2.2 Hz), 4.02–4.00 (1H, m), 3.96 (1H, dt, *J*=9.1, 2.9 Hz), 3.92–3.86 (2H, m), 3.83 (1H, d, *J*=12.0 Hz), 3.66 (1H, dd, *J*=2.2, 10.1 Hz), 3.58 (1H, t, *J*=2.9 Hz), 3.55 (1H, dt, *J*=2.8, 9.6 Hz), 3.48 (1H, ddd, *J*=2.2, 6.6, 8.8 Hz), 3.46 (1H, dd, *J*=2.2, 10.1 Hz), 3.37 (1H, dd, *J*=6.6, 10.1 Hz), 3.25 (1H, dt, *J*=2.7, 8.8 Hz),

2.98 (1H, ddd, *J*=2.9, 10.5, 13.3 Hz), 2.88–2.82 (1H, m), 2.44 (1H, ddd, *J*=2.9, 4.8, 13.5 Hz), 2.40–2.26 (3H, m), 1.99–1.82 (5H, m), 1.77 (1H, dt, *J*=14.6, 2.7 Hz), 1.46 (1H, ddd, *J*=6.1, 8.8, 14.6 Hz), 1.01 (3H, d, *J*=7.1 Hz), 0.99 (9H, s), 0.97 (3H, s), 0.14 (3H, s), 0.075 (3H, s); ^{13}C NMR (100 MHz, C_6D_6), δ (ppm) 139.40 (C), 139.35 (C), 138.2 (C), 138.1 (C), 137.2 (C), 134.1 (C), 133.6 (C), 131.61 (CH×2), 131.56 (CH×2), 129.7 (CH), 129.2 (CH×4), 128.6 (CH×2), 127.3 (CH×2), 126.4 (CH), 126.3 (CH×2), 125.9 (CH), 124.8 (CH), 121.5 (C), 121.4 (C), 100.5 (CH), 85.0 (CH), 79.9 (CH), 79.80 (CH), 79.77 (CH), 79.6 (CH), 78.3 (CH), 78.23 (CH), 78.20 (CH), 76.1 (CH), 74.8 (CH), 73.2 (CH_2), 72.7 (CH_2), 72.3 (CH_2), 71.3 (CH_2), 70.0 (CH_2), 69.6 (C), 68.7 (CH_2), 45.2 (CH_2), 39.5 (CH_2), 34.9 (CH_2), 34.1 (CH_2), 30.4 (CH_2), 29.0 (CH_2), 28.8 (CH), 26.9 (CH_3), 26.1 (CH_3×3), 18.3 (C), 17.2 (CH_3), −4.4 (CH_3), −4.7 (CH_3) (The signals of seven carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3063, 3025, 2956, 2853, 1593, 1487, 1471, 1453, 1405, 1375, 1359, 1326, 1256, 1214, 1202, 1174, 1098, 1012, 954, 836, 804, 755, 698; HR-FDMS, calcd for $C_{69}H_{84}^{79}Br_2O_{10}Si$ [M]$^+$: 1285.4200, found: 1258.4218.

7.1.39. (1*R*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,1″*S*,3″*R*,4″*S*,6″*S*,8″*R*,10″*S*,11″*S*)-2-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-{4″-(4-bromobenzyloxy)-3″-(4-bromobenzyloxymethy)-6″,10″-dimethyl-11″-(2-naphthylmethyl)-2″,9″-dioxabicyclo[6.4.0]dodecan-10″-yl}ethanol (49). To a solution of **48** (3.2 mg, 2.54 μmol) in DCM (0.70 ml) was added DIBAL (0.15 ml, 0.94 M in hexane, 141 μmol) at 0 °C. The reaction mixture was warmed to 10 °C and stirred for 3 h. Then, MeOH (0.1 ml) and saturated aqueous potassium sodium tartrate (1 ml) were added. The mixture was diluted with Et_2O (5 ml) and stirred at 25 °C for 18 h. The layers were separated and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=4) to give **49** (3.2 mg, ∼100%,). **49**: a colorless oil; $[\alpha]_D^{25}$ +7.40 (*c* 0.160, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.84 (1H, s), 7.72–7.62 (3H, m), 7.56 (1H, dd, *J*=1.6, 8.4 Hz), 7.41–7.39 (2H, m), 7.30–7.05 (14H, m), 6.92 (2H, d, *J*=8.5 Hz), 6.82 (2H, d, *J*=8.3 Hz), 5.96 (1H, dt, *J*=6.5, 10.7 Hz), 5.84 (1H, dt, *J*=5.7, 10.7 Hz), 4.65–4.62 (1H, m), 4.58 (1H, d, *J*=12.2 Hz), 4.474 (1H, d, *J*=12.2 Hz), 4.472 (1H, d, *J*=11.7 Hz), 4.284 (1H, d, *J*=11.8 Hz), 4.279 (1H, d, *J*=11.7 Hz), 4.20 (1H, d, *J*=11.8 Hz), 4.17 (2H, s), 4.11 (1H, dt, *J*=9.0, 3.5 Hz), 4.05 (1H, d, *J*=11.8 Hz), 3.96–3.87 (4H, m), 3.86 (1H, d, *J*=11.8 Hz), 3.70 (1H, ddd, *J*=2.1, 7.6, 9.1 Hz), 3.68–3.63 (2H, m), 3.61 (1H, dd, *J*=2.1, 9.9 Hz), 3.53 (1H, dt, *J*=9.0, 3.0 Hz), 3.39 (1H, dd, *J*=7.6, 9.9 Hz), 3.35 (1H, t, *J*=3.3 Hz), 3.25 (1H, dt, *J*=2.4, 9.1 Hz), 3.05 (1H, br s), 2.81 (1H, ddd, *J*=1.3, 10.7, 12.7 Hz), 2.68 (1H, ddd, *J*=3.0, 10.7, 13.4 Hz), 2.50 (1H, dt, *J*=13.9, 3.3 Hz), 2.28–2.22 (1H, m), 2.12 (1H, ddd, *J*=3.0, 5.7, 13.4 Hz), 1.91–1.69 (6H, m), 1.64–1.53 (2H, m), 1.22 (3H, s), 1.01 (3H, d, *J*=7.1 Hz), 0.97 (9H, s), 0.074 (6H, s); ^{13}C NMR (100 MHz, C_6D_6), δ (ppm) 139.6 (C), 139.3 (C), 138.1 (C), 137.9 (C), 136.7 (C), 133.9 (C), 133.4 (C), 131.6 (CH×4), 129.3 (CH×2), 129.2 (CH×2), 129.1 (CH), 128.5 (CH×4), 127.4 (CH), 126.8 (CH), 126.3 (CH×2), 126.2 (CH), 126.0 (CH×2), 121.6 (C), 121.5 (C), 85.7 (CH), 81.8 (CH), 81.2 (CH), 79.9 (CH), 79.5 (CH), 78.8 (C), 77.7 (CH), 77.5 (CH), 77.0 (CH), 74.2 (CH), 73.4 (CH_2), 73.1 (CH_2), 72.4 (CH_2), 71.5 (CH), 71.3 (CH_2), 71.0 (CH_2), 70.2 (CH_2), 70.1 (CH_2), 45.4 (CH_2), 39.7 (CH_2), 36.1 (CH_2), 31.6 (CH_2), 29.8 (CH_2), 28.8 (CH), 27.0 (CH_2), 26.8 (CH_3), 26.1 (CH_3×3), 18.3 (C), 15.6 (CH_3), −4.45 (CH_3), −4.50 (CH_3) (The signals of eight carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3584, 3503, 3061, 3025, 2925, 2854, 1593, 1509, 1487, 1454, 1404, 1375, 1360, 1337, 1298, 1256, 1203, 1100, 1012, 964, 946, 836, 804, 774, 751, 698; HR-FDMS, calcd for $C_{69}H_{87}^{79}Br_2O_{10}Si$ [M+H]$^+$: 1261.4430, found: 1261.4440.

7.1.40. (1*S*,3*R*,4*S*,6*S*,8*R*,10*S*,11*S*,1′*R*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{1′-Benzyloxy-2′-[8″-benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-11-(2-naphthymethyl)-2,9-dioxabicyclo[6.4.0]dodecane (50). To a suspension of **49** (3.8 mg, 3.01 μmol) and TBAI (3.0 mg, 8.12 μmol) in THF-DMF (5:1, v/v, 1.0 ml) was added NaH (17.4 mg, 435 μmol) at 0 °C and the mixture was stirred for 10 min. Then, benzyl bromide (20.0 μmol, 168 μmol) was added at 0 °C, the reaction mixture was warmed to 25 °C and stirred for 8 h. After that, H_2O (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=50 to 7) to give **50** (4.1 mg, ∼100%). **50**: a colorless oil; $[\alpha]_D^{23}$ −7.61 (*c* 0.205, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.95 (1H, s), 7.67–7.51 (6H, m), 7.35–7.03 (14H, m), 6.88 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.3 Hz), 5.88 (1H, dt, *J*=6.8, 10.5 Hz), 5.77 (1H, dt, *J*=5.7, 10.5 Hz), 5.50 (1H, d, *J*=12.2 Hz), 5.06 (1H, d, *J*=12.2 Hz), 4.68 (1H, t, *J*=5.7 Hz), 4.59 (1H, d, *J*=12.6 Hz), 4.48 (1H, d, *J*=12.6 Hz), 4.37–4.31 (2H, m), 4.22–4.16 (3H, m), 4.13 (2H, s), 4.01 (1H, d, *J*=12.0 Hz), 3.94 (1H, br d, *J*=8.7 Hz), 3.93–3.89 (1H, m), 3.86 (1H, d, *J*=12.0 Hz), 3.82 (1H, dd, *J*=1.7, 10.0 Hz), 3.76–3.69 (3H, m), 3.59 (2H, dd, *J*=2.6, 10.0 Hz), 3.40 (1H, dd, *J*=7.4, 10.0 Hz), 3.26 (1H, dt, *J*=2.4, 9.0 Hz), 3.14 (1H, br dd, *J*=10.5, 12.6 Hz), 2.54–2.44 (2H, m), 2.30 (1H, ddd, *J*=6.8, 8.7, 12.6 Hz), 2.01–1.90 (4H, m), 1.82 (1H, ddd, *J*=2.9, 5.7, 13.4 Hz), 1.76 (1H, ddd, *J*=2.6, 11.5, 13.9 Hz), 1.66 (1H, ddd, *J*=5.1, 9.0, 14.3 Hz), 1.41 (1H, t, *J*=5.7 Hz), 1.25 (3H, s), 1.00 (3H, d, *J*=5.9 Hz), 0.95 (9H, s), 0.061 (3H, s), 0.060 (3H, s); ^{13}C NMR (100 MHz, C_6D_6), δ (ppm) 141.5 (C), 139.8 (C), 139.4 (C), 138.1 (C), 137.9 (C), 136.9 (C), 133.9 (C), 133.3 (C), 131.64 (CH×2), 131.60 (CH×2), 129.4 (CH×2), 129.1 (CH×2), 128.5 (CH), 128.4 (CH×4), 127.5 (CH×2), 127.4 (CH), 127.2 (CH×3), 127.0 (CH), 126.8 (CH), 126.2 (CH), 125.8 (CH), 125.5 (CH×2), 121.6 (C), 121.5 (C), 85.8 (CH), 81.3 (CH), 81.0 (C), 80.7 (CH), 79.9 (CH), 79.3 (CH), 78.7 (CH), 78.1 (CH), 77.0 (CH), 76.1 (CH), 75.2 (CH_2), 74.0 (CH), 73.1 (CH_2×2), 72.3 (CH_2), 71.1 (CH_2), 70.8 (CH_2), 70.2 (CH_2), 69.7 (CH_2), 45.7 (CH_2), 39.9 (CH_2), 37.5 (CH_2), 31.4 (CH_2),

28.8 (CH), 27.9 (CH_2), 26.9 (CH_3), 26.2 (CH_2), 26.0 ($CH_3\times3$), 18.3 (C), 14.7 (CH_3), −4.6 (CH_3), −4.8 (CH_3) (The signals of seven carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3062, 3025, 2926, 2855, 1603, 1593, 1509, 1496, 1487, 1453, 1404, 1360, 1338, 1250, 1201, 1096, 1070, 1012, 946, 835, 774, 733, 697; HR-FDMS, calcd for $C_{76}H_{92}^{79}Br_2O_{10}Si$ $[M]^+$: 1350.4826, found: 1350.4854.

7.1.41. (1*S*,3*R*,4*S*,6*S*,8*R*,10*R*,11*S*,1′*R*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-{1′-Benzyloxy-2′-[8″-benzyloxy-9″-benzyloxymethyl-3″-(*tert*-butyldimethylsilyloxy)-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl]ethyl}-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-ol (51). To a solution of **50** (10.4 mg, 7.68 μmol) in DCM–pH 7 buffer (10:1, v/v, 0.90 ml) was added DDQ (10.7 mg, 47.1 μmol) at 0 °C and the mixture was stirred for 20 min. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30 to 5) to give **51** (8.5 mg, 91%). **51**: a colorless oil; $[\alpha]_D^{18}$ +11.8 (*c* 0.425, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.48 (1H, d, *J*=7.1 Hz), 7.37–7.21 (9H, m), 7.15–7.01 (10H, m), 6.84 (2H, d, *J*=8.3 Hz), 5.91–5.82 (2H, m), 5.28 (1H, d, *J*=12.0 Hz), 4.88 (1H, d, *J*=12.0 Hz), 4.40 (1H, d, *J*=11.8 Hz), 4.37 (1H, d, *J*=11.7 Hz), 4.30–4.16 (7H, m), 4.13 (1H, d, *J*=11.8 Hz), 4.04 (1H, ddd, *J*=4.5, 9.8, 10.9 Hz), 3.90 (1H, d, *J*=12.0 Hz), 3.82–3.75 (4H, m), 3.72–3.60 (4H, m), 3.47 (1H, dd, *J*=7.0, 10.0 Hz), 3.32 (1H, dt, *J*=2.4, 9.0 Hz), 2.90–2.84 (1H, m), 2.61 (1H, br dd, *J*=8.5, 13.2 Hz), 2.55 (1H, ddd, *J*=3.5, 4.5, 13.3 Hz), 2.38 (1H, ddd, *J*=3.8, 4.5, 13.5 Hz), 2.18 (1H, ddd, *J*=5.6, 8.7, 13.2 Hz), 2.03–1.83 (5H, m), 1.67 (1H, ddd, *J*=5.7, 9.0, 14.8 Hz), 1.45 (2H, dd, *J*=4.1, 6.1 Hz), 1.21 (3H, s), 1.03 (3H, d, *J*=6.8 Hz), 0.94 (9H, s), 0.035 (3H, s), 0.027 (3H, s); ^{13}C NMR (100 MHz, C_6D_6), δ (ppm) 140.9 (C), 139.2 (C), 139.0 (C), 138.3 (C), 138.1 (C), 131.6 (CH×4), 129.4 (CH×2), 129.3 (CH×2), 129.2 (CH), 128.5 (CH×2), 128.4 (CH×2), 127.51 (CH), 127.47 (CH), 127.3 (CH), 126.4 (CH), 121.6 (C), 121.4 (C), 85.6 (CH), 82.1 (CH), 82.0 (CH), 80.5 (CH, C), 79.8 (CH), 77.0 (CH), 76.9 (CH), 76.5 (CH), 75.7 (CH_2), 74.0 (CH), 73.3 (CH_2), 72.9 (CH_2), 72.3 (CH_2), 71.54 (CH_2), 71.48 (CH), 70.3 (CH_2), 69.3 (CH_2), 45.7 (CH_2), 40.1 (CH_2), 38.3 (CH_2), 35.7 (CH_2), 28.8 (CH), 27.6 (CH_2), 27.0 (CH_3), 26.4 (CH_2), 26.0 ($CH_3\times3$), 18.3 (C), 13.8 (CH_3), −4.77 (CH_3), −4.84 (CH_3) (The signals of eight carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3475, 3063, 3027, 2927, 2857, 1593, 1487, 1470, 1453, 1405, 1372, 1360, 1339, 1298, 1250, 1215, 1088, 1028, 1012, 940, 888, 836, 804, 755, 697; HR-FDMS, calcd for $C_{65}H_{84}^{79}Br_2O_{10}Si$ $[M]^+$: 1210.4200, found: 1210.4193.

7.1.42. (1*S*,3*R*,4*S*,6*S*,8*R*,10*S*,11*S*,1′*R*,2″*R*,3″*S*,5″*Z*,8″*R*,9″*S*)-10-[1′-Benzyloxy-2′-(8″-benzyloxy-9″-benzyloxymethyl-3″-hydroxy-2″,3″,4″,7″,8″,9″-hexahydrooxonin-2″-yl)ethyl]-4-(4-bromobenzyloxy)-3-(4-bromobenzyloxymethyl)-6,10-dimethyl-2,9-dioxabicyclo[6.4.0]dodecan-11-one (53). To a solution of **51** (7.5 mg, 6.18 μmol) in DCM (0.80 ml) were added $NaHCO_3$ (21.8 mg, 259 μmol) and DMPI (22.0 mg, 51.9 μmol) at 25 °C and the reaction mixture was stirred for 30 min. After the mixture was diluted with Et_2O (1 ml), saturated aqueous Na_2SO_3 (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/AcOEt=4) to give a crude product, and it was used in the next reaction without further purification. To a solution of the above crude product in THF (0.80 ml) was added HF·Py at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 2 d. After the reaction mixture was diluted with Et_2O and cooled to 0 °C, saturated aqueous $NaHCO_3$ (1 ml) was added and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with AcOEt (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=5 to 2) to give **53** (6.8 mg, ~100% from **51**). **53**: a colorless oil; $[\alpha]_D^{22}$ +15.6 (*c* 0.340, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6), δ (ppm) 7.45–7.43 (2H, m), 7.31–7.28 (5H, m), 7.21–7.04 (12H, m), 6.92 (2H, d, *J*=8.3 Hz), 6.81 (2H, d, *J*=8.5 Hz), 5.95 (1H, dt, *J*=5.9, 10.6 Hz), 5.88 (1H, dt, *J*=5.9, 10.6 Hz), 4.79 (1H, d, *J*=10.9 Hz), 4.60 (1H, d, *J*=10.9 Hz), 4.41 (1H, d, *J*=12.3 Hz), 4.39 (1H, d, *J*=11.7 Hz), 4.35 (1H, d, *J*=12.3 Hz), 4.18–4.13 (4H, m), 4.10 (1H, d, *J*=11.7 Hz), 3.85 (1H, d, *J*=12.0 Hz), 3.83–3.80 (2H, m), 3.74 (1H, dt, *J*=6.8, 4.9 Hz), 3.70–3.65 (2H, m), 3.58–3.54 (2H, m), 3.44 (1H, dd, *J*=2.7, 9.8 Hz), 3.40 (1H, dt, *J*=2.7, 6.8 Hz), 3.38 (1H, ddd, *J*=2.0, 6.6, 9.0 Hz), 3.28 (1H, dd, *J*=6.6, 9.8 Hz), 3.16 (1H, dt, *J*=2.4, 9.0 Hz), 3.09 (1H, dd, *J*=7.4, 16.8 Hz), 2.86 (1H, ddd, *J*=2.7, 10.6, 13.4 Hz), 2.75 (1H, ddd, *J*=2.7, 10.6, 13.7 Hz), 2.52 (1H, dd, *J*=6.6, 16.8 Hz), 2.34–2.24 (4H, m), 1.92–1.88 (1H, m), 1.78–1.62 (3H, m), 1.47 (1H, ddd, *J*=5.6, 9.0, 15.6 Hz), 1.22 (3H, s), 0.95 (3H, d, *J*=6.8 Hz); ^{13}C NMR (100 MHz, C_6D_6), δ (ppm) 211.2 (C), 139.1 (C), 139.0 (C), 138.8 (C), 138.0 (C), 137.8 (C), 131.73 (CH×2), 131.67 (CH×2), 129.4 (CH×2), 129.3 (CH×2), 128.64 (CH×2), 128.60 (CH×4), 128.5 (CH×4), 121.69 (C), 121.66 (C), 86.6 (C), 85.1 (CH), 83.0 (CH), 82.7 (CH), 82.6 (CH), 81.7 (CH), 79.6 (CH), 78.7 (CH), 75.6 (CH), 74.8 (CH), 74.7 (CH_2), 73.3 (CH_2), 72.5 (CH_2), 72.0 ($CH_2\times2$), 71.4 (CH_2), 70.3 (CH_2), 46.2 (CH_2), 44.1 (CH_2), 39.6 (CH_2), 36.6 (CH_2), 30.8 (CH_2), 28.6 (CH), 26.9 (CH_3, CH_2), 17.9 (CH_3) (The signals of seven carbons were undetected due to overlapping with solvent signal.); IR (film), ν (cm^{-1}) 3454, 3063, 3027, 2926, 2865, 1717, 1592, 1487, 1453, 1405, 1367, 1321, 1300, 1215, 1099, 1027, 1012, 911, 838, 804, 755, 698; HR-FDMS, calcd for $C_{59}H_{68}^{79}Br_2O_{10}$ $[M]^+$: 1094.3179, found: 1094.3174.

7.1.43. (1*R*,3*S*,5*Z*,8*R*,9*S*,11*R*,13*R*,14*S*,16*R*,18*S*,20*S*,21*R*,23*S*)-8,13-Dibenzyloxy-9-benzyloxymethyl-20-(4-bromobenzyloxy)-21-(4-bromobenzyloxymethyl)-14,18-dimethyl-2,10,15,22-tetraoxatetracyclo[12.10.0.0^{3,11}.0^{16,23}]-tetracos-5-ene (54). To a solution of **53** (6.0 mg, 5.47 μmol) in DCM–Et_3SiH (10:1, v/v, 0.80 ml) was added TMSOTf (3.0 μl, 16.6 μmol) at 0 °C and the mixture was stirred for 30 min. Then, saturated aqueous $NaHCO_3$ (1 ml) was added

and the aqueous layer was extracted with Et_2O (5 ml) and AcOEt (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=10 to 4) to give **54** (4.6 mg, 78%). **54**: a colorless oil; $[\alpha]_D^{23}$ −4.96 (*c* 0.025, $CHCl_3$); ^{1}H NMR (600 MHz, C_6D_6), δ (ppm) 7.45 (2H, d, *J*=7.3 Hz), 7.21–7.02 (17H, m), 6.94 (2H, d, *J*=8.1 Hz), 6.83 (2H, d, *J*=8.1 Hz), 5.93 (1H, dt, *J*=6.4, 10.0 Hz), 5.82 (1H, dt, *J*=5.4, 10.0 Hz), 4.85 (1H, d, *J*=12.1 Hz), 4.71 (1H, d, *J*=12.1 Hz), 4.39 (1H, d, *J*=12.3 Hz), 4.37 (2H, s), 4.25 (1H, d, *J*=12.9 Hz), 4.22 (1H, d, *J*=12.9 Hz), 4.21 (1H, d, *J*=11.7 Hz), 4.11 (1H, d, *J*=12.3 Hz), 3.87 (1H, d, *J*=11.7 Hz), 3.70 (1H, t, *J*=6.0 Hz), 3.66–3.53 (6H, m), 3.52–3.50 (1H, m), 3.49–3.47 (1H, m), 3.39 (1H, dd, *J*=7.1, 9.9 Hz), 3.26–3.20 (2H, m), 3.07 (1H, dd, *J*=4.6, 12.1 Hz), 2.94 (1H, ddd, *J*=5.0, 10.0, 13.9 Hz), 2.69–2.63 (2H, m), 2.40 (1H, dt, *J*=12.5, 4.6 Hz), 2.31–2.26 (3H, m), 2.00–1.84 (4H, m), 1.68–1.57 (2H, m), 1.44 (3H, s), 1.00 (3H, d, *J*=7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 139.5 (C), 138.3 (C), 138.2 (C), 137.4 (C), 137.2 (C), 131.5 (CH×2), 131.4 (CH×2), 129.4 (CH×4), 128.3 (CH×5), 128.1 (CH×2), 128.0 (CH×2), 127.8 (CH×3), 127.62 (CH), 127.59 (CH), 127.5 (CH×2), 127.1 (CH), 121.5 (C), 121.4 (C), 85.9 (CH), 84.7 (CH), 84.4 (CH), 83.7 (CH), 82.6 (CH×2), 80.4 (C), 79.7 (CH), 79.0 (CH), 78.0 (CH), 73.3 (CH_2), 73.1 (CH_2), 72.7 (CH_2), 72.1 (CH), 71.9 (CH_2), 71.4 (CH_2), 70.54 (CH_2), 70.49 (CH_2), 45.0 (CH_2), 40.6 (CH_2), 38.6 (CH_2), 35.4 (CH_2), 32.5 (CH_2), 27.9 (CH), 27.6 (CH_2), 27.0 (CH_3), 11.5 (CH_3); IR (neat), ν (cm^{-1}) 2954, 2923, 2853, 1594, 1487, 1462, 1376, 1287, 1260, 1204, 1096, 1070, 1027, 1012, 840, 803, 729, 697; HR-FDMS, calcd for $C_{59}H_{68}^{79}Br_2O_9$ $[M]^+$: 1078.3230, found: 1078.3217.

7.1.44. (1*R*,3*R*,4*S*,6*S*,8*S*,10*S*,12*R*,13*S*,15*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*)-4-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-13-(4-bromobenzyloxy)-12-(4-bromobenzyloxymethyl)-3,15-dimethyl-6-(2-naphthyl)-2,5,7,11-tetraoxatricyclo[8.6.0.0^{3,8}]hexadecane (55a) and (1*R*,3*R*,4*S*,6*R*,8*S*,10*S*,12*R*,13*S*,15*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*)-4-[8′-benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-13-(4-bromobenzyloxy)-12-(4-bromobenzyloxymethyl)-3,15-dimethyl-6-(2-naphthyl)-2,5,7,11-tetraoxatricyclo[8.6.0.0^{3,8}]hexadecane (55b). To a solution of **31** (17.3 mg, 15.4 μmol) in benzene (1.0 ml) were added 2-naphthaldehyde dimethyl acetal (33.3 mg, 165 μmol) and PPTS (6.0 mg, 23.9 μmol). The reaction mixture was heated to 80 °C and stirred for 2 h. Then, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=30 to 4) to give **55a** (17.3 mg, 89%,) and **55b** (2.1 mg, 11%). **55a**: a colorless oil; $[\alpha]_D^{24}$ −27.9 (*c* 0.750, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 8.29 (1H, s), 7.91 (1H, dd, *J*=1.5, 8.5 Hz), 7.75–7.72 (2H, m), 7.62–7.58 (1H, m), 7.34–6.95 (18H, m), 6.81 (2H, d, *J*=8.5 Hz), 6.05 (1H, s), 5.88 (1H, dt, *J*=5.2, 10.4 Hz), 5.83 (1H, dt, *J*=5.2, 10.4 Hz), 4.55 (1H, dd, *J*=2.8, 11.3 Hz), 4.47 (1H, d, *J*=12.0 Hz), 4.41 (1H, d, *J*=12.0 Hz), 4.38 (1H, d, *J*=12.0 Hz), 4.22–4.15 (4H, m), 4.11 (1H, d, *J*=12.0 Hz), 3.93 (1H, dt, *J*=4.8, 9.5 Hz), 3.86 (1H, d, *J*=12.2 Hz), 3.69–3.57 (7H, m), 3.53 (1H, dd, *J*=2.4, 10.0 Hz), 3.43–3.41 (1H, m), 3.40 (1H, dd, *J*=6.7, 10.0 Hz), 3.30 (1H, dt, *J*=2.6, 8.8 Hz), 2.71 (1H, ddd, *J*=2.8, 10.4, 13.2 Hz), 2.52–2.45 (2H, m), 2.30–2.24 (1H, m), 2.17 (1H, dt, *J*=13.2, 5.2 Hz), 2.05–1.82 (7H, m), 1.53 (1H, ddd, *J*=5.4, 8.8, 14.5 Hz), 1.07 (3H, s), 1.03 (3H, d, *J*=6.3 Hz), 0.97 (9H, s), 0.13 (3H, s), 0.063 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.6 (C), 138.4 (C), 137.5 (C), 137.4 (C), 136.3 (C), 133.6 (C), 133.0 (C), 131.41 (CH×2), 131.39 (CH×2), 129.2 (CH×4), 128.4 (CH), 128.2 (CH×5), 128.0 (CH×2), 127.8 (CH), 127.7 (CH×2), 127.6 (CH), 127.5 (CH), 127.4 (CH), 127.1 (CH), 125.9 (CH), 125.8 (CH×2), 124.6 (CH), 121.4 (C), 121.3 (C), 95.7 (CH), 84.9 (CH), 83.2 (CH), 82.8 (CH), 80.3 (CH), 79.0 (CH), 78.2 (CH), 77.2 (C), 75.5 (CH), 75.2 (CH), 74.3 (CH), 73.4 (CH), 73.2 (CH_2), 72.5 (CH_2), 72.0 (CH_2), 71.9 (CH_2), 71.2 (CH_2), 70.4 (CH_2), 45.20 (CH_2), 45.18 (CH_2), 40.0 (CH_2), 33.3 (CH_2), 32.2 (CH_2), 28.3 (CH), 27.0 (CH_2), 26.9 (CH_3), 25.9 (CH_3×3), 17.9 (C), 16.4 (CH_3), −4.3 (CH_3), −4.4 (CH_3); IR (film), ν (cm^{-1}) 3062, 3026, 2854, 1593, 1487, 1453, 1370, 1317, 1255, 1213, 1172, 1100, 941, 835, 776, 737, 697; HR-FDMS, calcd for $C_{69}H_{84}^{79}Br_2O_{10}Si$ $[M]^+$: 1258.4200, found: 1258.4202. **55b**: a colorless oil; $[\alpha]_D^{21}$ +12.2 (*c* 0.105, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 8.05 (1H, s), 7.81 (1H, d, *J*=8.1 Hz), 7.75 (1H, d, *J*=8.5 Hz), 7.68–7.65 (2H, m), 7.51 (2H, d, *J*=7.1 Hz), 7.35–6.95 (16H, m), 6.82 (2H, d, *J*=8.3 Hz), 6.20 (1H, s), 6.07 (1H, dt, *J*=5.0, 10.7 Hz), 6.00 (1H, dt, *J*=5.0, 10.7 Hz), 4.74 (1H, d, *J*=11.7 Hz), 4.62 (1H, t, *J*=7.0 Hz), 4.54 (1H, dt, *J*=8.1, 3.3 Hz), 4.52 (1H, d, *J*=11.7 Hz), 4.45 (1H, d, *J*=11.8 Hz), 4.20 (1H, d, *J*=12.3 Hz), 4.190 (1H, d, *J*=11.8 Hz), 4.185 (1H, d, *J*=12.1 Hz), 4.14 (1H, d, *J*=12.3 Hz), 4.00 (1H, dt, *J*=8.4, 3.3 Hz), 3.93 (1H, t, *J*=4.8 Hz), 3.88 (1H, d, *J*=12.1 Hz), 3.86 (2H, d, *J*=2.4 Hz), 3.80 (1H, dt, *J*=4.8, 9.1 Hz), 3.66–3.57 (3H, m), 3.53 (1H, dd, *J*=2.2, 10.0 Hz), 3.43–3.41 (1H, m), 3.39 (1H, dd, *J*=6.7, 10.0 Hz), 3.28 (1H, dt, *J*=2.4, 9.0 Hz), 3.09 (1H, ddd, *J*=3.3, 10.7, 13.1 Hz), 2.81 (1H, ddd, *J*=3.3, 10.7, 14.0 Hz), 2.48 (1H, dt, *J*=13.8, 4.8 Hz), 2.32–2.26 (1H, m), 2.21–2.14 (3H, m), 1.99–1.79 (5H, m), 1.61–1.55 (1H, m), 1.19 (3H, s), 1.06 (3H, d, *J*=7.6 Hz), 1.04 (9H, s), 0.24 (3H, s), 0.083 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) 138.61 (C), 138.56 (C), 137.4 (C), 137.3 (C), 136.5 (C), 133.4 (C), 132.9 (C), 131.44 (CH×2), 131.41 (CH×2), 129.3 (CH×2), 129.2 (CH×2), 128.3 (CH×3), 128.2 (CH×3), 127.9 (CH×3), 127.8 (CH×2), 127.7 (CH), 127.51 (CH), 127.47 (CH), 127.4 (CH), 126.09 (CH), 126.07 (CH), 125.0 (CH), 124.3 (CH), 121.5 (C), 121.3 (C), 97.9 (CH), 84.9 (CH), 84.5 (CH), 84.3 (CH), 81.0 (CH), 79.1 (CH), 78.3 (CH), 77.2 (CH), 75.4 (C), 74.8 (CH), 73.1 (CH_2), 72.55 (CH_2), 72.52 (CH), 71.8 (CH_2), 71.4 (CH_2), 71.3 (CH), 70.5 (CH_2), 70.3 (CH_2), 45.1 (CH_2), 40.1 (CH_2), 32.6 (CH_2), 32.1 (CH_2), 31.6 (CH_2), 29.7 (CH_2), 28.4 (CH), 26.9 (CH_3), 26.0 (CH_3×3), 18.0 (C), 16.1 (CH_3), −4.2 (CH_3), −4.4 (CH_3); IR (film), ν (cm^{-1}) 3062, 3025, 2924, 2853, 1593, 1507, 1487, 1454, 1371, 1299, 1257, 1214, 1172, 1096, 1012, 940, 836, 803, 776, 752, 697; HR-FDMS, calcd for $C_{69}H_{84}^{79}Br_2O_{10}Si$ $[M]^+$: 1258.4200, found: 1258.4213.

7.1.45. (1*S*,2′*R*,3′*S*,5′*Z*,8′*R*,9′*S*,1″*S*,3″*R*,4″*S*,6″*S*,8″*R*,10″*S*,11″*S*)-2-[8′-Benzyloxy-9′-benzyloxymethyl-3′-(*tert*-butyldimethylsilyloxy)-2′,3′,4′,7′,8′,9′-hexahydrooxonin-2′-yl]-1-{4″-(4-bromobenzyloxy)-3″-(4-bromobenzyloxymethy)-6″,10″-dimethyl-11″-(2-naphthylmethyl)-2″,9″-dioxabicyclo[6.4.0]dodecan-10″-yl}ethanol (56). To a solution of **55a** (26.4 mg, 20.9 μmol) in DCM (0.70 ml) was added DIBAL (0.15 ml, 0.94 M in hexane, 141 μmol) at 0 °C for 5.5 h. Then, MeOH (0.1 ml) and saturated aqueous potassium sodium tartrate (1 ml) were added. The mixture was diluted with Et_2O (5 ml) and stirred at 25 °C for 10 h. The layers were separated and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Since the resultant residue included **56** and unreacted **55a**, the residue was dissolved in DCM (0.80 ml) and treated again with DIBAL (0.25 ml, 0.94 M in hexane, 235 μmol) at 0 °C for 4.5 h. Then, the reaction was quenched with MeOH (0.1 ml) and saturated aqueous potassium sodium tartrate (1 ml). After the same work-up as described above, the resultant crude mixture was roughly purified by column chromatography (silica gel, hexane/AcOEt=15 to 7) to give **56** (13.6 mg) and a mixture of **55a** and **56** (11.5 mg). The mixture of **55a** and **56** was dissolved in DCM (0.7 ml) and treated with DIBAL (0.20 ml, 0.94 M in hexane, 188 μmol) at 0 °C for 2 h. Then the reaction mixture was warmed to 10 °C and stirred for 2 h. Then, the reaction was quenched with MeOH (0.1 ml) and saturated aqueous potassium sodium tartrate (1 ml). After the same work-up as described above, the resultant crude mixture was purified by column chromatography (silica gel, hexane/AcOEt=15 to 7) to give **56** (11.0 mg). Thus, total 24.6 mg (93%) of **56** was obtained. **56**: a colorless oil; $[\alpha]_D^{23}$ +17.0 (*c* 1.15, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.77 (1H, s), 7.66–7.58 (3H, m), 7.47 (1H, dd, *J*=1.6, 8.4 Hz), 7.30–7.00 (16H, m), 6.88 (2H, d, *J*=8.3 Hz), 6.82 (2H, d, *J*=8.3 Hz), 5.92 (1H, dt, *J*=6.2, 10.4 Hz), 5.84 (1H, dt, *J*=5.7, 10.4 Hz), 4.72–4.69 (2H, m), 4.66 (1H, d, *J*=12.4 Hz), 4.49 (1H, d, *J*=12.3 Hz), 4.36 (1H, d, *J*=12.0 Hz), 4.20 (1H, d, *J*=11.8 Hz), 4.17 (1H, d, *J*=12.4 Hz), 4.13 (1H, d, *J*=12.4 Hz), 4.12 (1H, d, *J*=12.0 Hz), 4.09 (1H, d, *J*=12.3 Hz), 4.01–3.91 (4H, m), 3.87 (1H, d, *J*=11.8 Hz), 3.82 (1H, d, *J*=2.4 Hz), 3.80–3.76 (3H, m), 3.71 (1H, ddd, *J*=2.1, 7.1, 8.8 Hz), 3.70–3.66 (1H, m), 3.64 (1H, dd, *J*=2.6, 10.4 Hz), 3.55 (1H, dd, *J*=2.1, 9.9 Hz), 3.38 (1H, dd, *J*=7.1, 9.9 Hz), 3.29 (1H, dt, *J*=2.6, 8.8 Hz), 2.88–2.79 (2H, m), 2.46 (1H, dt, *J*=13.7, 3.9 Hz), 2.29–2.16 (3H, m), 2.04–1.74 (6H, m), 1.63 (1H, ddd, *J*=5.5, 8.8, 14.0 Hz), 1.28 (3H, s), 1.09 (3H, d, *J*=6.3 Hz), 0.94 (9H, s), 0.060 (3H, s), 0.036 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 138.5 (C), 138.2 (C), 137.4 (C), 137.3 (C), 136.8 (C), 133.2 (C), 132.8 (C), 131.4 (CH×2), 131.3 (CH×2), 129.3 (CH×3), 129.1 (CH×2), 128.21 (CH×2), 128.17 (CH×2), 127.9 (CH), 127.8 (CH), 127.7 (CH×2), 127.6 (CH), 127.5 (CH×2), 127.4 (CH), 127.3 (CH), 126.1 (CH), 126.0 (CH), 125.8 (CH×2), 125.7 (CH), 121.5 (C), 121.2 (C), 85.5 (CH×2), 81.3 (CH), 79.0 (CH), 78.4 (CH), 77.6 (C), 77.2 (CH), 76.98 (CH), 75.9 (CH), 73.4 (CH), 72.9 (CH_2), 72.4 (CH_2×2), 72.3 (CH), 71.9 (CH_2), 71.4 (CH_2), 70.5 (CH_2), 67.6 (CH_2), 45.2 (CH_2), 40.3 (CH_2), 35.0 (CH_2), 32.1 (CH_2), 28.8 (CH_2), 28.3 (CH), 26.9 (CH_3), 26.4 (CH_2), 25.9 (CH_3×3), 18.0 (C), 14.6 (CH_3), −4.67 (CH_3), −4.72 (CH_3); IR (film), ν (cm^{-1}) 3584, 3497, 3061, 3026, 2926, 2857, 1593, 1509, 1487, 1453, 1405, 1360, 1337, 1256, 1205, 1099, 1012, 940, 836, 774, 735, 698; HR-FDMS, calcd for $C_{69}H_{86}^{79}Br_2O_{10}Si$ $[M]^+$: 1260.4357, found: 1260.4365.

7.1.46. (2*R*,3*S*,5*Z*,8*R*,9*S*,1′*S*,3′*R*,4′*S*,6′*S*,8′*R*,10′*R*,11′*S*)-[8-Benzyloxy-9-benzyloxymethyl-3-(*tert*-butyldimethylsilyloxy)-2,3,4,7,8,9-hexahydrooxonin-2-yl]methyl 4′-(4-bromobenzyloxy)-3′-(4-bromobenzyloxymethyl)-6′,10′-dimethyl-11′-(2-naphthylmethyl)-2′,9′-dioxabicyclo[6.4.0]dodecan-10′-yl ketone (57). To a solution of **56** (24.6 mg, 19.5 μmol) in DCM (1.0 ml) were added $NaHCO_3$ (24.6 mg, 293 μmol) and DMPI (28.3 mg, 117 μmol) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 8 h. After the mixture was diluted with Et_2O (5 ml), saturated aqueous Na_2SO_3 (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/AcOEt=7 to 5) to give **57** (22.1 mg, 90%). **57**: a colorless oil; $[\alpha]_D^{19}$ +40.3 (*c* 1.11, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6), δ (ppm) 7.75 (1H, d, *J*=8.1 Hz), 7.67 (1H, d, *J*=8.3 Hz), 7.64 (1H, s), 7.59 (1H, d, *J*=8.1 Hz), 7.43 (1H, dd, *J*=1.5, 8.3 Hz), 7.33–7.06 (16H, m), 6.90 (2H, d, *J*=8.3 Hz), 6.83 (2H, d, *J*=8.3 Hz), 5.99–5.91 (2H, m), 4.51 (1H, d, *J*=12.3 Hz), 4.49–4.45 (3H, m), 4.44 (1H, d, *J*=12.3 Hz), 4.36 (1H, dt, *J*=3.9, 6.3 Hz), 4.23 (1H, d, *J*=12.0 Hz), 4.21 (1H, d, *J*=11.3 Hz), 4.18 (1H, dt, *J*=8.7, 3.0 Hz), 4.17 (1H, d, *J*=12.4 Hz), 4.12 (1H, d, *J*=12.4 Hz), 4.04 (1H, ddd, *J*=2.8, 4.9, 6.3 Hz), 3.89 (1H, dt, *J*=8.7, 3.4 Hz), 3.86 (1H, d, *J*=11.3 Hz), 3.84 (1H, dt, *J*=4.4, 11.5 Hz), 3.73 (1H, t, *J*=2.4 Hz), 3.72 (1H, d, *J*=3.0 Hz), 3.71 (1H, ddd, *J*=2.0, 7.1, 9.3 Hz), 3.54 (1H, dd, *J*=2.0, 9.8 Hz), 3.53–3.50 (1H, m), 3.40–3.32 (3H, m), 3.24 (1H, dt, *J*=2.3, 9.3 Hz), 2.98 (1H, ddd, *J*=2.8, 9.4, 13.4 Hz), 2.85 (1H, ddd, *J*=3.4, 9.4, 13.4 Hz), 2.36–2.30 (2H, m), 2.14 (1H, dt, *J*=13.4, 4.9 Hz), 2.03–1.84 (4H, m), 1.63 (1H, ddd, *J*=5.2, 9.3, 14.3 Hz), 1.56 (1H, ddd, *J*=2.4, 11.5, 13.9 Hz), 1.15 (3H, s), 1.06 (3H, d, *J*=6.3 Hz), 0.89 (9H, s), 0.12 (3H, s), 0.019 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm) 212.6 (C), 138.8 (C), 138.6 (C), 137.4 (C), 137.2 (C), 135.8 (C), 133.1 (C), 132.9 (C), 131.5 (CH×2), 131.4 (CH×2), 129.3 (CH×2), 129.2 (CH×2), 128.22 (CH×2), 128.19 (CH×2), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.8 (CH×2), 127.7 (CH×4), 127.3 (CH×2), 126.5 (CH), 126.01 (CH), 125.96 (CH), 125.8 (CH), 121.5 (C), 121.4 (C), 85.7 (CH), 83.0 (C), 82.5 (CH), 80.5 (CH), 80.4 (CH), 79.01 (CH), 78.97 (CH), 78.0 (CH), 73.79 (CH), 73.75 (CH), 73.1 (CH_2), 72.5 (CH_2), 71.9 (CH_2×2), 71.3 (CH_2), 70.5 (CH_2), 70.2 (CH_2), 45.3 (CH_2), 44.3 (CH_2), 40.6 (CH_2), 31.7 (CH_2), 31.1 (CH_2), 28.1 (CH), 27.3 (CH_2), 27.0 (CH_3), 25.8 (CH_3×3), 18.3 (CH_3), 17.9 (C), −4.5 (CH_3), −4.7 (CH_3); IR (film), ν (cm^{-1}) 3061, 3026, 2926, 2857, 1718, 1593, 1509, 1487, 1453, 1361, 1338, 1257, 1214, 1172, 1101, 1012, 948, 836, 775, 735, 697; HR-FDMS, calcd for $C_{69}H_{84}^{79}Br_2O_{10}Si$ $[M]^+$: 1258.4200, found: 1258.4180.

7.1.47. Reduction of 57. To a solution of **57** (13.8 mg, 10.9 μmol) in THF–H_2O (3:1, v/v, 1.2 ml) were added

$CeCl_3 \cdot 7H_2O$ (12.8 mg, 34.4 μmol) and $NaBH_4$ (22.3 mg, 589 μmol) at 25 °C. During 8 d, $NaBH_4$ was added several times to the reaction mixture with stirring until the reaction was complete. After that, saturated aqueous $NaHCO_3$ (1 ml) was added and the aqueous layer was extracted with Et_2O (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/AcOEt=4) to give a mixture of **49** and **56** (13.3 mg, 96%, **49**:**56**>5:1 from ^{1}H NMR).

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References and notes

1. For reviews of ciguatoxins and related marine toxins, see: (a) Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, *93*, 1897; (b) Scheuer, P. J. *Tetrahedron* **1994**, *50*, 3; (c) Murata, M.; Yasumoto, T. *Nat. Prod. Rep.* **2000**, *17*, 293; (d) Yasumoto, T. *Chem. Rec.* **2001**, *1*, 228.
2. For recent reviews of ciguatera, see: (a) Lehane, L.; Lewis, R. J. *Int. J. Food Microbiol.* **2000**, *61*, 91; (b) Lewis, R. J. *Toxicon* **2001**, *39*, 97.
3. Yasumoto, T.; Nakajima, R.; Bagnis, R.; Adachi, R. *Bull. Jpn. Soc. Sci. Fish.* **1977**, *43*, 1021.
4. Recent reports for immunological detection of CTXs aiming at prevention of ciguatera intoxication: (a) Oguri, H.; Hirama, M.; Tsumuraya, T.; Fujii, I.; Maruyama, M.; Uehara, H.; Nagumo, Y. *J. Am. Chem. Soc.* **2003**, *125*, 7608; (b) Hokama, Y.; Whang, C.; Chun, K. F.; Suma, C.; Higa, N.; Or, B.; Cocchetto, A.; Kansky, G. *J. Toxcol., Toxin Rev.* **2003**, *22*, 547; (c) Nagumo, Y.; Oguri, H.; Shindo, Y.; Sasaki, S.; Oishi, T.; Hirama, M.; Tomioka, Y.; Mizugaki, M.; Tsumuraya, T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2037; (d) Pauillac, S.; Sasaki, M.; Inoue, M.; Naar, J.; Branaa, P.; Chinain, M.; Tachibana, K.; Legrand, A.-M. *Toxicon* **1999**, *38*, 669; (e) Oguri, H.; Tanaka, S.; Hishiyama, S.; Oishi, T.; Hirama, M.; Tsumuraya, T.; Tomioka, Y.; Mizugaki, M. *Synthesis* **1999**, 1431; (f) Hokama, Y.; Nishimura, K.; Takenaka, W.; Ebesu, J. S. M. *J. Nat. Toxins* **1998**, *7*, 1.
5. (a) Scheuer, P. J.; Takahashi, W.; Tsutsumi, J.; Yoshida, T. *Science* **1967**, *155*, 1267; (b) Tachibana, K. Ph.D. Thesis, University of Hawaii, 1980; (c) Nukina, M.; Koyanagi, L. M.; Scheuer, P. J. *Toxicon* **1984**, *22*, 169; (d) Tachibana, K.; Nukina, M.; Joh, Y. G.; Scheuer, P. J. *Biol. Bull.* **1987**, *172*, 122.
6. Satake, M.; Morohashi, A.; Oguri, H.; Oishi, T.; Hirama, M.; Harada, N.; Yasumoto, T. *J. Am. Chem. Soc.* **1997**, *119*, 11325.
7. Satake, M.; Murata, M.; Yasumoto, T. *Tetrahedron Lett.* **1993**, *34*, 1975.
8. For the bioactivities of ciguatoxins and related compounds, see: (a) Catterall, W. A.; Risk, M. *Mol. Pharmacol.* **1981**, *19*, 345; (b) Bidard, J.-N.; Vijverberg, H. P. M.; Frelin, C.; Chungue, E.; Legrand, A.-M.; Bagnis, R.; Lazdanski, M. *J. Biol. Chem.* **1984**, *259*, 8353; (c) Lombet, A.; Bidard, J.-N.; Lazdanski, M. *FEBS Lett.* **1987**, *219*, 355; (d) Dechraoui, M.-Y.; Naar, J.; Pauillac, S.; Legrand, A.-M. *Toxicon* **1999**, *37*, 125. See also references cited in Ref. 2.
9. For the total synthesis of CTX3C, see: (a) Hirama, M.; Oishi, T.; Uehara, H.; Inoue, M.; Maruyama, M.; Oguri, H.; Satake, M. *Science* **2001**, *294*, 1904; (b) Inoue, M.; Uehara, H.; Maruyama, M.; Hirama, M. *Org. Lett.* **2002**, *4*, 4551; (c) Maruyama, M.; Inoue, M.; Oishi, T.; Oguri, H.; Ogasawara, Y.; Shindo, Y.; Hirama, M. *Tetrahedron* **2002**, *58*, 1835; (d) Uehara, H.; Oishi, T.; Inoue, M.; Shoji, M.; Nagumo, Y.; Kosaka, M.; Brazidec, J.-Y. L.; Hirama, M. *Tetrahedron* **2002**, *58*, 6493; (e) Inoue, M.; Yamashita, S.; Tatami, A.; Miyazaki, K.; Hirama, M. *J. Org. Chem.* **2004**, *69*, 2797; (f) Kobayashi, S.; Takahashi, Y.; Komano, K.; Alizadeh, B. H.; Kawada, Y.; Oishi, T.; Tanaka, S.; Ogasawara, Y.; Sasaki, S.; Hirama, M. *Tetrahedron* **2004**, *60*, 8375; (g) Inoue, M.; Hirama, M. *Synlett* **2004**, 577; (h) Inoue, M.; Hirama, M. *Acc. Chem. Res.* **2004**, *37*, 961; (i) Inoue, M.; Miyazaki, K.; Uehara, H.; Maruyama, M.; Hirama, M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12013; (j) Hirama, M. *Chem. Rec.* **2005**, *5*, 240.
10. For recent synthetic studies on ciguatoxins from other groups, see: (a) Takai, S.; Ploypradith, P.; Hamajima, A.; Kira, K.; Isobe, M. *Synlett* **2002**, 588; (b) Takai, S.; Isobe, M. *Org. Lett.* **2002**, *4*, 1183; (c) Kira, K.; Hamajima, A.; Isobe, M. *Tetrahedron* **2002**, *58*, 1875; (d) Sasaki, M.; Ishikawa, M.; Fuwa, H.; Tachibana, K. *Tetrahedron* **2002**, *58*, 1889; (e) Takakura, H.; Sasaki, M.; Honda, S.; Tachibana, K. *Org. Lett.* **2002**, *4*, 2771; (f) Sasaki, M.; Noguchi, T.; Tachibana, K. *J. Org. Chem.* **2002**, *67*, 3301; (g) Candenas, M. L.; Pinto, F. J.; Cintada, C. G.; Morales, E. Q.; Brouard, I.; Díaz, M. T.; Rico, M.; Rodríguez, E.; Rodríguez, R. M.; Pérez, R.; Pérez, R. L.; Martín, J. D. *Tetrahedron* **2002**, *58*, 1921; (h) Bond, S.; Perlmutter, P. *Tetrahedron* **2002**, *58*, 1779; (i) Takai, S.; Sawada, N.; Isobe, M. *J. Org. Chem.* **2003**, *68*, 3225; (j) Baba, T.; Isobe, M. *Synlett* **2003**, 547; (k) Baba, T.; Huang, G.; Isobe, M. *Tetrahedron* **2003**, *59*, 6851; (l) Tatami, A.; Inoue, M.; Uehara, H.; Hirama, M. *Tetrahedron Lett.* **2003**, *44*, 5229; (m) Baba, T.; Takai, S.; Sawada, N.; Isobe, M. *Synlett* **2004**, 603; (n) Fuwa, H.; Fujikawa, S.; Tachibana, K.; Takakura, H.; Sasaki, M. *Tetrahedron Lett.* **2004**, *45*, 4795; (o) Kobayashi, S.; Alizadeh, B. H.; Sasaki, S.-y.; Oguri, H.; Hirama, M. *Org. Lett.* **2004**, *6*, 751; (p) Hamajima, A.; Isobe, M. *Org. Lett.* **2006**, *8*, 1205.
11. For convergent approaches to polycyclic ether systems, see: (a) Alvarez, E.; Díaz, M. T.; Hanxing, L.; Martín, J. D. *J. Am. Chem. Soc.* **1995**, *117*, 1437; (b) Nicolaou, K. C. *Angew. Chem., Int. Ed.* **1996**, *35*, 589; (c) Ravelo, J. L.; Regueiro, A.; Rodríguez, E.; de Vera, J.; Martín, J. D. *Tetrahedron Lett.* **1996**, *37*, 2869; (d) Alvarez, E.; Pérez, R.; Rico, M.; Rodríguez, R. M.; Martín, J. D. *Org. Chem.* **1996**, *61*, 3003; (e) Oishi, T.; Nagumo, Y.; Hirama, M. *Synlett* **1997**, 980; (f) Oishi, T.; Nagumo, Y.; Hirama, M. *Chem. Commun* **1998**, 1041; (g) Sasaki, M.; Inoue, M.; Noguchi, Y.; Takeichi, A.; Tachibana, K. *Tetrahedron Lett.* **1998**, *39*, 2783; (h) Sasaki, M.; Fuwa, H.; Inoue, M.; Tachibana, K. *Tetrahedron Lett.* **1998**, *39*, 9027; (i) Fujiwara, K.; Morishita, H.; Saka, K.; Murai, A. *Tetrahedron Lett.* **2000**, *41*, 503; (j) Matsuo, G.; Hinou, H.; Koshino, H.; Suenaga, T.; Nakata, T. *Tetrahedron Lett.* **2000**, *41*, 903; (k) Mori, Y.; Mitsuoka, S.; Furukawa, H. *Tetrahedron Lett.* **2000**, *41*, 4161; (l) Kadowaki, C.; Chan,

P. W. H.; Kadota, I.; Yamamoto, Y. *Tetrahedron Lett.* **2000**, *41*, 5769; (m) Díaz, M. T.; Pérez, R. L.; Rodríguez, L.; Ravelo, J. L.; Martín, J. D. *Synlett* **2001**, 345; (n) Suzuki, K.; Nakata, T. *Org. Lett.* **2002**, *4*, 2739; (o) Kadota, I.; Ohno, A.; Matsuda, K.; Yamamoto, Y. *J. Am. Chem. Soc.* **2002**, *124*, 3562; (p) Inoue, M.; Wang, G. X.; Wang, J.; Hirama, M. *Org. Lett.* **2002**, *4*, 3439; (q) Oishi, T.; Watanabe, K.; Murata, M. *Tetrahedron Lett.* **2003**, *44*, 7315; (r) Inoue, M.; Wang, J.; Wang, G.-X.; Ogasawara, Y.; Hirama, M. *Tetrahedron* **2003**, *59*, 5645; (s) Sasaki, M.; Fuwa, H. *Synlett* **2004**, 1851; (t) Kadota, I.; Yamamoto, Y. *Acc. Chem. Res.* **2005**, *38*, 423; (u) Inoue, M. *Chem. Rev.* **2005**, *105*, 4379.

12. Recent reviews for the synthesis of polycyclic ethers: (a) Nakata, T. *Chem. Rev.* **2005**, *105*, 4314; (b) Inoue, M. *Chem. Rev.* **2005**, *105*, 4379.
13. For the synthetic studies on ciguatoxins in our laboratory, see: (a) Oka, T.; Murai, A. *Chem. Lett.* **1994**, 1611; (b) Oka, T.; Fujiwara, K.; Murai, A. *Tetrahedron* **1996**, *52*, 12091; (c) Atsuta, H.; Fujiwara, K.; Murai, A. *Synlett* **1997**, 307; (d) Oka, T.; Fujiwara, K.; Murai, A. *Tetrahedron Lett.* **1997**, *38*, 8035; (e) Oka, T.; Murai, A. *Tetrahedron* **1998**, *54*, 1; (f) Oka, T.; Fujiwara, K.; Murai, A. *Tetrahedron* **1998**, *54*, 21; (g) Fujiwara, K.; Saka, K.; Takaoka, D.; Murai, A. *Synlett* **1999**, 1037; (h) Fujiwara, K.; Tanaka, H.; Murai, A. *Chem. Lett.* **2000**, 610; (i) Fujiwara, K.; Takaoka, D.; Kusumi, K.; Kawai, K.; Murai, A. *Synlett* **2001**, 691; (j) Fujiwara, K.; Koyama, Y.; Doi, E.; Shimawaki, K.; Ohtaniuchi, Y.; Takemura, A.; Souma, S.; Murai, A. *Synlett* **2002**, 1496; (k) Fujiwara, K.; Koyama, Y.; Kawai, K.; Tanaka, H.; Murai, A. *Synlett* **2002**, 1835; (l) Tanaka, H.; Kawai, K.; Fujiwara, K.; Murai, A. *Tetrahedron* **2002**, *58*, 10017; (m) Fujiwara, K.; Goto, A.; Sato, D.; Ohtaniuchi, Y.; Tanaka, H.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2004**, *45*, 7011; (n) Takemura, A.; Fujiwara, K.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2004**, *45*, 7567; (o) Takemura, A.; Fujiwara, K.; Shimawaki, K.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron* **2005**, *61*, 7392; (p) Domon, D.; Fujiwara, K.; Ohtaniuchi, Y.; Takezawa, A.; Takeda, S.; Kawasaki, H.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2005**, *46*, 8279; (q) Domon, D.; Fujiwara, K.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2005**, *46*, 8285; (r) Takizawa, A.; Fujiwara, K.; Doi, E.; Murai, A.; Kawai, H.; Suzuki, T. *Tetrahedron Lett.* **2006**, *47*, 747.
14. (a) Ogura, K.; Tsuchihashi, G. *Tetrahedron Lett.* **1972**, *13*, 2681; (b) Herrmann, J. L.; Richman, J. E.; Wepplo, P. J.; Schlessinger, R. H. *Tetrahedron Lett.* **1973**, *14*, 4707.
15. The position numbering is according to that of CTX3C.
16. Nicolaou, K. C.; Hwang, C. K.; Nugiel, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 4136.
17. A similar ring closure of the G-ring part by reductive etherification was reported by the Isobe group, where the cyclization of a tricyclic compound corresponding to the EF–H-ring part having 26-keto and 31-hydroxy groups was successfully performed: Takai, S.; Sawada, N.; Isobe, M. *J. Org. Chem.* **2003**, *68*, 3225.
18. (a) Okude, Y.; Hirano, S.; Hiyama, T.; Nozaki, H. *J. Am. Chem. Soc.* **1977**, *99*, 3179; (b) Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644.
19. For successful synthesis of a *Z*-iodoolefin, see: (a) Smith, A. B., III; Beauchamp, T. J.; LaMarche, M. J.; Kaufman, M. D.; Qiu, Y.; Arimoto, H.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc.* **2000**, *122*, 8654; (b) Sawada, D.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2000**, *122*, 10521; See, also: (c) Tanino, K.; Arakawa, K.; Satoh, M.; Iwata, Y.; Miyashita, M. *Tetrahedron Lett.* **2006**, *47*, 861.
20. Mori, Y.; Yaegashi, K.; Furukawa, H. *J. Am. Chem. Soc.* **1996**, *118*, 8158.
21. (a) Carling, R. W.; Holmes, A. B. *Tetrahedron Lett.* **1986**, *27*, 6133; (b) Kotsuki, H.; Kadota, I.; Ochi, M. *Tetrahedron Lett.* **1990**, *31*, 4609.
22. (a) Negishi, E.; Yoshida, T. *Tetrahedron Lett.* **1980**, *21*, 1501; (b) For the hydrozirconation reaction with the Schwartz reagent (Cp_2ZrHCl), see: Kiyotsuka, Y.; Igarashi, J.; Kobayashi, Y. *Tetrahedron Lett.* **2002**, *43*, 2725 (c) Hart, D. W.; Schwartz, J. *J. Am. Chem. Soc.* **1974**, *96*, 8115; (d) Schwartz, J.; Labinger, J. A. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 333.
23. cf. Nocolaou, K. C.; Veale, C. A.; Hwang, C.-K.; Hutchinson, J.; Prasad, C. V. C.; Ogilvie, W. W. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 299.
24. (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155; (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
25. (a) Corey, E. J.; Narasaka, K.; Shibasaki, M. *J. Am. Chem. Soc.* **1976**, *98*, 6417; (b) Crimmins, M. T.; Tabet, E. A. *J. Am. Chem. Soc.* **2000**, *122*, 5473.
26. Since the *R* configuration at C31 of **22** was proved by modified Mosher's method, the C31 configuration of the other diastereomer **21** was determined as S. Preparation and ^{1}H NMR data of MTPA esters of **22** are described in Section 7. Ohtani, I.; Kusumi, T.; Kashuman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.
27. Kim, S,; Ahn, K. H. *J. Org. Chem.* **1984**, *49*, 1717.
28. Gemal, A. L.; Luche, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 5454.
29. Krishnamurthy, S.; Brown, H. C. *J. Am. Chem. Soc.* **1976**, *98*, 3383.
30. Tanaka, S,; Yamamoto, H.; Nozaki, H.; Sharpless, K. B.; Michaelson, R. C.; Cutting, J. D. *J. Am. Chem. Soc.* **1974**, *96*, 5254.
31. Miyashita, M.; Yoshikoshi, A.; Grieco, P. A. *J. Org. Chem.* **1977**, *42*, 3772.
32. Nicolaou, K. C.; Prasad, C. V. C.; Hwang, C.-K.; Duggan, M. E.; Veale, C. A. *J. Am. Chem. Soc.* **1989**, *111*, 5321.
33. Fujiwara, K.; Saka, K.; Takaoka, D.; Murai, A. *Synlett* **1999**, 1037.
34. Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
35. Evans, D. A.; Gauchet-Prunet, J. A.; Carreira, E. M.; Charette, A. B. *J. Org. Chem.* **1991**, *56*, 741.
36. Chen, K.-M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155.
37. (a) Gaunt, M. J.; Yu, J.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172; (b) Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169; (c) Wright, J. A.; Yu, J.; Spencer, J. B. *Tetrahedron Lett.* **2001**, *42*, 4033.

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Alternative syntheses of the D_{2d} symmetric 1,3,5,7-tetraiodotricyclo[3.3.0.0^{3,7}]octane

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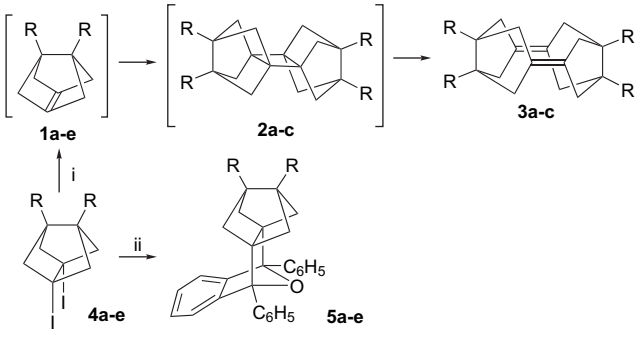
Abstract—Three alternative syntheses of 1,3,5,7-tetraiodotricyclo[3.3.0.0^{3,7}]octane are described. Reaction of this tetraiodide with sodium amalgam in the presence of dienes or with molten sodium in boiling 1,4-dioxane in the absence of trapping agents led to very complex mixtures of products, presumably due to competitive 1,2- and 1,3-deiodination reactions.

1. Introduction

As a part of our continuing research on pyramidalized alkene chemistry,[1] we have previously described the generation of highly pyramidalized tricyclo[3.3.0.0^{3,7}]oct-1(5)-ene derivatives (**1**) (Scheme 1) by reaction of 1,2-diiodo precursors (**4**) with molten sodium in boiling 1,4-dioxane, sodium amalgam in THF at room temperature or with *t*-BuLi in anhydrous THF at low temperature.[2] All these alkenes were trapped as Diels–Alder adducts with reactive dienes such as 1,3-diphenylisobenzofuran or 11,12-dimethylene-9,10-dihydro-9,10-ethanoanthracene.[3] When the pyramidalized alkenes were generated in the absence of a trapping diene, usually, but not always,[2e] diene dimers (**3**) via cyclobutane dimers (**2**) were obtained.



Scheme 1. Reactivity of highly pyramidalized alkenes **1a–e**, where **a**, R=H; **b**, R=Me; **c**, R=–OC(CH$_3$)$_2$O–; **d**, R=–OS(O)$_2$O–; **e**, R=-*o*,*o*′-biphenyl-. (i) Molten sodium, 1,4-dioxane, reflux; (ii) *t*-BuLi, 1,3-diphenylisobenzofuran, THF, −78 °C.

2. Results and discussion

In this article we describe three different syntheses of the D_{2d} symmetric 1,3,5,7-tetraiodotricyclo[3.3.0.0^{3,7}]octane, **6**, a potential precursor of tetraene **12**. Considering our previous experience with derivatives **1**, we reasoned that, as shown in Scheme 2, generation of highly pyramidalized alkene **7**, followed by dimerization to **8**, thermal [2+2] retrocycloaddition to **9**, and further reduction could lead to tetraene **12**. Alternatively, tricyclo[3.3.0.0^{3,7}]oct-1(5),3(7)-diene, **10**, might be generated, which on dimerization might give **11** and after [2+2] retrocycloaddition might lead to the targeted compound **12**. We were aware, however that in the present case, competitive 1,3-deiodination reactions leading to a propellane structure, **13**, could compete with the 1,2-deiodination leading to the pyramidalized alkene **7** (Fig. 1). In fact, UB3LYP/LANL2DZ theoretical calculations predict that propellane **13** is 1.5 kcal mol^{-1} more stable than the highly pyramidalized alkene **7**. In comparing the parent hydrocarbons, UB3LYP/6-31G(d) calculations predict that propellane **14** is 9.0 kcal mol^{-1} more stable than the highly pyramidalized alkene **1a**. However, MP2/6-31G(d) shows that **10** is an energy minimum with pyramidalization angles (Φ=64.1°) and carbon–carbon double bond distance (1.428 Å), quite similar to the previously calculated for alkenes **1a–e**.[1a]

We considered of interest to study the possible formation of tetraene **12**, no matter if it took place via the double highly

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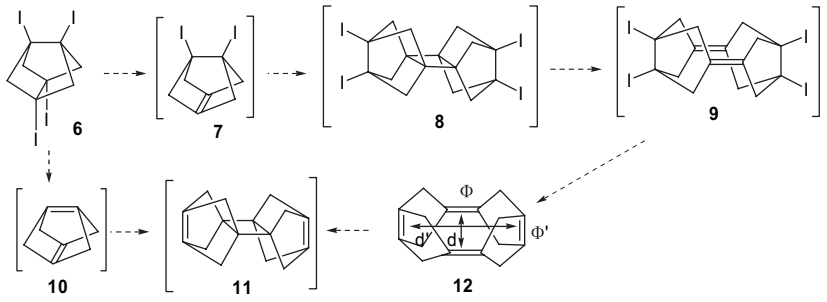


Scheme 2. Possible pathways from **6** to tetraene **12**.

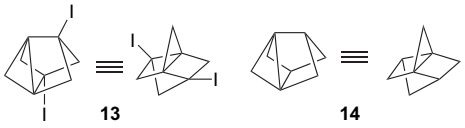
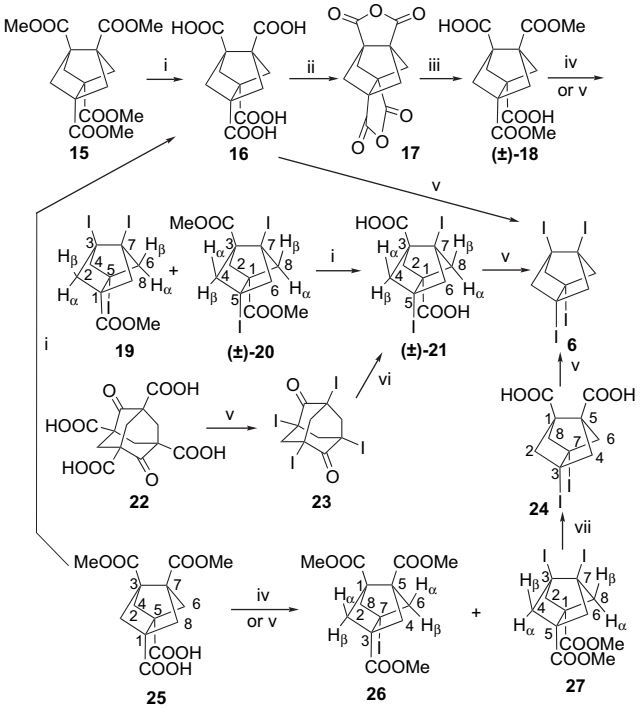


Figure 1. Structures of propellanes **13** and **14**.

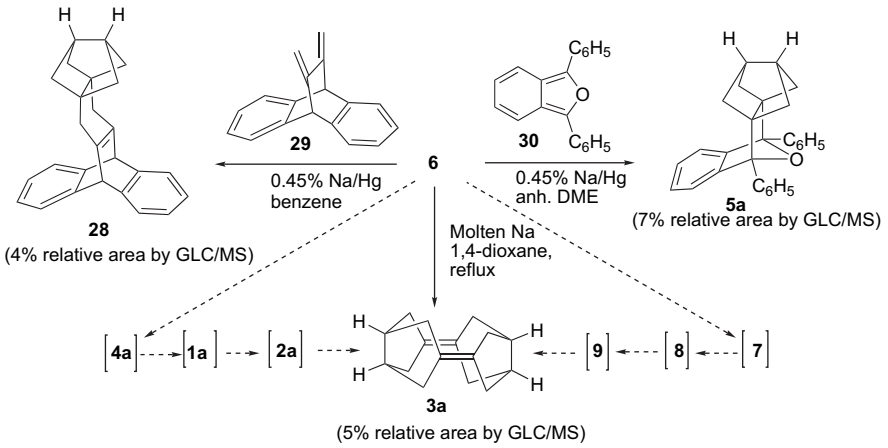
pyramidalized alkene **10** or via the pyramidalized alkene **7**. Tetraene **12**, with its four pyramidalized carbon–carbon double bonds, is a very interesting target. In addition to the pyramidalization-related issues [Φ=26.3° and Φ'=34.1°, as calculated by B3LYP/6-31G(d)], **12** has two pairs of proximal (d=3.619 Å, d'=3.789 Å), parallel double bonds, and π–π orbital interactions are therefore expected to occur.[4] The values of the pyramidalization angles of tetraene **12** suggest that it might be in the limit of isolable pyramidalized alkenes.[1a]

Initially, we used the recently described tetramethyl tricyclo[3.3.0.0^{3,7}]octane-1,3,5,7-tetracarboxylate, **15**, as a potential precursor of tetraiodo derivative **6**.[5] Although, the hydrolysis of tetraester **15** led to **16** in good yield, initial attempts to carry out the iododecarboxylation of tetraacid **16** using the Moriarty modification of the Suárez iododecarboxylation reaction [iodosobenzene diacetate (IBDA), and iodine in benzene or CH_2Cl_2 solution],[6] did not give the expected **6**, most of the starting acid being recovered unchanged. This result was ascribed to the low solubility of tetraacid **16** in both solvents. As an alternative, a stepwise iododecarboxylation reaction was planned. To this end, tetraacid **16** was transformed into bis-anhydride **17** by reaction with acetic anhydride, which on reaction with sodium methoxide in anhydrous methanol gave in good yield diester diacid (±)-**18** (Scheme 3). Iododecarboxylation of (±)-**18** in CH_2Cl_2 provided diiodo diester (±)-**20** in only 16% yield. When diiodo diacid (±)-**21**, obtained in high yield by saponification of (±)-**20**, was subjected to iododecarboxylation, tetraiodo derivative **6** was obtained in 70% yield. While this work was in progress, we found that acetonitrile could be advantageously used as a solvent in the Suárez iododecarboxylation reaction.[2e] When these conditions were applied to diester diacid (±)-**18**, the yield of (±)-**20** rose to 32%. Worthy of note, from this reaction, a small amount (5% yield) of triiodo ester **19** was also isolated, probably formed from the corresponding ester tricarboxylic acid. Moreover, using acetonitrile as a solvent, the direct conversion of tetraacid **16** to **6** was achieved in a modest 15.5% yield. To the best of our knowledge, this is the first time this kind of reaction has been applied to a tetracarboxylic acid. Overall, the direct transformation from **16** to **6** gave a slightly better yield than the five-step sequence going through dianhydride **17** (14.3% yield).



Scheme 3. (i) Concd HCl, AcOH, reflux, **16**: 94% from **15**; 95% from **25**; (±)-**21**: 91%; (ii) Ac_2O, reflux, 77%; (iii) NaOMe, anhyd methanol, reflux, 91%; (iv) IBDA, I_2, CH_2Cl_2, reflux, hν, (±)-**20**: 16%; **26**: 4%; **27**: 16%; (v) IBDA, I_2, acetonitrile, reflux, hν, (±)-**20**: 32%; **19**: 5%; **6**: 15.5% from **16**; 70% from (±)-**21**; 50% from **24**; **23**: 48%; **27**: 49%; (vi) KOH, ethanol, water, reflux, 85.5%; (vii) KOH, methanol, water, reflux, 98%.

As a shorter and higher yielding alternative sequence to tetraiodide **6**, we investigated a second route from the known diester diacid **25**.[5] Double iododecarboxylation of **25** was initially attempted using the Moriarty modification of the Suárez iododecarboxylation reaction in CH_2Cl_2 leading to diiodo diester **27** in 16% yield. Interestingly, iodo triester **26** was also isolated in 4% yield. This compound may arise from partial esterification of **25** by adventitious methyl iodide formed upon iododecarboxylation of acetic acid originated from IBDA. When the reaction was carried out in acetonitrile as the solvent, **27** was isolated in 49% yield. Saponification of **27** gave diacid **24** that on double iododecarboxylation in acetonitrile led to tetraiodo derivative **6**. The overall yield of this second route was 24%.



Scheme 4. Reactions of tetraiodide **6** with sodium amalgam in the presence of dienes **29** and **30** and with molten sodium in boiling 1,4-dioxane.

Unsatisfied with these lengthy and low yield syntheses of tetraiodide **6**, we undertook a third alternative synthesis of this compound from diketo tetraacid **22**, readily obtainable from the well-known Meerwein's ester, a compound easily available in multigram quantities.[7] Fourfold iododecarboxylation of **22** using IBDA and iodine under irradiation in acetonitrile as solvent furnished tetraiodo diketone **23** in 48% (about 84% yield per individual step).

Double Favorskii rearrangement of **23**, under similar conditions to those employed in a related case,[8] gave diiodo diacid (±)-**21** in high yield. Overall, the yield of tetraiodide **6** from diketo tetraacid **22** was 28.7% (11% from Meerwein's ester). Altogether, in spite of the lower yield of the last procedure, it is advantageous over the previous ones due to the availability of the starting Meerwein's ester.

With grams of compound **6** in hand, and in spite of its low solubility in most organic solvents, such as THF or DME, we carried out a preliminary study on the possible generation, trapping, and dimerization of the double highly pyramidalized alkene **10**. Reaction of tetraiodide **6** with *t*-BuLi in anhydrous THF at −78 °C in the presence of 11,12-dimethylene-9,10-dihydro-9,10-ethanoanthracene, **29**, gave a complex mixture of products (GC–MS), most of them derived from the reaction of diene **29** with *t*-BuLi. No product derived from **6** was observed in this mixture. Reaction of **6** with 0.45% sodium amalgam in benzene at room temperature in the presence of diene **29** gave a mixture containing mainly the starting diene and a small amount [4% relative area (r.a.)] of a compound of molecular mass 336, which could correspond to compound **28**. Similarly, reaction of **6** with 0.45% sodium amalgam in anhydrous DME at room temperature in the presence of 1,3-diphenylisobenzofuran, **30**, gave a complex mixture of products, containing mainly (GC–MS), starting diene (12% r.a.), products derived from it, such as 1,3-dihydro-1,3-diphenylisobenzofuran[9] (23% r.a.) and 2-benzoylbenzophenone (8% r.a.),[10] and compound **5a** (7% r.a.). Compound **5a** had been previously obtained by reaction of 1,5-diiodotricyclo[3.3.0.0^{3,7}]octane with *t*-BuLi in the presence of diene **30**.[2b] Also, compound **6** was reacted with molten sodium in boiling 1,4-dioxane in the absence of any trapping agent, thus giving rise to a very complex mixture of products (GC–MS), in which the known compound **3a** was present (5% r.a.).[2b]

These results may be explained by reduction of tetraiodide **6** to the known diiodide **4a**, followed by deiodination to give the highly pyramidalized alkene **1a**, which, as previously described, may be trapped with dienes to give the corresponding Diels–Alder adducts, such as **28** or **5a** or, in the absence of dienes, it may dimerize to give the cyclobutane dimer **2a**, which is then transformed into **3a** (Scheme 4). Alternatively, compound **3a** might be obtained by reduction of tetraiodo dimer **9**, formed as shown in Scheme 2 via the pyramidalized alkene **7** and the corresponding cyclobutane dimer **8**. Similarly, compounds **28** and **5a** could be obtained by reduction of Diels–Alder adducts derived from the highly pyramidalized alkene **7** and dienes **29** and **30**, respectively. We have no evidence in favor of the intermediate formation of the double highly pyramidalized alkene **10**.

The complexity of these reactions could be related to the fact that initial deiodination of **6** could take place in two competitive ways: (i) 1,2-deiodination to give the pyramidalized alkene **7** and (ii) 1,3-deiodination to give propellane **13** (Fig. 1). A study on the reaction of 1,3-diiodotricyclo[3.3.0.0^{3,7}]octane with molten sodium will be published elsewhere.

All of the new compounds herein described were fully characterized by spectroscopic means (IR, ^{1}H, ^{13}C NMR, and MS) and elemental analysis or HRMS. Assignments given for the NMR spectra are based on DEPT, COSY ^{1}H/^{1}H, HETCOR ^{1}H/^{13}C (HSQC and HMBC sequences for one bond and long range heterocorrelations, respectively), and NOESY experiments for selected compounds. Moreover, for tetraiodide **6**, an X-ray diffraction analysis was carried out.[11] Interestingly, **6** has a density of 3.22 g cm^{-3} and a bond length between the vicinal bridgehead carbon atoms of 1.625(7) Å.

3. Conclusions

In conclusion, three different approaches to the D_{2d} symmetric 1,3,5,7-tetraiodotricyclo[3.3.0.0^{3,7}]octane, **6**, have been developed, the key-step being a modified iododecarboxylation procedure, which uses acetonitrile as the solvent and has allowed the tetraiododecarboxylation of tetraacids **16** and **22**, the last one in an acceptable 48% yield. Reactions

of **6** with *t*-BuLi at low temperature have the drawback of low solubility of this compound in most organic solvents. Reactions of **6** with sodium amalgam or molten sodium seem to have generated a pyramidalized alkene, probably **1a**, which was trapped as Diels–Alder adducts and dimerized to **3a**. The complexity of these reactions may be understood by taking into account competitive 1,3-deiodination and 1,2-deiodination processes.

4. Computational details

Quantum-mechanical calculations were carried out at the unrestricted Becke's three-parameter hybrid functional with Lee, Yang and Parr correlation functional (UB3LYP) level,[12] using the 6-31G(d) basis set for **12**,[13] and the LANL2DZ basis set for **7** and **13**,[14] or at the restricted Møller–Plesset (MP2) level,[15] using the 6-31G(d) basis set for **10**, as implemented in Gaussian 03 on a Compaq HPC320 computer.[16] Geometry optimizations were undertaken using appropriate symmetry constraints and default convergence limits. The minimum energy nature of the optimized structures was verified from vibrational frequency analysis.

5. Experimental

5.1. General

Melting points were determined with a MFB 595010 M Gallenkamp melting point apparatus. Unless otherwise stated, NMR spectra were recorded in $CDCl_3$ in the following spectrometers: ^1H NMR (500 MHz, Varian VXR 500), ^{13}C NMR (75.4 MHz, Varian Gemini 300). ^1H and ^{13}C NMR chemical shifts (δ) are reported in parts per million with respect to internal tetramethylsilane (TMS). The multiplicity of the signals is: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; or their combinations. Assignments given for the NMR spectra are based on DEPT, COSY $^1H/^1H$, HETCOR $^1H/^{13}C$ (HSQC and HMBC sequences for one bond and long range heterocorrelations, respectively) and NOESY experiments for selected compounds. Diastereotopic methylene protons in tricyclo[3.3.0.0^{3,7}]octane derivatives are referred as H_α/H_β as shown in the corresponding structures. IR spectra were recorded on a FT–IR Perkin–Elmer spectrometer, model 1600; only the more intense absorption bands are given. Routine MS spectra were taken on a Hewlett–Packard 5988A spectrometer, the sample was introduced directly or through a gas chromatograph, Hewlett–Packard model 5890 Series II, equipped with a 30-meter HP-5 (5% diphenyl/95% dimethyl-polysiloxane) column [conditions: 10 psi; initial temperature: 35 °C (2 min); then heating at a rate of 8 °C min^{-1} till 300 °C, then isothermic] and the electron impact technique (70 eV). Only significant ions are given: those with higher relative abundance, except for the ions with higher *m*/*z* values. HRMS were performed on a Micromass Autospec spectrometer. Neutral aluminum oxide (MN), Brockmann activity 1 or silica gel SDS 60 (35–70 μm) was utilized for the standard and flash column chromatography, respectively. NMR and routine MS spectra were performed at the *Serveis Científico-Tècnics* of the University of Barcelona, while high resolution mass spectra and elemental analyses were carried out at the Mass Spectrometry Laboratory of the University of Santiago de Compostela (Spain) and at the Microanalysis Service of the IIQAB (C.S.I.C, Barcelona, Spain), respectively.

5.2. Tricyclo[3.3.0.0^{3,7}]octane-1,3,5,7-tetracarboxylic acid (16)

5.2.1. From tetramethyl tricyclo[3.3.0.0^{3,7}]octane-1,3,5,7-tetracarboxylate (15). A mixture of tetraester **15** (2.41 g, 7.08 mmol), concd HCl (57 mL), and glacial AcOH (57 mL) was heated under reflux for 15 h. The mixture was allowed to cool to room temperature and the precipitated solid was filtered under vacuum and washed with AcOEt (40 mL) to give tetraacid **16** (1.80 g) as a white solid. The combined filtrate and washings were concentrated in vacuo to give a residue (1.36 g), which was washed with AcOEt (20 mL) to give more tetraacid **16** (98 mg, global yield 94%), mp>300 °C (dec); IR (KBr) ν 3500–2300 (max at 3008, 2910, 2722, 2631, 2563), 1690, 1418, 1316, 1272, 1256 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ: 2.23 [s, 8H, 2(4,6,8)-H_2], 4.96 [br s, 4H, 1(3,5,7)-COO*H*]; ^{13}C NMR (CD_3OD) δ: 53.5 [CH_2, C2(4,6,8)], 59.1 [C, C1(3,5,7)], 174.7 (C, *C*OOH). MS (EI), *m*/*z* (%): 267 [(M−OH)$^+$, 2], 248 [(M−2H_2O)$^{\bullet+}$, 3], 238 [(M−HCO_2H)$^{\bullet+}$, 3], 220 [(M−HCO_2H−H_2O)$^{\bullet+}$, 66], 192 [(M−2HCO_2H)$^{\bullet+}$, 21], 181 (35), 176 (33), 175 [(M−2HCO_2H−OH)$^+$, 24], 150 (27), 149 (39), 148 [(M−2HCO_2H−CO_2)$^{\bullet+}$, 49], 147 (24), 137 (45), 132 (30), 131 (28), 119 (33), 105 (54), 104 (52), 103 (83), 91 (50), 79 (39), 78 (43), 77 (96), 65 (100). Elemental analysis calcd for $C_{12}H_{12}O_8$ (284.22): C 50.71, H 4.26. Found: C 50.51, H 4.30.

5.2.2. From 3,7-bis(methoxycarbonyl)tricyclo[3.3.0.0^{3,7}]-octane-1,5-dicarboxylic acid (25). Following the above procedure, starting from diacid **25** (801 mg, 2.57 mmol), tetraacid **16** (675+18 mg, 95% total yield) was obtained.

5.3. 5,11-Dioxapentacyclo[5.5.1.1^{3,9}.0^{1,9}.0^{3,7}]tetradecane-4,6,10,12-tetrone (17)

A mixture of tetraacid **16** (1.67 g, 5.88 mmol) and Ac_2O (70 mL) was heated under reflux for 2 h. The mixture was allowed to cool to room temperature, concentrated under reduced pressure to give anhydride **17** as a brown solid (1.43 g), which was sublimed at 240–250 °C/1 Torr, providing pure **17** (1.12 g, 77% yield) as a white solid, mp>300 °C (dec); IR (KBr) ν 1835, 1783, 1271, 1248, 939 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 2.77 [s, 2(8,13,14)-H_2]; ^{13}C NMR (DMSO-d_6) δ: 50.2 [C, C1(3,7,9)], 58.9 [CH_2, C2(8,13,14)], 168.1 [C, C4(6,10,12)]. MS (EI), *m*/*z* (%): 249 [(M+H)$^+$, 1], 204 [(M−CO_2)$^{\bullet+}$, 95], 176 [(M−CO_2−CO)$^{\bullet+}$, 24], 150 (37), 148 (22), 132 [(M−2CO_2−CO)$^{\bullet+}$, 33], 131 (26), 105 (26), 104 [(M−2CO_2−2CO)$^{\bullet+}$, 96], 103 (69), 92 (31), 91 (30), 78 (90), 77 (54), 63 (45), 52 (65), 51 (100). Elemental analysis calcd for $C_{12}H_8O_6$ (248.19): C 58.07, H 3.25. Found: C 57.75, H 3.25.

5.4. 5,7-Bis(methoxycarbonyl)tricyclo[3.3.0.0^{3,7}]octane-1,3-dicarboxylic acid [(±)-18]

To a mixture of anhydride **17** (1.08 g, 4.35 mmol) in anhydrous MeOH (70 mL), solid NaOMe (2.35 g, 43.5 mmol)

was added and the mixture was heated under reflux for 19 h. The solution was concentrated in vacuo to dryness and the residue was taken in water (50 mL). The aqueous solution was washed with AcOEt (2×9 mL), made acidic with concd HCl (5 mL), and the precipitated solid was filtered, washed with water (3×3 mL), and dried under vacuum to constant weight, to give pure bis-hemiester (±)-**18** (988 mg). The filtrate was extracted with AcOEt (4×30 mL), and the combined organic extracts were dried with anhydrous Na_2SO_4, and concentrated under reduced pressure to give more product (330 mg), which was crystallized from a mixture of AcOEt/*n*-pentane to give pure (±)-**18** (247 mg, total yield 91%), mp 219–221 °C (AcOEt/*n*-pentane); IR (KBr) ν 3700–2400 (max at 3549, 3004, 2958, 2845, 2710, 2640, 2565), 1712, 1443, 1335, 1309, 1281, 1239, 1145, 1089 cm^{-1}; ^1H NMR (CD_3OD) δ: 2.19–2.27 [complex signal, 8H, 2-H_2, 4(8)-H_2, and 6-H_2], 3.68 [s, 6H, 5(7)-$COOCH_3$], 4.86 [br s, 2H, 1(3)-COOH]; ^{13}C NMR (CD_3OD) δ: 52.4 [CH_3, 5(7)-COO*C*H_3], 52.9 (CH_2, C6), 53.2 [CH_2, C4(8)], 53.6 (CH_2, C2), 58.6 [C, C1(3)], 59.3 [C, C5(7)], 173.1 [C, 5(7)-*C*$OOCH_3$], 174.3 [C, 1(3)-*C*OOH]. MS (EI), *m/z* (%): 313 [(M+H)$^+$, 2], 295 [(M−OH)$^+$, 6], 281 [(M−CH_3O)$^+$, 34], 248 [(M−2CH_3OH)$^{\bullet+}$, 26], 234 [(M−HCOOCH_3−H_2O)$^{\bullet+}$, 100], 220 [(M−HCOOCH_3−CH_3OH)$^{\bullet+}$, 54], 209 (43), 195 (44), 175 (43), 151 (43), 147 (33), 119 (44), 103 (57), 91 (31), 77 (58), 65 (39), 59 [($COOCH_3$)$^+$, 52]. Elemental analysis calcd for $C_{14}H_{16}O_8\cdot 0.5H_2O$ (321.28): C 52.34, H 5.33. Found: C 52.28, H 5.19.

5.5. Dimethyl 5,7-diiodotricyclo[3.3.0.0^{3,7}]octane-1,3-dicarboxylate [(±)-20] and methyl 3,5,7-triiodotricyclo[3.3.0.0^{3,7}]octane-1-carboxylate (19)

A mixture of bis-hemiester (±)-**18** (700 mg, 2.24 mmol), iodine (1.25 g, 4.93 mmol), and iodosobenzene diacetate (IBDA, 1.62 g, 98% content, 4.93 mmol) in anhydrous acetonitrile (50 mL) was irradiated under reflux with a tungsten 100 W lamp in an argon atmosphere for 4 h. More iodine (1.25 g, 4.93 mmol) and IBDA (1.62 g, 4.93 mmol) were added and irradiation under reflux was continued for 20 h more. The resulting solution was concentrated under reduced pressure to dryness, the residue was taken in CH_2Cl_2 (100 mL), and the organic solution was washed with aqueous $Na_2S_2O_3$ solution (10%, 3×50 mL), saturated aqueous $NaHCO_3$ solution (3×50 mL), and brine (2×50 mL). Evaporation of the solvent from the dried organic phase (anhydrous Na_2SO_4) under reduced pressure gave a residue (430 mg), which was subjected to column chromatography [silica gel (21 g), hexane/AcOEt mixture]. On elution with hexane/AcOEt in the ratio of 45:1 (80 mL), triiodo ester **19** (60 mg, 5% yield) was obtained as a white solid. On elution with hexane/AcOEt in the ratio of 30:1 (400 mL), diiodo diester (±)-**20** (337 mg, 32% yield) was obtained as a white solid. The analytical sample of (±)-**20** was obtained by crystallization from AcOEt, mp 154–155 °C; TLC (silica gel), R_f 0.44 [hexane/AcOEt (3:1)]; IR (KBr) ν 1727, 1320, 1269, 1228 cm^{-1}; ^1H NMR δ: 2.180 [dt, J=10.2 Hz, J'=2.0 Hz, 2H, 4(8)-H_β], 2.182 (t, J=2.0 Hz, 2H, 2-H_2), 2.54 [dt, J=10.2 Hz, J'=2.0 Hz, 2H, 4(8)-H_α], 2.55 (t, J=2.0 Hz, 2H, 6-H_2), 3.78 [s, 6H, 1(3)-$COOCH_3$]; ^{13}C NMR δ: 28.5 [C, C5(7)], 50.4 (CH_2, C2), 52.2 [CH_3, 1(3)-COO*C*H_3], 60.0 [CH_2, C4(8)], 60.8 [C, C1(3)], 68.7 (CH_2, C6), 169.8 [C, 1(3)-*C*$OOCH_3$]. MS (EI), *m/z* (%): 349 [(M−I)$^+$, 6], 317 [(M−I−CH_3OH)$^+$, 38], 289 [(M−I−HCOOCH_3)$^+$, 45], 190 (28), 189 (25), 162 [(M−HCOOCH_3−2I)$^{\bullet+}$, 76], 131 (25), 104 (31), 103 [(M−HCOOCH_3−2I−COOCH_3)$^+$, 100], 102 (45), 91 (33), 78 (36), 77 (75), 59 [($COOCH_3$)$^+$, 83]. MS (CI, CH_4), *m/z* (%): 477 [(M+H)$^+$, 44], 445 [(M−CH_3O)$^+$, 17], 417 [(M−$COOCH_3$)$^+$, 15], 350 (18), 349 [(M−I)$^+$, 69], 318 (32), 317 [(M−I−CH_3OH)$^+$, 82], 291 (21), 290 (57), 289 [(M−I−HCOOCH_3)$^+$, 100], 221 [(M−I−IH)$^+$, 46], 191 (35), 190 (48), 189 [(M−I−IH−CH_3OH)$^+$, 48], 163 [(M−$COOCH_3$−2I)$^+$, 49], 162 (52), 103 [(M−HCOOCH_3−2I−COOCH_3)$^+$, 42]. HRMS calcd for ($C_{12}H_{14}I_2O_4$+H)$^+$: 476.9060. Found: 476.9071. The analytical sample of **19** was obtained by crystallization from diethyl ether, mp 120–122 °C; IR (KBr) ν 1732, 1314, 1265, 1227 cm^{-1}; ^1H NMR δ: 2.31 [m, 2H, 2(8)-H_β], 2.53 [m, 2H, 4(6)-H_α], 2.56 [m, 2H, 2(8)-H_α], 2.65 [m, 2H, 4(6)-H_β], 3.79 [s, 3H, $COOCH_3$]; ^{13}C NMR δ: 27.9 (C, C5), 39.9 [C, C3(7)], 52.3 [CH_3, COO*C*H_3], 60.5 [CH_2, C2(8)], 61.2 (C, C1), 69.1 [CH_2, C4(6)], 168.7 [C, *C*$OOCH_3$]. MS (EI), *m/z* (%): 544 (M$^{\bullet+}$, <1), 417 [(M−I)$^+$, 1], 389 [(M−I−CO)$^+$, 2], 357 [(M−I−HCOOCH_3)$^+$, 2], 290 (27), 289 [(M−I−IH)$^+$, 99], 163 (50), 162 [(M−HI−2I)$^{\bullet+}$, 90], 104 (51), 103 [(M−HCOOCH_3−3I)$^+$, 100], 78 (31), 77 (49), 59 [($COOCH_3$)$^+$, 25]. HRMS calcd for ($C_{10}H_{11}I_3O_2$)$^{\bullet+}$: 543.7893. Found: 543.7896. When this reaction was carried out in CH_2Cl_2 as solvent, the yield of (±)-**20** was 16%.

5.6. 5,7-Diiodotricyclo[3.3.0.0^{3,7}]octane-1,3-dicarboxylic acid [(±)-21]

5.6.1. From diiodo diester (±)-20. Hydrolysis of diiodo diester (±)-**20** was carried out as described before for the preparation of tetraacid **16**, from (±)-**20** (357 mg, 0.75 mmol), concd HCl (3 mL), and glacial AcOH (3 mL). Diiodo diacid (±)-**21** (204 mg of precipitated material and 102 mg of extracted product, 91% total yield) was obtained as a white solid, mp>300 °C (dec); IR (KBr) ν 3500–2200 (max at 3456, 3005, 2896, 2718, 2609, 2522), 1698, 1423, 1314, 1271, 1237 cm^{-1}; ^1H NMR (CD_3OD) δ: 2.16 (t, J=2.0 Hz, 2H, 2-H_2), 2.20 [dt, J=10.0 Hz, J'=2.0 Hz, 2H, 4(8)-H_β], 2.50 [dt, J=10.0 Hz, J'=2.0 Hz, 2H, 4(8)-H_α], 2.55 (br s, 2H, 6-H_2), 4.86 [br s, 2H, 1(3)-COOH]; ^{13}C NMR (CD_3OD) δ: 29.3 [C, C5(7)], 51.5 (CH_2, C2), 61.5 [CH_2, C4(8)], 62.4 [C, C1(3)], 70.5 (CH_2, C6), 173.0 [C, 1(3)-COOH]. MS (EI), *m/z* (%): 448 (M$^{\bullet+}$, 1), 430 [(M−H_2O)$^{\bullet+}$, 2], 321 [(M−I)$^+$, 4], 303 [(M−I−H_2O)$^+$, 100], 275 [(M−HCO_2H−I)$^+$, 64], 176 [(M−2I−H_2O)$^{\bullet+}$, 39], 175 (23), 149 (32), 148 [(M−HCO_2H−2I)$^{\bullet+}$, 78], 147 (21), 105 (34), 104 (47), 103 (54), 77 (61). Elemental analysis calcd for $C_{10}H_{10}I_2O_4$ (447.99): C 26.81, H 2.25, I 56.65. Found: C 26.80, H 2.07, I 56.26.

5.6.2. From 1,3,5,7-tetraiodoadamantane-2,6-dione (23). Tetraiodo tetrone **23** (9.66 g, 14.5 mmol) was added to a solution of 85% KOH (11.1 g, 168 mmol) in EtOH (30 mL) and water (30 mL) and the mixture was heated under reflux for 4 h. The resulting brown solution was cooled in an ice-bath, made acidic to pH ≈ 1–2 with 5 N HCl (30 mL), with formation of a white solid. The mixture was concentrated

to dryness under reduced pressure and the brown residue was dissolved in a mixture of AcOEt (600 mL) and water (150 mL). The organic phase was separated and the aqueous one was extracted with AcOEt (3×100 mL). The combined organic phase and extracts were dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give an orange residue (6.03 g), which was washed with a small amount of AcOEt to give pure diiodo diacid (±)-**21** (5.54 g, 85.5% yield), as a white solid.

5.7. 1,3,5,7-Tetraiodotricyclo[3.3.0.0^{3,7}]octane (6)

5.7.1. From tetraacid 16. This reaction was carried out in a similar manner to that described for the preparation of diiodo diester (±)-**20**. From **16** (129 mg, 0.45 mmol), iodine [2×(506 mg, 1.99 mmol)], and IBDA [2×(655 mg, 1.99 mmol)] in anhydrous acetonitrile (10 mL), tetraiodo derivative **6** (43 mg, 15.5% yield) was obtained, after washing the obtained yellow solid (71 mg) with diethyl ether, as a white solid very poorly soluble in most of the common organic solvents. When this reaction was carried out in CH_2Cl_2, no tetraiodo compound was isolated, the starting tetraacid being mainly recovered.

5.7.2. From diiodo diacid (±)-21. This reaction was carried out as described for the preparation of diiodo diester (±)-**20**. From (±)-**21** (244 mg, 0.54 mmol), iodine [2×(304 mg, 1.2 mmol)], and IBDA [2×(393 mg, 1.2 mmol)] in anhydrous acetonitrile (11 mL), tetraiodo derivative **6** (230 mg, 70% yield) was obtained, as a white solid.

5.7.3. From diiodo diacid 24. This reaction was carried out in a similar manner to that described for the preparation of diiodo diester (±)-**20**, using a lower excess of iodine (2.5+0.55 equiv) and IBDA (2.5+0.55 equiv). From diiodo diacid **24** (500 mg, 1.11 mmol), after the usual workup, a residue (460 mg) was obtained, which was subjected to column chromatography [flash silica gel (10 g), hexane/AcOEt mixture]. On elution with hexane, tetraiodo compound **6** (340 mg, 50% yield) was obtained as a white solid. The analytical sample was obtained by crystallization from CH_2Cl_2, mp 305–306 °C; IR (KBr) ν 1469, 1263, 1227, 989, 952, 867 cm^{-1}; ^{1}H NMR (300 MHz) δ: 2.64 [s, 2(4,6,8)-H_2]; ^{13}C NMR δ: 39.7 [C, C1(3,5,7)], 69.5 [CH_2, C2(4,6,8)]. MS (EI), *m/z* (%): 612 ($M^{\bullet+}$, 1), 358 [$(M-2I)^{\bullet+}$, 39], 231 [$(M-3I)^+$, 42], 104 [$(M-4I)^{\bullet+}$, 100], 103 (29), 78 (23), 77 (20). Elemental analysis calcd for $C_8H_8I_4$ (611.77): C 15.71, H 1.32, I 82.98. Found: C 15.87, H 1.34, I 83.03.

5.8. 1,3,5,7-Tetraiodoadamantane-2,6-dione (23)

A mixture of 2,6-dioxoadamantane-1,3,5,7-tetracarboxylic acid (**22**) (4.74 g, 13.93 mmol), iodine (15.56 g, 61.3 mmol), and iodosobenzene diacetate (IBDA, 20.14 g, 98% content, 61.3 mmol) in anhydrous acetonitrile (280 mL) was irradiated under reflux with two 100 W tungsten lamps in an argon atmosphere for 4 h. More iodine (15.56 g, 61.3 mmol) and IBDA (20.14 g, 61.3 mmol) were added and irradiation under reflux was continued for 20 h more. The mixture was allowed to cool to room temperature and the solvent was eliminated to dryness under reduced pressure. The residue was taken in AcOEt (450 mL) and the organic solution was washed with aqueous $Na_2S_2O_3$ solution (10%, 3×150 mL), saturated aqueous $NaHCO_3$ solution (3×150 mL), and brine (2×150 mL). Evaporation of the solvent from the dried organic phase (anhydrous Na_2SO_4) under reduced pressure gave a residue from which iodobenzene was distilled off at 100 °C/1–2 Torr. The residue (6.68 g) was taken in AcOEt (250 mL) and the organic solution was dried overnight with P_2O_5 (15 g). The mixture was filtered, the solid material was washed with AcOEt (200 mL), and the solvent was eliminated from the combined filtrate and washing under reduced pressure to give a light orange solid. Washing this solid with a small amount of diethyl ether, tetraiodo dione **23** (4.45 g, 48% yield) was obtained as a white solid. An analytical sample was obtained by sublimation at 220–230 °C/1 Torr, mp 307–308 °C; IR (KBr) ν 1736, 765, 661, 634 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.72 [s, 4(8,9,10)-H_2]; ^{13}C NMR (DMSO-d_6) δ: 46.6 [C, C1(3,5,7)], 62.2 [CH_2, C4(8,9,10)], 193.9 [C, C2(6)]. MS (EI), *m/z* (%): 668 ($M^{\bullet+}$, 6), 541 [$(M-I)^+$, 29], 513 [$(M-I-CO)^+$, 5], 386 [$(M-2I-CO)^{\bullet+}$, 37], 259 [$(M-3I-CO)^+$, 52], 231 [$(M-3I-2CO)^+$, 12], 132 [$(M-4I-CO)^{\bullet+}$, 32], 104 [$(M-4I-2CO)^{\bullet+}$, 100], 103 (76), 78 (82), 77 (74), 63 (45), 52 (44), 51 (86). HRMS calcd for $(C_{10}H_8I_4O_2)^{\bullet+}$: 667.6703. Found: 667.6706.

5.9. Dimethyl 3,7-diiodotricyclo[3.3.0.0^{3,7}]octane-1,5-dicarboxylate (27) and trimethyl 7-iodotricyclo-[3.3.0.0^{3,7}]octane-1,3,5-tricarboxylate (26)

This reaction was carried out as described for the preparation of diiodo diester (±)-**20**. From the known[5] diacid **25** (3.90 g, 12.5 mmol), iodine [2×(6.99 g, 27.5 mmol)], and IBDA [2×(9.03 g, 27.5 mmol)], after the usual workup, a residue (9.19 g) was obtained, which was subjected to column chromatography [flash silica gel (30 g), hexane/AcOEt mixture]. On elution with hexane/AcOEt in the ratio of 80:20, diiodo diester **27** (2.90 g, 49% yield) was obtained as a white solid. An analytical sample was obtained by crystallization from diethyl ether, mp 165.6–166.4 °C; TLC (silica gel), R_f 0.35 [hexane/AcOEt (3:1)]; IR (KBr) ν 1746, 1729 1435, 1304, 1270, 1215, 1131, 1081, 963, 946 cm^{-1}; ^{1}H NMR (300 MHz) δ: 2.48 [s, 8H, 2(4,6,8)-H_α and 2(4,6,8)-H_β], 3.70 [s, 6H, 1(5)-$COOCH_3$]; ^{13}C NMR δ: 41.0 [C, C3(7)], 52.3 [CH_3, 1(5)-$COOCH_3$], 58.8 [C, C1(5)], 61.4 [CH_2, C2(4,6,8)], 168.9 [C, 1(5)-*C*$OOCH_3$]. MS (EI), *m/z* (%): 476 ($M^{\bullet+}$, 1), 444 [$(M-CH_3OH)^{\bullet+}$, 14], 317 [$(M-I-CH_3OH)^+$, 23], 289 [$(M-I-HCOOCH_3)^+$, 71], 163 (22), 162 [$(M-HCOOCH_3-2I)^{\bullet+}$, 100], 150 (23), 131 (16), 104 (20), 103 (68), 102 (23), 77 (39), 59 [$(COOCH_3)^+$, 39]. Elemental analysis calcd for $C_{12}H_{14}I_2O_4$ (476.05): C 30.28, H 2.96, I 53.32. Found: C 30.22, H 2.94, I 53.42.

When the above reaction was carried out in a similar way but using CH_2Cl_2 instead of acetonitrile as the solvent, from diacid **25** (189 mg, 0.61 mmol), after the usual workup, a residue (123 mg) was obtained, which was subjected to column chromatography [silica gel (3.5 g), hexane/AcOEt mixture]. On elution with hexane/AcOEt in the ratio of 30:1 (240 mL), diiodo diester **27** (46 mg, 16% yield) was obtained as a white solid. On elution with hexane/AcOEt in the ratio of 10:1 (110 mL), iodo triester **26** (10 mg, 4% yield) was obtained as a white solid. An analytical sample of **26** was obtained by crystallization from diethyl ether, mp 134–135 °C; TLC

(silica gel), R_f 0.19 [hexane/AcOEt (3:1)]; IR (KBr) ν 1731, 1437, 1336, 1299, 1272, 1219 cm^{-1}; ^1H NMR δ: 2.10 [m, 2H, 2(4)-H_α], 2.36 [m, 2H, 2(4)-H_β], 2.39 [m, 2H, 6(8)-H_β], 2.49 [m, 2H, 6(8)-H_α], 3.70 [s, 6H, 1(5)-$COOCH_3$], 3.80 (s, 3H, 3-$COOCH_3$); ^{13}C NMR (50.3 MHz) δ: 30.0 (C, C7), 51.3 [CH_2, C2(4)], 52.1 (CH_3, 3-$COOCH_3$), 52.2 [CH_3, 1(5)-$COOCH_3$], 58.1 [C, C1(5)], 60.5 (C, C3), 60.9 [CH_2, C6(8)], 170.1 [C, 1(5)-$COOCH_3$], 170.8 (C, 3-$COOCH_3$). MS (EI), *m/z* (%): 377 [$(M-CH_3O)^+$, 11], 348 [$(M-HCO_2CH_3)^{\bullet+}$, 21], 316 [$(M-CH_3OH-HCOOCH_3)^{\bullet+}$, 84], 309 (75), 289 [$(M-COOCH_3-HCOOCH_3)^+$, 18], 277 (29), 221 [$(M-HCOOCH_3-I)^+$, 51], 189 [$(M-HCOOCH_3-CH_3OH-I)^+$, 100], 182 (47), 162 (54), 161 [$(M-2HCOOCH_3-I)^+$, 88], 150 (49), 133 (38), 119 (30), 103 [$(M-HCOOCH_3-2I-COOCH_3)^{\bullet+}$, 75], 91 (34), 77 (64), 59 [$(COOCH_3)^+$, 100]. HRMS calcd for $(C_{14}H_{17}IO_6)^{\bullet+}$: 408.0070. Found: 408.0063. From the aqueous phase, after acidification with 10% HCl (5 mL), extraction with AcOEt (7×10 mL), and concentration of the dried organic extracts, starting diacid **25** (83 mg, 44% yield) was recovered.

5.10. 3,7-Diiodotricyclo[3.3.0.0^{3,7}]octane-1,5-dicarboxylic acid (24)

A mixture of diester **27** (730 mg, 1.53 mmol) and a solution of KOH (10%) in MeOH (9 mL) was heated under reflux for 3 h. Water (7 mL) was added and heating under reflux was continued for 3 h more. The organic solvent was distilled off under reduced pressure and the aqueous phase was made acidic with aqueous HCl (10%, 8 mL). The precipitated solid was filtered, thoroughly washed with water (3×8 mL), and dried with P_2O_5 under reduced pressure to give diiodo diacid **24** (680 mg, 98% yield). The analytical sample was obtained by crystallization from a mixture of AcOEt/*n*-pentane in the ratio of 1:1, mp>280 °C (dec). IR (KBr) 3400−2300 (max at 3092, 2992, 2942, 2713, 2613), 1715 cm^{-1}; ^1H NMR (CD_3OD) δ: 2.43 [d, J=9.0 Hz, 4H], 2.49 [d, J=9.0 Hz, 4H, 2(4,6,8)-H_α and 2(4,6,8)-H_β], 4.90 [s, 2H, 1(5)-COOH]; ^{13}C NMR (CD_3OD) δ: 42.3 [C, C3(7)], 60.4 [C, C1(5)], 62.7 [CH_2, C2(4,6,8)], 172.2 [C, 1(5)-COOH]. MS (EI), *m/z* (%): 449 [$(M+H)^+$, 2], 448 ($M^{\bullet+}$, 1), 430 [$(M-H_2O)^{\bullet+}$, 8], 403 [$(M-COOH)^+$, 5], 358 [$(M-2COOH)^{\bullet+}$, 14], 304 (16), 303 [$(M-I-H_2O)^+$, 18], 276 (77), 275 [$(M-I-HCOOH)^+$, 89], 231 [$(M-I-2COOH)^+$, 31], 150 (33), 149 (76), 148 (76), 105 (42), 104 [$(M-2I-2COOH)^{\bullet+}$, 100], 103 (56), 78 (51), 77 (63). Elemental analysis calcd for $C_{10}H_{10}I_2O_4$ (448.00): C 26.81, H 2.25, I 56.65. Found: C 27.19, H 2.32, I 56.13.

5.11. Reaction of tetraiodide 6 with *t*-BuLi in the presence of 11,12-dimethylene-9,10-dihydro-9,10-ethanoanthracene (29)

To a stirred and cold (−78 °C) suspension of tetraiodide **6** (100 mg, 0.163 mmol) and diene **29** (83 mg, 0.36 mmol) in anhydrous THF (5 mL) maintained under an argon atmosphere, a solution of *t*-BuLi in pentane (1.5 M, 0.4 mL, 0.6 mmol) was added dropwise. After stirring for 30 min at −78 °C, the mixture was allowed to heat to room temperature. Methanol (1 mL) and water (5 mL) were added and the mixture was extracted with diethyl ether (3×10 mL). The combined organic phases were dried (anhydrous Na_2SO_4) and concentrated in vacuo to give a yellowish residue (172 mg), which when analyzed by GC–MS showed the presence of four components: (i) starting diene **29** [molecular ion: *m/z*=230, rt 23.2 min, 14.3% relative area (r.a.)]; (ii) product of addition of C_4H_{10} to the starting diene (rt 25.3 min, 29.6% r.a.), MS *m/z* (%): 288 ($M^{\bullet+}$, 46), 232 (50), 231 [$(M-C_4H_9)^+$, 100], 217 (61), 216 (69), 215 (60), 178 [$(C_{14}H_{10})^{\bullet+}$, 46], 57 (20); (iii) product of double addition of C_4H_9 to the starting diene (rt 27.4 min, 46.9% r.a.), MS *m/z* (%): 344 ($M^{\bullet+}$, 24), 288 (13), 232 (29), 231 (94), 178 [$(C_{14}H_{10})^{\bullet+}$, 31], 57 (100); (iv) product of addition of C_4H_9 and C_4H_7O (tetrahydrofuryl) to the starting diene (rt 25.3 min, 9.2% r.a.), MS *m/z* (%): 358 ($M^{\bullet+}$, 5), 274 (5), 231 (7), 178 [$(C_{14}H_{10})^{\bullet+}$, 12], 71 [$(C_4H_7O)^+$, 100].

5.12. Reaction of tetraiodide 6 with 0.45% sodium amalgam in the presence of diene 29

To a mixture of 0.45% sodium amalgam [from Na (97 mg, 4.24 mmol) and Hg (21.6 g, 108 mmol)] and diene **29** (90 mg, 0.39 mmol) in anhydrous benzene (10 mL) kept under an argon atmosphere, solid tetraiodide **6** (100 mg, 0.163 mmol) was added at once and the reaction mixture was stirred overnight at room temperature. The mixture was filtered through a pad of Celite® and the residue was thoroughly washed with AcOEt (3×10 mL). Concentration of the combined filtrate and washings under reduced pressure gave a residue (194 mg) containing inorganic material, which was extracted with CH_2Cl_2 (20 mL). Elimination of the solvent from the extract in vacuo gave a new residue (113 mg), which when analyzed by GC–MS showed the presence of two main components: (i) starting diene **29** (rt 23.2 min, 95% r.a.) and (ii) a product (rt 31.5 min, 4% r.a.), whose MS spectrum is compatible for compound **28**, MS *m/z* (%): 337 (24), 336 ($M^{\bullet+}$, 81), 293 (17), 267 (18), 265 (17), 253 (18), 252 (19), 217 (34), 216 (100), 215 (62), 203 (61), 202 (60), 178 [$(C_{14}H_{10})^{\bullet+}$, 78].

5.13. Reaction of tetraiodide 6 with 0.45% sodium amalgam in the presence of 1,3-diphenylisobenzofuran (30)

To 0.45% sodium amalgam [from Na (600 mg, 26 mmol) and Hg (133 g, 663 mmol)] a solution of diene **30** (650 mg, 2.4 mmol) and tetraiodide **6** (611 mg, 1.0 mmol) in anhydrous 1,2-dimethoxyethane (DME, 65 mL) was added and the reaction mixture was stirred overnight at room temperature, under an argon atmosphere and protected from light. The mixture was filtered through a pad of Celite® and the filtrate was analyzed by GC–MS showing the presence of many components, those with higher r.a. being: (i) dihydro-1,3-diphenylisobenzofuran[9] (rt 23.5 min, 23% r.a.), (ii) 2-benzoylbenzophenone[10] (rt 25.0 min, 8% r.a.), (iii) starting diene **30** (rt 26.6 min, 12% r.a.), and (iv) compound **5a**[2b] (rt 28.5 min, 7% r.a.).

5.14. Reaction of tetraiodide 6 with molten sodium in boiling 1,4-dioxane

Solid tetraiodide **6** (1.22 g, 2.0 mmol) was added to molten sodium (920 mg, 40 mmol) in boiling 1,4-dioxane and the mixture was heated under reflux for 4 h. The mixture was filtered through a pad of Celite® and the residue was washed

with Et_2O (3×20 mL). Distillation of the combined filtrate and washings at atmospheric pressure using a 10 cm Vigreux column left a residue (650 mg), still containing 1,4-dioxane, which when analyzed by GC–MS showed the presence of many components, among them: (i) and (ii) products **A** and **B**: diastereomeric 2-(1,4-dioxan-2-yl)-1,4-dioxane (1,4-dioxane dimers)[17] (molecular ions: *m*/*z*=174, rt's 11.5 and 11.9 min, 9 and 10% r.a.'s, respectively); (iii) product **C** (rt 14.2 min, 36% r.a.), MS *m*/*z* (%): 194 ($M^{\bullet+}$, 8), 117 (16), 113 (15), 112 (12), 107 [$(M-C_4H_7O_2)^+$, 12], 91 (29), 87 [$(C_4H_7O_2)^+$, 32], 86 (21), 79 (48), 73 (51), 67 (100); (iv) product **D** (rt 16.4 min, 10% r.a.), MS *m*/*z* (%): 214 ($M^{\bullet+}$, 1), 213 (1), 171 (18), 143 (20), 131 (25), 129 (30), 117 (21), 106 (21), 105 (34), 91 (58), 80 (22), 79 (46), 77 (24), 67 (100); (v) compound **3a**[2b] (rt 19.1 min, 5% r.a.), (vi) product **E** (rt 21.4 min, 12% r.a.), MS *m*/*z* (%): 280 ($M^{\bullet+}$, 6), 218 (14), 205 (25), 193 [$(M-C_4H_7O_2)^+$, 33], 132 (44), 131 (49), 107 [$(M-C_4H_7O_2-C_4H_6O_2)^+$, 82], 91 (49), 86 (48), 79 (57), 73 (100), 67 (55); (vii) product **F** (rt 23.3 min, 8% r.a.), MS *m*/*z* (%): 300 ($M^{\bullet+}$, 1), 259 (10), 213 [$(M-C_4H_7O_2)^+$, 44], 171 (20), 131 (37), 129 (31), 117 (25), 105 (33), 91 (69), 87 (37), 81 (27), 79 (74), 77 (33), 73 (47), 67 (100); (viii) product **G** (rt 25.4 min, 8% r.a.), MS *m*/*z* (%): 299 (24), 298 ($M^{\bullet+}$, 87), 143 (23), 129 (24), 117 (26), 115 (22), 107 (25), 106 (28), 105 (27), 93 (26), 91 (78), 87 (40), 79 (59), 77 (41), 73 (100).

5.15. X-ray crystal structure determination of 6[11]

A prismatic crystal (0.1×0.1×0.2 mm) was selected and mounted on a MAR345 diffractometer with an image plate detector. Unit-cell parameters were determined from 9482 reflections ($3<\theta<31^\circ$) and refined by least-squares method. Intensities were collected with graphite monochromatized Mo Kα radiation. Reflections (9891) were measured in the range $3.29\leq\theta\leq31.72^\circ$. Reflections (1945) of which were nonequivalent by symmetry [R_{int}(on *I*)=0.046]. Reflections (1842) were assumed as observed applying the condition $I>2\sigma(I)$. Lorentz polarization and absorption corrections were made. The structure was solved by direct methods, using SHELXS computer program[18] and refined by full matrix least-squares method with SHELX-97 computer program,[19] using 1945 reflections (very negative intensities were not assumed). The function minimized was $\sum w||F_o|^2-|F_c|^2|^2$, where $w=[\sigma^2(I)+(0.0482P)^2+11.4234P]^{-1}$, and $P=(|F_o|^2+2|F_c|^2)/3$, *f*, *f*′ and *f*″ were taken from the literature.[20] All H atoms were located from a difference synthesis and refined with an isotropic temperature factor. The final *R*(on *F*) factor was 0.046, $wR(\text{on}|F|^2)=0.114$ and goodness of fit=1.095 for all observed reflections. Number of refined parameters was 73. Max shift/esd=0.00, mean shift/esd=0.00. Max and min peaks in final difference synthesis was 0.732 and −0.753 e Å^{-3}, respectively. [$C_8H_8I_4$], M_r=611.74, orthorhombic, space group *Pnca*, *a*=9.7270(10), *b*=12.2340(10), *c*=10.6080(10), α=90, β=90, γ=90°, *V*=1262.4(2) Å^3, *Z*=4, *F*(000)=1072, ρ_{calcd}=3.219 g cm^{-3}; crystal dimensions (mm), 0.1×0.1×0.2 mm; μ (Mo Kα) linear absorption coefficient=9.828 mm^{-1}, *T*=293(2) K, 1945 reflections and 73 parameters were used for the full matrix (Fig. 2).

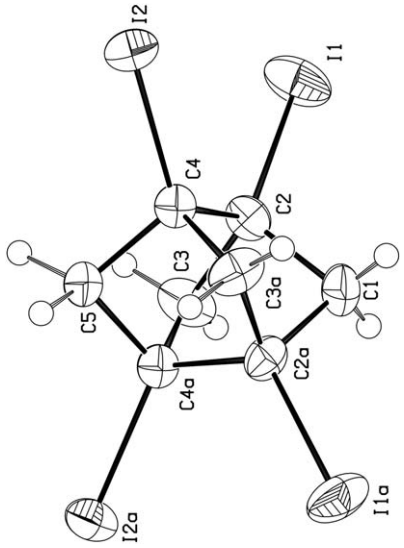


Figure 2. Crystal structure (ORTEP) of adduct **6**.

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References and notes

1. For a very recent review on pyramidalized alkenes see: (a) Vázquez, S.; Camps, P. *Tetrahedron* **2005**, *61*, 5147–5208; See also: (b) Borden, W. T. *Chem. Rev.* **1989**, *89*, 1095–1109.
2. (a) Camps, P.; Font-Bardia, M.; Pérez, F.; Solans, X.; Vázquez, S. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 912–914; (b) Camps, P.; Font-Bardia, M.; Pérez, F.; Solà, Ll.; Solans, X.; Vázquez, S. *Tetrahedron Lett.* **1996**, *37*, 8601–8604; (c) Camps, P.; Luque, F. J.; Orozco, M.; Pérez, F.; Vázquez, S. *Tetrahedron Lett.* **1996**, *37*, 8605–8608; (d) Camps, P.; Pujol, X.; Vázquez, S. *Tetrahedron* **2002**, *58*, 10081–10086; (e) Camps, P.; Muñoz, M. R.; Vázquez, S. *J. Org. Chem.* **2005**, *70*, 1945–1948.
3. For the use of 2,5-dimethylfuran or furan as trapping agents, see: Camps, P.; Fernández, J. A.; Font-Bardia, M.; Solans, X.; Vázquez, S. *Tetrahedron* **2005**, *61*, 3593–3603.
4. (a) Gleiter, R.; Schäfer, W. *Acc. Chem. Res.* **1990**, *23*, 369–375; (b) Lange, H.; Schäfer, W.; Gleiter, R.; Camps, P.; Vázquez, S. *J. Org. Chem.* **1998**, *63*, 3478–3480.
5. Ayats, C.; Camps, P.; Duque, M. D.; Font-Bardia, M.; Muñoz, M. R.; Solans, X.; Vázquez, S. *J. Org. Chem.* **2003**, *68*, 8715–8718.
6. (a) Concepción, J. I.; Francisco, C. G.; Freire, R.; Hernández, R.; Salazar, J. A.; Suárez, E. *J. Org. Chem.* **1986**, *51*, 402–404; (b) Moriarty, R. M.; Khosrowshahi, J. S.; Dalecki, T. M. *J. Chem. Soc., Chem. Commun.* **1987**, 675–676.

7. (a) Böttger, O. *Chem. Ber.* **1937**, *70*, 314–325; (b) Newkome, G. R.; Nayak, A.; Behera, R. K.; Moorefield, C. N.; Baker, G. R. *J. Org. Chem.* **1992**, *57*, 358–362.
8. Webster, O. W.; Sommer, L. H. *J. Org. Chem.* **1964**, *29*, 3103–3105.
9. Adams, R.; Gold, M. H. *J. Am. Chem. Soc.* **1940**, *62*, 56–61.
10. Rieche, A.; Schulz, M. *Ann.* **1962**, *653*, 32–45.
11. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 604057. 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].
12. (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652; (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
13. (a) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939–947; (b) Gordon, M. S.; Binkley, J. S.; Pople, J. A.; Pietro, W. J.; Hehre, W. J. *J. Am. Chem. Soc.* **1982**, *104*, 2797–2803.
14. The LANL2DZ basis set consists of the valence double-zeta D95V basis set for first-row atoms and the Los Alamos effective core potential for I. See: (a) Dunning, T. H., Jr.; Hay, P. J. *Modern Theoretical Chemistry*; Schaefer, H. F., III, Ed.; Plenum: New York, NY, 1976; Vol. 3, pp 1–28; (b) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 270–283; (c) Wadt, W. R.; Hay, P. J. *J. Chem. Phys.* **1985**, *82*, 284–298; (d) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 299–310.
15. (a) Cremer, D. Møller–Plesset Perturbation Theory. *Encyclopedia of Computational Chemistry*; Schleyer, P. v. R., Ed.; Wiley: Chichester, UK, 1998; Vol. 3, pp 1706–1735; (b) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, NY, 1986; (c) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618–622.
16. Ab initio calculations were carried out using the Gaussian program. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreve, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03, Rev. B.02*; Gaussian: Pittsburgh, PA, 2003.
17. Watanabe, Y.; Otha, K.; Suyama, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2063–2066.
18. Sheldrick, G. M. *A Program for Automatic Solution of Crystal Structures*; University of Göttingen: Germany, 1997.
19. Sheldrick, G. M. *SHELX-97: A Program for Crystal Structure Refinement*; University of Göttingen: Germany, 1997.
20. *International Tables of X-ray Crystallography*; Ibers, J. A., Hamilton, W. C., Eds.; Kynoch: Birmingham, UK, 1974; Vol. IV, pp 99–100 and 149.

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Synthesis of α-amino tetrahydropyranyl-, tetrahydrothiopyranyl-, 4- and 3-piperidinyl-phosphonic acids via phosphite addition to iminium ions☆

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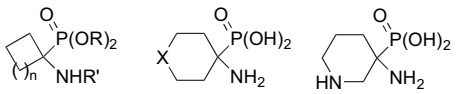
Abstract—α-Amino cyclobutyl-, cyclopentyl-, cyclohexyl- and 4- and 3-heterocyclohexyl-phosphonates were efficiently prepared from carbocyclic and heterocyclic ketones, by nucleophilic addition of phosphite to the iminium ion formed by in situ condensation of these ketones with benzylic amines. Cleavage of the benzyl groups and acidic hydrolysis of the resulting α-amino heterocyclohexyl-phosphonates gave, in a three-step sequence from ketones, new 4- and 3-heterocyclohexyl-phosphonic acids.

1. Introduction

Due to their potential biological activity[1] and their use as building blocks for phosphorus-containing peptide mimetics, derivatives of phosphonic acid analogues of α-amino acids are of considerable current interest. Several α-aminophosphonic acid derivatives show activity as enzyme inhibitors, antibacterials, herbicides, fungicides or plant growth regulators.[2] Other α-aminophosphonic acid derivatives, in particular phosphorus-containing peptide mimetics, have been prepared. These acid derivatives, in which the tetrahedral phosphorus moiety acts as a transition-state analogue of peptide bond cleavage, selectively inhibit peptidases and proteinases (e.g., HIV protease,[3] serine protease[4]). Such an impressive array of applications has recently stimulated considerable effort towards the asymmetric synthesis of α-aminophosphonic acids.[5–7]

In addition, cyclic or heterocyclic rings introduced into the molecular skeleton increase its rigidity and modify electronic effects. Thus in recent years, many cyclic α-aminophosphonic acids **1** (R=H) or -phosphonates **1** (R=alkyl) have been prepared, such as derivatives of α-aminocyclopropylphosphonic acid,[8–10] as well as their cyclobutyl,[10b] cyclopentyl[11] and cyclohexyl analogues.[12] These compounds were prepared either from cycloalkanones or derivatives by Mannich-type reactions,[13] or from cycloalkylphosphonates by electrophilic azidation.[10b]

However, very few examples of heterocyclic α-aminophosphonic acids **2** or the corresponding phosphonates have been reported in the literature; only the 4-aminobutyric acid (GABA)[14] analogue **2a** (X=NH),[15] the pyran phosphonate derivative of **2b** (X=O)[16] and thiopyran phosphonate derivative of **2c** (X=S) are described.[17] Among these synthetic approaches, only one describes a total synthesis and isolation of free α-amino(4-pyrrolidine)phosphonic acid **2a** (X=NH).[15c] In contrast, pyran acid **2b**, thiopyran acid **2c** and especially 3-pyrrolidine acid analogue **3** are still unknown. In all these synthetic approaches, the Kabachnick–Fields reaction[16a] was used from heterocyclohexanones, to provide α-aminophosphonates with moderate to good yields, accompanied, in some cases, with α-hydroxyphosphonate derivatives as byproducts (Scheme 1).



Scheme 1.

We have previously reported a simple and convenient synthesis of 1-aminocyclopropanephosphonic acids **4** (ACC analogues), in three steps, starting from cyclopropanone

☆ Part of this study was previously reported at the Organic Chemistry Symposium at Marseille (GECO 46, September 2005), France.

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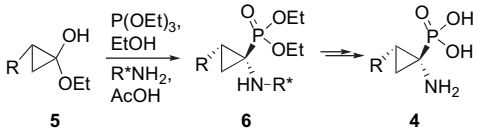
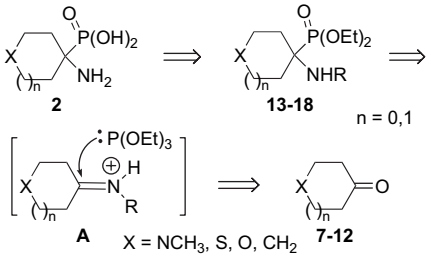
hemi-acetals **5** and proceeding via aminophosphonates **6** (Scheme 2).[9]



Scheme 2.

As part of our ongoing programme in this area, we decided to study the addition of triethyl phosphite to the iminium ions **A** of readily available heterocyclic ketones **7–12**. This one-pot reaction, involving the iminium species **A** as intermediate, should occur in the presence of benzylamine derivatives to give the desired aminophosphonates **13–18** (Scheme 3).



Scheme 3.

2. Results and discussion

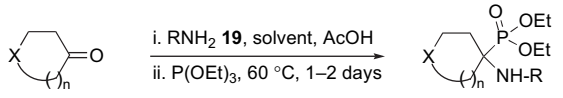
The reactions were carried out using a one-pot procedure. Benzylamine derivatives, α-methylbenzylamine **19a**, benzylamine **19b**, α-phenylglycinol **19c** and *p*-methoxybenzylamine **19d**, were selected for their efficient reaction to form the iminium ions **A**, and in particular for their straightforward cleavage by catalytic hydrogenolysis at the end of the synthetic sequence. In addition, the phenylglycinol derivative can be cleaved by an oxidative degradation with $NaIO_4$, whereas, the *p*-methoxybenzyl group can be cleaved by a DDQ oxidation followed by basic hydrolysis.

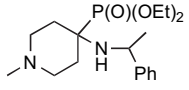
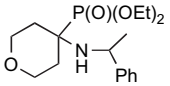
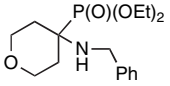
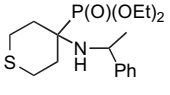
The standard one-pot procedure of ketones **7–12** with amine derivatives **19** was carried out in EtOH in the presence of 2 equiv of AcOH, 1 equiv of $MgSO_4$ and 1.5 equiv of triethyl phosphite at 50–60 °C for 1 day. The commercially available heterocyclic ketones **7–9** were reacted with benzylamine derivatives **19a–d**, to give the iminium ion intermediates **A** before triethyl phosphite addition to furnish aminophosphonates **13–15** in good yields (46–92%) (Table 1, entries 1–7).

By comparison, conducting the same reaction with cyclic ketones (cyclohexanone **10** and cyclobutanone **12**), in EtOH for 1–4 days, gave the corresponding aminophosphonates **16** and **18** in good yields (93% and 50%, respectively) (Table 1, entries 8 and 10). However, lower yields were obtained for aminophosphonates **16–18** in DMSO as a solvent in the presence of $MgSO_4$ at 55 °C for 2 days (Table 1, entries 8–10). These new aminophosphonates **16–18** are of particular interest, since the dibutyl phosphonate analogue of **16**, known as Trakephon®, is a highly efficient herbicide.[12a,b]

On the other hand, the commercially available *N*-Boc-3-piperidone **20a**, in which the heteroatom is in the 3-position to the carbonyl function, underwent a one-pot reaction with amine **19a** to give aminophosphonate **21a**, for the first time, in 54% yield as a mixture of two diastereoisomers. However, with the benzylamine **19b** aminophosphonate **21a′** was

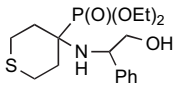
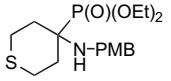
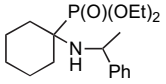
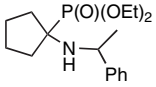
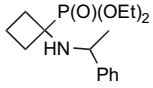
Table 1. Preparation of aminophosphonates **13–18** from ketones **7–12**[a]



Entry	Ketone 7–12			RNH_2 19a–d[b]	Solvent	Time (h)	Yield (%) EtOH (DMSO)[c]	Phosphonates 13–18	
		n	X						
1	**7**	2	N-Me	**19a**	EtOH	18	71 (36)	**13**	
2	**7**	2	N-Me	**19a**	Toluene/CH_3CN[d]	18	30	**13**	
3	**8**	2	O	**19a**	EtOH	30	66	**14a**	
4	**8**	2	O	**19b**	EtOH	22	55	**14b**	
5	**9**	2	S	**19a**	EtOH	18	92	**15a**	

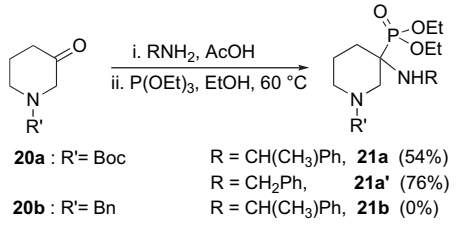
(*continued*)

Table 1. *(continued)*

Entry		Ketone **7–12**		RNH_2 **19a–d**[b]	Solvent	Time (h)	Yield (%) EtOH (DMSO)[c]		Phosphonates **13–18**
		n	X						
6	**9**	2	S	**19c**	EtOH	39	46	**15b**	
7	**9**	2	S	**19d**	EtOH	24	80	**15c**	
8	**10**	2	CH_2	**19a**	EtOH	20	93 (43)	**16**	
9	**11**	1	CH_2	**19a**	DMSO	48	NR[e] (76)	**17**	
10	**12**	0	CH_2	**19a**	EtOH	100	50 (30)	**18**	

[a] Reactions of ketones **7–12** with amines **19** were carried out in the presence of $MgSO_4$.
[b] Amines, **19a**: H_2N–$CH(CH_3)Ph$; **19b**: H_2N–CH_2Ph; **19c**: H_2N–$CH(CH_2OH)Ph$; **19d**: H_2N–$CH_2C_6H_4$–*p*OMe.
[c] Reactions in DMSO were run at 55 °C for 2 days.
[d] The reaction was run with $HP(O)(OEt)_2$ and catalyzed with 5 mol % of Me_2AlCl (Ref. 18).
[e] NR=not run.

obtained in 76% yield. In contrast, the *N*-benzyl-3-piperidone **20b** did not lead to the expected phosphonate **21b**, but to a mixture of degradation products (Scheme 4).

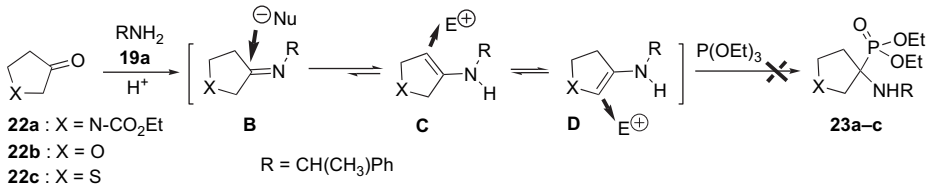


Scheme 4.

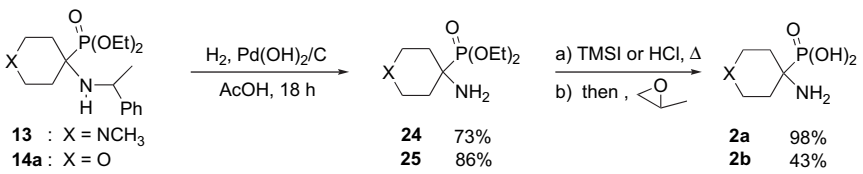
To better understand this behaviour, we attempted to react α-methylbenzylamine **19a** with 3-heterocyclopentanones **22a–c**, but none among them gave the corresponding aminophosphonates **23a–c** under these conditions. We can tentatively explain this by the fact that the iminium intermediate **B** is not favoured, and undergoes a double bond migration to the heterocyclic enamines **C** and **D**, thus preventing the nucleophilic attack of the phosphite on imine **B**. Such migration has already been reported,[19] and could allow electrophilic reaction of enamine **C** or **D** (Scheme 5).[20]

We then submitted the heterocyclic phosphonates **13–15** to the deprotection sequence. The aminophosphonates **13** and **14a** reacted under mild conditions (20% $Pd(OH)_2$/C, and 1 atm H_2) to cleave the benzyl group affording free aminophosphonates **24** and **25**, in a good yield (Scheme 6).

Subsequent hydrolysis of aminophosphonate **24** was accomplished by 6 N HCl solution at reflux for 7 h, followed by



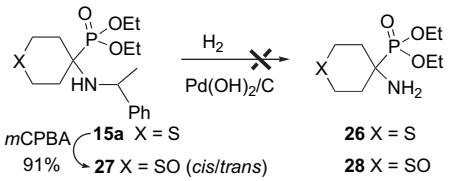
Scheme 5.



Scheme 6.

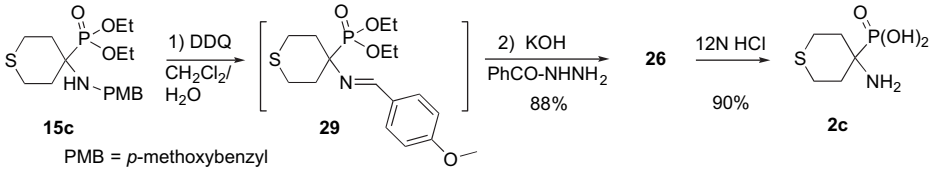
treatment with propylene oxide to provide, the previously unknown *N*-methyl-4-aminopiperidine-phosphonic acid **2a**. Surprisingly, hydrolysis of the phosphonate moiety of **24** with TMSI led only to the formation of byproducts with a small amount of the desired acid **2a**. On the other hand, treatment of aminophosphonate **25** with the trimethylsilyl iodide in dichloromethane followed by the addition of propylene oxide in ethanol furnished, for the first time, aminophosphonic acid **2b** in 43% yield.

N-Benzyl cleavage of **15a** (X=S) was unsuccessful under the same conditions (H_2, $Pd(OH)_2$/C, AcOH), or with other known debenzylation procedures (H_2, Pd/C, HCl; Na/NH_3 liq. or Na/naphthalene). Knowing that the sulfur group could poison the palladium catalyst, we oxidized **15a** by *m*CPBA (1 equiv) to afford a mixture of unseparable sulfoxides **27** (cis/trans: 37/63) in 91% yield. Subsequent hydrogenolysis (H_2, $Pd(OH)_2$/C) of this cis/trans **27** mixture did not furnish the expected free amine **28** (cis/trans), returning the starting sulfoxides unreacted (Scheme 7). Similarly, an attempted cleavage of the phenylglycinol group of **15b** by a known oxidative degradation with $Pb(OAc)_4$[21] did not furnish **26**.



Scheme 7.

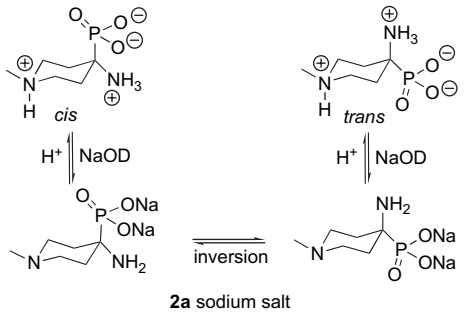
Nevertheless, cleavage of **15c** (Table 1, entry 6) by DDQ (H_2O/CH_2Cl_2: 1/9) at 20 °C,[22] provided, after purification on silica gel, the corresponding free aminophosphonate **26**, accompanied by imine **29** (Scheme 8). However, treatment



Scheme 8.

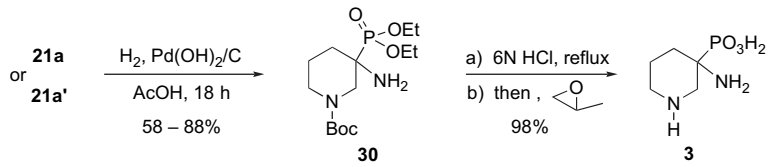
of the reaction mixture with 2 N KOH in the presence of benzoyl hydrazine, to trap the highly reactive *p*-anisaldehyde, furnished exclusively the desired aminophosphonate **26** in 88% yield.

Hydrolysis of **26** with 12 N HCl solution at reflux, followed by treatment with propylene oxide provided the new 4-tetrahydrothiopyranylphosphonic acid **2c**, in quantitative yield. We found that hydrolysis of phosphonate **26** with TMSI or TMSBr followed by propylene oxide treatment gave a mixture of acid **2c** and degradation products. It is worthy of note that the NMR spectra of **2a** in D_2O solution showed two diastereomers, due to the pyramidalization of the nitrogen atom in acidic medium, as previously described by Kafarski.[15c] Deprotection of the ring nitrogen in neutral or basic solution speeds up the nitrogen inversion and makes the whole process faster. Thus, the coalescence of the two singlets in ^{31}P NMR was observed on addition of NaOD to the solution, and forming **2a** sodium salt (Scheme 9, see also Section 4).



Scheme 9.

On the other hand, in the 3-heterocyclic system, cleavage of the benzyl group of compounds **21a** or **21a′** under mild conditions (20% $Pd(OH)_2$/C and 1 atm H_2) furnished the corresponding free amine **30** in good yield 76% or 88%, respectively. Phosphonate hydrolysis and Boc deprotection of **30** were accomplished simultaneously in 6 N aq HCl solution at reflux, to give quantitatively the amino acid hydrochloride **3·2HCl**. Treatment of this amino acid salt with propylene oxide provided for the first time the racemic free aminophosphonic acid **3** in excellent yield. However, the use of TMSI for hydrolysis of **30** was ineffective once more (Scheme 10).



Scheme 10.

3. Conclusion

We have developed an easy and efficient three-step synthesis of new heterocyclic α-aminophosphonic acids **2** and **3**. Thus,

starting from readily available cyclic and heterocyclic ketones **7**–**12** and **20**, we have demonstrated that the iminium ion formed from these ketones undergoes nucleophilic addition of phosphite to give the cyclic and heterocyclic aminophosphonates **13**–**18** and **21** in good yield. Subsequent hydrogenolysis of the benzyl group (for tetrahydropyran **14** and piperidine derivatives **13** and **21**) and hydrolysis of phosphonate functions were accomplished with good yields. However, deprotection of the *N*-PMB group (for the tetrahydrothiopyran derivative) required oxidation with DDQ. The stable cyclic aminophosphonates **16**–**18**, analogues of the biologically active molecule Trakephon®, were not transformed into their corresponding aminophosphonic acids, but may be performed straightforwardly using a reported method.[9c] We are currently developing an asymmetric version to prepare the homochiral 3-heterocyclic aminophosphonic acids.

4. Experimental

4.1. General

Except where otherwise indicated, all reactions were carried out under argon with magnetic stirring. Di- and triethylphosphite were distilled at reduced pressure and stored under argon (see Table 1). R_f values refer to TLC on 0.25 mm silica gel plates (Merck F_{254}). Flash chromatography (FC) was performed on silica gel 60 (0.040–0.063 mm). Yields refer to chromatographically and spectroscopically pure compounds, except where noted. IR spectra were recorded on a Perkin–Elmer (spectrum one) spectrophotometer. Melting points were determined on a Büchi B-545 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on a Bruker AM250 (250 MHz) or Bruker AC360 (360 MHz) spectrometer. Chemical shifts were recorded in parts per million from the solvent resonance ($CDCl_3$ at 7.27 ppm and D_2O at 4.8 ppm). ^{13}C NMR spectra were measured on a Bruker AM250 (62.9 MHz), or Bruker AC360 (90.56 MHz) spectrometer. Chemical shifts were recorded in parts per million from the solvent resonance ($CDCl_3$ at 77.16 ppm). ^{31}P NMR spectra were recorded on a Bruker AM250 (101.25 MHz), and chemical shifts were quoted relative to internal 85% H_3PO_4 (δ=0 ppm). Mass spectra were recorded on a Finnigan DSQ-Thermo. High-resolution mass spectra were recorded on a Finnigan MAT 95S. All new compounds were determined to be >95% pure by ^{1}H NMR spectroscopy.

4.2. General procedure A

To a solution of heterocyclic ketones **7**–**12** or **20** (5 mmol) in EtOH (10 mL), was added benzylamine **19** (7.50 mmol), AcOH (600 μL, 10 mmol) and $MgSO_4$ (420 mg, 3.50 mmol). After stirring and heating at 55 °C for 4–5 h, $P(OEt)_3$ (1.25 g, 1.31 mL, 7.50 mmol) was added. The mixture was heated at 55 °C for 1–3 days. It was then concentrated in vacuo, concd aq ammonia (2 mL) was added and the resulting mixture was filtered through a 3 cm pad of silica gel eluting with ethyl acetate (50 mL). The filtrate was concentrated in vacuo to give the crude phosphonate. Purification by flash chromatography (FC) on silica gel (MeOH/CH_2Cl_2: 1/9) gave pure aminophosphonates **13**–**18** or **21**.

4.2.1. Diethyl 4-(1′-methylbenzyl)amino-1-methylpiperidin-4-yl-phosphonate (13). Following procedure A: reaction of *N*-methylpiperidin-4-one **7** (545 mg, 5 mmol), EtOH (10 mL), α-methylbenzylamine **19a** (910 mg, 7.50 mmol), $MgSO_4$ (420 mg), AcOH (600 μL, 10 mmol) and $P(OEt)_3$ (1.25 g, 7.5 mmol) for 18 h at 55 °C gave, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 2/98/1), 1.260 g (71%) of aminophosphonate **13** as a colourless oil. R_f=0.65 (MeOH/CH_2Cl_2: 50/50+2% NH_3 aq). IR (neat) ν: 3449, 3353, 2932, 1235 (P═O), 1050 and 1025 (P–O), 957 cm^{-1}. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 1.30 (t, J=7.2 Hz, 3H, CH_3–CH_2O), 1.33 (t, J=7.2 Hz, CH_3–CH_2O), 1.33 (d, J=6.8 Hz, 3H, CH_3–$C_{1'}$), 1.42–1.90 (m, 5H, 4H$_{cycle}$ and NH), 1.90–2.20 (m, 2H$_{cycle}$), 2.10 (s, 3H, CH_3N), 2.30–2.60 (m, 2H, 1H–C_2 and 1H–C_6), 4.10 (qd, J=7.2 Hz, $^2J_{PC}$=7.2 Hz, 2H, CH_2OP), 4.13 (qd, J=7.2 Hz, $^2J_{PC}$=7.2 Hz, 2H, CH_2OP), 4.44 (qd, J=6.8 Hz, $^4J_{PH}$=2.5 Hz, 1H–$C_{1'}$), 7.08–7.48 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.6 (*C*H_3–CH_2O), 16.7 (*C*H_3–CH_2O), 27.0 (CH_3–$C_{1'}$), 27.9 (d, $^2J_{PC}$=4.1 Hz, C_3 or C_5), 32.6 (s, C_5 or C_3), 46.2 (CH_3N), 49.4 (d, $^3J_{PC}$=12.1 Hz, C_2 or C_6), 49.55 (d, $^3J_{PC}$=9.8 Hz, C_6 or C_2), 52.5 ($C_{1'}$), 54.8 (d, $^1J_{PC}$=143.1 Hz, C_4), 61.7 (d, $^2J_{PC}$=8.0 Hz, *C*H_2OP), 61.9 (d, $^2J_{PC}$=8.0 Hz, *C*H_2OP), [6 arom C: 126.3 (1C), 126.8 (2C), 128.0 (2C), 148.4 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 29.51. ES$^+$ MS, *m*/*z*: 377.2 [M+Na]$^+$. HRMS data were not obtained.[23]

4.2.2. Diethyl 4-(1′-methylbenzyl)amino-tetrahydro-2*H*-pyran-4-yl-phosphonate (14a). Following procedure A: reaction of tetrahydropyran-4-one **8** (172 mg, 1.72 mmol), EtOH (4.5 mL), α-methylbenzylamine **19a** (330 μL, 2.68 mmol), $MgSO_4$ (155 mg), AcOH (200 μL, 3.43 mmol) and $P(OEt)_3$ (442 μL, 2.58 mmol) for 30 h at 55 °C furnished, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 2/98/0.5), 387 mg (66%) of tetrahydropyranphosphonate **14a** as a colourless oil. R_f=0.35 (MeOH/CH_2Cl_2: 5/95). IR (neat) ν: 3468, 3333, 1233 (P═O), 1047 and 1026 (P–O), 950. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.37 (t, J=7.0 Hz, 3H, CH_3–CH_2O), 1.40 (t, J=7.0 Hz, CH_3–CH_2O), 1.41 (d, J=6.8 Hz, 3H, CH_3), 1.30–1.70 (m, 3H, 2H$_{cycle}$ and NH), 1.70–1.90 (m, 1H$_{cycle}$), 1.90–2.28 (m, 1H$_{cycle}$), 3.06–3.35 (m, 2H$_{cycle}$, CH_2O), 3.61–3.77 (m, 1H$_{cycle}$, CH_2O), 3.98 (tt, J=10.7 Hz, J=1.9 Hz, 1H$_{cycle}$, CH_2O), 4.16 (qd, J=7.3 Hz, $^3J_{PH}$=7.0 Hz, 2H, CH_2OP), 4.22 (qd, J=7.3 Hz, $^3J_{PH}$=7.0 Hz, 2H, CH_2OP), 4.49 (qd, J=6.8 Hz, $^4J_{PH}$=2.8 Hz, 1H–$C_{1'}$), 7.10–7.25 (m, 1H), 7.25–7.36 (m, 2H), 7.36–7.50 (m, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.5 (q, *C*H_3–CH_2O), 16.6 (q, *C*H_3–CH_2O), 26.8 (CH_3-$C_{1'}$), 27.8 (d, $^2J_{PC}$=2.2 Hz, C_3 or C_5), 32.8 (C_5 or C_3), 52.8 ($C_{1'}$), 55.0 (d, $^1J_{PC}$=144.3 Hz, C_4), 61.7 (d, $^2J_{PC}$=7.3 Hz, *C*H_2OP), 61.8 (C_2 or C_6), 61.9 (C_6 or C_2), 62.0 (d, $^2J_{PC}$=7.6 Hz, CH_2OP), [6 arom C: 126.4 (d), 126.6 (d, 2C), 128.1 (d, 2C), 148.0 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 28.35. HRMS (ESI, *m*/*z*): calcd mass for $C_{17}H_{28}O_4NPNa$, [M+Na]$^+$: 364.1648. Found: 364.1653.

4.2.3. Diethyl 4-(benzylamino)-tetrahydro-2*H*-pyran-4-yl-phosphonate (14b). Following procedure A: reaction of tetrahydropyran-4-one **8** (200 mg, 2.0 mmol), EtOH (5.0 mL), benzylamine **19b** (325 μL, 2.98 mmol), $MgSO_4$ (180 mg), AcOH (230 μL, 3.96 mmol) and $P(OEt)_3$

(493 μL, 2.97 mmol) for 22 h at 50 °C provided, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 2/98/0.5), 354 mg (55%) of tetrahydropyran-phosphonate **14b** as a colourless oil. R_f=0.42 (MeOH/CH_2Cl_2: 10/90). IR (neat) ν: 3468, 3312, 1240 (P═O), 1047 and 1027 (P–O), 958. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.37 (t, J=7.0 Hz, 6H, CH_3), 1.50–1.70 (m, 2H, 1H–C_3 and 1H–C_5), 1.94–2.17 (m, 3H, 1H–C_3, 1H–C_5 and 1NH), 3.54–3.71 (m, 2H, 1H–C_2 and 1H–C_6), 3.77–3.95 (m, 4H, $2H_{benzyl}$, 1H–C_2 and 1H–C_6), 3.95–4.24 (m, 4H, 2CH_2OP), 7.10–7.43 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.5 (q, CH_3), 16.6 (q, CH_3), 29.8 (C_3 and C_5), 47.1 (CH_2–N), 53.4 (d, $^1J_{PC}$=146.7 Hz, C_4), 61.6 (d, $^2J_{PC}$=2.7 Hz, 2CH_2OP), 61.8 (C_2 and C_6), [6 arom C: 126.8 (d), 128.0 (2C), 128.2 (2C), 140.8 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 27.60. HRMS (ESI, m/z): calcd mass for $C_{16}H_{26}NO_4PNa$, $[M+Na]^+$: 350.1492. Found: 350.1496.

4.2.4. Diethyl 4-(1′-methylbenzyl)amino-tetrahydro-2*H*-thiopyran-4-yl-phosphonate (15a). Following procedure A: reaction of tetrahydrothiopyran-4-one **9** (430 mg, 4.75 mmol), EtOH (12 mL), α-methylbenzylamine **19a** (910 μL, 7.13 mmol), $MgSO_4$ (420 mg, 3.5 mmol), AcOH (520 μL, 9.50 mmol) and $P(OEt)_3$ (1.22 g, 7.13 mmol) for 20 h at 50 °C gave, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 1/99/0.5), 1.560 g (92%) of thiopyran phosphonate **15a** as a colourless oil. R_f=0.57 (MeOH/CH_2Cl_2: 5/95). IR (neat) ν: 3457, 3333, 2982, 1210 (P═O), 1055 and 1026 (P–O), 954. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.38 (t, J=7.0 Hz, 3H, CH_3–CH_2O), 1.39 (t, J=6.8 Hz, 3H, CH_3–$C_{1'}$), 1.40 (t, J=7.0 Hz, 3H, CH_3–CH_2O), 1.67 (br s, 1H, NH), 1.78–2.24 (m, $5H_{cycle}$), 2.24–2.50 (m, $2H_{cycle}$), 3.28 (tt, J=12.7 Hz, J=2.0 Hz, 1H–C_2 or 1H–C_6), 4.16 (qd, J=7.0 Hz, $^2J_{PH}$=7.0 Hz, 2H, CH_2OP), 4.22 (qd, J=7.0 Hz, $^2J_{PH}$=6.5 Hz, 2H, CH_2OP), 4.46 (qd, J=6.8 Hz, $^4J_{PH}$=2.5 Hz, 1H–$C_{1'}$), 7.14–7.24 (m, 1H), 7.24–7.35 (m, 2H), 7.35–7.50 (m, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.5 (CH_3–C–O), 16.6 (CH_3–C–O), 21.5 (d, $^2J_{PC}$=13.7 Hz, C_2 or C_6), 21.7 (d, $^2J_{PC}$=10.6 Hz, C_6 or C_2), 26.8 (CH_3–$C_{1'}$), 29.0 (C_3 or C_5), 33.7 (C_5 or C_3), 52.4 ($C_{1'}$), 56.6 (d, $^1J_{PC}$=141.0 Hz, C_4), 61.7 (d, $^2J_{PC}$=7.5 Hz, CH_2OP), 61.9 (d, $^2J_{PC}$=7.7 Hz, CH_2OP), [6 arom C: 126.5 (d, 2C), 128.2 (d, 3C), 148.1 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 28.87. HRMS (ESI, m/z): calcd mass for $C_{17}H_{28}NO_3PSNa$, $[M+Na]^+$: 380.1420. Found: 380.1424.

4.2.5. Diethyl 4-[(1′-hydroxymethyl)benzylamino]tetrahydro-2*H*-thiopyran-4-yl-phosphonate (15b). Following procedure A: reaction of tetrahydrothiopyran-4-one **9** (210 mg, 1.8 mmol), EtOH (4.5 mL), α-hydroxymethylbenzylamine **19c** (370 mg, 2.7 mmol), $MgSO_4$ (162 mg), AcOH (200 μL, 3.62 mmol) and $P(OEt)_3$ (465 μL, 2.70 mmol), for 40 h at 50 °C furnished, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 1/99/0.5), 311 mg (46%) of aminophosphonate **15b** as a colourless oil. R_f=0.45 (MeOH/CH_2Cl_2: 5/95). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.31 (t, J=7.0 Hz, 3H, CH_3–CH_2O), 1.32 (t, J=7.0 Hz, CH_3–CH_2O), 1.70–2.18 (m, 5H, 2H–C_3, 2H–C_5 and 1H–C_2 or 1H–C_6), 2.18–2.46 (m, 2H, 1H–C_2 and 1H–C_6), 3.20 (br t, J=12.5 Hz, 1H–C_6 or 1H–C_2), 3.42 (dd, J=9.0 Hz, J=11.0 Hz, 1H, CH_2–$C_{1'}$), 3.63 (dd, J=4.2 Hz, J=11.0 Hz, 1H, CH_2–$C_{1'}$), 4.12 (dq, $^3J_{PH}$=7.2 Hz, J=7.0 Hz, 2H, CH_2O), 4.15 (dq, J=7.2 Hz, J=7.0 Hz, 2H, CH_2O), 4.30–4.46 (m, 1H–$C_{1'}$), 7.08–7.50 (m, 5H arom). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.5 (CH_3), 16.55 (CH_3), 21.5 (d, $^2J_{PC}$=14.1 Hz, C_2 or C_6), 21.7 (d, $^2J_{PC}$=10.8 Hz, C_6 or C_2), 29.1 (d, $^3J_{PC}$=4.0 Hz, C_3 or C_5), 33.6 (C_5 or C_3), 56.7 (d, $^1J_{PC}$=141.0 Hz, C_4), 58.9 ($C_{1'}$), 61.9 (d, $^2J_{PC}$=8.2 Hz, CH_2O), 62.4 (d, $^2J_{PC}$=8.2 Hz, CH_2O), 68.1 (CH_2OH), [6 arom C: 127.0 (1C), 127.2 (2C), 128.2 (2C), 143.4 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 29.58. HRMS (ESI, m/z): calcd mass for $C_{17}H_{28}NO_4PSNa$, $[M+Na]^+$: 396.1369. Found: 396.1363.

4.2.6. Diethyl 4-(4-methoxybenzyl)amino-tetrahydro-2*H*-thiopyran-4-yl-phosphonate (15c). Following procedure A: condensation reaction of tetrahydrothiopyran-4-one **9** (618 mg, 5.39 mmol), EtOH (14 mL), *p*-methoxybenzylamine **19d** (1.04 mL, 8.0 mmol), $MgSO_4$ (480 mg) and AcOH (590 μL, 10.66 mmol) was stirred and heated for 17 h). Then addition of $P(OEt)_3$ (1.37 mL, 8.0 mmol), (24 h at 50 °C) gave, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 2/98/0.5), 1.60 g (80%) of PMB aminophosphonate **15c** as a colourless oil. R_f=0.74 (MeOH/CH_2Cl_2: 10/90). IR (neat) ν: 3463, 3318, 2977, 1510, 1243 (P═O), 1044, 954. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.35 (t, J=7.0 Hz, 3H, CH_3), 1.80 (br s, NH), 2.00–2.24 (m, 4H, 2H–C_3 and 2H–C_5), 2.29 (d, J=12.7 Hz, 2H, 1H–C_2 and 1H–C_6), 3.26 (ddd, J=2.5 Hz, J=12.2 Hz, J=12.7 Hz, 2H, 1H–C_2 and 1H–C_6), 3.83 (s, 3H, OMe), 3.87 (d, $^4J_{PH}$=3.0 Hz, 2H, CH_2–N), 4.14 (dq, $^3J_{PH}$=7.2 Hz, J=7.0 Hz, 2H, CH_2O), 4.17 (dq, $^3J_{PH}$=7.2 Hz, J=7.0 Hz, 2H, CH_2O), 6.87 (like d, J=8.5 Hz, $2H_{aryl}$), 7.32 (like d, J=8.5 Hz, $2H_{aryl}$). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.45 (CH_3), 16.5 (CH_3), 21.2 (C_2 or C_6), 21.4 (C_6 or C_2), 30.5 (C_3 and C_5), 46.0 (CH_2–N), 54.8 (d, $^1J_{PC}$=141.6 Hz, C_4), 55.0 (CH_3O), 61.7 (CH_2O), 61.8 (CH_2O), [6 arom C: 113.6 (2C), 129.1 (2C), 132.7 (s), 158.5 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 28.22. HRMS (ESI, m/z): calcd mass for $C_{17}H_{28}NO_4PSNa$, $[M+Na]^+$: 396.1369. Found: 396.1365.

4.2.7. Diethyl 1-[(1′-methylbenzyl)amino]cyclohexane-phosphonate (16). Following procedure A: reaction of cyclohexanone **10** (294 mg, 3 mmol), EtOH (6 mL), α-methylbenzylamine **19a** (410 mg, 4.5 mmol), $MgSO_4$ (250 mg), AcOH (360 μL, 6 mmol) and $P(OEt)_3$ (750 mg, 4.5 mmol), for 20 h at 55 °C furnished, after standard work-up and purification by FC (eluent, ether), 930 mg (93%) of pure aminophosphonate **16** as a colourless oil. R_f=0.40 (MeOH/CH_2Cl_2: 5/95). IR (neat) ν: 3463, 3061, 2932, 1232 (P═O), 1062 and 1025 (P–O), 955. ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 0.92–1.21 (m, $3H_{cycle}$), 1.30 (t, J=7.0 Hz, 3H, CH_3), 1.31 (d, J=6.9 Hz, CH_3–$C_{1'}$), 1.325 (t, J=7.0 Hz, 3H, CH_3), 1.38–1.90 (m, $7H_{cycle}$ and 1NH), 4.10 (qd, J=7.0 Hz, $^3J_{PH}$=7.0 Hz, 2H, CH_2O), 4.12 (qd, J=7.0 Hz, $^3J_{PH}$=7.0 Hz, 2H, CH_2O), 4.37 (qd, J=6.9 Hz, $^4J_{PH}$=2.2 Hz, 1H–$C_{1'}$), 7.00–7.40 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.35 (CH_3–CH_2O), 16.4 (CH_3–CH_2O), 19.55 (d, $^2J_{PC}$=12.2 Hz, C_2), 19.85 (d, $^2J_{PC}$=9.8 Hz, C_6), 25.3 (C_4), 26.8 (CH_3–C_1), 27.9 (d, $^3J_{PC}$=4.1 Hz, C_5), 32.4 (C_3), 52.2 ($C_{1'}$), 57.1 (d, $^1J_{PC}$=137.6 Hz, C_1), 61.25 (d, $^2J_{PC}$=7.8 Hz, CH_2O), 61.5 (d, $^2J_{PC}$=7.9 Hz, CH_2O), [6 arom C: 125.8, 126.3 (2C), 127.7 (2C), 148.5

(s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 31.21. ES$^+$ MS, *m/z*: 362.2 [M+Na]$^+$. HRMS data were not obtained.[23]

4.2.8. Diethyl 1-[(1′-methylbenzyl)amino]cyclopentanephosphonate (17). Following procedure A, with DMSO as solvent: reaction of cyclopentanone **11** (252 mg, 3 mmol), DMSO (6 mL), α-methylbenzylamine **19a** (545 mg, 4.5 mmol), $MgSO_4$ (250 mg), AcOH (360 μL, 6 mmol) and $P(OEt)_3$ (750 mg, 4.5 mmol), for 48 h at 55 °C furnished, after standard work-up and purification by FC (eluent, EtOAc/hexane: 20/80), 740 mg (76%) of pure aminophosphonate **17** as a colourless oil. R_f=0.47 (EtOAc/CH_2Cl_2: 15/85). ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 1.31 (d, J=6.8 Hz, CH_3–$C_{1'}$), 1.33 (t, J=7.0 Hz, CH_3–CH_2O), 1.36 (t, J=7.0 Hz, CH_3–CH_2O), 1.20–1.50 (m, 2H$_{cycle}$), 1.50–1.70 (m, 4H$_{cycle}$), 1.70–2.10 (m, 3H, 2H$_{cycle}$ and NH), 4.06–4.24 (m, J=7.0 Hz, 4H, CH_2O), 4.32 (qd, J=6.8 Hz, $^4J_{PH}$=2.3 Hz, 1H–$C_{1'}$), 7.10–7.45 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.7 (d, $^3J_{PC}$=4.2 Hz, CH_3–CH_2O), 16.75 (d, $^3J_{PC}$=4.2 Hz, CH_3–CH_2O), 24.1 (dd, $^2J_{PC}$=11.1 Hz, d, $^3J_{PC}$=4.2 Hz, C_3), 27.1 (CH_3–$C_{1'}$), 29.7 (C_4), 31.9 (d, $^3J_{PC}$=9.1 Hz, C_2), 37.4 (d, $^3J_{PC}$=7.4 Hz, C_5), 53.4 ($C_{1'}$), 61.6 (d, $^2J_{PC}$=7.7 Hz, CH_2O), 62.0 (d, $^2J_{PC}$=7.5 Hz, CH_2O), 64.5 (d, $^1J_{PC}$=144.0 Hz, C_1), [6 arom C: 126.2 (3C), 128.1 (2C), 149.1 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 31.90. ES$^+$ MS, *m/z*: 348.2 [M+Na]$^+$. HRMS data were not obtained.[23]

4.2.9. Diethyl 1-[(1′-methylbenzyl)amino]cyclobutanephosphonate (18). Following procedure A, with DMSO as solvent: reaction of cyclobutanone **12** (210 mg, 3 mmol), DMSO (6 mL), α-methylbenzylamine **19a** (545 mg, 4.50 mmol), $MgSO_4$ (250 mg), AcOH (360 μL, 6 mmol) and $P(OEt)_3$ (750 mg, 4.5 mmol), for 3 days at 55 °C gave, after standard work-up and purification by FC (eluent, EtOAc/petroleum ether, 40/60→60/40), 470 mg (51%) of aminophosphonate **18**. Mp 93.6 °C. R_f=0.30 (EtOAc/CH_2Cl_2: 3/7). IR (neat) ν: 3420, 3320 (NH), 1250 and 1200 (P═O), 1050 (P–O). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.35 (d, J=6.8 Hz, 3H–$C_{1'}$), 1.36 (t, J=7.1 Hz, CH_3–CH_2O), 1.37 (t, J=7.1 Hz, CH_3–CH_2O), 1.70 (br s, NH), 1.70–2.10 (m, 4H$_{cycle}$), 2.10–2.50 (m, 2H$_{cycle}$), 4.00–4.40 (m, J=7.1 Hz, 4H, CH_2O), 7.10–7.55 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 15.4 (d, $^2J_{PC}$=7.1 Hz, C_4), 16.6 (d, $^3J_{PC}$=2.4 Hz, CH_3–C–O), 16.65 (d, $^3J_{PC}$=2.4 Hz, CH_3–C–O), 26.0 (CH_3), 28.0 (C_3), 30.6 (C_2), 53.5(d, $^3J_{PC}$=5.2 Hz, $C_{1'}$), 57.9 (d, $^1J_{PC}$=147.2 Hz, C_1), 61.9 (d, $^2J_{PC}$=7.6 Hz, CH_2–O), 62.2 (d, $^2J_{PC}$=7.6 Hz, CH_2–O), [6 arom C: 126.4 (2C), 126.5, 128.2 (2C), 148.1 (s)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 28.70. MS (*m/z*): 311 (M$^+$, 0.4), 111 (12), 105 (100), 104 (11), 70 (20). HRMS (EI, *m/z*): calcd mass for $C_{16}H_{26}NO_3P$: 311.1650. Found: 311.1653.

4.2.10. Diethyl 1-(*tert*-butyloxycarbonyl)-3-(1-methylbenzyl)amino-piperidin-3-yl-phosphonate (21a). Following procedure A: reaction of *N*-Boc-piperidone **20a** (600 mg, 3 mmol), EtOH (6 mL), α-methylbenzylamine **19a** (410 mg, 4.5 mmol), $MgSO_4$ (250 mg), AcOH (360 μL, 6 mmol) and $P(OEt)_3$ (750 mg, 4.5 mmol), for 14 h at 55 °C furnished, after standard work-up and purification by FC (eluent, MeOH/CH_2Cl_2: 5/95), 680 mg (54%) of piperidinephosphonate **21a** as a mixture of two diastereoisomers in 63/37 ratio. R_f=0.29 (EtOH/petroleum ether: 50/50). IR (neat) ν: 3468, 3979, 2929, 1694 (CON), 1427, 1276, 1245, 1054, 1024, 964. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: two diastereomers **a/b** (63/37): 1.14 (t, J=7.0 Hz, 3H, **b**), 1.20–1.45 (m, 9H, 6H **a/b** CH_3–$C_{1'}$ and CH_3–CH_2–, 1NH **a/b**, and 3H **a**), 1.50 (s, 9H *t*-Bu, **a/b**), 1.45–2.10 (m, 3.4H, 2H–C_5 **a/b**, 1H–C_4 **a/b**, and 1H–C_4, **b**), 2.60 (ddd, J=3.2 Hz, J=12.7 Hz, 0.6H–C_4 **a**), 2.80–3.65 (m, 2H–C_6, **a/b**), 2.65–3.97 (m, 2H–C_2 **a/b**), 3.97–4.22 (m, 4H, CH_2O, **a/b**), 4.22–4.42 (m, 1H–$C_{1'}$, **a/b**), 7.03–7.20 (m, 5H arom, **a/b**). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: two diastereomers **a/b** (63/37): 16.4–16.6 (d, $^3J_{PC}$=5.5 Hz, 2CH_3–CH_2O, **b/a**), 19.4 (d, $^3J_{PC}$=10.4 Hz, C_5, **a**), 20.0 (d, J=8.2 Hz, C_5, **b**), 26.5 (C_4, **a/b**), 27.1 (CH_3–$C_{1'}$, **a/b**), 28.3/28.5 (C(CH_3)$_3$, **b/a**), 43.5/44.6 (C_6, **b/a**), 49.0/50.2 (d, J=9.2 Hz, C_2, **a/b**), 52.3 ($C_{1'}$, **a**), 52.6 ($C_{1'}$, **b**), 56.5 (d, J=145.4 Hz, C_3, **b**), 57.5 (d, J=141.5 Hz, C_3, **a**), 61.7 (OCH_2, **a**), 62.1 (d, J=7.7 Hz, CH_2O, **b**), 79.3 (C(CH_3)$_3$, **a/b**), [6 arom C: 126.3, 126.4 (2C), 128.1 (2C), 148.1/148.7 (s, **a/b**)], 155.1/155.6 (COO, **a/b**). ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: two diastereoisomers **a/b** (63/37): 27.48/27.78 (**b/a**). HRMS (ESI, *m/z*): calcd mass for $C_{22}H_{37}N_2O_5PNa$, [M+Na]$^+$: 463.2332. Found: 463.2348.

4.2.11. Diethyl 3-benzylamino-1-(*tert*-butyloxycarbonyl)-piperidin-3-yl-phosphonate (21a′). Following procedure A: condensation reaction of 255 mg (1.62 mmol) of ketone **20a**, benzylamine **19b** (174 mg, 1.62 mmol), AcOH (200 μL) and $MgSO_4$ (164 mg) was heated and stirred at 50 °C for 4 h. Then heating with $P(OEt)_3$ (410 μL, 2.43 mmol) at 50 °C overnight, gave after usual work-up and purification by FC (eluent, MeOH/CH_2Cl_2/NH_3: 3/97/0.5), 530 mg (76%) of pure aminophosphonate **21a′** as a colourless oil. R_f=0.65 (MeOH/CH_2Cl_2/NH_3: 5/95/0.5). IR (neat) ν: 3560, 2980, 1693 (CON), 1428, 1276 and 1246 (P═O), 1161, 1028. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.36 (t, J=7.2 Hz, 6H, CH_{3ester}), 1.44 (s, 9H, *t*-Bu), 1.20–2.10 (m, 5H, 4H$_{cycle}$ and NH), 2.40–3.70 (m, 2H$_{cycle}$), 3.87 (d, J=12.2 Hz, 1H$_{benzyl}$), 4.08 (d, J=12.2 Hz, 1H$_{benzyl}$), 4.40–4.90 (m, 5H, 2CH_2O and 1H$_{cycle}$), 7.10–7.50 (5H arom). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.6 (d, $^3J_{PC}$=5.2 Hz, CH_{3ester}), 19.5 (d, $^3J_{PC}$=10.0 Hz, C_5), 27.6 (C_4), 28.3 (CH_3)$_3$C), 43.5 (C_6), 47.2 ($CH_{2benzyl}$), 47.8 (C_2), 56.0 (d, $^1J_{PC}$=146.6 Hz, C_3), 61.9 (d, $^2J_{PC}$=7.7 Hz, CH_2O), 62.2 (d, $^2J_{PC}$=7.4 Hz, CH_2O), 79.6 (C(CH_3)$_3$), [6 arom C: 126.7 (1C), 128.0 (2C), 128.1 (2C), 141.0 (s)], 155.2 (CON). ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 26.78. HRMS (ESI, *m/z*): calcd mass for $C_{21}H_{35}N_2O_5PNa$, [M+Na]$^+$: 449.2176. Found: 449.2182.

4.3. Diethyl [1-oxido-4-(1′-methylbenzyl)amino-tetrahydro-2*H*-thiopyran-4-yl-phosphonate (27)

To a solution of aminophosphonate **15a** (159 mg, 0.415 mmol) in 2 mL of CH_2Cl_2, was added at 0 °C *m*CPBA (77%, 146 mg, 0.415 mmol). The mixture was stirred at 0 °C for 20 min then 5 mL of saturated solution of $Na_2S_2O_3$/$NaHCO_3$ (1/1) was added, vigorously stirred for 1 h, then extracted with CH_2Cl_2 (3×50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum to furnish 168 mg (100%) of clean sulfoxide **27** as a colourless oil mixture of two isomers (40/60, cis/trans or trans/cis). R_f=0.44 (MeOH/CH_2Cl_2: 10/90). IR (neat) ν: 3447, 3354, 2925, 1266 and 1233 (P═O), 1199, 1163 (S═O), 1029

(P–O), 962. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: (two isomers **a/b**: 40/60): 1.25–1.40 (m, 9H, CH_3, **a/b**), 1.50–1.67 (m, 0.6H–C_3, **b**), 1.67–2.20 (m, 4.4H, $3H_{cycle}$ **a/b** and NH and 1H **a**), 2.25–2.85 (m, 2.6H, 2H **a/b** and 0.6H **b**), 2.90–3.10 (m, 1H, **a/b**), 3.10–3.26 (m, 0.4H, **a**), 4.02–4.24 (m, 4H, CH_2OP, **a/b**), 4.39 (qd, J=6.8 Hz, $^3J_{PH}$=2.3 Hz, 0.4H, H–$C_{1'}$, **a**), 4.46 (qd, J=6.8 Hz, $^3J_{PH}$=2.5 Hz, 0.6H, H–$C_{1'}$, **b**), 7.08–7.40 (m, 5H, **a/b**). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: (two isomers, **a/b**: 40/60): 16.6 (CH_3–CH_2O, **a/b**), 16.7 (CH_3–CH_2O, **a/b**), 17.1 (d, $^2J_{PC}$=6.5 Hz, C_3 or C_5, **b**), 21.8 (d, $^2J_{PC}$=3.2 Hz, C_5 or C_3, **b**), 24.5 (d, $^2J_{PC}$=7.6, C_3 or C_5, **a**), 26.7 (CH_3–$C_{1'}$, **b**), 26.8 (CH_3–$C_{1'}$, **a**), 28.5 (d, $^2J_{PC}$=2.8 Hz, C_5 or C_3, **a**), 39.2 (d, $^3J_{PC}$=10.4 Hz, C_2 or C_6, **b**), 39.5 (d, $^3J_{PC}$=13.8 Hz, C_6 or C_2, **b**), 44.3 (C_2 or C_6, **a**), 44.45 (C_6 or C_2, **a**), 52.7 ($C_{1'}$, **a/b**), 55.1 (d, $^1J_{PC}$ 142.4 Hz, C_4, **a**), 55.3 (d, $^1J_{PC}$=143.1 Hz, C_4, **b**), 62.2 (d, $^2J_{PC}$=7.8 Hz, CH_2OP, **b**), 62.5 (d, $^2J_{PC}$=7.7 Hz, CH_2OP, **a**), [6 arom C: 126.4/126.5 (2C, **b/a**), 126.8/126.9 (1C, **b/a**), 128.5 (2C, **a/b**), 147.3/147.9 (s, **a/b**)]. ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: (**a/b**: 40/60): 28.44/27.54. HRMS (EI, *m/z*): calcd mass for $C_{17}H_{28}NO_4PSNa$, $[M+Na]^+$: 396.1369. Found: 396.1361.

4.4. Hydrogenolysis: general procedure B

To a solution of aminophosphonates **13**–**18** or **21** (1 mmol) in 5 mL of AcOH, was added 20% $Pd(OH)_2$/C (Pearlman's catalyst, 150 mg (40% w/w)). The flask was connected to a hydrogenation apparatus equipped with a graduated burette containing water that allowed the uptake of hydrogen to be monitored. TLC control showed that under 1 atm for 18 h, the reaction was complete. Then degassed under a stream of argon, filtered through paper and the collected solid was washed with EtOH (2×10 mL). The combined filtrate and washings were concentrated and purified by FC on silica gel (20 g), eluent ($MeOH/CH_2Cl_2/NH_3$: 10/90/0.5) to give free amines **24**–**26** or **30**.

4.4.1. Diethyl 4-amino-1-methylpiperidin-4-yl-phosphonate (24). Following procedure B: reaction of phosphonate **13** (274 mg, 0.773 mmol), AcOH (4 mL) and 20% $Pd(OH)_2$/C (132 mg) under H_2 (1 atm) for 18 h followed by FC ($CH_2Cl_2/MeOH/NH_3$: 90/10/0.5→80/20) gave 140 mg (73%) of aminophosphonate **24** as a colourless oil. R_f=0.20 ($MeOH/CH_2Cl_2$: 10/90). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.25 (t, J=7.0 Hz, 6H, CH_3–CH_2O), 1.46–1.61 (m, 2H, 1H–C_3 and 1H–C_5), 1.94–2.12 (m, 2H, 1H–C_3 and 1H–C_5), 2.27 (s, 3H, CH_3N), 2.47 (br t, J=11.3 Hz, 1H–C_2 and 1H–C_6), 2.66 (br d, J=11.3 Hz, 2H, 1H–C_2 and 1H–C_6), 3.12 (br s, 2HN), 4.07 (qd, J=7.0 Hz, $^3J_{PH}$=7.0 Hz, 4H, CH_2OP). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.5 (d, $^3J_{PC}$=5.3 Hz, $2CH_3$–CH_2O), 30.6 (C_3 and C_5), 45.4 (CH_3-N), 48.7 (d, $^1J_{PC}$=154.9 Hz, C_4), 48.8 (d, $^2J_{PC}$=11.6 Hz, $2CH_2OP$), 62.4 (C_2 or C_6), 62.5 (C_6 or C_2). ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 29.50. HRMS (ESI, *m/z*): calcd mass for $C_{10}H_{23}N_2O_3PNa$, $[M+Na]^+$: 273.1339. Found: 273.1349.

4.4.2. Diethyl 4-amino-tetrahydro-2*H*-pyran-4-yl-phosphonate (25). Following procedure B: reaction of phosphonate **14a** (140 mg, 0.410 mmol), AcOH (2.40 mL) and 10% $Pd(OH)_2$/C (70 mg) under H_2 (1 atm) for 18 h followed by FC gave 83 mg (86%) of aminophosphonate **25** as a colourless oil. R_f=0.23 ($MeOH/CH_2Cl_2$: 10/90). IR (neat) ν: 3457, 3380, 2951, 1235 (P=O), 1049 and 1026 (P–O), 959. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.28 (t, J=7.0 Hz, 6H, CH_3), 1.30–1.45 (m, 2H, 1H–C_3 and 1H–C_5), 1.74 (br s, 2HN), 1.90–2.10 (m, 2H, 1H–C_3 and 1H–C_5), 3.62–3.72 (m, $2H_{cycle}$, CH_2O), 3.76–3.88 (m, $2H_{cycle}$, CH_2O), 4.08 (q, J=7.0 Hz, 2H, CH_2OP), 4.11 (q, J=7.0 Hz, 2H, CH_2OP). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.5 (d, $^3J_{PC}$=5.2 Hz, $2CH_3$–CH_2O), 31.4 (C_3 and C_5), 49.2 (d, $^1J_{PC}$=155.0 Hz, C_4), 61.7 (C_2), 61.77 (d, $^2J_{PC}$=7.7 Hz, CH_2OP), 61.85 (C_6), 62.4 (d, J=7.7 Hz, CH_2OP). ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 28.61. HRMS (ESI, *m/z*): calcd mass for $C_9H_{20}NO_4PNa$, $[M+Na]^+$: 260.1022. Found: 260.1030.

4.4.3. Diethyl 3-amino-1-(*tert*-butyloxycarbonyl)-piperidin-3-yl-phosphonate (30). Following procedure B: reaction of *N*-Boc phosphonate **21a′** (350 mg, 0.82 mmol), AcOH (6 mL) and 20% $Pd(OH)_2$/C (140 mg) under H_2 (1 atm) for 18 h followed by FC (eluent: $MeOH/CH_2Cl_2/NH_3$: 5/93/2), gave 285 mg (88%) of aminophosphonate **30** as a colourless oil. R_f=0.63 (ether). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.34 (t, J=7.0 Hz, 6H, CH_3–CH_2O), 1.46 (s, 9H, *t*-Bu), 1.40–2.00 (m, 6H, 2H–C_4, 2H–C_5, and NH_2), 2.67–2.90 (m, 1H, 1H–C_6), 3.04–3.32 (m, 1H–C_6), 3.80–4.10 (m, 2H–C_2), 4.05–4.37 (m, 4H, CH_2OP). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.7 ($2CH_3$–CH_2O), 19.5 (C_5), 28.5 ((CH_3)$_3$–C), 30.5 (d, $^2J_{PC}$=2.4 Hz, C_4), 51.6 (d, $^1J_{PC}$=155.8 Hz, C_3), 62.8 (C_6), 76.6 (C_2), 77.2 (CH_2OP), 77.6 (CH_2OP), 79.9 (s, $C(CH_3)_3$), 155.7 (COO). ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 27.84. HRMS (ESI, *m/z*): calcd mass for $C_{14}H_{29}N_2O_5PNa$, $[M+Na]^+$: 359.1706. Found: 359.1723.

4.5. Diethyl 4-amino-tetrahydro-2*H*-thiopyran-4-yl-phosphonate (26)

To a solution of *N*-PMB aminophosphonate **15c** (240 mg, 0.64 mmol) in 2.5 mL of a mixture of CH_2Cl_2/H_2O (9/1), was added DDQ (16.2 mg, 0.70 mmol). The mixture was stirred at rt for 3 h. Then 350 μL of 2 N KOH solution was added and stirred for 1 h. The reaction mixture was filtered over Celite® and concentrated, and the residue was purified by FC (eluent: $MeOH/CH_2Cl_2/NH_3$ aq: 1/99/0.5) to afford 65 mg (40%) of the free aminophosphonate **26** as a yellow viscous oil accompanied with 120 mg (50%) of imine intermediate **29**. Or treatment of the crude mixture (**26** and **29**) with KOH (2 N) in the presence of benzoyl hydrazine (1 equiv) gave after FC the desired free amine **26** (143 mg, 88%).

4.5.1. Data for free amine (26). R_f=0.42 ($MeOH/CH_2Cl_2$+NH_3: 10/90). IR (neat) ν: 3468, 2980, 1605, 1248, 1026, 965. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.34 (t, J=7.0 Hz, 6H, CH_3), 1.40–1.90 (br s, 2H–N), 1.83–2.01 (m, 2H, 1H–C_3 and 1H–C_5), 2.01–2.22 (m, 2H, 1H–C_3 and 1H–C_5), 2.37 (br d, J=12.7 Hz, 2H, 1H–C_2 and 1H–C_6), 3.13 (dddd, J=2.2 Hz, J=2.2 Hz, J=12.7 Hz, J=12.2 Hz, 2H, 1H–C_2 and 1H–C_6), 4.13 (q, J=7.0 Hz, 2H, CH_2O), 4.16 (q, J=7.0 Hz, 2H, CH_2O). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.7 (d, $^3J_{PC}$=5.2 Hz, $2CH_3$), 21.7 (C_2 or C_6), 21.9 (C_6 or C_2), 32.3 (C_3 and C_5), 50.7 (d, J=151.2 Hz, C_4), 62.6 (d, J=7.7 Hz, $2CH_2O$). ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 29.36. HRMS (ESI, *m/z*): calcd mass for $C_9H_{20}NO_3PSNa$, $[M+Na]^+$: 276.0794. Found: 276.0800.

4.5.2. (*E*)-Diethyl 3-(4′-methoxybenzylideneamino)tetrahydro-2*H*-thiopyran-4-yl-phosphonate (29). R_f=0.83 (MeOH/CH_2Cl_2+NH_3: 5/95+0.5). IR (neat) ν: 3044, 2982, 1630 (C=N), 1605, 1575, 1512, 1245, 1029, 967. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.30 (t, J=7.0 Hz, 6H, $2CH_3$), 2.20–2.50 (m, 4H, 1H–C_3, 1H–C_5 and 1H–C_2, 1H–C_6), 2.50–2.78 (m, 2H, 1H–C_3 and 1H–C_5), 2.78–3.10 (m, like t, 2H, 1H–C_2 and 1H–C_6), 3.86 (s, 3H, OCH_3), 4.06 (q, J=7.0 Hz, 2H, CH_2O), 4.09 (q, J=7.0 Hz, 2H, CH_2O), 6.95 (d, J=8.7 Hz, $2H_{aryl}$), 7.77 (d, J=8.7 Hz, $2H_{aryl}$), 8.52 (d, $^4J_{PH}$=4.7 Hz, $1H_{imine}$). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 16.6 (d, $^3J_{PC}$=5.5 Hz, $2CH_{3ester}$), 22.3 (C_2), 22.5 (C_6), 32.5 (C_3 and C_5), 55.5 (OCH_3), 61.3 (d, $^1J_{PC}$=146.7 Hz, C_4), 62.9 (d, $^2J_{PC}$=7.2 Hz, $2CH_2O$), [6 arom C: 144.1 (2C), 129.6 (s), 130.0 (2C), 161.1 (*C*–OCH_3)], 163.4 (d, $^3J_{PC}$=10.4 Hz, N=C), ^{31}P NMR ($CDCl_3$, 101.25 MHz) δ: 25.26. HRMS data were not obtained.[23]

4.6. Hydrolysis of aminophosphonates

4.6.1. 4-Amino-1-methylpiperidin-4-yl-phosphonic acid (2a). General procedure C—Hydrolysis of aminophosphonates with HCl: A solution of diethylphosphonate **24** (119 mg, 0.38 mmol) in aq 6 N HCl (3 mL) was heated at reflux for 7 h. The solvent was evaporated under reduced pressure to dryness. The residue was dissolved in 4 mL of CH_2Cl_2, then concentrated to dryness to give the crude aminophosphonic acid·*x*HCl, hydrochloride. The crude hydrochloride aminophosphonic acid·*x*HCl was dissolved in minimum amount of EtOH (3 mL), then to which was added dropwise an excess of propylene oxide (5 mL) and stirring at rt for 18 h. The volatile compounds were removed by evaporation under vacuum, to give 74 mg of phosphonic acid **2a** quantitatively. Mp>250 decomp. IR (KBr) ν cm^{-1}: 3413, 2925, 1617, 1206, 1080, 1057, 923. ^{1}H NMR (D_2O, 360 MHz) δ: two diastereomers **a/b** (in 55/45 ratio): 1.87–2.10 (m, 2H, 1H–C_3 and 1H–C_5, **a**), 2.10–2.25 (m, 2H, 1H–C_3 and 1H–C_5, **b**), 2.25–2.38 (m, 2H, 1H–C_3 and 1H–C_5, **b**), 2.38–2.54 (m, 2H, 1H–C_3 and 1H–C_5, **a**), 2.81/2.83 (s, CH_3, **a/b**), 3.02–3.20 (m, 2H, 1H–C_2 and 1H–C_6, **b**), 3.33–3.65 (m, 6H, 4H **a** and 2H **b**); in (NaOD/D_2O, 250 MHz) δ: 1.28–1.47 (m, 2H, 1H–C_3 and 1H–C_5), 1.67–1.94 (m, 2H, 1H–C_3 and 1H–C_5), 2.06 (s, CH_3), 2.08–2.30 (m, 2H, 1H–C_2 and 1H–C_6), 2.37–2.60 (m, 2H, 1H–C_2 and 1H–C_6). ^{13}C NMR (D_2O, 62.9 MHz) δ: two diastereomers, **a/b** (55/45): 26.4 (C_3 and C_5, **b**), 28.6 (C_3 and C_5, **a**), 43.0 (CH_3, **a/b**), 48.2 (C_2 and C_6, **a**), 48.4 (C_2 and C_6, **b**), 50.2 (d, $^1J_{PC}$=147.2 Hz, C_4, **a**), 50.4 (d, $^1J_{PC}$=141.6 Hz, C_4, **b**), 50.8 (C_2 and C_6, **a**); in (D_2O+NaOD): 31.3 (C_3 and C_5), 44.8 (CH_3), 48.44 (d, $^1J_{PC}$=144.1 Hz, C_4), 49.1 (C_2 or C_6), 49.2 (C_6 or C_2). ^{31}P NMR (D_2O, 101.25 MHz) δ: two diastereomers, **a/b** (55/45): 12.09/12.85 (**a/b**); in (DMSO-d_6): 14.91/15.72 (**b/a**); in (D_2O+NaOD): 23.33 only one. HRMS (ESI, *m/z*): calcd mass for $C_6H_{16}N_2O_3P$, $[M+H]^+$: 195.0893. Found: 195.0899.

4.6.2. 4-Amino-tetrahydro-2*H*-pyran-4-yl-phosphonic acid (2b). Hydrolysis of aminophosphonates with TMSI according to our reported method:[9b] Reaction of diethylphosphonate **25** (62 mg, 0.26 mmol), CH_2Cl_2 (3 mL), TMSI (78 mg, 0.55 mmol), 6 h then EtOH (1.3 mL) and propylene oxide (1 mL), for 18 h at rt gave, after usual work-up, 20 mg (43%) of pure aminophosphonic acid **2b** as a white solid. Mp 237 °C decomp. ^{1}H NMR (D_2O, 250 MHz) δ: 1.60–1.77 (m, 2H, 1H–C_3 and 1H–C_5), 2.01–2.23 (m, 2H, 1H–C_3 and 1H–C_5), 3.60 (ddd, J=2.8 Hz, J=10.3 Hz, J=12.3 Hz, 2H, 1H–C_2 and 1H–C_6), 3.87 (ddd, J=4.7 Hz, J=4.7 Hz, J=12.3 Hz, 2H, 1H–C_2 and 1H–C_6). ^{13}C NMR (D_2O, 62.9 MHz) δ: 30.0 (C_3 and C_5), 52.9 (d, $^1J_{PC}$=138.5 Hz, C_4), 62.4 (C_2 or C_6), 62.5 (C_6 or C_2). ^{31}P NMR (D_2O, 101.25 MHz) δ: 13.31. ES$^+$ MS, *m/z*: 204.1 $[M+Na]^+$. HRMS data were not obtained.[23]

4.6.3. 4-Amino-tetrahydro-2*H*-thiopyran-4-yl-phosphonic acid (2c). Following procedure C: reaction of diethylphosphonate **26** (127 mg, 0.50 mmol), 12 N HCl (2 mL) at reflux for 5 h, then EtOH (2.5 mL) and propylene oxide (2 mL) for 17 h at rt gave, after usual work-up, 92 mg (90%) of aminophosphonic acid **2c**. Mp 242 °C decomp. IR (KBr) ν cm^{-1}: 3338, 3230, 3140, 2920, 1617, 1545, 1203, 1070, 1038, 912. ^{1}H NMR (D_2O, 250 MHz) δ: 1.90–2.14 (m, 2H, 1H–C_3 and 1H–C_5), 2.14–2.35 (m, 2H, 1H–C_3 and 1H–C_5), 2.60–2.85 (m, 4H, 2H–C_2 and 2H–C_6). ^{1}H NMR (D_2O+NaOD, 360 MHz) δ: 1.00–1.32 (m, 4H, 2H–C_3 and 2H–C_5), 1.60–1.80 (m, 2H, 1H–C_2 and 1H–C_6), 2.13–2.34 (m, 2H, 1H–C_2 and 1H–C_6). ^{13}C NMR (D_2O+NaOD, 90.6 MHz) δ: 21.3 (C_2), 21.5 (C_6), 32.0 (C_3 and C_5), 49.0 (d, $^1J_{PC}$=142.6 Hz, C_4). ^{31}P NMR (D_2O, 101.25 MHz) δ: 16.25; in (D_2O+NaOD) δ: 23.13.

Purification of a sample by FC (eluent, EtOH/H_2O/concd NH_4OH: 30/3/10): ^{1}H NMR (D_2O, 360 MHz) δ: 2.06–2.22 (m, 2H), 2.22–2.37 (m, 2H), 2.72–2.91 (m, 4H). ^{31}P NMR (D_2O, 101.25 MHz) δ: 13.78. HRMS data were not obtained.[23]

4.6.4. 3-Amino-piperidin-3-yl-phosphonic acid hydrochloride (3·2HCl). Following procedure C: reaction of *N*-Boc phosphonate **30** (122 mg, 0.36 mmol), 6 N HCl (4 mL) at reflux for 12 h, gave after usual work-up 99 mg (100%) of crude aminophosphonic acid hydrochloride **3·2HCl**. IR (KBr) ν cm^{-1}: 3390, 2926, 1613, 1204, 1153, 1073, 982. ^{1}H NMR (D_2O, 250 MHz) δ: 1.70–2.27 (m, 3H, 2H–C_5 and 1H–C_4), 2.27–2.57 (m, 1H, C_4), 2.92–3.57 (m, 3H, 2H–C_6+1H–C_2), 3.57–3.88 (m, 1H–C_2). ^{1}H NMR (D_2O+NaOD, 250 MHz) δ: 1.20–1.41 (m, 1H–C_5), 1.41–1.64 (m, 2H), 1.64–1.85 (m, 1H–C_4), 2.20–2.44 (m, 1H–C_6), 2.44–2.88 (m, 3H, 2H–C_2 and 1H–C_6). ^{13}C NMR (D_2O, 62.9 MHz) δ: 18.4 (C_5), 27.7 (C_4), 43.5 (C_6), 46.0 (C_2), 50.7 (d, $^1J_{PC}$=138.5 Hz, C_3).

4.6.5. 3-Amino-piperidin-3-yl-phosphonic acid (3). Following procedure C: The crude aminophosphonic acid hydrochloride **3·2HCl** (80 mg) and propylene oxide (5 mL) gave after concentration 74 mg (100%) of free aminophosphonic acid **3** as colourless solid. Mp >250 °C decomp. ^{1}H NMR (D_2O, 250 MHz) δ: 1.60–2.17 (m, 3H, 2H–C_5 and 1H–C_4), 2.17–2.37 (m, 1H–C_4), 2.96 (m, 1H–C_6), 3.13–3.04 (d, J=12.7 Hz, 1H–C_2), 3.32 (m, 1H–C_6), 3.57–3.54 (dd, J=1.5 Hz, J=12.7 Hz, 1H–C_2); in (D_2O+NaOD) δ: 1.22–1.30 (m, 1H–C_5), 1.36–1.58 (m, 2H, 1H–C_5 and 1H–C_4), 1.58–1.80 (m, 1H–C_4), 2.20–2.34 (m, 1H–C_6), 2.42–2.58 (m, 1H–C_2), 2.58–2.78 (m, 2H, 1H–C_6 and 1H–C_6). ^{13}C NMR (D_2O+NaOD, 62.9 MHz) δ: 20.6 (d, $^3J_{PC}$=8.0 Hz, C_5), 30.3 (C_4), 44.7 (C_6), 50.15 (d, $^1J_{PC}$=140.1 Hz, C_3), 50.9 (d, $^2J_{PC}$=5.3 Hz, C_2). ^{31}P NMR

(D_2O, 101.25 MHz) δ: 11.81; in (D_2O+NaOD) δ: 21.97. ES^+ MS, *m/z*: 203.1 $[M+Na]^+$. HRMS data were not obtained.[23]

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References and notes

1. Kafarski, P.; Lejczak, B. *Phosphorus, Sulfur Silicon Relat. Elem.* **1991**, *63*, 193–215.
2. (a) Maier, L. *Phosphorus Sulfur* **1983**, *14*, 295–322; (b) Baylis, E. K.; Campbell, C. D.; Dingwall, J.-G. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2845–2853; (c) Maier, L. *Phosphorus, Sulfur Silicon Relat. Elem.* **1990**, *53*, 43–67; (d) Hudson, H. R.; Pianka, M. *Phosphorus, Sulfur Silicon Relat. Elem.* **1996**, *109–110*, 345–348.
3. Peyman, A.; Budt, K. H.; Spanig, J.; Ruppert, D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1720–1722.
4. (a) Cheng, L.; Goodwin, C. A.; Scully, M. F.; Kakkar, V. V.; Claeson, G. *Tetrahedron Lett.* **1991**, *32*, 7333–7336; (b) Bergin, C.; Hamilton, R.; Walker, B.; Walker, B. J. *Chem. Commun.* **1996**, 1155–1156.
5. Kukhar, V. P.; Hudson, H. R. *Aminophosphonic and Aminophosphinic Acids*; Wiley: New York, NY, 2000.
6. For recent enantioselective synthesis of α-aminophosphonic acids, see: (a) Dhawan, B.; Redmore, D. *Phosphorus Sulfur* **1987**, *32*, 119–144; (b) Kukhar, V. P.; Soloshonok, V. A. *Phosphorus, Sulfur Silicon Relat. Elem.* **1994**, *92*, 239–264; (c) Bennani, Y. L.; Hanessian, S. *Chem. Rev.* **1997**, *97*, 3161–3195; (d) Kolodiazhnyi, O. I. *Tetrahedron: Asymmetry* **1998**, *9*, 1279–1332 and references cited therein.
7. Lefebvre, I. M.; Evans, S. A., Jr. *J. Org. Chem.* **1997**, *62*, 7532–7533; (b) Vercruysse, K.; Dejugnat, C.; Munoz, A.; Etemad-Moghadam, G. *Eur. J. Org. Chem.* **2000**, 281–289 and references cited therein; (c) Dejugnat, C.; Etemad-Moghadam, G.; Rico-Lattes, I. *Chem. Commun.* **2003**, 1858–1859; (d) Joly, G. D.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2004**, *126*, 4102–4103 and references cited therein; (e) Maury, C.; Gharbaoui, T.; Royer, J.; Husson, H.-P. *J. Org. Chem.* **1996**, *61*, 3687–3693; (f) Smith, A. B., III; Yager, K. M.; Taylor, C. M. *J. Am. Chem. Soc.* **1995**, *117*, 10879–10888.
8. (a) Diel, P. J.; Maier, L. *Phosphorus Sulfur* **1984**, *20*, 313–321; (b) Groth, U.; Lehmann, L.; Richter, L.; Schöllkopf, U. *Liebigs Ann. Chem.* **1993**, 427–431; (c) Yamazaki, S.; Takada, T.; Jmanishi, T.; Moriguchi, Y.; Yamabe, S. *J. Org. Chem.* **1998**, *63*, 5919–5928.
9. (a) Fadel, A. *J. Org. Chem.* **1999**, *64*, 4953–4955; (b) Fadel, A.; Tesson, N. *Eur. J. Org. Chem.* **2000**, 2153–2159; (c) Fadel, A.; Tesson, N. *Tetrahedron: Asymmetry* **2000**, *11*, 2023–2031; (d) Tesson, N.; Dorigneux, B.; Fadel, A. *Tetrahedron: Asymmetry* **2002**, *13*, 2267–2276.
10. (a) Hercouet, A.; Le Corre, M.; Carboni, B. *Tetrahedron Lett.* **2000**, *41*, 197–199; (b) Guéguen, C.; About-Jaudet, E.; Collignon, N.; Savignac, Ph. *Synth. Commun.* **1996**, *26*, 4131–4143.
11. (a) Ranu, B. C.; Hajra, A. *Green Chem.* **2002**, *4*, 551–554; (b) Wieczorek, J. C.; Gancarz, R.; Bielecki, K.; Grzys, E.; Sarapuk, J. *Phosphorus, Sulfur Silicon Relat. Elem.* **2001**, *174*, 119–128; (c) Stoikov, I. I.; Repejkov, S. A.; Antipin, I. S.; Konovalov, A. I. *Heteroat. Chem.* **2000**, *11*, 518–527.
12. (a) Günter, E.; Löhge, W. German Patent 1991; 2,022,228; *Chem. Abstr.* **1971**, *74*, 100219; (b) Dedek, W.; Partisch, M.; Grahl, R. *Biochem. Physiol. Pflanz.* **1978**, *173*, 70–81; (c) Soroka, M. *Liebigs Ann. Chem.* **1990**, 331–334; (d) See Ref. 10b; (e) Wieczorek, J. S.; Gancarz, R.; Bielecki, K.; Grzys, E.; Sarapuk, J. *Phosphorus, Sulfur Silicon Relat. Elem.* **2000**, *166*, 111–123; (f) Yadav, J. S.; Subba Reddy, B. V.; Madan, Ch. *Synlett* **2001**, 1131–1133; (g) Matveeva, E. D.; Podrugina, T. A.; Tishkovskaya, E. V.; Tomilova, L. G.; Zefirov, N. S. *Synlett* **2003**, 2321–2324; (h) Ravinder, K.; Reddy, A. V.; Krishnaiah, P.; Venkataramana, G.; Reddy, V. L. N.; Venkateswarlu, Y. *Synth. Commun.* **2004**, *34*, 1677–1683; (i) Firouzabadi, H.; Iranpoor, N.; Sobhani, S. *Synthesis* **2004**, 2692–2696; (j) Ghosh, R.; Maiti, S.; Chakraborty, A.; Maiti, D. K. *J. Mol. Catal. A: Chem.* **2004**, *210*, 53–57.
13. For reaction of diethylphosphite with ketones, lower reactive than aldehydes, necessitated either harsh conditions, the use of various catalysts, or microwave assistance, see: Kabachnick, M. M.; Zobnina, E. V.; Belatskaya, I. P. *Synlett* **2005**, 1393–1396 and references cited therein.
14. GABA-related drugs has shown promising properties as anti-leptic, antispastic and antidepressive agents: Fonnum, F. *Psychopharmacology*; Meltzer, H., Ed.; Raven: New York, NY, 1987; Vol. 18, pp 173–182.
15. (a) Tukanova, S. K.; Dzhiembaev, B. Zh.; Butin, B. M. *Zh. Obshch. Khim.* **1991**, *61*, 1118–1120; (b) Dzhiembaev, B. Zh.; Abiyurov, B. D.; Zaporozhskaya, N. I.; Minbaev, B. U. *Zh. Obshch. Khim.* **1989**, *59*, 77–80; (c) Gancarz, R.; Latajka, R.; Halama, A.; Kafarski, P. *Magn. Reson. Chem.* **2000**, *38*, 867–871; (d) For a substituted piperidine and thio-pyran aminophosphonic acid derivatives, see: Bosyakov, Y. G.; Maishinova, G. T.; Logunov, A. P.; Revenko, G. P. *Izv. Nats. Akad. Nauk. Resp. Kaz., Ser. Khim.* **1993**, *6*, 72–76.
16. For a substituted pyran-aminophosphonates see: (a) Kabatschnik, M. *Izv. Akada. Nauk SSSR, Ser. Khim.* **1957**, 1357 and 1362; Engl. Ausg. S 1375 and 1379; (b) For unsubstituted pyran derivatives see Ref. 17a.
17. (a) Tukanova, S. K.; Dzhiembaev, B. Zh.; Khalilova, S. F.; Butin, B. M. *Zh. Obshch. Khim.* **1993**, *63*, 938–939; (b) Kiyashev, D. K.; Turisbekova, L. K.; Sadykov, T. S.; Erzhanov, K. B.; Nurakhov, D. B. *Izv. Akad. Nauk. Kaz. SSR, Ser. Khim.* **1990**, *6*, 82–84; (c) See Ref. 15d.
18. Haak, E.; Bytschkov, I.; Doye, S. *Eur. J. Org. Chem.* **2002**, 457–463.
19. (a) Curphey, T. J.; Hung, J.-C.; Chian Chu, C. C. *J. Org. Chem.* **1975**, *40*, 607–614; (b) Taylor, E. C.; Macor, J. E. *J. Org. Chem.* **1989**, *54*, 1249–1264.
20. The investigation on reactivity in the five-membered ring series is currently under way.
21. Wu, M.-J.; Pridgen, L. N. *Synlett* **1990**, 636–637.
22. Strekowski, L.; Cegla, M. T.; Harden, D. B.; Kong, S.-B. *J. Org. Chem.* **1989**, *54*, 2464–2466.
23. Unfortunately, HRMS were not obtained, even by using various kind of measure: electro spray, electronic impact or chemical ionisation.

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Synthesis of 6-amino-6-deoxy-D-gulono-1,6-lactam and L-gulono-1,6-lactam derived from corresponding 5,6-*O*-sulfinyl hexono-1,4-lactones

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Abstract—Syntheses of 6-amino-6-deoxy-2,3-*O*-isopropylidene-D-gulono- and L-gulono-1,6-lactams **3** and **4** from corresponding glycono-1,4-lactones are described. Activation of the primary hydroxyl group requires 5,6-cyclic sulfite intermediate to obtain 6-azido-6-deoxy derivatives, which are cyclized after reduction.

1. Introduction

Intracyclic nitrogen-based sugar analogues named azasugars or iminosugars have been discovered as natural products. These compounds have been of considerable interest because many of them show specific inhibition against glycosidases and glycosyltransferases, being potential therapeutic agents for viral, proliferative and metabolic diseases.[1] Thus, these properties have stimulated intensive work directed on their synthesis, usually towards the five- and six-member iminosugars.

Nevertheless, only few reports have appeared on the synthesis of seven-member iminosugars (polyhydroxyazepanes), which have also been shown to possess potent inhibitory activities.[2] In some of those reported, they have often been obtained in admixture with their corresponding six-member ring derivatives, requiring separation.[3]

Polyhydroxyheptonolactams have been reported as tetrahydroxycaprolactam derived, potentially useful monomers for the preparation of novel oligomeric and polymeric materials.[4a]

Polyhydroxyheptonolactams are also precursors of polyhydroxyazepanes, and have been described from protected lactones as starting materials.[4] Our group has recently described an improved synthesis of 6-amino-6-deoxy-D-galactono- and D-mannono-1,6-lactams **1** and **2** via corresponding 6-bromo-6-deoxy-1,4-lactones (Fig. 1).[5]

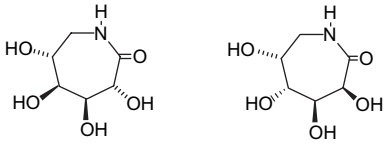

Figure 1.

In previous work, we have shown the usefulness of cyclic sulfites for stereoselective *N*-glycosylation, *O*-glycosylation and *C*-glycosylation reactions of hexoses and pentoses.[6] In the continuation of our interest in the synthesis of azasugars,[5,7] we now describe a synthetic route to 6-amino-6-deoxy-D- and L-gulono-1,6-lactams **3** and **4** (Fig. 2) from D- and L-gulono-1,4-lactones involving 5,6-cyclic sulfite as activating group of the primary hydroxyl.

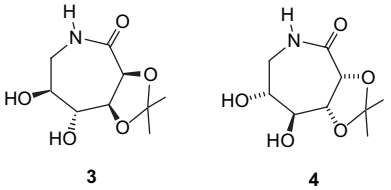

Figure 2.

Keywords: D-Gulono-1,4-lactone; L-Gulono-1,4-lactone; 5,6-*O*-Sulfinyl-1,4-lactone; 6-Amino-6-deoxy-D-gulonolactam; 6-Amino-6-deoxy-L-gulonolactam.
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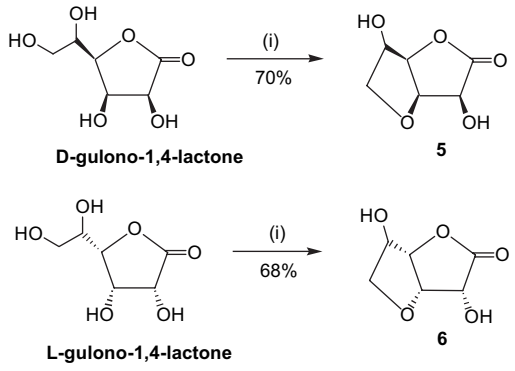
2. Results and discussion

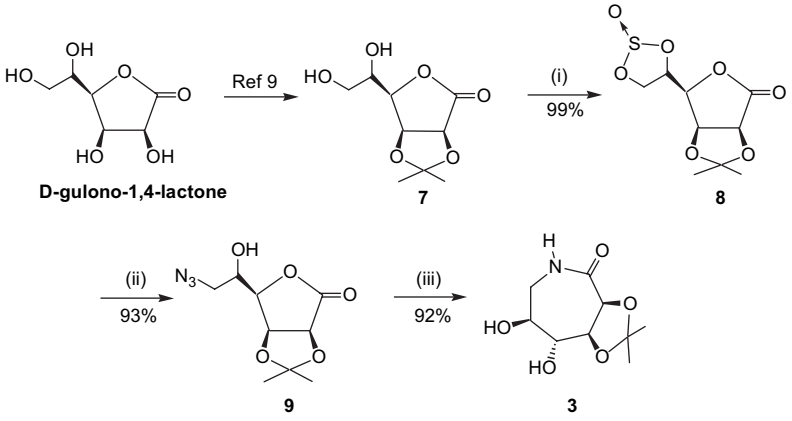
We first selected the D-gulono-1,4-lactone as starting material. D-Gulono-1,4-lactone was subjected to bromination, with the intention to prepare 6-bromo-6-deoxy-D-gulono-1,4-lactone, by using carbon tetrabromide–triphenylphosphine system in pyridine.[5] In spite of many attempts with other solvents such as DMF or higher temperatures, we never obtained the 6-bromo-6-deoxy derivative. With this reagent, D-gulono-1,4-lactone gave a 3,6-anhydro derivative **5** (70%). This reaction applied to L-gulono-1,4-lactone gave the same result, the 3,6-anhydro compound **6** was isolated in only 38% yield, in this case more starting lactone was retrieved (Scheme 1). The formation of 3,6-anhydro compound has already been described in the literature for structures with cis-relationship between the 3-OH group and the side chain at C-4. This anhydridation was observed by heating 6-bromo-6-deoxy-D-mannono and D-idono-1,4-lactone.[8]



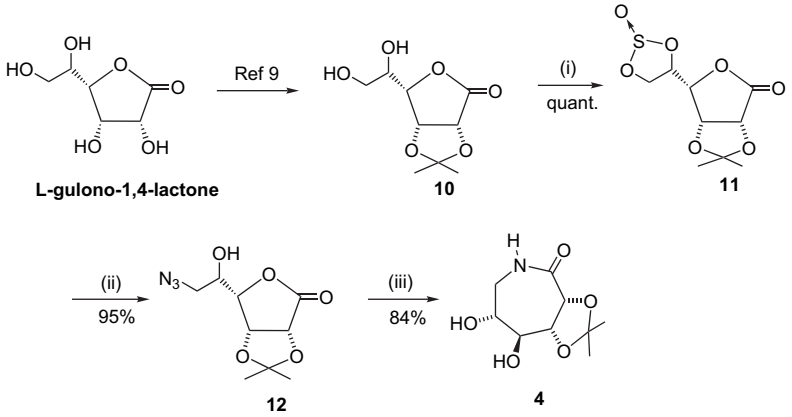
Scheme 1. (i) Conditions: PPh_3, CBr_4, pyridine.

Therefore, we turned to the cyclic sulfite as activating group. 5,6-Cyclic sulfite derivative **8** of D-gulono-1,4-lactone was obtained from 2,3-*O*-isopropylidene-D-gulono-1,4-lactone **7**,[9] which was synthesized in two steps from commercial D-gulono-1,4-lactone (Scheme 2). Sulfinylation of **7** by thionyl chloride in THF in the presence of pyridine afforded 5,6-cyclic sulfite D-gulono-1,4-lactone **8** in 99% in 5–10 min. Treatment of **8** with sodium azide in DMF gave the corresponding 6-deoxy-6-azido compound **9** in 93% yield. The one-pot reduction of the azido group and subsequent N-heterocyclization was realized by catalytic hydrogen transfer with ammonium formate as hydrogen donor and palladium on charcoal (10%) as catalyst in ethyl acetate at 70 °C.[10] The 6-amino-6-deoxy-D-gulono-1,6-lactam **3** was isolated in 92% yield.

This strategy was also applied to the L-gulono-1,4-lactone. 2,3-*O*-Isopropylidene-L-gulono-1,4-lactone **10** was prepared as described in the literature.[9] Sulfinylation of **10** was quantitative. Then azidation of **11** afforded the product **12** in 95% yield. The reduction–cyclization steps by catalytic hydrogen transfer produced 6-amino-6-deoxy-L-gulono-1,6-lactam **4** in 84% yield from **12** (Scheme 3).



Scheme 2. (i) Conditions: $SOCl_2$, pyridine, THF; (ii) NaN_3, DMF; (iii) Pd/C (10%), HCO_2NH_4, EtOAc.



Scheme 3. (i) Conditions: $SOCl_2$, pyridine, THF; (ii) NaN_3, DMF; (iii) Pd/C (10%), HCO_2NH_4, EtOAc.

In summary, we have described an access to 6-amino-6-deoxy-D- and L-gulono-1,6-lactam derived **3** and **4** from corresponding 2,3-*O*-isopropylidene-hexono-1,4-lactones. Attempts at 6-bromination of D- and L-gulono-1,4-lactones were unsuccessful. The azidation was efficient with a 5,6-cyclic sulfite as an alternative intermediate. Overall yields for the sulfinylation, azidation and reduction–cyclization are 84 and 80%, respectively, for the D- and L-isomers **3** and **4**.

3. Experimental

3.1. General

All chemicals were purchased from Aldrich or Acros (France). Carbon tetrabromide furnished by Aldrich was sublimed before use. Melting points were determined on a Büchi 535 apparatus and are uncorrected. Optical rotations were determined with a Jasco Dip 370 electronic micropolarimeter (10 cm cell) at 24 °C. ^{1}H NMR spectra were recorded on a Bruker 300 WB spectrometer at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz.

Thin layer chromatography (TLC) was performed on Merck glass plates silica gel 60 F254 stained with phosphomolybdic acid–H_2SO_4 reagent in ethanol. Column chromatography was carried out on silica gel (Merck, 230–400 mesh). All solvents were distilled before use.

3.1.1. 3,6-Anhydro-D-gulono-1,4-lactone (5). A solution of D-gulono-1,4-lactone (0.5 g, 2.8 mmol) in pyridine (10 mL) was treated with triphenylphosphine (1.47 g, 2 equiv) and carbon tetrabromide (0.93 g, 1 equiv). The mixture was stirred at room temperature for 2 h, then concentrated in vacuo and the residue was purified by column chromatography (EtOAc) to give **5** (0.314 g, 70%) as white solid. R_f 0.5 (EtOAc–MeOH 9:1), mp 146–147 °C, $[\alpha]_D$ −5.9 (*c* 0.4, MeOH), ^{1}H NMR (MeOD, 300 MHz) δ 4.52 (d, 1H, H-2, $J_{2,3}$=4.6 Hz), 4.43 (dd, 1H, H-3, $J_{3,4}$=2.9 Hz), 4.38 (dd, 1H, H-4, $J_{4,5}$=7.7 Hz), 4.15 (m, 1H, H-5), 3.78 (dd, 1H, H-6a, $J_{5,6a}$=4.2 Hz, $J_{6a,6b}$=11.5 Hz), 3.70 (dd, 1H, H-6b, $J_{5,6b}$=4.9 Hz). ^{13}C NMR (MeOD, 75 MHz) δ 176.4 (C-1), 85.0 (C-4), 78.7 (C-3), 75.2 (C-2), 74.2 (C-5), 69.9 (C-6).

3.1.2. 3,6-Anhydro-L-gulono-1,4-lactone (6). L-Gulono-1,4-lactone (0.5 g, 2.8 mmol) in pyridine treated with triphenylphosphine (2 equiv) and carbon tetrabromide as described above for **5**, gave **6** as white solid (0.172 g, 38%). R_f 0.5 (EtOAc–MeOH 9:1), mp 150–151 °C, $[\alpha]_D$ +5 (*c* 0.5, MeOH), ^{1}H NMR (MeOD, 300 MHz) δ 4.46 (dd, 1H, H-4, $J_{3,4}$=6.5 Hz), 4.39 (d, 1H, H-2, $J_{2,3}$=3.1 Hz), 4.32 (dd, 1H, H-3), 4.17 (m, 1H, H-5, $J_{4,5}$=5.0 Hz), 3.82 (d, 2H, H-6, $J_{5,6}$=7.2 Hz). ^{13}C NMR (MeOD, 75 MHz) δ 173.4 (C-1), 80.4 (C-3), 72.8 (C-6), 72.3 (C-4), 71.4 (C-5), 70.0 (C-2).

3.1.3. 2,3-*O*-Isopropylidene-5,6-*O*-sulfinyl-D-gulonolactone (8). To a solution of 2,3-*O*-isopropylidene-D-gulono-1,4-lactone **7**[9] (1 g, 4.6 mmol) in THF (20 mL) precooled to 0 °C, were added anhydrous pyridine (2.4 equiv) then dropwise thionyl chloride (1.2 equiv). The mixture was stirred at 0 °C under argon atmosphere for 10 min and pyridinium salts were filtrated and washed with cooled THF (3 mL). The filtrate was concentrated to give a yellow syrup, which was purified by flash chromatography on silica gel (EtOAc). The 5,6-*O*-sulfinyl derivative **8** was isolated as a mixture of *endo*/*exo* diastereoisomers (1.2 g, 99%) as a colourless syrup. R_f 0.46 (hexane–EtOAc 1:1), *endo*/*exo* mixture ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.16 (dd, 2H, H-1, H-3), 4.87 (m, 7H), 4.53–4.31 (2 dd, 4H, H-6 *endo*/*exo*), 1.40 (s, 3H, CH_3), 1.38 (s, 6H, $2CH_3$), 1.31 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.4 (C-1), 115.4 (C-*iso*), 82.1, 81.0 (C-4), 79.7, 79.0 (C-2), 76.0 (C-3), 75.8 (C-5), 68.5, 67.7 (C-6), 26.8, 25.8 ($2CH_3$).

3.1.4. 6-Azido-6-deoxy-2,3-*O*-isopropylidene-D-gulonolactone (9). To a solution of compound **8** (1 g, 3.78 mmol) in DMF (10 mL) was added sodium azide (369 mg, 1.5 equiv). The mixture was heated at 80 °C for 24 h under argon atmosphere. After evaporation of DMF under reduced pressure, the residue was purified by flash chromatography on silica gel (acetone) to furnish **9** (920 mg, 93%) as a syrup. R_f 0.7 (hexane–EtOAc 1:1), $[\alpha]_D$ −31 (*c* 1, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.97 (d, 1H, H-2, $J_{2,3}$=5.32 Hz), 4.87 (dd, 1H, H-3, $J_{3,4}$=3.45 Hz), 4.61 (dd, 1H, H-4, $J_{4,5}$=7.2 Hz), 4.08 (m, 1H, H-5, $J_{5,6a}$=5.5 Hz), 3.52 (dd, 1H, H-6a, $J_{6a,6b}$=13.06 Hz), 3.42 (dd, 1H, H-6b, $J_{5,6b}$=3.1 Hz), 1.42 (s, 3H, CH_3), 1.32 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.2 (C-1), 114.1 (C-*iso*), 81.1 (C-4), 77.0 (C-2), 76.3 (C-3), 70.2 (C-5), 53.0 (C-6), 26.1, 25.0 ($2CH_3$).

3.1.5. 2,3-*O*-Isopropylidene-D-gulono-1,6-lactam (3). Compound **9** (200 mg, 0.822 mmol) in ethyl acetate (15 mL) was treated with palladium on charcoal (10%) in the presence of ammonium formate (10 equiv). The mixture was heated under argon atmosphere for 18 h, then filtered through a layer of Celite and concentrated in vacuo to give **3** as white crystals (165 mg, 92%). R_f 0.36 (hexane–EtOAc 4:6), $[\alpha]_D$ −15.5 (*c* 0.88, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.9 (d, 1H, H-2, $J_{2,3}$=5.34 Hz), 4.8 (dd, 1H, H-3, $J_{3,4}$=3.52 Hz), 4.55 (dd, 1H, H-4, $J_{4,5}$=7.28 Hz), 4.15 (m, 1H, H-5, $J_{5,6a}$=4.27 Hz), 3.53 (dd, 1H, H-6a, $J_{6a,6b}$= 12.9 Hz), 3.42 (dd, 1H, H-6b, $J_{5,6b}$=1.24 Hz), 1.32 (s, 3H, CH_3), 1.22 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.2 (C-1), 114.1 (C-*iso*), 81.1 (C-4), 77.9 (C-2), 77.0 (C-3), 70.2 (C-5), 53.5 (C-6), 26.1, 25.0 ($2CH_3$). Anal. Calcd for $C_9H_{15}O_5N$: C 49.76; H 6.96; N 6.45. Found (%): C 49.71; H 6.91; N 6.43.

3.1.6. 2,3-*O*-Isopropylidene-5,6-*O*-sulfinyl-L-gulonolactone (11). Reaction of 2,3-*O*-isopropylidene-L-gulono-1,4-lactone **10**[9] (1 g, 4.61 mmol) in THF (20 mL), with anhydrous pyridine (2.4 equiv) and thionyl chloride (1.2 equiv), as in case of **7**, gave quantitatively **11** as a colourless syrup in *endo*/*exo* mixture. R_f 0.46 (hexane–EtOAc 1:1), *endo*/*exo* mixture ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.23 (dd, 1H, H-3), 4.76 (m, 6H), 4.46–4.35 (2 dd, 4H, H-6 *endo*/*exo*), 1.42 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.31 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.4 (C-1), 115.4 (C-*iso*), 82.3, 82.1 (C-4), 79.6, 79.1 (C-2), 76.4 (C-3), 75.8 (C-5), 68.5, 67.7 (C-6), 26.9, 26.0 ($2CH_3$).

3.1.7. 6-Azido-6-deoxy-2,3-*O*-isopropylidene-L-gulonolactone (12). Reaction of **11** (720 mg, 2.724 mmol) with sodium azide (1.5 equiv) in DMF, as in case of **8**, gave **12**

as a colourless syrup (630 mg, 95%). R_f 0.73 (hexane–EtOAc 1:1), $[\alpha]_D$ +23 (*c* 1, $CHCl_3$), ^1H NMR ($CDCl_3$, 300 MHz) δ 4.9 (d, 1H, H-2, $J_{2,3}$=5.34 Hz), 4.9 (dd, 1H, H-3, $J_{3,4}$=3.52 Hz), 4.55 (dd, 1H, H-4, $J_{4,5}$=7.28 Hz), 4.15 (m, 1H, H-5, $J_{5,6a}$=4.27 Hz), 3.53 (dd, 1H, H-6a, $J_{6a,6b}$=12.9 Hz), 3.42 (dd, 1H, H-6b, $J_{5,6b}$=2.98 Hz), 1.42 (s, 3H, CH_3), 1.34 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.0 (C-1), 114.9 (C-*iso*), 80.2 (C-4), 76.8 (C-2), 76.3 (C-3), 70.3 (C-5), 52.8 (C-6), 27.1, 26.1 ($2CH_3$).

3.1.8. 2,3-*O*-Isopropylidene-L-gulono-1,6-lactam (4). Reaction of **12** (200 mg, 0.822 mmol) in ethyl acetate with palladium on charcoal (10%) in the presence of ammonium formate (10 equiv), as in the case of **9**, gave **4** as white crystals (150 mg, 84%). R_f 0.36 (hexane–EtOAc 4:6), $[\alpha]_D$ +17 (*c* 2.5, $CHCl_3$), ^1H NMR (MeOD, 300 MHz) δ 4.3 (d, 1H, H-2, $J_{2,3}$=7.8 Hz), 3.9 (dd, 1H, H-3, $J_{3,4}$=4.1 Hz), 3.45 (dd, 1H, H-4, $J_{4,5}$=7.28 Hz), 4.15 (m, 1H, H-5, $J_{5,6a}$= 4.27 Hz), 3.53 (dd, 1H, H-6a, $J_{6a,6b}$=12.9 Hz), 3.42 (dd, 1H, H-6b, $J_{5,6b}$=1.24 Hz), 1.32 (s, 3H, CH_3), 1.22 (s, 3H, CH_3). ^{13}C NMR (MeOD, 75 MHz) δ 175.2 (C-1), 114.1 (C-*iso*), 81.1 (C-4), 77.9 (C-2), 77.0 (C-3), 70.2 (C-5), 53.5 (C-6), 26.1, 25.0 ($2CH_3$). Anal. Calcd for $C_9H_{15}O_5N$: C 49.76; H 6.96; N 6.45. Found (%): C 49.73; H 6.89; N 6.40.

References and notes

1. (a) *Carbohydrate Mimics, Concepts and Methods*; Chapleur, Y., Ed.; Wiley-VCH: Weinheim, 1998; (b) *Iminosugars as Glycosidase Inhibitors*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999.
2. (a) Poitout, L.; Le Merrer, Y.; Depezay, J. C. *Tetrahedron Lett.* **1994**, *35*, 3293–3296; (b) Qian, X.; Moris, F. V.; Wong, C. H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1117–1122; (c) Moris, F. V.; Qian, X. H.; Wong, C. H. *J. Am. Chem. Soc.* **1996**, *118*, 7647–7662; (d) Painter, G. F.; Eldridge, P. J.; Falshaw, A. *Bioorg. Med. Chem.* **2004**, *12*, 225–232.
3. (a) Dax, K.; Graigg, V.; Koblinger, B.; Stütz, A. E. *J. Carbohydr. Chem.* **1990**, *9*, 479–499; (b) Lohray, B. B.; Jayamma, Y.; Chatterjee, M. *J. Org. Chem.* **1995**, *60*, 5958–5960; (c) Poitout, L.; Le Merrer, Y.; Depezay, J. C. *Tetrahedron Lett.* **1996**, *37*, 1613–1616; (d) Le Merrer, Y.; Poitout, L.; Depezay, J. C.; Dosbaa, I.; Geoffroy, S.; Foglietti, M. J. *Bioorg. Med. Chem.* **1997**, *5*, 519–533.
4. (a) Long, D. D.; Stetz, R. J. E.; Nash, R. J.; Marquess, D. G.; Lloyd, J. D.; Winters, A. L.; Asano, N.; Fleet, G. W. J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 901–908; (b) Joseph, C. C.; Regeling, H.; Zwanenburg, B.; Chittenden, G. J. F. *Tetrahedron* **2002**, *58*, 6907–6911.
5. Chaveriat, L.; Stasik, I.; Demailly, G.; Beaupere, D. *Tetrahedron* **2004**, *60*, 2079–2081.
6. (a) Aouad, M. E.; El Meslouti, A.; Uzan, R.; Beaupere, D. *Tetrahedron Lett.* **1994**, *35*, 6279–6282; (b) El Meslouti, A.; Beaupere, D.; Demailly, G.; Uzan, R. *Tetrahedron Lett.* **1994**, *35*, 3913–3916; (c) Beaupere, D.; El Meslouti, A.; Lelievre, P.; Uzan, R. *Tetrahedron Lett.* **1995**, *36*, 5347–5348; (d) Roussel, F.; Wadouachi, A.; Beaupere, D. *Carbohydr. Lett.* **2000**, *3*, 397–404; (e) Benksim, A.; Beaupere, D.; Wadouachi, A. *Org. Lett.* **2004**, *6*, 3913–3915.
7. Bouchez, V.; Stasik, I.; Beaupere, D.; Uzan, R. *Tetrahedron Lett.* **1997**, *38*, 7733–7736.
8. Lundt, I.; Frank, H. *Tetrahedron* **1994**, *50*, 13285–13298.
9. Fleet, G. W. J.; Ramsden, N. G.; Witty, D. R. *Tetrahedron* **1989**, *45*, 319–326.
10. Glaçon, V.; El Meslouti, A.; Uzan, R.; Demailly, G.; Beaupere, D. *Tetrahedron Lett.* **1996**, *37*, 3683–3686.

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Syntheses of enantio-enriched chiral building blocks from L-glutamic acid

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Abstract—Starting from lactone-amide **8**, easily derived from L-glutamic acid, enantioselective syntheses of (*S*)-tetrahydrofuran 2-carboxamide derivative **2** and a protected (*S*)-3-hydroxypiperidin-2-one (**3**) are reported. The building block **3** was converted to (2*S*,3*R*)-3-hydroxypipecolamide (**6**) by a three-step procedure. A solvent altered H-bonding capacity leading to a highly chemoselective tosylation of the primary hydroxyl group in the presence of an α-hydroxy-carboxamide was observed.

1. Introduction

Tetrahydrofuran 2-carboxylic acid derivatives such as **1**[1] (Fig. 1) are key structural units used in designing peptidomimetics. Protected 3-hydroxypiperidin-2-ones such as **3** may serve as useful building blocks,[2] while substituted piperidin-3-ols are key components found in a number of natural products and bioactive compounds.[3] For example, *cis*-3-hydroxypipecolic acid[4] **4** constitutes a part of antitumor antibiotic tetrazomine;[4b] (2*S*,3*R*)-**5**[5] has been served as a key intermediate in the asymmetric synthesis[5c] of (−)-swainsonine. In addition, the N-*tert*-butyl derivative of 3-hydroxypipecolamide (**6**) is an isomer of **7**, the 4-hydroxypipecolamide moiety presented in a class of highly potent HIV proteases inhibitors such as palinavir.[6] Carboxamide **6** may also be useful as an organocatalyst.[7]

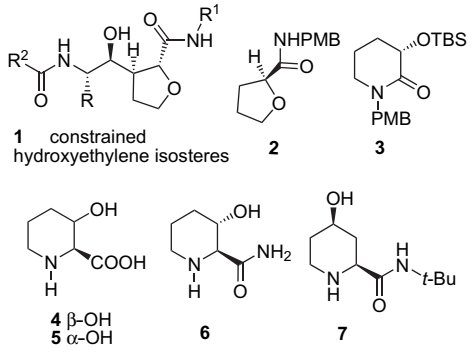

Figure 1.

In continuation of our ongoing program aimed at the development of new multifunctional building blocks starting from L-glutamic acid,[8] we now report the enantioselective syntheses of (*S*)-tetrahydrofuran 2-carboxamide **2**, protected (*S*)-3-hydroxypiperidin-2-one **3** and (2*S*,3*R*)-3-hydroxypipecolamide (**6**).

2. Results and discussion

We first investigated the synthesis of tetrahydrofuran 2-carboxamide (*S*)-**2** by the route depicted in Scheme 1. Lactone-amide[8d] (*S*)-**8**, easily available in multigram quantity from L-glutamic acid in 70% yield, was chemoselectively reduced with sodium borohydride (0 °C–rt) to give diol (*S*)-**9** in 89% yield. For the chemoselective *p*-tosylation of the primary hydroxyl group, (*S*)-**9** was treated with *p*-toluenesulfonyl chloride at low temperature (*p*-TsCl, py, −35 °C, 2 h, then −5 °C, overnight). With pyridine as the base, formation of the expected monotosylate **10** was observed from the TLC analysis. However, this was unstable and cyclized to tetrahydrofuran carboxamide[9] **2** on standing as well as during concentration of the extract. Thus, **2** was obtained in 58% isolated yield along with starting material (30%) and the ditosylate **12** (5%). HPLC analysis of **2** on a chiral column revealed its ee as 92%. On the other hand, with triethylamine as the base,[9g,10] although the yield of **2** was marginally better (62%), its ee was only 8% indicating extensive

Keywords: 3-Hydroxypipecolamide; 3-Hydroxypiperidin-2-one; Building block; Hydrogen bond; Chemoselective reaction.
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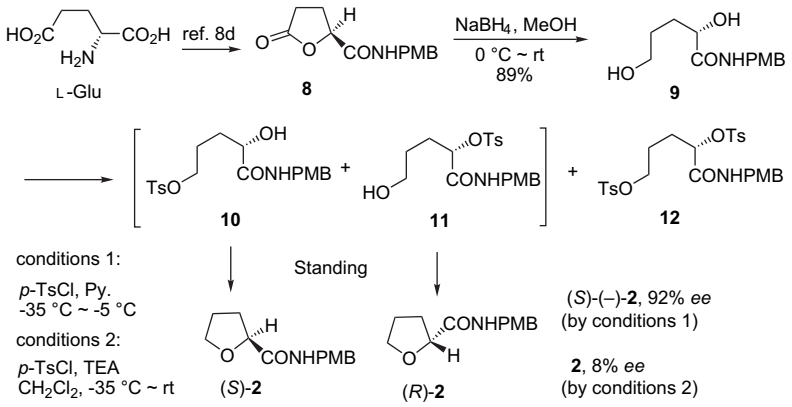


Scheme 1.

racemization. In this case, more ditosylate **12** (12%) was also formed, while 20% of the starting material was recovered. These results demonstrated that, attempted selective monotosylation of the primary hydroxyl group in diol **9** was always accompanied by tosylation of the secondary hydroxyl group and both monotosylated isomers **10** and **11** cyclized easily to give the corresponding enantiomer (*S*)-**2** or (*R*)-**2**.

It is worth mentioning that, although partial racemization has been observed in a diethoxytriphenylphosphorane mediated regioselective cyclodehydration of (*R*)-1,4-pentanediol,[11] many *p*-TsCl activated one-pot intramolecular etherification of chiral 1,4-diols have been reported to proceed with complete retention of configuration at the chiral carbinol center.[11] Our observations indicated that caution must be taken when performing a *p*-TsCl activated one-pot intramolecular etherification of chiral 1,4-diols, because tosylation of the secondary hydroxyl group may occur, leading to either racemic ethers or diastereomeric mixtures, in particular when using triethylamine as a base.

The easy tosylation of the secondary hydroxyl group of diol **9** may be explained by H-bonding effects as shown in **13a** and **13b** (Fig. 2).[12] Quantum chemistry calculation showed that **13a** is more stable than **13b** by 2.90 kcal/mol (Fig. 2). The higher stability of **13a** and thus lower reactivity of the primary hydroxyl group toward tosylation can be attributed to the strong hydrogen-bond formation between the primary hydroxyl group and the carbonyl of the amide functional group in **13a**. In more polar and more basic medium, such as when using pyridine as the solvent, formation of the intramolecular hydrogen bonds is efficiently inhibited,[13] thus the primary hydroxyl group shows normally higher reactivity during the tosylation. The phenomena of weakening of hydrogen bonds by polar solvents have been noted previously.[12f,13e]

Next, we turned our attention to the synthesis of protected 3-hydroxypiperidin-2-one **3**. We decided to protect firstly the latent hydroxyl group in **8**. Thus, stirring a methanolic solution of **8** in the presence of a catalytic amount of concentrated H_2SO_4 at rt for 1.5 h led smoothly to the ring-opening product **14**. TLC monitoring of the reaction showed that a small amount of the starting material remained intact even after prolongation of the reaction time. This might reflect that an equilibrium has been established between

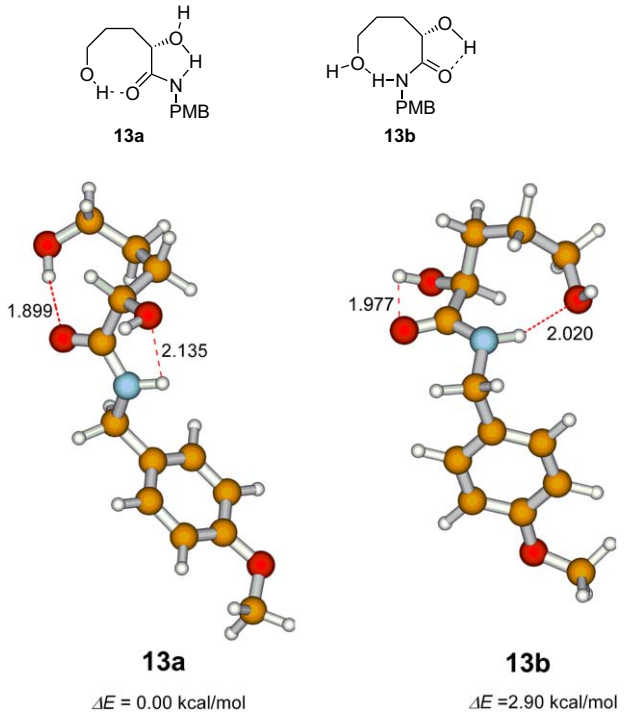
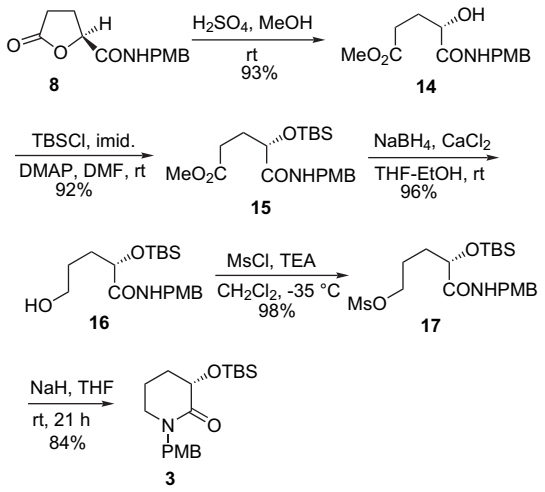


Figure 2. Energy-minimized structures of **13a** and **13b**.

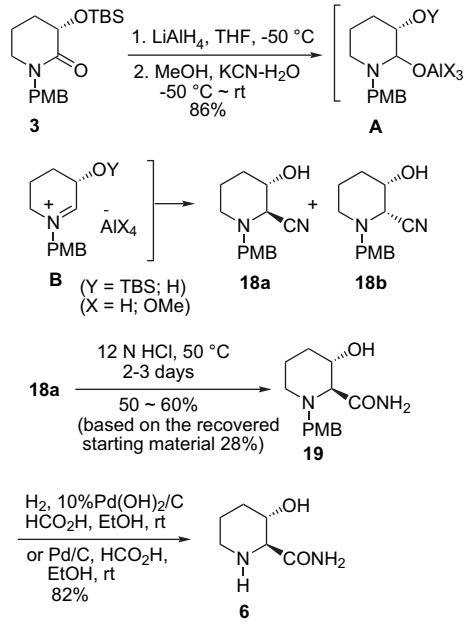
compounds **8** and **14**. Compounds **8** and **14** showed similar R_f and were difficult to separate by column chromatography on silica gel. To our delight, pure **14** could be obtained by recrystallization from EtOAc/Et_2O. The subsequent silylation[14] (TBSCl, imid., DMAP, DMF, rt, 3 h) proceeded smoothly to give compound **15** in 92% yield. Chemoselective ester reduction[15] ($NaBH_4$, $CaCl_2$, THF–EtOH, rt, overnight) followed by mesylation (MsCl, NEt_3) at −35 °C for 15 min furnished **17** in excellent overall yield. Treatment of **17** with NaH at rt for 21 h afforded the desired piperidine-2-one **3** in 84% yield. HPLC analysis on a chiral column showed that the ee of **3** was 94% (Scheme 2).

To demonstrate the utility of **3** as a building block, we sought the synthesis of **6** (Scheme 3). Thus, **3** was subjected to one-pot reductive cyanation,[16,17] which consisted in controlled chemoselective partial reduction of the amide by $LiAlH_4$ (3 molar equiv, THF, −50 °C, 15 min) followed by addition



Scheme 2.

of MeOH, and treating the in situ formed *N*,*O*-semiacetal intermediate with aqueous KCN at rt for 1 h. Under such conditions, concomitant *O*-desilylation[6,18] occurred and a separable diastereomeric mixture of cyanides **18a** and **18b** was obtained in 72:28 ratio with a combined yield of 86%. The α-amino nitriles **18a/18b** were assumed to be formed via the intermediacy of the *N*,*O*-acetal **A** and the *N*-acyliminium ion **B**. The stereochemistry of the major diastereomer was tentatively assigned as *trans* (**18a**) in analogy with its lower homologous.[17]



Scheme 3.

In pursuing the cyano group hydrolysis, both acidic and basic conditions were investigated,[17] and at the best case (12 N HCl, 50 °C, 2–3 days), amide **19** was obtained from **18a** in 50–74% yields based on the recovered starting material (ca. 20%). A single crystal X-ray crystallographic analysis of amide **19**,[19] derived from the major diastereomer **18a**, revealed its *trans*-stereochemistry, which confirmed our previous stereochemical assumption. Cleavage of PMB was performed under catalytic hydrogenolytic conditions (20% $Pd(OH)_2/C$, H_2, HCO_2H (cat.), rt, 5 h, EtOH, yield: 82%), or catalytic transfer hydrogenolytic conditions (10% Pd/C, HCO_2H (cat.), rt, 3 h, EtOH, yield: 79%). The 3-hydroxypipecolamide (**6**) thus obtained may find application in the asymmetric organocatalysis.[7,13a–d,20]

3. Conclusion

In summary, we have shown that by proper selection of synthetic procedures and reaction conditions, one can achieve chemoselective manipulation of multifunctional chiral building block **8**. The compounds thus obtained are also useful motifs. The usefulness of the protected 3-hydroxypiperidin-2-one **3** as a new building block was demonstrated by its conversion into 3-hydroxypipecolamide **6**. Importantly, the observations made during the synthesis of (*S*)-tetrahydrofuran 2-carboxamide derivative **2** from diol **9** indicated that H-bonding may affect the chemoselectivity of the tosylation reaction, which can be tuned by changing the reaction medium.

4. Experimental

4.1. General

Melting points were determined (uncorrected) on a Yanaco MP-500 micro melting point apparatus. Infrared spectra were measured with a Nicolet Avatar 360 FTIR spectrometer. ^{1}H NMR spectra were recorded on a Varian unity +500 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are expressed in δ (ppm) units downfield from TMS. Mass spectra were recorded on a Bruker Dalton Esquire 3000 plus LC–MS apparatus (ESI direct injection). Optical rotations were measured with Perkin–Elmer 341 automatic polarimeter. THF used in the reactions were dried by distillation over metallic sodium and benzophenone; dichloromethane were distilled over P_2O_5. Silica gel (Zhifu, 300–400 mesh) was used for column chromatography, eluting (unless otherwise stated) with ethyl acetate/petroleum ether (PE) (60–90 °C) mixtures.

The calculations were performed with the GAUSSIAN 98 package. The hybrid density functional method including Becke's 3-parameter non-local-exchange functional with the correlation functional of Lee–Yang–Parr (B3LYP) was employed. The basis set used is 6-31G* including the polarization d-function on non-hydrogen atoms. Geometry optimizations and vibrational analyses were performed without any constraint. The optimized structures of compounds **13a** and **13b** are characterized by none imaginary frequency. Reported energies are ZPE (zero-point energy)-corrected.

4.1.1. (*S*)-2,5-Dihydroxy-*N*-(4-methoxybenzyl)pentanamide 9. To a methanolic solution (16 mL) of **8**[8] (1.000 g, 4.02 mmol) was added $NaBH_4$ (473 mg, 12.45 mmol) at 0 °C. The mixture was allowed to warm to rt and stirred for 1 h. The reaction mixture was quenched by addition of brine (5 mL) and aqueous $NaHCO_3$ (5 mL) at 0 °C. MeOH was removed under reduced pressure. The mixture was

diluted with water (6 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification on silica gel (eluent: EtOAc/MeOH) provided **9** (903 mg, yield: 89%) as a colorless solid. Mp 103 °C (EtOAc/PE); $[\alpha]_D^{20}$ −24.3 (*c* 1.1, $CHCl_3$); IR (film) 3394, 3301, 2950, 2932, 2913, 2866, 1617, 1532, 1507, 1430, 1314, 1109, 1085, 1021 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.60–1.80 (m, 3H, $CH_2CH_2CH_2OH$), 1.97–2.05 (m, 1H, $CH_2CH_2CH_2OH$), 3.20 (br s, 1H, OH, D_2O exchangeable), 3.60–3.75 (m, 2H, CH_2OH), 3.78 (s, 3H, OCH_3), 4.17–4.20 (m, 1H, COCH), 4.28–4.40 (m, 2H, $PhCH_2N$), 4.85 (br s, 1H, OH, D_2O exchangeable), 6.86 (d, *J*=8.5 Hz, 2H, Ar–H), 7.20 (d, *J*=8.5 Hz, 2H, Ar–H), 7.18 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.2, 32.4, 42.5, 55.3, 62.5, 72.0, 114.1 (2C), 129.0, 130.1 (2C), 159.0, 174.0; MS (ESI) *m*/*z* 254 ($M+H^+$, 100). Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 62.05; H, 7.44; N, 5.47.

4.1.2. (*S*)-*N*-(4-Methoxybenzyl)-tetrahydrofuran-2-carboxamide 2. Procedure 1: To a solution of **9** (200 mg, 0.79 mmol) in pyridine (1 mL) was rapidly added *p*-TsCl (166 mg, 0.87 mmol) at about −35 °C under nitrogen atmosphere. The mixture was kept at about −20 °C for 2 h, then at −5 °C overnight before quenched by addition of ice-water (1 mL). The mixture was washed with 1 N aqueous HCl (2×1 mL) and extracted with ether (3×1.5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification of the residue on silica gel (eluent: EtOAc/PE = 1:1) provided, besides the recovered starting material (ca. 30%), ditosylated product **12** (19 mg, yield: 5%) and a mixture of monotosylated product **10** and (*S*)-**2** (132 mg). The latter was formed during the purification and concentration. Upon standing at rt, a complete transformation of **10** to (*S*)-**2** was observed, giving (*S*)-**2** (104 mg) in 58% yield. The enantiomeric excess (ee) of (*S*)-**2** was determined to be 92% by HPLC analysis using a chiral column OD-H (4.6 mm×250 mm, eluting with hexane:2-propanol = 9:1, 1.0 mL/min; detected at 225 nm. $[\alpha]_D^{20}$ −9.6 (*c* 1.3, $CHCl_3$).

Procedure 2: To a solution of **9** (200 mg, 0.79 mmol) in CH_2Cl_2 (1.5 mL) was added Et_3N (0.15 mL, 1.03 mmol). *p*-TsCl (166 mg, 0.87 mmol) in CH_2Cl_2 (0.5 mL) was dropped into the solution at about −35 °C. The reaction temperature was allowed to rise to rt, then stirred at rt for 36 h. The reaction mixture was quenched by addition of 1 mL ice-water and the resulting mixture was extracted with CH_2Cl_2 (3×1.5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification of the residue on silica gel (eluent: EtOAc/PE = 1:1) provided, besides the recovered starting material (ca. 20%), ditosylated product **12** (53 mg, yield: 12%) and a mixture of two monotosylated products **10/11** and **2** (138 mg). The latter was formed during the purification and concentration. Compound **2** obtained at this stage showed $[\alpha]_D^{20}$ −7.0 (*c* 1.2, $CHCl_3$) and 52% ee. Upon standing at rt, a complete transformation of **10/11** to **2** was observed, giving **2** (115 mg) in 62% yield, which showed $[\alpha]_D^{20}$ 0 (*c* 1.3, $CHCl_3$) and 8% ee.

Compound **2**: colorless oil. IR (film) 3349, 2923, 2853, 1665, 1513, 1247, 1175, 1073, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.82–1.96 (m, 2H, $CH_2CH_2CH_2O$), 2.05–2.14 (m, 1H, $CH_2CH_2CH_2O$), 2.27–2.36 (m, 1H, $CH_2CH_2CH_2O$), 3.80 (s, 3H, OCH_3), 3.82–3.94 (m, 2H, $CH_2CH_2CH_2O$), 4.34–4.44 (m, 3H, COCHO, $NHCH_2$), 6.86 (d, *J*=8.5 Hz, 2H, Ar–H), 7.20 (d, *J*=8.5 Hz, 2H, Ar–H), 6.92 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.5, 30.2, 42.3, 55.3, 69.4, 78.5, 114.1 (2C), 129.0, 130.2 (2C), 159.0, 173.0; MS (ESI) *m*/*z* 236 ($M+H^+$, 100); HR-MALDIMS calcd for $C_{13}H_{17}NO_3Na$ $(M+Na)^+$: 258.1106; found: 258.1110.

4.1.3. Methyl (*S*)-4-hydroxy-5-(4-methoxybenzylamino)-5-oxopentanoate 14. To a solution of **8** (2.045 g, 8.03 mmol) in MeOH (10 mL) was added a catalytic amount of concentrated H_2SO_4. After stirred for 1.5 h at rt, the mixture was neutralized with solid $CaCO_3$ and filtered through Celite. Flash chromatographic purification of the residue on silica gel (eluent: EtOAc/PE = 1.5:1) provided **14** (2.152 g, yield: 93%) as a colorless solid. Mp 97–98 °C (EtOAc/Et_2O); $[\alpha]_D^{20}$ −16.4 (*c* 1.0, $CHCl_3$); IR (film) 3366, 2928, 1735, 1650, 1513, 1438, 1248, 1176, 1103, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.08–2.26 (m, 1H, $COCH_2CH_2CH_2$), 2.19–2.27 (m, 1H, $COCH_2CH_2CH_2$), 2.48–2.63 (m, 2H, $COCH_2CH_2CH_2$), 3.70 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.15 (d, *J*=3.5 Hz, 1H, OH, D_2O exchangeable), 4.19 (ddd, *J*=7.8, 3.5, 3.2 Hz, 1H, CHOH), 4.38 (dd, *J*=15.4, 5.9 Hz, 1H, $PhCH_2N$), 4.41 (dd, *J*=15.4, 5.9 Hz, 1H, $PhCH_2N$), 6.84 (d, *J*=8.5 Hz, 2H, Ar–H), 7.18 (d, *J*=8.5 Hz, 2H, Ar–H), 7.03 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.2, 30.6, 42.7, 52.2, 55.3, 72.0, 114.1 (2C), 129.1 (2C), 130.1, 159.1, 172.9, 175.8; MS (ESI) *m*/*z* 282 ($M+H^+$, 100). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98. Found: C, 60.06; H, 6.84; N, 4.76.

4.1.4. Methyl (*S*)-4-(*tert*-Butyldimethylsilyloxy)-5-(4-methoxybenzylamino)-5-oxopentanoate 15. A mixture of **14** (1.711 g, 6.09 mmol), imidazole (1.029 g, 15.23 mmol), TBSCl (1.120 g, 7.31 mmol), and a catalytic amount of DMAP in dry DMF (12 mL) was stirred at rt for 3 h and then quenched by the addition of water (50 mL). The mixture was extracted with Et_2O (6×10 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification of the residue on silica gel (eluent: EtOAc/PE = 1:5) provided **15** (2.112 g, yield: 92%) as a colorless oil. $[\alpha]_D^{20}$ −22.0 (*c* 1.0, $CHCl_3$); IR (film) 3427, 2953, 2930, 1740, 1678, 1613, 1514, 1464, 1439, 1250, 1174, 1107, 1034 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.22 (s, 3H, $Si(CH_3)_2$), 0.23 (s, 3H, $Si(CH_3)_2$), 0.92 (s, 9H, $SiC(CH_3)_3$), 2.02–2.15 (m, 2H, $COCH_2CH_2CH_2$), 2.30–2.44 (m, 2H, $COCH_2CH_2CH_2$), 3.62 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.24 (dd, *J*=5.2, 5.2 Hz, 1H, CHCON), 4.30 (dd, *J*=5.8, 14.5 Hz, 1H, $PhCH_2N$), 4.45 (dd, *J*=5.8, 14.5 Hz, 1H, $PhCH_2N$), 6.79 (br s, 1H, NH), 6.88 (d, *J*=8.5 Hz, 2H, Ar–H), 7.20 (d, *J*=8.5 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −5.0, 17.9, 25.6 (3C), 28.9, 30.3, 42.5, 51.6, 55.3, 72.4, 114.1 (2C), 129.0 (2C), 130.0, 159.1, 172.6, 173.5; MS (ESI) *m*/*z* 396 ($M+H^+$, 100). Anal. Calcd for $C_{20}H_{33}NO_5Si$: C, 60.73; H, 8.41; N, 3.54. Found: C, 60.41; H, 8.08; N, 3.79.

4.1.5. (*S*)-2-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-*N*-(4-methoxybenzyl)pentanamide 16. To a mixture of **15** (1.400 g, 3.54 mmol) and $CaCl_2$ (1.690 g, 14.89 mmol) in EtOH (4.7 mL)/THF (9.4 mL) was added $NaBH_4$ (1.030 g, 27.11 mmol) in one portion at 0 °C. The mixture was allowed to warm to rt and stirred overnight. The reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ (10 mL) and brine (20 mL) at 0 °C. The mixture was extracted with EtOAc (6×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification of the residue on silica gel (eluent: EtOAc/PE = 1:1) provided **16** (1.303 g, yield: 96%) as a colorless oil. $[\alpha]_D^{20}$ −25.2 (*c* 1.0, $CHCl_3$); IR (film) 3423, 2953, 2930, 2857, 1664, 1613, 1514, 1464, 1250, 1176, 1111, 1037 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.30 (s, 3H, $Si(CH_3)_2$), 0.32 (s, 3H, $Si(CH_3)_2$), 0.85 (s, 9H, $SiC(CH_3)_3$), 1.55–1.70 (m, 2H, $OCH_2CH_2CH_2$), 1.74–1.95 (m, 2H, $OCH_2CH_2CH_2$), 2.10 (br s, 1H, OH, D_2O exchangeable), 3.62 (dd, *J*=6.2, 6.2 Hz, 2H, CH_2OH), 3.80 (s, 3H, OCH_3), 4.25 (dd, *J*=5.1, 5.1 Hz, 1H, CHCON), 4.31 (dd, *J*=5.8, 14.6 Hz, 1H, $PhCH_2N$), 4.45 (dd, *J*=6.2, 14.6 Hz, 1H, $PhCH_2N$), 6.79 (br s, 1H, NH), 6.85 (d, *J*=8.5 Hz, 2H, Ar–H), 7.20 (d, *J*=8.5 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −4.9, 17.9, 25.6 (3C), 27.5, 31.7, 42.5, 55.3, 62.5, 73.1, 114.1 (2C), 129.0 (2C), 130.0, 159.0, 173.4; MS (ESI) *m*/*z* 368 ($M+H^+$, 100). Anal. Calcd for $C_{19}H_{33}NO_4Si$: C, 62.09; H, 9.05; N, 3.81. Found: C, 61.91; H, 9.35; N, 3.78.

4.1.6. (*S*)-4-(*tert*-Butyldimethylsilyloxy)-5-(4-methoxybenzylamino)-5-oxopentyl methanesulfonate 17. To a solution of **16** (814 mg, 2.22 mmol) and Et_3N (0.46 mL, 3.33 mmol) in CH_2Cl_2 (8.9 mL) was added dropwise MsCl (0.22 mL, 2.88 mmol) at −35 °C. The mixture was stirred at the same temperature for 15 min and then quenched with water (6 mL). The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification on silica gel (eluent: EtOAc/PE = 1:2) provided **17** (970 mg, yield: 98%) as a colorless oil. $[\alpha]_D^{20}$ −6.6 (*c* 1.0, $CHCl_3$); IR (film) 3424, 2955, 2932, 2857, 1673, 1613, 1515, 1467, 1354, 1250, 1174, 1111, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, two rotamers in a ratio of 4.5:1) δ 0.40 (s, 3H, $Si(CH_3)_2$, m+M), 0.45 (s, 3H, $Si(CH_3)_2$, M+m), 0.90 (s, 9H, $SiC(CH_3)_3$, m+M), 1.70–1.95 (m, 4H, $OCH_2CH_2CH_2$, m+M), 3.01, 3.03 (s, 3H, OCH_3, M+m), 3.79, 3.81 (s, 3H, CH_3SO_2, m+M), 4.18–4.24 (m, 3H, $MsOCH_2$, COCH, M+m), 4.32 (dd, *J*=5.4, 14.6 Hz, 1H, $PhCH_2N$, M+m), 4.44 (dd, *J*=6.3, 14.6 Hz, 1H, $PhCH_2N$, M+m), 6.80 (br s, 1H, NH, M+m), 6.86 (d, *J*=8.5 Hz, 2H, Ar–H, M+m), 7.20 (d, *J*=8.5 Hz, 2H, Ar–H, M+m); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −4.9, 17.9, 24.1, 25.6 (3C), 31.1, 37.4, 42.5, 55.3, 69.6, 72.6, 114.1 (2C), 129.1 (2C), 129.9, 159.1, 172.7; MS (ESI) *m*/*z* 446 ($M+H^+$, 100). The product is too labile to perform the required elementary analysis or HRMS measurement.

4.1.7. (*S*)-3-(*tert*-Butyldimethylsilyloxy)-1-(4-methoxybenzyl)piperidin-2-one 3. To a cooled (0 °C) solution of **17** (608 mg, 1.37 mmol) in dry THF (3 mL) was added dropwise a suspension of NaH (137 mg, 60% w/w) in anhydrous THF (2.5 mL) over a period of 20 min. The mixture was allowed to warm to rt and stirred overnight. The reaction mixture was quenched with water (30 mL) at 0 °C. The organic layer was separated and the aqueous layer extracted with Et_2O (3×20 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification on silica gel (eluent: EtOAc/PE=1:8) provided **3** (400 mg, yield: 84%) as a colorless oil. $[\alpha]_D^{20}$ −34.0 (*c* 1.1, $CHCl_3$). The enantiomeric excess (ee) of **3** was determined to be 94% by HPLC analysis using a chiral column OD-H (4.6 mm×250 mm, eluting with hexane:2-propanol = 99:1, 0.75 mL/min; detected at 254 nm). IR (film) 2952, 2928, 2854, 1654, 1611, 1512, 1489, 1247, 1172, 1148, 1109, 1039 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.48 (s, 3H, $Si(CH_3)_2$), 0.51 (s, 3H, $Si(CH_3)_2$), 0.92 (s, 9H, $SiC(CH_3)_3$), 1.63–1.71 (m, 1H, H-5), 1.82–1.91 (m, 1H, H-5), 1.90–2.02 (m, 2H, H-4), 3.08–3.21 (m, 2H, H-6), 3.79 (s, 3H, OCH_3), 4.16 (dd, *J*=7.1, 4.6 Hz, 1H, H-3), 4.46 (d, *J*=14.4 Hz, 1H, $PhCH_2N$), 4.53 (d, *J*=14.4 Hz, 1H, $PhCH_2N$), 6.90 (d, *J*=8.5 Hz, 2H, Ar–H), 7.20 (d, *J*=8.5 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4, −4.5, 18.3, 19.0, 25.8 (3C), 30.8, 46.7, 49.4, 55.2, 69.6, 113.9 (2C), 129.3, 129.4 (2C), 158.9, 170.0; MS (ESI) *m*/*z* 350 ($M+H^+$, 100); HR-MALDIMS calcd for $C_{19}H_{31}NO_3Si$ $(M+H)^+$: 350.2151; found: 350.2153.

4.1.8. (2*RS*,3*S*)-2-Cyano-3-hydroxy-1-(4-methoxybenzyl) piperidines 18a and 18b. To a cooled (−50 °C) suspension of $LiAlH_4$ (332 mg, 2.81 mmol) in anhydrous THF (18 mL) was added a solution of **3** (980 mg, 2.81 mmol) in anhydrous THF (10 mL) over a period of 20 min. After stirred at the same temperature for 15 min, the mixture was quenched with MeOH (2.2 mL) and a solution of KCN (732 mg, 11.24 mmol) in water (2.2 mL) was added. The mixture was allowed to warm to rt and stirred for another 1 h. After diluted with water (10 mL), the organic layer was separated and the aqueous layer extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification on silica gel (eluent: EtOAc/PE = 1:2) provided (2*R*,3*S*)-**18a** and (2*S*,3*S*)-**18b** in 72:28 ratio with a combined yield of 86%.

Compound (2*R*,3*S*)-**18a**: colorless crystals, mp 72–73 °C (CH_2Cl_2/PE); $[\alpha]_D^{20}$ −133.4 (*c* 1.0, $CHCl_3$); IR (film) 3493, 2946, 2835, 2219, 1616, 1513, 1465, 1442, 1249, 1178, 1132, 1109, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.56–1.70 (m, 2H, H-5), 1.78–1.90 (m, 2H, H-4), 2.48–2.56 (m, 1H, H-6), 2.76–2.84 (m, 2H, H-6, OH, D_2O exchangeable), 3.49 (d, *J*=12.7 Hz, 1H, $PhCH_2N$), 3.70 (d, *J*=4.0 Hz, 1H, H-2), 3.71 (d, *J*=12.7 Hz, 1H, $PhCH_2N$), 3.80 (s, 3H, OCH_3), 3.97–4.20 (m, 1H, H-3), 6.95 (d, *J*=8.5 Hz, 2H, Ar–H), 7.23 (d, *J*=8.5 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.2, 27.2, 49.0, 55.3, 56.7, 59.6, 66.1, 114.1 (2C), 115.0, 128.0, 130.3 (2C), 159.4; MS (ESI) *m*/*z* 247 ($M+H^+$, 100). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.44; H, 7.69; N, 11.15.

Compound (2*S*,3*S*)-**18b**: colorless oil; $[\alpha]_D^{20}$ +97.2 (*c* 1.0, $CHCl_3$); IR (film) 3435, 2934, 2835, 2226, 1612, 1585,

1513, 1465, 1248, 1174, 1128, 1104, 1064, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.48–1.64 (m, 2H, H-5), 1.70–1.76 (m, 1H, H-4), 1.97–2.03 (m, 1H, H-4), 2.35 (dt, J=11.8, 2.9 Hz, 1H, H-6), 2.72–2.77 (m, 1H, H-6), 3.49 (d, J=12.8 Hz, 1H, $PhCH_2N$), 3.69 (d, J=12.8 Hz, 1H, $PhCH_2N$), 3.74 (ddd, J=15.4, 9.3, 4.6 Hz, 1H, H-3), 3.80 (s, 3H, OCH_3), 3.89 (d, J=4.6 Hz, 1H, H-2), 6.95 (d, J=8.5 Hz, 2H, Ar–H), 7.23 (d, J=8.5 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.0, 30.0, 48.3, 55.3, 59.4 (2C), 67.8, 113.9 (2C), 115.0, 128.5, 130.2 (2C), 159.2; MS (ESI) m/z 247 ($M+H^+$, 100).

4.1.9. (2*S*,3*S*)-2-Aminocarbonyl-3-hydroxy-1-(4-methoxybenzyl)piperidine 19. A solution of **18a** (100 mg, 0.41 mmol) in 12 N aqueous HCl (25 mL) was stirred at 60 °C for 2 days and then neutralized with solid Na_2CO_3. The mixture was extracted with EtOAc (6×25 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatographic purification on silica gel (eluent: EtOAc/MeOH = 30: 1) provided **19** (50 mg, yield: 46%) and the recovered starting material **18a** (28 mg, 28%). Compound **19**: colorless solid, mp 159–160 °C (EtOAc/PE); $[\alpha]_D^{20}$ −77.8 (*c* 0.6, $CHCl_3$); IR (film) 3379, 3200, 2959, 2927, 2855, 2789, 1688, 1662, 1614, 1513, 1444, 1381, 1258, 1113, 1067, 1034 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.25–1.55 (m, 2H, H-5), 1.62–1.72 (m, 1H, H-4), 1.95–2.05 (m, 1H, H-4), 2.04–2.14 (m, 1H, H-6), 2.70 (d, J=8.7 Hz, 1H, H-2), 2.83–2.88 (m, 1H, H-6), 3.22 (d, J=13.6 Hz, 1H, $PhCH_2N$), 3.67 (ddd, J=10.8, 8.7, 4.6 Hz, 1H, H-3), 3.79 (s, 3H, OCH_3), 3.85 (d, J=13.6 Hz, 1H, $PhCH_2N$), 6.10 (br s, 1H, OH, D_2O exchangeable), 6.80 (br s, 2H, NH_2, D_2O exchangeable), 6.95 (d, J=8.5 Hz, 2H, Ar–H), 7.23 (d, J=8.5 Hz, 2H, Ar–H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.0, 32.0, 50.6, 55.3, 59.7, 70.5, 73.5, 113.9 (2C), 129.4, 129.8 (2C), 158.9, 176.5; MS (ESI) m/z 265 ($M+H^+$, 100); HR-MALDIMS calcd for $C_{14}H_{20}N_2O_3$ $(M+H)^+$: 265.1552; found: 265.1557.

4.1.10. (2*S*,3*S*)-3-Hydroxy-2-piperidine-carboxamide 6. To a suspension of 20% $Pd(OH)_2/C$ (26 mg) in EtOH (1 mL) were added a solution of **19** (52 mg) in EtOH (1 mL) and a catalytic amount of HCO_2H. The mixture was stirred at rt and under an atmosphere of H_2 for 5 h. After filtration of the catalyst, the filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel (eluent: MeOH/EtOAc/aqueous NH_3 = 1:4:0.1) to provide **6** (23 mg, yield: 82%) as a colorless solid. Mp 149–150 °C (MeOH/Et_2O); $[\alpha]_D^{20}$ +43.8 (*c* 0.4, 10% HCl); IR (KBr) 3376, 3317, 3198, 2947, 2857, 1773, 1677, 1635, 1507, 1077 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.42–1.62 (m, 2H, H-4, H-5), 1.76–1.86 (m, 1H, H-5), 2.10–2.18 (m, 1H, H-4), 2.56 (dt, J=12.9, 2.8 Hz, 1H, H-6), 3.04 (dd, J=12.9, 1.7 Hz, 1H, H-6), 3.14 (d, J=9.6 Hz, 1H, H-2), 3.66 (ddd, J=10.7, 9.6, 4.4 Hz, H-3); ^{13}C NMR (125 MHz, D_2O) δ 27.0, 35.0, 46.7, 67.4, 71.4, 178.2; MS (ESI) m/z 145 ($M+H^+$, 100); HR-EIMS calcd for $[C_6H_{12}N_2O_2]^+$: 144.0899; found: 144.0892.

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References and notes

1. (a) Hanessian, S.; Brassard, M. *Tetrahedron* **2004**, *60*, 7621–7628; (b) Hanessian, S.; Hou, Y.; Bayrakdarian, M.; Tintelnot-Blomley, M. *J. Org. Chem.* **2005**, *70*, 6735–6745.
2. (a) Gibbs, G.; Hateley, M. J.; McLaren, L.; Welham, M.; Willis, C. L. *Tetrahedron Lett.* **1999**, *40*, 1069–1072; (b) Kamal, A.; Ramana, K. V.; Ramana, A. V.; Babu, A. H. *Tetrahedron: Asymmetry* **2003**, *14*, 2587–2594.
3. For two reviews on the piperidine alkaloids, see: (a) Schneider, M. Pyridine and Piperidine Alkaloids: An Update. In *Alkaloids: Chemical and Biochemical Perspectives*; Pelletier, S. W., Ed.; Elsevier Science: Oxford, 1996; Vol. 10, pp 155–299; (b) Plunkett, O.; Sainsbury, M. Pyridine and Piperidine Alkaloids. In *Second Supplements to the 2nd Edition of Rodd's Chemistry of Carbon Compounds*; Sainsbury, M., Ed.; Elsevier Science: Amsterdam, 1998; Vol. IV F/G, pp 365–421.
4. (a) Roemmele, R. C.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1866–1875; (b) Scott, J. D.; Tippie, T. N.; Williams, R. M. *Tetrahedron Lett.* **1998**, *39*, 3659–3662; (c) Horikawa, M.; Busch-Petersen, J.; Corey, E. J. *Tetrahedron Lett.* **1999**, *40*, 3843–3846.
5. (a) Greck, C.; Ferreira, F.; Genêt, J. P. *Tetrahedron Lett.* **1996**, *37*, 2031–2034; (b) Agami, C.; Couty, F.; Mathieu, H. *Tetrahedron Lett.* **1996**, *37*, 4001–4002; (c) Ferreira, F.; Greck, C.; Genêt, J. P. *Bull. Soc. Chim. Fr.* **1997**, *134*, 615–621; (d) Battistini, L.; Zanardi, F.; Rassu, G.; Spanu, P.; Pelosi, G.; Fava, G. G.; Ferrari, M. B.; Casiraghi, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2975–2987; (e) Jourdant, A.; Zhu, J. *Tetrahedron Lett.* **2000**, *41*, 7033–7036; (f) Haddad, M.; Larchevêque, M. *Tetrahedron Lett.* **2001**, *42*, 5223–5225; (g) Kumar, P.; Bodas, M. S. *J. Org. Chem.* **2005**, *70*, 360–363.
6. Gillard, J.; Abraham, A.; Anderson, P. C.; Beaulieu, P. L.; Bogri, T.; Bousquet, Y.; Grenier, L.; Guse, I.; Lavallée, P. *J. Org. Chem.* **1996**, *61*, 2226–2231.
7. (a) Wang, Z.; Ye, X.; Wei, S.; Wu, P.; Zhang, A.; Sun, J. *Org. Lett.* **2006**, *8*, 999–1001; (b) For a use of pipecolic acid as a superior organocatalyst, see: Aroyan, C. E.; Vasbinder, M. M.; Miller, S. J. *Org. Lett.* **2005**, *7*, 3849–3851 (c) Cheong, P. H.-Y.; Zhang, H.; Thayumanavan, R.; Tanaka, F.; Houk, K. N.; Barbas, C. F., III. *Org. Lett.* **2006**, *8*, 811–814; For related examples, see: (d) Kawabata, T.; Stragies, R.; Fukaya, T.; Nagaoka, Y.; Schedel, H.; Fuji, K. *Tetrahedron Lett.* **2003**, *44*, 1545–1548; (e) Cobb, A. J. A.; Shaw, D. M.; Longbottom, D. A.; Gold, J. B.; Ley, S. V. *Org. Biomol. Chem.* **2005**, *3*, 84–96; (f) Wang, W.; Mei, Y.; Li, H.; Wang, J. *Org. Lett.* **2005**, *7*, 601–604; (g) Tang, Z.; Yang, Z. H.; Chen, X. H.; Cun, L. F.; Mi, A. Q.; Jiang, Y. Z.; Gong, L. Z. *J. Am. Chem. Soc.* **2005**, *127*, 9285–9289; (h) Chen, J.-R.; Lu, H.-H.; Li, X.-Y.; Cheng, L.; Wan, J.; Xiao, W.-J. *Org. Lett.* **2005**, *7*, 4543–4545.
8. For an account, see: (a) Huang, P.-Q. *Synlett* **2006**, 1133–1149; For other papers, see: (b) Huang, P.-Q.; Liu, L.-X.; Wei, B.-G.; Ruan, Y.-P. *Org. Lett.* **2003**, *5*, 1927–1929; (c) Huang, P.-Q.; Wei, B.-G.; Ruan, Y.-P. *Synlett* **2003**, 1663–1667; (d) Liu, L. X.; Ruan, Y.-P.; Guo, Z. Q.; Huang, P.-Q. *J. Org. Chem.* **2004**, *69*, 6001–6009; (e) Ruan, Y.-P.; Wei, B.-G.; Xu, X.-Q.; Liu, G.; Yu, D.-S.; Liu, L.-X.; Huang, P.-Q. *Chirality* **2005**, *17*, 595–599; (f) Wei, B. G.; Chen, J.; Huang, P.-Q.

Tetrahedron **2006**, *62*, 190–198; (g) Huang, P.-Q.; Guo, Z.-Q.; Ruan, Y.-P. *Org. Lett.* **2006**, *8*, 1435–1438.

9. (a) Buchanan, J. G.; Dunn, A. D.; Edgar, A. R. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1943–1949; (b) Buchanan, J. G.; Dunn, A. D.; Edgar, A. R. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1191–1200; (c) Bukownik, R. R.; Wilcox, C. S. *J. Org. Chem.* **1988**, *64*, 463–471; (d) Lehmann, T. E.; Berkessel, A. *J. Org. Chem.* **1997**, *62*, 302–309; (e) Kelly, D. K.; Nally, J. *Tetrahedron Lett.* **1999**, *40*, 2209–2212; (f) Ninomiya, M.; Hirohara, H.; Onishi, J.; Kusumi, T. *J. Org. Chem.* **1999**, *64*, 5436–5440; (g) Paquette, L. A.; Ohmori, N.; Lowinger, T. B.; Rogers, R. D. *J. Org. Chem.* **2000**, *65*, 4303–4308; (h) Ref. 10; For a method of selective monotosylation of symmetric diols, see: (i) Bouzide, A.; Sauvé, G. *Org. Lett.* **2002**, *4*, 2329–2332.
10. Jirgensons, A.; Marinozzi, M.; Pellicciari, R. *Tetrahedron* **2005**, *61*, 373–377.
11. For the regioselective cyclodehydration of a chiral 1,4-diol, see: (a) Robinson, P. L.; Barry, C. N.; Bass, S. W.; Jarvis, S. E.; Evans, S. A., Jr. *J. Org. Chem.* **1983**, *48*, 5396–5398; (b) Robinson, P. L.; Evans, S. A., Jr. *J. Org. Chem.* **1985**, *50*, 3860–3863; (c) Robinson, P. L.; Barry, C. N.; Kelly, J. W.; Evans, S. A., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 5210–5219; (d) Sharma, G. V. M.; Kumar, K. R.; Sreenivas, P.; Krishna, P. R.; Chorghade, M. S. *Tetrahedron: Asymmetry* **2002**, *13*, 687–690; For the regioselective cyclodehydration of a chiral 1,5-diol, see: (e) Flamme, E. M.; Roush, W. R. *Beilstein J. Org. Chem.* **2005**, 1–5; http://bjoc.beilstein-journals.org/.
12. For selected examples of formation of intramolecular hydrogen bond in hydroxyketones, see: (a) Heathcock, C. H.; Pirrung, M. C.; Sohn, J. E. *J. Org. Chem.* **1979**, *44*, 4294–4299; (b) Battaglia, A.; Barbaro, G.; Giorgianni, P.; Guerrini, A.; Bertucci, C.; Geremia, S. *Chem.—Eur. J.* **2000**, *6*, 3551–3557; For selected examples of formation of hydrogen bonds involving amides, see: (c) Johansson, A.; Kollman, P. A. *J. Am. Chem. Soc.* **1972**, *94*, 6196–6198; (d) Bennes, R.; Philp, D.; Spencer, N.; Kariuki, B. M.; Harris, K. D. M. *Org. Lett.* **1999**, *1*, 1087–1090; (e) Salas-Coronado, R.; Vasquez-Badillo, A.; Medina-Garcia, M.; Garcia-Colon, J. G.; Noth, H.; Contreras, R.; Flores-Parra, A. *J. Mol. Struct. (Theochem)* **2001**, *543*, 259–275; (f) Kim, K. M.; Park, H.; Kim, H.-J.; Chin, J.; Nam, W. *Org. Lett.* **2005**, *7*, 3525–3527.
13. For reviews on the hydrogen bonding controlled selective reactions, see: (a) Schreiner, P. R. *Chem. Soc. Rev.* **2003**, *32*, 289–296; (b) Pihko, P. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 2062–2064; (c) Dalko, P. I.; Moisan, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 5138–5175; (d) Bolm, C.; Rantanen, T.; Schiffers, I.; Zani, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1758–1763; For selective examples, see: (e) Thevenet, S.; Wernicke, A.; Belniak, S.; Descotes, G.; Bouchu, A.; Queneau, Y. *Carbohydr. Res.* **1999**, *318*, 52–66; (f) Huang, Y.; Unni, A. K.; Thadani, A. N.; Rawal, V. H. *Nature* **2003**, *424*, 146; (g) Cook, G. R.; Yu, H.; Sankaranarayanan, S.; Shanker, P. S. *J. Am. Chem. Soc.* **2003**, *125*, 5115–5120; (h) Du, H. F.; Zhao, D. B.; Ding, K. L. *Chem.—Eur. J.* **2004**, *10*, 5964–5970.
14. Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190–6191.
15. Lewis, N.; McKillop, A.; Taylor, R. J. K.; Watson, R. J. *Synth. Commun.* **1995**, *25*, 561–568.
16. (a) Gless, R. D.; Rapoport, H. *J. Org. Chem.* **1979**, *44*, 1324–1336; (b) Compernolle, F.; Saleh, M. A.; Branden, S. V. D.; Toppet, S.; Hoornaert, G. *J. Org. Chem.* **1991**, *56*, 2386–2390.
17. Huang, P.-Q.; Huang, H. Y. *Synth. Commun.* **2004**, *34*, 1377–1382.
18. de Vries, E. F. J.; Brussee, J.; van der Gen, A. *J. Org. Chem.* **1994**, *59*, 7133–7137.
19. Feng, C.-G.; Fang, H.; Huang, P.-Q. *Acta Crystallogr.* **2004**, *E60*, o1075–o1077.
20. For selected reviews on the asymmetric organocatalysis, see: (a) Jarvo, E. R.; Miller, S. J. *Tetrahedron* **2002**, *58*, 2481–2495; (b) Clarke, M. L. *Lett. Org. Chem.* **2004**, *1*, 292–296; (c) Saito, S.; Yamamoto, H. *Acc. Chem. Res.* **2004**, *37*, 570–579. See also Refs. 13a–d.

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$CeCl_3 \cdot 7H_2O$–NaI catalyzed intramolecular addition reactions of 7-hydroxy-1,3-dienes: a facile approach to hexahydrobenzofurans and tetrahydrofurans

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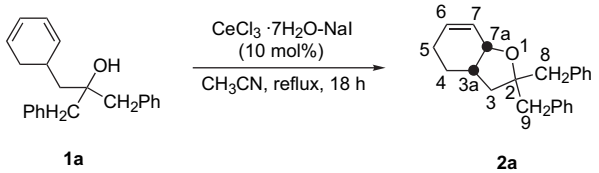
Abstract—$CeCl_3 \cdot 7H_2O$–NaI effectively catalyzed intramolecular cyclization of cyclic 7-hydroxy-1,3-dienes, yielding hexahydrobenzofurans in diastereoselective fashion. This cyclization has been applied to synthesize tetrahydrofurans from acyclic 7-hydroxy-1,3-dienes.

1. Introduction

Condensed heterocycles are widespread in nature, and many of these compounds show interesting biological activities.[1] The benzo[*b*]furan[2] and tetrahydrofuran rings[3,4] are often incorporated in pharmaceutical agents as a core structural motif.[5] Due to the high stereo- and regiochemical control, transition metals such as palladium,[6] molybdenum,[7] and indium[8] have been used to promote the furan-ring formation across unsaturated carbon–carbon bonds and a tethered hydroxyl group. However, many of these catalysts suffer from some drawbacks, which include use of expensive reagents under dry conditions. Therefore, the preparation of benzo[*b*]furan and tetrahydrofuran skeletons is still a challenge for synthetic chemists in order to find safer and milder conditions utilizing more 'friendly' reagents. Recently, cerium(III) chloride has emerged as a very cheap, water-tolerant, and safe reagent and is able to catalyze various selective chemical transformations and cyclizations.[9] In most cases, the activity of $CeCl_3$ can be increased in combination with NaI.[10] The cyclization of unsaturated 3-hydroxy esters to tetrahydrofuranacetic acid esters and tetrahydropyranacetic acid esters catalyzed by $CeCl_3 \cdot 7H_2O$–NaI has been previously reported.[9a] We now report that $CeCl_3 \cdot 7H_2O$–NaI (10 mol %) catalyzes (Scheme 1) intramolecular cyclization of 7-hydroxy-1,3-dienes under mild reaction conditions to afford hexahydrobenzofurans and tetrahydrofurans.



1a **2a**

Scheme 1.

2. Results and discussion

The starting material of 7-hydroxy-1,3-dienes **1a–i** (entries 1–9, Table 1) was prepared by addition of 2.5 equiv of Grignard reagents to the corresponding ester-functionalized 1,3-dienes according to the literature procedures.[11] The primary alcohol **1j** (entry 10, Table 1) was synthesized by addition of $LiAlH_4$ to the corresponding ester at 0 °C in diethyl ether. Secondary alcohol **1k** (entry 11, Table 1) was obtained from addition of $BrZnCH_2CO_2Et$/CuCN to the corresponding aldehyde at −78 °C in THF.[11c]

Our $CeCl_3 \cdot 7H_2O$–NaI catalyzed cyclization study was first carried out by using alcohol **1a**. Treatment of **1a** with 10 mol % equiv of $CeCl_3 \cdot 7H_2O$–NaI in boiling acetonitrile under nitrogen for 18 h afforded, after flash column chromatography, a 58% yield of 2,2-dibenzylhexabenzofuran derivative **2a** as the major product (Scheme 1). The structure for **2a** was established by comparing its ^1H and ^{13}C NMR spectral data with those of related compounds known in the literature.[12] Moreover, the relative stereochemistry of the ring juncture of **2a** was determined as cis on the basis of comparing the coupling constant (4.4 Hz) for hydrogen atoms at C(**3a**) and C(**7a**) to those of related compounds.[12] In order to gain more insights on the intramolecular cyclization of alcohol **1a**, anhydrous $CeCl_3$ (0.1 equiv) and NaI (0.1 equiv) were used. Thus, reaction of **1a** with $CeCl_3$ and NaI in boiling acetonitrile for 18 h produced **2a** in 51% yield. Therefore, water is not needed for the cyclization. However,

Keywords: Cerium chloride; 7-Hydroxy-1,3-diene; Hydroalkoxylation.
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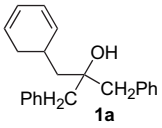
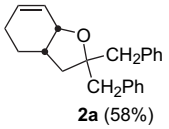
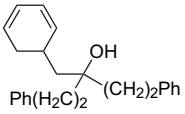
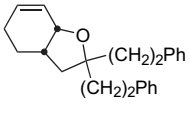
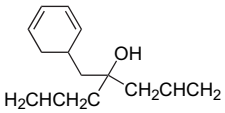
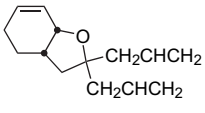
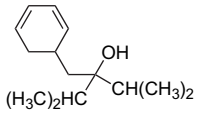
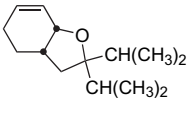
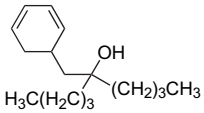
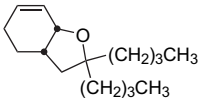
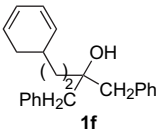
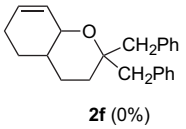
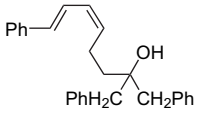
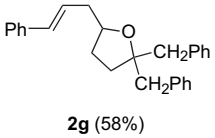
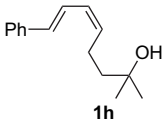
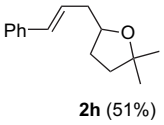
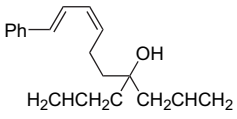
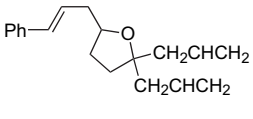
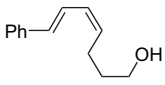
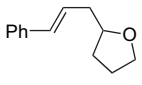
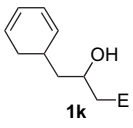
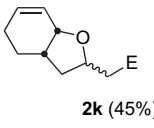
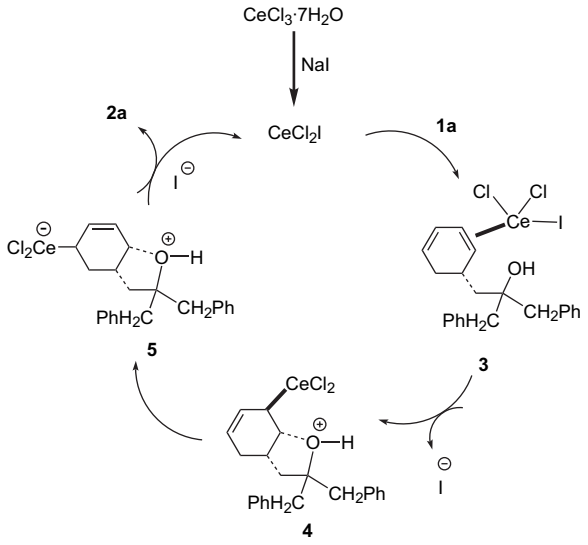
Table 1. Intramolecular addition reactions of 7-hydroxy-1,3-dienes via Scheme 1[a]

Entry	7-Hydroxy-1,3-dienes	Products (yield[b])
1	 **1a**	 **2a** (58%)
2	 **1b**	 **2b** (48%)
3	 **1c**	 **2c** (56%)
4	 **1d**	 **2d** (22%)
5	 **1e**	 **2e** (12%)
6	 **1f**	 **2f** (0%)
7	 **1g**	 **2g** (58%)
8	 **1h**	 **2h** (51%)
9	 **1i**	 **2i** (48%)
10	 **1j**	 **2j** (70%)
11	 **1k**	 **2k** (45%)

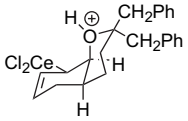
[a] All intramolecular addition reactions were performed in refluxing CH_3CN using 10 mol % of $CeCl_3\cdot 7H_2O$–NaI as the catalyst.
[b] Isolated yields after silica-gel column chromatography.

reaction of **1a** with $CeCl_3\cdot 7H_2O$ alone failed to produce **2a** and alcohol **1a** was recovered almost quantitatively. This is consistent with the failure of cyclization of unsaturated 3-hydroxy esters using $CeCl_3\cdot 7H_2O$ as the sole catalyst reported in the literature.[9a] Based upon the above results, it is reasonable to state that both $CeCl_3$ and NaI are required in the catalytic process. Our proposed reaction mechanism for the $CeCl_3\cdot 7H_2O$–NaI-mediated hydroalkoxylation is shown in Scheme 2. Reaction of $CeCl_3$ with NaI would give $CeCl_2I$. The catalyst $CeCl_2I$ coordinated on the β-face of the proximal double bond of **1a** to give **3**, which was then attacked by the oxygen-nucleophile on the opposite face. This afforded the postulated η^1-allylic intermediate **4** with the newly formed carbon–oxygen bond positioned trans to the cerium–carbon bond. Due to the steric congestion caused by the cerium fragment adjacent to the bicyclic ring juncture, intermediate **4** may undergo η^1–η^3–η^1 allylic rearrangement to the η^1-allylic intermediate **5**. Subsequent protonation of **5** resulted in formation of the 1,4-hydroalkoxylation product **2a** and regeneration of the $CeCl_2I$ catalyst. The addition of an oxygen and a metal across a double bond was found for indium,[8a] palladium,[6b] and cerium[9a] in the literature.



Scheme 2.

Under the same reaction conditions, intramolecular addition reactions of tertiary alcohols **1b–e** using 10 mol % equiv of $CeCl_3\cdot 7H_2O$–NaI in boiling acetonitrile gave hexahydrobenzofurans **2b–e** as single diastereomer in each case (entries 2–5, Table 1). In general, yields of hexahydrobenzofurans are fair (ca. 50%). The fair yields might be due to the fact that $CeCl_3\cdot 7H_2O$–NaI is an efficient reagent for the conversion of tertiary alcohols into alkyl iodides. Moreover, the problem of the competing elimination found in tertiary alcohols reduced the yield of cyclization.[9d] It is important to mention that unlike successful formation of tetrahydrofuran ring, six-membered ring of tetrahydropyran cannot be formed. Thus, intramolecular addition reaction of a substrate with one more methylene unit on the tethered failed and 8-hydroxy-1,3-diene **1f** (entry 6, Table 1) was recovered quantitatively even after refluxing in acetonitrile for 24 h. The failure in the formation of tetrahydropyranyl rings might be attributed to unfavorable formation of the *cis*-decalin intermediate **6**, which contained the bulky cerium fragment adjacent to the bicyclic ring juncture (Chart 1). It is important to mention that cyclization of 3-hydroxy esters

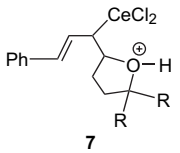


6

Chart 1.

containing a disubstituted olefin using $CeCl_3 \cdot 7H_2O$–NaI as the catalyst led to both terahydro-furanyl and -pyranyl rings.[9a]

Next, the analogous reactions of acyclic 7-hydroxy-1,3-dienes **1g–j** were examined, and the results are listed in entries 7–10, Table 1. Reactions of acyclic substrates **1g–j** with $CeCl_3 \cdot 7H_2O$–NaI (10 mol % equiv) under the same reaction conditions provided the 1,2-hydroalkoxylation products **2g–j** in 48–70% yields. The better yield observed for intramolecular cyclization of the primary alcohol **1j** to give **2j** might be attributed to unfavorable formation of the primary carbocation, which may lead to a primary iodide and/or an olefin via elimination. The 1,2-hydroalkoxylation products **2g–j** apparently derived from protonation of the η^1-allylcerium intermediate **7** (Chart 2). The isolation of 1,2-hydroalkoxylation products from acyclic precursors may suggest that the protonation of Ce–C bond occurred from intermediate **7** at a rate that was faster than η^1–η^3–η^1 allylic rearrangement. The difference in the formation of hydroalkoxylation products (1,4- vs 1,2-hydroalkoxylation) between cylic and acyclic substrates could be explained as follows. The η^1–η^3–η^1 allylic isomerization may be faster in the cyclic intermediate **4** than in the acyclic intermediate **7** for steric reasons. For example, intermediate **7** has more conformational flexibility to minimize unfavorable interactions via the σ-bond (Ce–C) rotation, whereas in **4**, the cerium fragment is close to the bicyclic ring juncture, and the η^1–η^3–η^1 allylic isomerization would place the cerium further away from the tertiary carbon center to give **5**. The η^1-allylcerium intermediate **5** led to 1,4-hydroalkoxylation products **2a–e**. The different reaction paths observed between cyclic and acyclic substrates (1,4- vs 1,2-hydroalkoxylation) were also found for arylalkoxylation of diene alcohols using $Pd(PPh_3)_4$ and aryl bromides in the literature.[12a] It is important to mention that the secondary alcohol **1k** also underwent intramolecular hydroalkoxylation to give **2k** as a mixture of diastereomers in a 1:1 ratio and in 45% total isolated yield (entry 11, Table 1).



7

Chart 2.

3. Conclusion

The reaction outlined herein demonstrates that the $CeCl_3 \cdot 7H_2O$–NaI catalyzed intramolecular addition reaction of an oxygen nucleophile to a conjugated diene can be an effective method for the formation of hexahydrobenzofurans and tetrahydrofurans. With cyclic 7-hydroxy-1,3-dienes, the reaction led to 1,4-hydroalkoxylation products after allylic isomerization of the initial formed η^1-allylcerium intermediate. In contrast to the reactions of cyclic precursors, reactions of acyclic 7-hydroxy-1,3-dienes afforded 1,2-hydroalkoxylation products after protonation of the initial formed η^1-allylcerium intermediate. The use of cerium is economic as compared to catalytic amounts of expensive transition metals employed previously.

4. Experimental

4.1. General methods

All reactions were run using oven-dried glassware under a nitrogen atmosphere unless otherwise indicated. 7-Hydroxy-1,3-dienes **1a–i** were synthesized by addition of 2.5 mol equiv of methyl-, phenyl-, or benzylic magnesium halides to the corresponding esters.[11,12a] The primary dienol **1j** (entry 10, Table 1) was synthesized by addition of $LiAlH_4$ to the corresponding ester at 0 °C in diethyl ether. Secondary dienol **1k** (entry 11, Table 1) was obtained from addition of $BrZnCH_2CO_2Et$/CuCN to the corresponding aldehyde at −78 °C in THF. Anhydrous solvents or reaction mixtures were transferred via an oven-dried syringe or cannula. Tetrahydrofuran (THF) and acetonitrile (CH_3CN) were dried by molecular sieves and then passed through an Al_2O_3 column.[13] Flash column chromatography, following the method of Still, was carried out with E. Merck silica gel (Kieselgel 60, 230–400 mesh) using the indicated solvents.[14] ^1H nuclear magnetic resonance (NMR) spectra were obtained with Bruker-AC 400 (400 MHz) and Bruker-AV 500 (500 MHz) spectrometers. The chemical shifts are reported in parts per million with either tetramethylsilane (0.00 ppm) or $CDCl_3$ (7.26 ppm) as internal standard. ^{13}C NMR spectra were recorded with Bruker-AC 400 (100.4 MHz) spectrometer with $CDCl_3$ (77.0 ppm) as the internal standard. Infrared (IR) spectra were recorded with a JASCO IR-700 spectrometer. Mass spectra were acquired on a JEOL JMS-D 100 spectrometer at an ionization potential of 70 eV and are reported as mass/charge (*m*/*e*) with percent relative abundance. High-resolution mass spectra were obtained with an AEI MS-9 double-focusing mass spectrometer and a JEOL JMS-HX 110 spectrometer at the Department of Chemistry, Central Instrument Center, Taichung, Taiwan.

4.2. General procedure for the intramolecular cyclization of 7-hydroxy-1,3-dienes catalyzed by $CeCl_3 \cdot 7H_2O$–NaI

A mixture of 7-hydroxy-1,3-diene (1.0 mmol), $CeCl_3 \cdot 7H_2O$ (0.1 mmol), and NaI (0.1 mmol) in acetonitrile (10 mL) under nitrogen was stirred at reflux temperature for 18 h (ca. 82 °C). The reaction mixture was extracted with ethyl acetate, and the combined organic layers were washed with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (silica gel, hexanes/ethyl acetate).

4.2.1. (3a*S,7a*R**)-2,2-Dibenzyl-2,3,3a,4,5,7a-hexahydrobenzofuran 2a.** This compound was prepared from

1a (0.29 g, 0.95 mmol): yield 0.17 g (0.55 mmol, 58%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (m, 10H), 5.64 (m, 2H), 4.19 (m, 1H), 2.87 (s, 2H), 2.76 (m, 2H), 1.85 (m, 2H), 1.76 (m, 3H), 1.39 (m, 1H), 1.16 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.2, 130.9, 130.7, 128.5, 128.0, 127.8, 127.7, 126.0, 126.0, 84.9, 74.7, 47.8, 46.0, 37.3, 36.23, 23.2, 21.5; IR (CH_2Cl_2) 3048, 3029, 2989, 2927, 1602, 1495, 1454, 1374, 1237, 1033 cm^{-1}; MS (20 eV) *m/e* 304.2 (M^+), 213.1, 135.1, 91.0, 79.0, 61.0; HRMS (EI) *m/e* calcd for $C_{22}H_{24}O$ 304.1827. Found 304.1835.

4.2.2. (3a*S,7a*R**)-2,2-Diphenethyl-2,3,3a,4,5,7a-hexahydrobenzofuran 2b.** This compound was prepared from **1b** (0.63 g, 1.9 mmol): yield 0.30 g (0.9 mmol, 48%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (m, 10H), 5.87 (m, 1H), 5.81 (m, 1H), 4.36 (m, 1H), 2.68 (m, 4H), 2.42 (m, 1H), 1.95 (m, 7H), 1.72 (m, 2H), 1.58 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.7, 142.6, 129.8, 128.4, 128.3, 128.3, 127.5, 125.7, 83.9, 73.7, 41.9, 40.9, 40.2, 36.7, 31.1, 30.6, 24.4, 22.6; IR (CH_2Cl_2) 3693, 3601, 3039, 2993, 2941, 2863, 2340, 1603, 1495, 1453, 1433 cm^{-1}; MS (EI) *m/e* (%) 332.6 (M^+, 9), 228.4 (16), 227.4 (86), 105.2 (52), 91.2 (100), 79.2 (32); HRMS calcd for $C_{24}H_{28}O$ (M^+) 332.2140. Found 332.2148; Anal. Calcd for $C_{24}H_{28}O$: C, 86.70; H, 8.49. Found C, 86.99; H, 8.56.

4.2.3. (3a*S,7a*R**)-2,2-Diallyl-2,3,3a,4,5,7a-hexahydrobenzofuran 2c.** This compound was prepared from **1c** (0.50 g, 2.65 mmol): yield 0.28 g (1.42 mmol, 56%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.82 (m, 4H), 5.06 (m, 4H), 4.32 (b, 1H), 2.30 (m, 5H), 2.05 (m, 1H), 1.90 (m, 2H), 1.66 (m, 2H), 1.54 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.8, 134.6, 129.8, 127.4, 117.6, 117.4, 83.5, 74.0, 45.2, 43.8, 38.8, 36.5, 24.2, 22.6; IR (CH_2Cl_2) 3686, 2928, 2843, 2366, 2333, 1642, 1609 cm^{-1}; MS (20 eV) *m/e* 163.3 (78), 93.2 (18), 91.2 (12), 85.2(15), 80.2 (12), 79.2 (100), 77.2 (18), 69.2 (58); HRMS (EI) *m/e* calcd for $C_{14}H_{20}O$ 204.1514. Found 204.1520.

4.2.4. (3a*S,7a*R**)-2,2-Diisopropyl-2,3,3a,4,5,7a-hexahydrobenzofuran 2d.** This compound was prepared from **1d** (0.86 g, 4.18 mmol): yield 0.19 g (0.9 mmol, 22%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.76 (m, 2H), 4.45(b, 1H), 2.54 (m, 1H), 2.03 (m, 1H), 1.93 (m, 2H), 1.85 (m, 1H), 1.78 (m, 3H), 1.56 (m, 1H), 0.95 (d, J=6.85 Hz, 6H), 0.88 (d, J=6.85 Hz, 3H), 0.85 (d, J=6.90 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 129.0, 128.2, 89.6, 75.4, 36.7, 35.1, 33.4, 32.9, 24.4, 21.3, 18.9, 18.7, 18.3, 18.0; IR (CH_2Cl_2) 3693, 2961, 2935, 2346, 1609, 1469 cm^{-1}; MS (20 eV) *m/e* 166.3 (13), 165.3 (98), 121.2 (12), 87.2 (49), 80.2 (14), 79.2 (63), 77.2 (12), 71.2 (100), 69.2 (20); HRMS (EI) *m/e* calcd for $C_{14}H_{24}O$ 208.1827. Found 208.1818.

4.2.5. (3a*S,7a*R**)-2,2-Dibutyl-2,3,3a,4,5,7a-hexahydrobenzofuran 2e.** This compound was prepared from **1e** (0.33 g, 1.40 mmol): yield 39 mg (0.16 mmol, 12%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85 (m, 1H), 5.78 (m, 1H), 4.24 (b, 1H), 2.31 (m, 1H), 2.03 (m, 1H), 1.92 (m, 1H), 1.84 (dd, J=12.55, 8.35 Hz, 1H), 1.44 (m, 15H), 0.89 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 129.8, 127.6, 84.4, 73.2, 40.6, 40.0, 38.3, 36.7, 27.0, 26.4, 24.7, 23.4, 23.3, 23.00, 14.1, 14.1; IR (CH_2Cl_2) 3686, 3601, 2954, 2856, 2719, 2359, 1769, 1609, 1589, 1440, 1371 cm^{-1}; MS (20 eV) *m/e* 236.5 (M^+, 1), 180.4 (14), 179.4 (100), 101.3 (40), 85.2 (78), 79.2 (47); HRMS (EI) *m/e* calcd for $C_{16}H_{28}O$ 236.2140. Found 236.2147.

4.2.6. 2,2-Dibenzyl-5-(*trans*-3-phenylallyl)tetrahydrofuran 2g. This compound was prepared from **1g** (0.37 g, 1.0 mmol): yield 0.21 g (0.58 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18–7.42 (m, 15H), 6.67 (d, J=16.0 Hz, 1H), 6.24 (dd, J=16.0, 5.7 Hz, 1H), 4.25 (m, 1H), 3.30 (d, J=14.1 Hz, 1H), 2.28 (dd, J=13.9, 12.6 Hz, 2H), 2.6 (d, J=13.8 Hz, 1H), 1.86 (m, 1H), 1.68 (d, J=11.2 Hz, 2H), 1.39 (m, 2H), 1.16 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 137.9, 137.2, 131.5, 131.2, 130.5, 129.3, 128.5, 128.1, 127.5, 127.3, 126.4, 126.1, 125.9, 71.0, 46.1, 39.2, 31.7, 30.6, 19.5; IR (CH_2Cl_2) 3691, 3569, 3084, 3053, 2945, 2869, 2410, 1952, 1733, 1601, 1495, 1453, 1424, 1366, 1263, 1192, 1084, 967 cm^{-1}; MS (20 eV) *m/e* 368.2 (M^+, 4) 277.1 (54), 259.1 (30), 157.1 (15), 143.1 (100), 129.0 (32), 128.0 (33), 91.0 (62); HRMS (EI) *m/e* calcd for $C_{27}H_{28}O$ 368.2140. Found 368.2143.

4.2.7. 2,2-Dimethyl-5-(*trans*-3-phenylallyl)tetrahydrofuran 2h. This compound was prepared from **1h** (0.37 g, 1.71 mmol): yield 0.19 g (0.88 mmol, 51%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18–7.37 (m, 5H), 6.55 (d, J=16.0 Hz, 1H), 6.20 (dd, J=16.0, 6.2 Hz, 1H), 4.22 (m, 1H), 1.27 (s, 6H), 1.29–1.50 (m, 3H), 1.67–1.75 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.1, 131.7, 129.8, 128.4, 127.3, 126.4, 72.1, 71.5, 35.9, 31.9, 31.9, 22.00, 19.90; IR (CH_2Cl_2) 3691, 3589, 3073, 2987, 2935, 2306, 1733, 1601, 1493, 1449, 1374, 1277, 1212, 1036, 967 cm^{-1}; MS (20 eV) *m/e* 216.1 (M^+, 85), 198.1 (14), 159.1 (16), 143.1 (31), 133.0 (91), 131.1 (86), 130.1 (69), 129.1 (50), 128.0 (44), 115.0 (49), 111.1 (51), 105.0 (68), 104.0 (100), 91.0 (64), 57.0 (54); HRMS (EI) *m/e* calcd for $C_{15}H_{20}O$ 216.1514. Found 216.1516.

4.2.8. 2,2-Diallyl-5-(*trans*-3-phenylallyl)tetrahydrofuran 2i. This compound was prepared from **1i** (0.27 g, 1.0 mmol): yield 0.13 g (0.48 mmol, 48%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18–7.37 (m, 5H), 6.54 (d, J=16.0 Hz, 1H), 6.18 (dd, J=16.0, 6.0 Hz, 1H), 5.87 (m, 2H), 5.09 (m, 4H), 4.23 (m, 1H), 2.67 (dd, J=14.4, 6.2 Hz, 1H), 2.27 (d, J=7.9 Hz, 2H), 2.20 (dd, J=14.4, 8.2 Hz, 1H), 1.67–1.76 (m, 2H), 1.31–1.48 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.1, 134.2, 133.9, 131.5, 129.5, 128.4, 127.3, 126.4, 117.5, 117.5, 75.2, 70.8, 45.2, 36.0, 31.8, 31.7, 19.3; IR (CH_2Cl_2) 3691, 3568, 3078, 3047, 2939, 2870, 2304, 1733, 1639, 1444, 1279, 1248, 1071, 998, 936 cm^{-1}; MS (20 eV) *m/e* 268.1 (M^+, 5) 227.1 (23), 209.1 (18), 157.1 (15), 143.1 (100), 129.1 (28), 128.0 (35), 120.1 (22), 115.0 (20), 91.0 (27); HRMS (EI) *m/e* calcd for $C_{19}H_{24}O$ 268.1827. Found 268.1826.

4.2.9. 2-(*trans*-3-Phenylallyl)tetrahydrofuran 2j. This compound was prepared from **1j** (0.23 g, 1.22 mmol): yield 0.16 g (0.85 mmol, 70%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J=7.4 Hz, 2H), 7.28 (t, J=7.4 Hz, 2H), 7.20 (t, J=7.4 Hz, 1H), 6.59 (d, J=15.9 Hz, 1H), 6.20 (dd, J=15.9, 5.7 Hz, 1H), 4.05 (dt,

J=11.3, 2.0 Hz, 1H), 3.95 (m, 1H), 3.52 (td, J=11.4, 2.2 Hz, 1H), 1.86 (d, J=10.8 Hz, 1H), 1.70 (d, J=12.2 Hz, 1H), 1.47–1.61 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.9, 129.5, 128.3, 127.3, 126.3, 130.7, 77.8, 68.2, 32.1, 25.7, 23.3; IR (CH_2Cl_2) 3028, 2941, 2850, 1951, 1881, 1732, 1600, 1495, 1449, 1373, 1277, 1203, cm^{-1}; MS (70 eV) *m/e* (rel intensity) 188.1 (M^+, 100), 187.1 (17), 131.0 (56), 129.1 (16), 115.0 (21), 104.1 (89), 103.0 (23), 91.0 (26), 77.0 (17), 55.0 (39); HRMS (EI) *m/e* calcd for $C_{13}H_{16}O$ 188.1201. Found 188.1199.

4.2.10. (2,3,3a,4,5,7a-Hexahydrobenzofuran-2-yl)acetic acid ethyl ester 2k. This compound was prepared from **1k** (0.20 g, 0.95 mmol): yield 0.09 g (0.43 mmol, 45%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.96 (m, 1H), 5.81 (m, 1H), 4.47 (m, 1H), 4.27 (m, 1H), 4.15 (q, J=7.1 Hz, 2H), 2.64 (dd, J=15, 6.8 Hz, 1H), 2.45 (dd, J=15.4, 6.5 Hz, 1H), 2.31 (m, 1H), 2.07 (m, 1H), 1.96 (m, 2H), 1.82 (dt, J=15.4, 7.7 Hz, 1H), 1.67 (m, 1H), 1.46 (m, 1H), 1.26 (t, J=7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 131.1, 126.3, 74.0, 73.5, 60.4, 41.4, 37.1, 36.7, 24.0, 23.2, 14.2; IR (CH_2Cl_2) 3058, 3048, 2929, 1730, 1422, 1280, 1249 cm^{-1}; MS (20 eV) *m/e* 210.1 (M^+, 2), 131.1 (33), 96.1 (38), 94.0 (19), 80.1 (88), 79.1 (100), 77 (21); HRMS (EI) *m/e* calcd for $C_{12}H_{18}O_3$ 210.1256. Found 210.1259.

Acknowledgements

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References and notes

1. *Naturstoffe*; Steglich, W., Fugmann, B., Lang-Fugmann, S., Eds.; Thieme: Stuttgart/New York, NY, 1997.
2. Hou, X.-L.; Yang, Z.; Wong, H. N. C. *Prog. Heterocycl. Chem.* **2003**, *15*, 167; Kraus, G. A.; Kim, I. *Org. Lett.* **2003**, *5*, 1191; Kao, C.-L.; Chern, J.-W. *J. Org. Chem.* **2002**, *67*, 6772; Macleod, C.; McKiernan, G. J.; Guthrie, E. J.; Farrugia, L. J.; Hamprecht, D. W.; Macritchie, J.; Hartley, R. C. *J. Org. Chem.* **2003**, *68*, 387.
3. (a) Ueda, K.; Hu, Y. *Tetrahedron Lett.* **1990**, *40*, 6305; (b) Kobayashi, J.; Kubota, T.; Endo, T.; Tsuda, M. *J. Org. Chem.* **2001**, *66*, 134; (c) Satake, M.; Ofuji, K.; Naoki, H.; James, K. J.; Furey, A.; Macmahon, T.; Silke, J.; Yasumoto, T. *J. Am. Chem. Soc.* **1998**, *120*, 9967.
4. Oberlies, N. H.; Jones, J. L.; Corbett, T. H.; Fotopoulos, S. S.; Mclaughlin, J. L. *Cancer Lett.* **1995**, *96*, 55.
5. (a) Elliot, M. C. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1291; (b) Gasparski, C. M.; Herrinton, P. M.; Overman, L. E.; Wolfe, J. P. *Tetrahedron Lett.* **2000**, *41*, 9431; (c) Micalizio, G. C.; Roush, W. R. *Org. Lett.* **2000**, *2*, 461; (d) Sutterer, A.; Moeller, K. D. *J. Am. Chem. Soc.* **2000**, *122*, 5636.
6. (a) Hay, M. B.; Hardin, A. R.; Wolfe, J. P. *J. Org. Chem.* **2005**, *70*, 3099; (b) Wolfe, J. P.; Rossi, M. A. *J. Am. Chem. Soc.* **2004**, *126*, 1620; (c) Liao, Y.; Smith, J.; Fathi, R.; Yang, Z. *Org. Lett.* **2005**, *7*, 2707; (d) Willis, M. C.; Taylor, D.; Gillmore, A. T. *Org. Lett.* **2004**, *6*, 4755.
7. Pearson, A. J.; Mesaros, E. F. *Org. Lett.* **2001**, *3*, 2665.
8. (a) Loh, T.-P.; Hu, Q.-Y.; Ma, L.-T. A. *J. Am. Chem. Soc.* **2001**, *123*, 2450; (b) Loh, T.-P.; Hu, Q.-Y.; Tan, K.-T.; Cheng, H.-S. *Org. Lett.* **2001**, *3*, 2669.
9. (a) Marotta, E.; Foresti, E.; Marcelli, T.; Peri, F.; Righi, P.; Scardovi, N.; Rosini, G. *Org. Lett.* **2002**, *4*, 4451; (b) Marcantoni, E.; Nobili, F.; Bartoli, G.; Bosco, M.; Sambri, L. *J. Org. Chem.* **1997**, *62*, 4183; (c) Cappa, A.; Marcantoni, E.; Torregiani, E.; Bartoli, G.; Bellucci, M. C.; Bosco, M.; Sambri, L. *J. Org. Chem.* **1999**, *64*, 5696; (d) Deo, M. D.; Marcantoni, E.; Torregiani, E.; Bartoli, G.; Bellucci, M. C.; Bosco, M.; Sambri, L. *J. Org. Chem.* **2000**, *65*, 2830; (e) Liu, H. S.; Shia, K. S.; Shang, X.; Zhu, B. Y. *Tetrahedron* **1999**, *55*, 3803.
10. (a) Bartoli, G.; Bellucci, M. C.; Bosco, M.; Marcantoni, E.; Sambri, L.; Torregiani, E. *Eur. J. Org. Chem.* **1999**, 617; (b) Bartoli, G.; Bosco, M.; Marcantoni, E.; Sambri, L.; Torregiani, E. *Synlett* **1998**, 209; (c) Marcantoni, E.; Nobili, F.; Bartoli, G.; Bosco, M.; Sambri, L. *J. Org. Chem.* **1997**, *62*, 4183.
11. (a) Yeh, M. C. P.; Liang, J. H.; Jiang, Y. L.; Tsai, M. S. *Tetrahedron* **2003**, *59*, 3409; (b) Yeh, M. C. P.; Sheu, B. A.; Fu, H. W.; Tau, S. I.; Chuang, L. W. *J. Am. Chem. Soc.* **1993**, *115*, 5941; (c) Wang, J. L.; Ueng, C. H.; Yeh, M. C. P. *J. Chin. Chem. Soc.* **1994**, *41*, 129.
12. (a) Yeh, M. C. P.; Tsao, W. C.; Tu, L. H. *Organometallics* **2005**, *24*, 5909; (b) Larock, R. C.; Oertle, K.; Potter, G. F. *J. Am. Chem. Soc.* **1980**, *102*, 190; (c) Yang, C. G.; Reich, N. W.; Shi, Z.; He, C. *Org. Lett.* **2005**, *7*, 4553.
13. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.
14. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

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Toluene dioxygenase-mediated oxidation of dibromobenzenes. Absolute stereochemistry of new metabolites and synthesis of (−)-conduritol E

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Abstract—Dibromobenzenes (*o*-, *m*-, and *p*-isomers) were converted to the corresponding *cis*-cyclohexadiene diols by whole-cell fermentation with *Escherichia coli* JM 109 (pDTG601A), an organism over-expressing the enzyme toluene dioxygenase (TDO). Absolute stereochemistry of new metabolites was determined, and (−)-conduritol was synthesized.

1. Introduction

Oxidation of aromatic compounds to the corresponding *cis*-cyclohexadiene diols with toluene dioxygenase represents a reaction that has, as yet, no equivalent in synthetic methodology. To date over 400 metabolites have been isolated[1] and many served as optically pure material in total synthesis of natural products.[2,3] The whole-cell fermentation of aromatic compounds with the recombinant organism *Escherichia coli* JM 109 (pDTG601)[4] produces moderate to excellent yields of the corresponding diols, which are easily extracted from the fermentation broth using base-washed ethyl acetate.[3a]

The mechanism of the enzymatic oxidation remains unknown although some predictive models have been proposed regarding the expected regio- and stereochemistry of oxidation in single-ring, disubstituted aromatic compounds.[5] For several such compounds the fate of oxidation is known for all three regioisomers: *ortho*, *meta*, and *para*. The metabolites of these are shown in Table 1. A large number of diene diols is known for at least two of the three possible isomers, and their metabolites have been listed in several recent compilations.[1,2] Many diol metabolites have been observed in variously substituted biphenyls but were not rigorously characterized. A total of nearly 100 such compounds has been noted.[1] This manuscript reports the results of oxidation of a series of dibromobenzenes as well as the determination of absolute stereochemistry for a new diol derived from *o*-dibromobenzene by chemical conversion to (−)-conduritol E.

2. Results and discussion

Of the three isomers of dibromobenzene, only one, *m*-dibromobenzene (**28**), had previously been subjected to

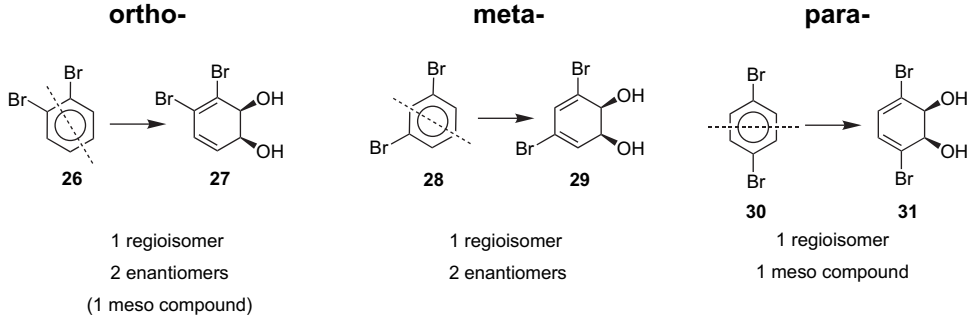

Figure 1. Possible modes of oxidation for the series of dibromobenzenes.

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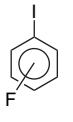
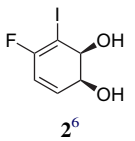
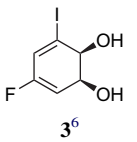
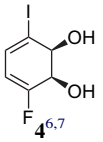
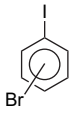
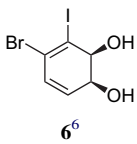
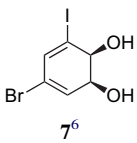
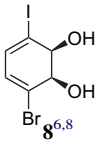
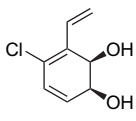
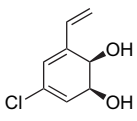
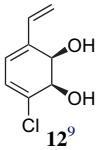
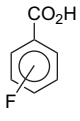
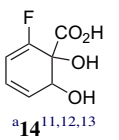
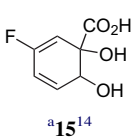
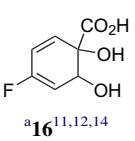
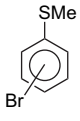
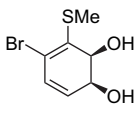
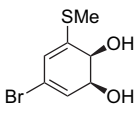
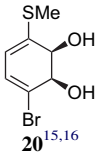
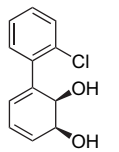
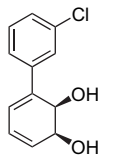
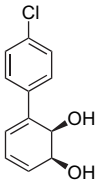
Table 1. Oxidation of isomeric series of disubstituted arenes

Substrate	Products (references in superscript)		
 **1**	 **2**[6]	 **3**[6]	 **4**[6,7]
 **5**	 **6**[6]	 **7**[6]	 **8**[6,8]
 **9**	 **10**[9,10]	 **11**[9]	 **12**[9]
 **13**	 [a]**14**[11,12,13]	 [a]**15**[14]	 [a]**16**[11,12,14]
 **17**	 **18**[15,16]	 **19**[15,16]	 **20**[15,16]
 **21**	 **22**[17,18]	 **23**[17]	 **24**[18]

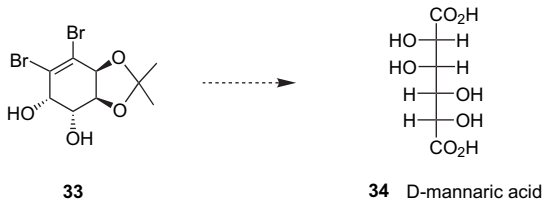
[a] Absolute stereochemistry not determined.

toluene dioxygenase-mediated oxidation. A single isomer, **29**, was produced in a yield of 4 g L^{-1} [3a] and served as a convenient starting material for a short synthesis of the amaryllidaceae constituent narciclasine.[19]

Because of the symmetry of dibromobenzenes, only one regioisomer of a diol is possible, as shown in Figure 1. In principle, *o*-dibromobenzene could also form a *meso* compound if the oxidation took place with a 3,4-regiochemistry, however, TDO oxidations of disubstituted arenes yield exclusively 1,2-regioisomers with respect to the directing group. Whole-cell oxidation of *m*-dibromobenzene with *E. coli* JM 109 (pDTG601) produced a single enantiomer (>99% ee). Similarly, the *o*-isomer provided diol **27** in a yield of 4.1 g L^{-1}, and the *p*-isomer furnished the *meso*-diol **31** in 55 mg L^{-1}.[20] Though this particular metabolite is *meso*, it may find application in asymmetric synthesis through further desymmetrization.[21] Procedures such as lipase resolution have been used to enrich enantiomeric excesses of those diols that are produced as scalemic mixtures. For example, *p*-bromoiodobenzene provides the corresponding diol with only 20% ee and may be enriched by either subsequent lipase resolution of acetyl derivatives or by resubmission of the scalemic diol to further fermentation with a wild *Pseudomonas* strain in which one of the enantiomer is consumed. Such procedures have been applied in the preparation of *ent*-diene diols[22] and *ent*-7-deoxypancratistatin.[8] The symmetrical nature of the molecule may be exploited through the use of double radical or Heck-type cyclizations from the vinyl bromides to appropriately tethered functionalities.

The absolute configuration of **27** was established by its conversion to conduritol E,[23] as shown in Scheme 1, and its enantiomeric excess was conveniently determined by ^{19}F NMR evaluation of the Mosher ester derived from monoprotected diol **38**, (Scheme 2). The Mosher ester **39** was previously prepared from homochiral and racemic alcohols **38** in connection with a study on the oxidation of a series of methylsulfanyl bromobenzenes.[15,16] Analysis of the crude ^{19}F NMR spectrum indicated a single peak corresponding to an enantioselectivity of greater than 95% in the enzymatic oxidation of *o*-dibromobenzene.

Enantiomerically pure diol **33** can easily be converted into D-mannaric acid by ozonolysis according to established procedures for the conversion of vinyl bromides of this type into hexoses.[24] The provision of isomeric sugars D-glucaric acid and D-altaric acid is also possible, as all four diastereoisomers at C-4 and C-5 are accessible by directed hydroxylation or epoxidation procedures. Diol **27** would appear to be suitable for synthesis of such acids (Fig. 2).



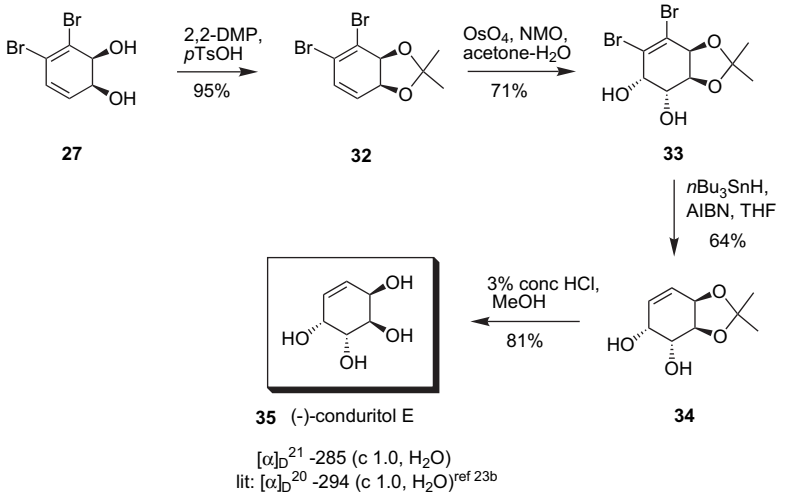
33 **34** D-mannaric acid

Figure 2.

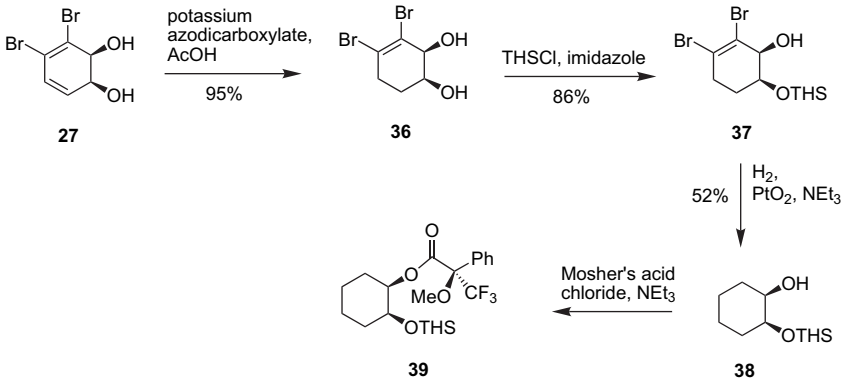
3. Experimental

3.1. General

All non-hydrolytic reactions were carried out under an argon atmosphere. Glassware used for moisture-sensitive reactions was flame-dried under vacuum and subsequently purged with argon. THF was distilled from potassium/benzophenone. Methylene chloride was distilled from calcium hydride. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh). Analytical thin-layer chromatography was performed using silica gel 60-F_{254} plates. Melting points are reported uncorrected. IR spectra were recorded as a film, unless otherwise specified. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker instrument at 300 and 75 MHz, respectively. Specific rotation measurements are given in deg cm^3 g^{-1} dm^{-1}. Ultraviolet spectroscopy was performed using a diode array spectrophotometer. Large-scale fermentation was performed in a 15-L B. Braun Biostat C-15 Fermentor. All biological media was purchased through Fisher Canada. Combustion analyses



Scheme 1. Correlation of absolute configuration by conversion to (−)-conduritol E.



Scheme 2. Synthesis of Mosher's ester **39**.

were performed by Atlantic Microlabs, Norcross, Georgia, USA.

3.2. General biotransformation procedure

3.2.1. Small-scale fermentation with *E. coli* JM 109 (pDTG601).

3.2.1.1. Growth of colonies. Agar plates consisted of bactotryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (5 g L^{-1}), agar (30 g L^{-1}), and ampicillin (100 mg L^{-1}). *E. coli* JM 109 (pDTG601) cells were streaked onto a plate and were incubated at 35 °C for 12–24 h. A single bacterial colony was selected for preculture preparations as described in the following section.

3.2.1.2. Preparation of preculture. Luria Bertani (LB) liquid medium consisted of bactotryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (5 g L^{-1}), and ampicillin (100 mg L^{-1}). The preculture medium (3 mL) was inoculated with a single colony of *E. coli* JM 109 (pDTG601) and the resulting inoculum was grown at 35 °C on an orbital shaker (200 rpm) for 6 h.

3.2.1.3. Fernbach flask preparation. LB liquid medium consisted of bactotryptone (10 g L^{-1}), yeast extract (5 g L^{-1}), NaCl (5 g L^{-1}), glucose (5 g L^{-1}), and ampicillin (100 mg L^{-1}). LB medium (500 mL) was inoculated with 1 mL of *E. coli* JM 109 (pDTG601) of the preculture medium. This inoculum was grown at 35 °C on an orbital shaker (180 rpm) for 5 h. A chemical inducer, isopropyl-1-thio-β-D-galactopyranoside (IPTG) (10 mg L^{-1}), was added via sterile filter and the cells were grown for an additional 7 h at 35 °C on an orbital shaker (200 rpm).

3.2.1.4. Substrate addition. The supernatant was separated from the cells by centrifugation at 7000 rpm for 15 min. The cell pellet was re-suspended in 500 mL of 0.1 M phosphate buffer consisting of KH_2PO_4 (6.8 g L^{-1}), K_2HPO_4 (8.7 g L^{-1}), and glucose (2 g L^{-1}). The aromatic substrate (400 mg L^{-1}) was added as a solution in isopropyl alcohol. Product formation was monitored by thin-layer chromatography (silica gel, hexane/ethyl acetate, 1:1).

3.2.1.5. Product isolation. After 5 h of incubation with substrate the pH of the culture medium was adjusted with 6 M NaOH to 8.5, and a cell pellet was obtained by centrifugation at 7000 rpm and 4 °C for 20 min. The supernatant liquid was extracted with acid-free ethyl acetate, prepared by stirring the organic solvent with a saturated solution of Na_2CO_3 and separation of the organic layer from the aqueous layer. The extract was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed under reduced pressure.

The crude material was purified by crystallization or flash column chromatography (silica gel deactivated with 10% distilled water) immediately after concentration of the solvent in order to minimize decomposition of the unstable dienediols.

3.2.2. Large-scale fermentations. Large-scale fermentations were carried out in a 15-L (8-L working volume) B. Braun Fermentor according to a published procedure.[3]

3.2.3. Extraction of products. Dienediols obtained from large-scale (8-L fermentation) were extracted from the aqueous fermentation broth into ethyl acetate either by standard manual extraction or by continuous extraction. Large-scale manual extraction requires up to 20 L of ethyl acetate, whereas the use of a rotary-evaporator-driven continuous extractor facilitates the extraction of up to 9 L of aqueous broth using as little as 3 L of ethyl acetate. The diene diols derived from small-scale fermentations (1-L) were extracted manually. Progress of either manual or continuous extraction was monitored by thin-layer chromatographic analysis of the aqueous layer.

3.2.3.1. (1*S*,2*S*)-3,4-Dibromo-cyclohexa-3,5-diene-1,2-diol (27). The biooxidation of *o*-dibromobenzene was performed according to the general procedure for large-scale fermentation.[3] *o*-Dibromobenzene **26** (60 g) was added dropwise over 45 min to a 15-L fermentor containing a growing culture of *E. coli* JM 109 (pDTG601). After stirring the media for an additional 1 h, the cell broth was separated from the cells by centrifugation. The broth was extracted using a rotary-evaporator-driven continuous extractor over a three-days period with 3 L of ethyl acetate. The combined organic layers were washed twice with approximately 10% w/v sodium carbonate solution to remove any phenolic residue. The organic extracts of the fermentation broth were concentrated in vacuo and the dienediol precipitated by addition of pentane. Recrystallization from ethyl acetate/pentane provided the title compound as a white solid (32.8 g, 4.1 g L^{-1}). Mp 144–146 °C (from ethyl acetate/pentane); $[\alpha]_D^{22}$ +104 (*c* 0.15, diethyl ether); R_f 0.36 (hexanes/ethyl acetate, 1:1); IR (film) ν 3175, 1621 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 6.03 (dd, *J*=9.9, 2.1 Hz, 1H), 6.00 (dd, *J*=6.6, 2.4 Hz, 1H), 4.62 (d, *J*=6.9 Hz, 1H), 4.55 (m, 1H), 4.28 (m, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 133.4, 126.5, 126.2, 120.6, 74.2, 69.0; HRMS (EI) Calcd for $C_6H_6Br_2O_2$ (M^+), 267.8731; Found, 267.8733; Anal. Calcd for $C_6H_6Br_2O_2$: C, 26.70; H, 2.24. Found: C, 27.04; H, 2.32.

3.2.3.2. (3a*S*,7a*S*)-2,2-Dimethyl-4,5-dibromo-4,6-benzo[1,3]dioxole (32). Diene diol **27** (3.0 g, 11.1 mmol, 1 equiv) was transferred to a 100-mL round-bottomed flask and suspended in 5 mL acetone and 15 mL of 2,2-dimethoxypropane. A few crystals of *p*-toluenesulfonic acid were added, and the reaction mixture was stirred at rt for 3 h. The reaction was quenched with 12 mL of 10% aq sodium hydroxide solution, and the acetone was removed under reduced pressure. The residue was diluted with ethyl acetate, and the layers were separated. The aqueous layer was then extracted with several portions of ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. The solvent was removed under vacuum to provide a light oil (3.21 g, 95%). An analytical sample was obtained after chromatography over 10% deactivated silica gel to afford the title compound as a clear oil. $[\alpha]_D^{22}$ +101 (*c* 0.75, $CHCl_3$); R_f 0.50 (50% ethyl acetate in hexanes); IR (film) ν 2988, 2933, 2896, 1634 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (d, *J*=9.9 Hz, 1H), 5.93 (dd, *J*=9.9, 3.6 Hz, 1H), 4.82–4.72 (m, 2H), 1.45 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.7, 125.6, 123.8, 120.3, 106.9, 77.3, 71.3, 26.6, 25.0; HRMS (EI) Calcd for $C_9H_{10}Br_2O_2$, 307.9047; Found, 307.9037.

3.2.3.3. (3a*S*,4*R*,5*S*,7a*S*)-2,2-Dimethyl-6,7-dibromo-4,5-dihydroxybenzo[1,3]dioxole (33). The protected dienediol **32** (3.1 g, 10.0 mmol, 1 equiv) was suspended in 60 mL of 8:1 (by volume) mixture of acetone/water. *N*-Methylmorpholine-*N*-oxide (2.34 g, 20.0 mmol, 2 equiv) was added followed by the addition of four crystals of osmium tetraoxide. The reaction mixture darkened slightly and was stirred for 18 h until consumption of starting material was complete. The reaction mixture was quenched by addition of 5 mL satd aq sodium bisulfite and 2 g solid sodium bisulfite, and the pH of the mixture was adjusted by addition of concd HCl. The mixture was stirred for 15 min, and the acetone was removed under reduced pressure. The aqueous portion was extracted repeatedly with ethyl acetate, and the combined organic extracts were washed with 1 N HCl, 20% aq solution of KOH, and brine before being dried over anhydrous magnesium sulfate. The extracts were filtered through a short column of silica gel and the solvent evaporated to furnish 2.4 g of a white crystalline solid, 71% yield, which required no further purification for the subsequent reaction. An analytically pure sample was obtained by recrystallization from ethyl acetate/pentane. Mp 155–156 °C; $[\alpha]_D^{22}$ +5.47 (*c* 0.75, MeOH); R_f 0.3 (50% ethyl acetate in hexanes); IR (KBr) ν 3435, 3360, 2994, 2905, 1606 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.77 (dd, *J*=1.2, 5.4 Hz, 1H), 4.50–4.45 (m, 2H), 4.34 (m, 1H), 2.68 (br s, 2H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 127.6, 125.6, 77.6, 75.3, 70.9, 69.1, 27.6, 26.1; HRMS (EI) Calcd for $C_8H_9O_4Br$, 326.8867; Found, 326.8863; Anal. Calcd for $C_9H_{12}Br_2O_4$: C, 31.42; H, 3.52. Found: C, 31.59; H, 3.53.

3.2.3.4. (−)-Conduritol E (35). Dibromide **33** (0.50 g, 1.4 mmol, 1 equiv) was dissolved in 50 mL distilled THF and transferred to a flame-dried 100-mL round-bottomed flask equipped with a reflux condenser. The solution was degassed in an ultrasound bath and under positive argon pressure for 10 min. Azoisobutyronitrile (23 mg, 0.14 mmol, 0.1 equiv) was added, and the solution was heated to steady reflux (83 °C external temp). At this time, tributyl tin hydride (1.0 mL, 3.36 mmol, 2 equiv) was added in portion. Reflux was maintained for 1.5 h until complete consumption of starting material was witnessed by TLC analysis. The reaction mixture was cooled, and potassium fluoride (2 g) was added. The resulting precipitate was filtered, and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography using 4:1 hexanes/ethyl acetate to 100% ethyl acetate to provide 170 mg (64%) of the de-brominated material. The solid was dissolved in 5 mL of methanol and to this solution was added 2 mL of a 3% (by volume) solution of concd HCl in methanol and the resulting solution was stirred for 40 h after which time the solvent was removed under reduced pressure to

provide a crude white solid. The solid was purified by flash column chromatography (4:1 chloroform/methanol) to give conduritol E as a white crystalline solid (78 mg, 81%). Mp 194–195 °C (lit.[23b] mp 193 °C); $[\alpha]_D^{20}$ −285 (*c* 1.0, H_2O), lit.[23b]: $[\alpha]_D^{20}$ −294 (*c* 1.0, H_2O); R_f 0.18 (chloroform/methanol, 4:1); IR (film) ν 3434, 1634 cm^{-1}; ^1H NMR (300 MHz, MeOD) δ 5.79 (d, *J*=2.1 Hz, 2H), 4.27 (s, 2H), 3.93 (d, *J*=0.9 Hz, 2H); ^{13}C NMR (75 MHz, MeOD) δ 130.7, 70.9, 67.6; HRMS (EI) Calcd for $C_6H_8O_3$ (M^+-H_2O), 128.0473; Found, 128.0455.

3.2.3.5. (1*S*,2*S*)-3,4-Dibromo-cyclohexa-3-ene-1,2-diol (36). Diol **27** (0.38 g, 1.47 mmol, 1 equiv) was dissolved in 6 mL MeOH, and the round-bottomed flask containing the solution was subsequently placed into an ice/NaCl bath. Potassium azodicarboxylate (0.93 g, 4.27 mmol, 3 equiv) was added in two portions to the methanolic solution. Acetic acid (0.85 mL, 12.78 mmol, 9 equiv) in 2 mL MeOH was added dropwise over 40 min. The reaction flask was allowed to warm to room temperature overnight (15 h). The reaction was quenched by adding 2 mL saturated Na_2CO_3 solution and stirring for 20 min. Methanol was removed under reduced pressure and the residue diluted with 10 mL EtOAc. The layers were separated and the aqueous phase was extracted with 3×10 mL EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and treated with activated charcoal. Filtration and concentration of the filtrate under reduced pressure afforded **36** as a white crystalline solid (0.367 g, 95%). Mp 175–176 °C; $[\alpha]_D^{21}$ −50.4 (*c* 0.75, MeOH); R_f 0.23 (Hex/EtOAc, 1:1); IR (KBr pellet) ν 3246, 1626 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 4.61 (d, *J*=6 Hz, 1H), 4.26 (s, 1H), 3.96–3.84 (m, 2H), 2.79–2.51 (m, 2H), 2.05–1.92 (m, 1H), 1.85–1.73 (m, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 127.1, 124.9, 73.5, 68.25, 35.2, 26.9; HRMS (EI) Calcd for $C_6H_8Br_2O_2$, 271.8872; Found, 271.8871; Anal. Calcd for $C_6H_8Br_2O_2$: C, 26.50; H, 2.97. Found: C, 27.34; H, 3.16.

3.2.3.6. (1*S*,2*S*)-1-[(Hexyldimethylsilyl)oxy]-3,4-dibromocyclohexa-3-ene-2-ol (37). A 5-mL round-bottom flask was charged with diol **36** (200 mg, 0.74 mmol, 1 equiv), imidazole (65 mg, 0.96 mmol, 1.3 equiv), and 1 mL anhydrous dimethylformamide. The flask was cooled externally to −30 °C, then hexyldimethylsilyl chloride (0.15 mL, 0.78 mmol, 1.05 equiv) was added. The mixture was stirred at −30 °C for 1 h and then the reaction flask was placed in a freezer (−18 °C) for 21 h. The mixture was allowed to warm to room temperature and diluted with 50 mL ether, washed with 10×1 mL distilled H_2O, brine, and dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure. The crude silyl ether was purified by flash column chromatography (pentane/Et_2O, 10:1) to give **37** as a clear and colorless oil (0.26 g, 86%). $[\alpha]_D^{21}$ −41.1 (*c* 0.75, MeOH); R_f 0.23 (pentane/Et_2O, 10:1); IR (film) ν 3547, 2958, 2868, 1628 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.26–4.17 (m, 1H), 4.03–3.93 (m, 1H), 2.84 (d, *J*=4 Hz, 1H), 2.77–2.64 (m, 1H), 2.63–2.48 (m, 1H), 2.10–1.9 (m, 1H), 1.77–1.57 (m, 2H), 0.95–0.84 (m, 13H), 0.17 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.8, 127.8, 122.9, 74.1, 73.6, 69.6, 35.1, 34.1, 27.2, 24.8, 20.2, 20.0, 18.5, 18.4; HRMS (EI) Calcd for $C_{14}H_{26}Br_2O_2Si$, 328.9032; Found, 328.9026; Anal. Calcd for $C_{14}H_{26}Br_2O_2Si$: C, 40.59; H, 6.33. Found: C, 40.96; H, 6.36.

3.2.3.7. (1*R*,2*S*)-2-[(Hexyldimethylsilyl)oxy]cyclohexan-1-ol (38). A flask containing a magnetic stirring bar was charged with dibromide **37** (0.219 g, 0.53 mmol, 1 equiv), triethylamine (0.5 mL, 3.56 mmol, 7 equiv), platinum oxide (Adam's catalyst, 24 mg, 0.11 mmol, 0.2 equiv), and 0.5 mL MeOH. The reaction flask was evacuated, flushed with hydrogen via a balloon (1 atm), and stirred until total consumption of starting material as was observed by TLC control (6 h). The crude mixture was filtered through a short plug of Celite, and the solvent was removed under reduced pressure. The crude residue was purified by flash column chromatography (pentane/Et_2O, 10:1) to give the title compound as a clear and colorless oil (71 mg, 52%) with spectral data matching that of previously reported compound **38**. $[\alpha]_D^{23}$ +3.4 (*c* 1.0, $CHCl_3$), lit.[16]: $[\alpha]_D^{23}$ +3.3 (*c* 1.0, $CHCl_3$).

3.3. General procedure for the formation of Mosher ester derivative 39

Alcohol **38** (20 mg, 0.076 mmol, 1 equiv) was transferred to a flame-dried round-bottomed flask containing magnetic stirring bar under an argon atmosphere. Anhydrous triethylamine (17 μL) was added followed by 4-dimethylaminopyridine (4.8 mg, 0.038 mmol, 0.5 equiv). (*R*)-(−)-α-Methoxy-α-trifluoromethyl-phenylacetic acid chloride (23 μL, 0.11 mmol, 1.5 equiv) was added dropwise. Within minutes a white precipitate was observed. The reaction was stirred overnight. The reaction mixture was then diluted with 5 mL methylene chloride, transferred to a separatory funnel, and washed with 5 mL saturated solution of sodium bicarbonate. The layers were separated, the organic layer dried ($MgSO_4$), and the solvent evaporated to provide the ester as a crude oil. The compound was purified by flash column chromatography (pentane/Et_2O, 10:1) to afford the ester as a clear and colorless oil (20 mg, 57%). Physical and spectral data matched that for the compound previously reported.[16]

R_f 0.46 (pentane/Et_2O, 10:1); IR (film) ν 2948, 2867, 1745, 1463, 1450, 1379, 1263, 1169 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.60 (m, 2H), 7.40 (m, 3H), 5.12 (m, 1H), 3.80 (m, 1H), 3.55 (m, 3H), 1.75–1.55 (m, 7H), 1.5–1.2 (m, 2H), 0.88 (dd, *J*=6.8, 5.2 Hz, 6H), 0.81 (d, *J*=4.1 Hz, 6H), 0 (s, 3H), −0.10 (s, 3H); ^{19}F NMR (188 MHz, $CDCl_3$) δ −72.4 ppm. Lit.[15]: ^{19}F NMR (188 MHz, $CDCl_3$) δ −72.8 ppm.

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References and notes

1. For a listing of known metabolites see: Johnson, R. A. *Org. React.* **2004**, *63*, 117.
2. Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35.
3. (a) Endoma, M.; Bui, V. P.; Hansen, J.; Hudlicky, T. *Org. Process Res. Dev.* **2002**, *6*, 525; (b) Brown, S. M.; Hudlicky, T. *Organic Synthesis: Theory and Applications*; Hudlicky, T., Ed.; JAI: Greenwich, CT, 1993; Vol. 2, p 113; (c) Hudlicky, T.; Reed, J. W. *Advances in Asymmetric Synthesis*; Hassner, A., Ed.; JAI: Greenwich, CT, 1995; p 271.
4. Zylstra, G.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.
5. Boyd, D. R.; Sheldrake, G. N. *Nat. Prod. Rep.* **1998**, *15*, 309.
6. Boyd, D. R.; Sharma, N. D.; Bart, S. A.; Dalton, H.; Chima, J.; Whited, G.; Seemeyer, R. *J. Am. Chem. Soc.* **1994**, *116*, 1147.
7. Boyd, D. R.; Sharma, N. D.; Hand, M. V.; Groocock, M. R.; Kerley, N. A.; Dalton, H.; Chima, J.; Sheldrake, G. N. *J. Chem. Soc., Chem. Commun.* **1993**, 974.
8. Hudlicky, T.; Akgün, H. *Tetrahedron Lett.* **1999**, *40*, 3081.
9. Hudlicky, T.; Boros, E. E.; Boros, C. H. *Tetrahedron: Asymmetry* **1993**, *4*, 1365.
10. Hudlicky, T.; Boros, E. E.; Boros, C. H. *Synlett* **1992**, 391.
11. Reineke, W.; Otting, W.; Knackmuss, H.-J. *Tetrahedron* **1978**, *34*, 1707.
12. Reineke, W.; Knackmuss, H.-J. *Biochim. Biophys. Acta* **1978**, *542*, 412.
13. Engesser, K. H.; Schmidt, E.; Knackmuss, H.-J. *Appl. Environ. Microbiol.* **1980**, *39*, 68.
14. Reiner, A. M.; Hegeman, G. D. *Biochemistry* **1971**, *10*, 2530.
15. Finn, K. J.; Cankař, P.; Jones, R. T. B.; Hudlicky, T. *Tetrahedron: Asymmetry* **2004**, *15*, 2833.
16. Finn, K. J.; Pavlyuk, O.; Hudlicky, T. *Collect. Czech. Chem. Commun.* **2005**, *70*, 1709.
17. Haddock, J. D.; Horton, J. R.; Gibson, D. T. *J. Bacteriol.* **1995**, *177*, 20.
18. Raschke, H.; Meier, M.; Burken, J. G.; Hany, R.; Muller, M. D.; van der Meer, J. R.; Kohler, H.-P. E. *Appl. Environ. Microbiol.* **2001**, *67*, 3333.
19. (a) Gonzalez, D.; Martinot, T.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3077; (b) Hudlicky, T.; Rinner, U.; Gonzalez, D.; Akgun, H.; Schilling, S.; Siengalewicz, P.; Martinot, T. A.; Pettit, G. R. *J. Org. Chem.* **2002**, *67*, 8726.
20. The transformation of *p*-dibromobenzene was carried out according to the general procedure for a small scale (shake-flask) fermentation. Product yields of shake-flask transformations are, on the average, significantly lower than transformations performed in a bio-reactor.
21. (a) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769; (b) Garcia-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev.* **2005**, *105*, 313.
22. Allen, C. C. R.; Boyd, D. R.; Dalton, H.; Sharma, N. D.; Brannigan, I.; Kerley, N. A.; Sheldrake, G.; Taylor, S. C. *J. Chem. Soc., Chem. Commun.* **1995**, 117.
23. (a) Hudlicky, T.; Luna, H.; Olivo, H. F.; Andersen, C.; Nugent, T.; Price, J. D. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2907; (b) Dumortier, L.; Liu, P.; Dobbelaere, S.; Van der Eycken, J.; Vandewalle, M. *Synlett* **1992**, 243.
24. Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195.

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Total synthesis of prolycopene, a novel 7,9,7′,9′-tetra-*cis*(*Z*) carotenoid and main pigment of the tangerine tomato *Lycopersicon esculentum*

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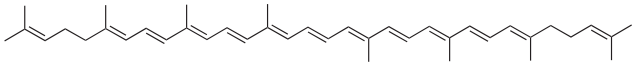
This paper is dedicated to the memory of Basil C. L. Weedon, 1923–2003

Abstract—A total synthesis of the 7,9,7′,9′-tetra-*cis*(*Z*) isomer of lycopene, also known as 'prolycopene', produced as the major carotenoid pigment in fruits of the tangerine tomato *Lycopersicon esculentum* ('Tangella') is described. The synthesis is based on: (i) a modified Sonogashira coupling reaction between the *E*-alkenyl bromide **6** and the *Z*-enynol **7**, leading to the 2*Z*-trienynol **8**, followed by (ii) a Wittig reaction between the phosphonium salt **4** and the C_{10}-triene dialdehyde **5** producing the symmetrical 9,9′-*Z* isomer of the bis-acetylene **3** and (iii) semi-hydrogenation of **3** in the presence of Lindlar's catalyst, and chromatography.

1. Introduction

Prolycopene **2** is the tetra-*cis*(*Z*) isomer of the more familiar carotenoid, all-*E*-lycopene **1**, found in commercial tomatoes. It was first isolated by Zechmeister et al. in 1941[1] from the tangerine tomato *Lycopersicon esculentum* var, known as 'Tangella'. Although the stereochemistry of prolycopene was investigated extensively by Zechmeister et al. over two decades, it was not until NMR spectroscopy, and particularly carbon NMR spectroscopy, became routinely available that the intriguing tetra-*Z* geometry of the pigment was established independently by Englert et al.[2] and ourselves[3] in 1979. In contemporaneous studies we also separated and characterised the *Z*-isomers of the carotenoid pigments phytoene, phytofluene, ζ-carotene and neurosporene, congeners to prolycopene, in *L. esculentum*.[4] Later we described the total synthesis of prolycopene,[5] Hengartner, Englert and co-workers, in 1992, presented the syntheses of six bis- and three tris-*Z*-isomers of lycopene.[6] In this paper we describe full details of our synthesis of natural (tetra-*Z*) prolycopene **2**.



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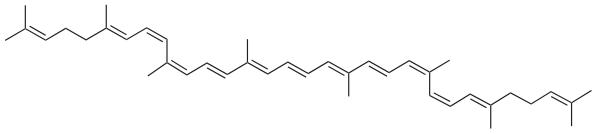

2

The synthesis of conjugated polyisopenoids with one or more *Z*-double bonds has been a formidable challenge since the early beginnings of carotenoid chemistry.[7] Without doubt, the Wittig reaction and its variants have been the cornerstone of polyene synthesis in recent years.[8] This is in spite of the fact that the stereochemical outcomes of these reactions are less predictable, and often lead to mixtures of *Z*- and *E*-isomers at the newly introduced double bonds. Prolycopene **2** has a symmetrical structure and accommodates two *Z*-disubstituted and two *Z*-trisubstituted double bonds. This led us to design a synthesis of the compound based on semi-hydrogenation of the bis-acetylenic precursor **3**, which we planned to elaborate from the *Z*-allylphosphonium salt **4** and the known C_{10}-triene dialdehyde **5**. There is considerable precedent for the formation of *Z*-disubstituted bonds from semi-hydrogenations of acetylene precursors in the carotenoid field, since the early work of Lindlar in

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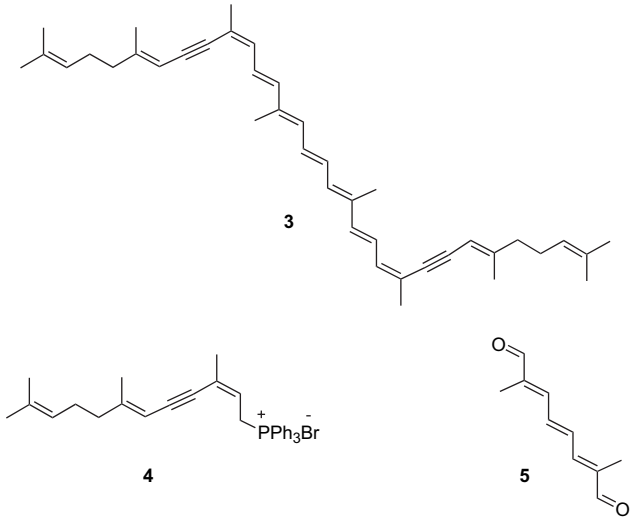
1952, i.e., Pd on $CaCO_3$ deactivated by quinoline.[9] Furthermore, the *Z*-pentenynol **7** is readily available, and we planned to use this precursor in a palladium-catalysed coupling reaction with the *E*-vinyl bromide **6**, to produce the C_{15}-2*Z*,6*E*-trienynol intermediate **8**, en route to the corresponding phosphonium salt **4**.



2. Results and discussion

Thus, a Wittig reaction between 6-methyl-5-hepten-2-one and bromomethyltriphenylphosphonium bromide, in the presence of $KOBu^t$ at −60 °C to 25 °C gave a 3:1 mixture of *E*- and *Z*-isomers of the vinyl bromide **6**,[10] from which the *E*-isomer was easily separated by gas chromatography (Scheme 1). An sp^2–sp coupling reaction between the vinyl bromide **6** and the enynol **7** under modified Sonogashira conditions, i.e., Ph_4Pd–CuI–$PrNH_2$,[11] next gave the 2*Z*-trienynol **8** in 72% yield. The stereochemistry assigned to **8** followed from analysis and comparison of its PMR and CMR spectroscopic data with those of its 2*Z*,6*Z*-, 2*E*,6*Z*- and 2*E*,6*E*-isomers, which were prepared from corresponding sp^2–sp coupling reactions involving **6** and the *Z*-vinyl bromide **10**, and **7** and the *E*-allyl alcohol **11**. The relevant diagnostic ^{13}C NMR chemical shift data for each of these isomers are collected on structures **12**, **13**, **14** and **15** (Fig. 1).

Bromination of the 2*Z*-trienynol **8**, using dibromotetrachloroethane in the presence of triphenylphosphine,[12] next gave the 2*Z*-allyl bromide **9**, which was immediately reacted with triphenylphosphine leading to the corresponding phosphonium salt **4**. The salt **4** was obtained as colourless crystals, which were very hygroscopic (Scheme 1). A Wittig reaction between molar equivalents of the phosphonium salt **4** and the C_{10} triene dial **5**,[13] using aq NaOH as base

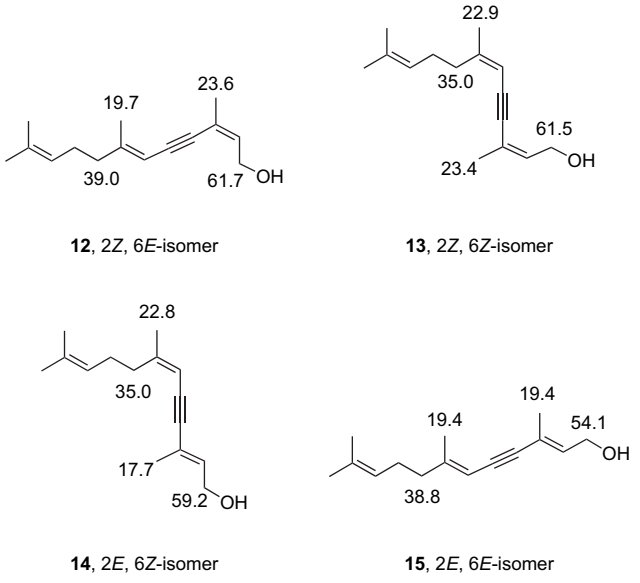
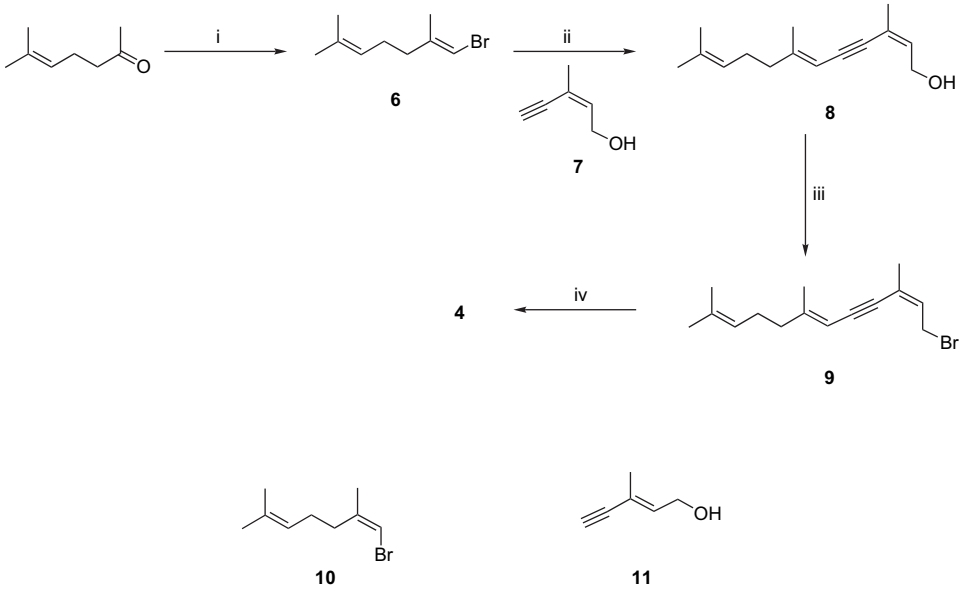
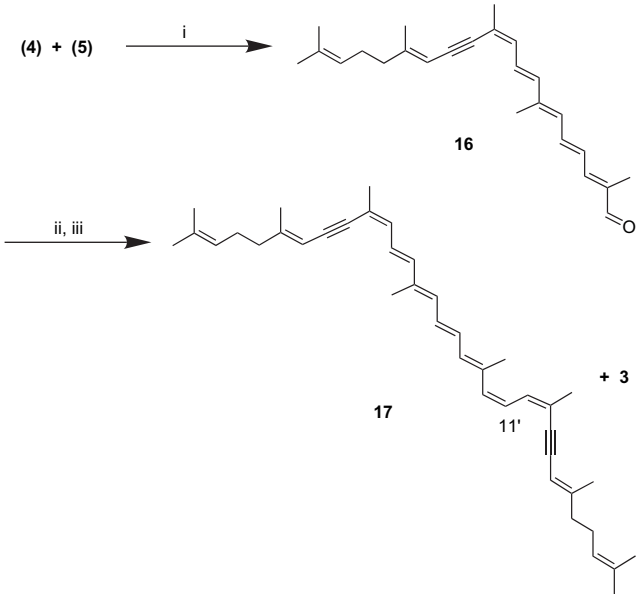


Figure 1. Pertinent ^{13}C NMR chemical shift data for the four isomers of the substituted 2,4,10-trien-4-yn-1-ol (**8**).



Scheme 1. Reagents and conditions: (i) $BrCH_2\overset{+}{P}Ph_3\bar{B}r$, $KOBu^t$, THF, −60 °C, 81%, 3:1 *E*/*Z* mixture; (ii) $(PPh_3)_4Pd$, $PrNH_2$, then **7**, CuI, 70%; (iii) $BrCl_2CCCl_2Br$, PPh_3, Et_2O, 0 °C, 98% and (iv) PPh_3, C_6H_6, 25 °C, 85%.

in $ClCH_2CH_2Cl$, followed by chromatography, led cleanly to the 9*Z*-heptenynal **16** (Scheme 2). The geometry of **16** followed from analysis of its CMR spectroscopic data[14] and comparison with similar data recorded for the related 9*Z*- and 9*E*-polyenynals **18** and **19**,[15] respectively (Fig. 2).† A second Wittig reaction between the salt **4** and the 9*Z*-heptenynal **16**, using aq NaOH in $ClCH_2CH_2Cl$, then gave the symmetrical 9,9′-*Z* isomer of the bis-acetylene **3**, which was obtained as a deep red solid. HPLC analysis demonstrated that the bis-acetylene **3** was produced as a mixture of two 11′*Z*,*E*-isomers in the ratio 2:1, identified as the required 9*Z*,11*E*,9′*Z*,11′*E*-isomer **3** (major product) and the isomeric 9*Z*,11*E*,9′*Z*,11′*Z* compound **17**.

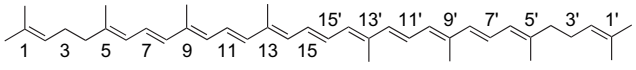


Scheme 2. Reagents and conditions: (i) aq NaOH, $ClCH_2CH_2Cl$, 25 °C, 36%; (ii) **4**, aq NaOH, $ClCH_2CH_2Cl$, 25 °C, then **16**, 61%, 2:1 mixture of **3** and **17** and (iii) I_2, C_6H_6, 90%.

Rather than separating the two isomers at this stage, a solution containing a mixture of the isomers in hexane was treated with a very dilute solution of iodine in benzene, and the progress of the isomerisation of the 9*Z*,11*E*,9′*Z*,11′*Z*-isomer **17** to the isomer **3** was monitored by HPLC analysis. After washing with aqueous sodium thiosulfate solution, work up and crystallisation gave the symmetrical 9,9′-*Z* isomer of the bis-acetylene **3** as minute orange crystals, mp 110 °C.

Consistent with its symmetrical structure only 20 carbon signals were observed in the CMR spectrum of **3**, which also showed diagnostic resonances at δ 39.0 and δ 19.7 (5*E* and 5′*E* double bonds), δ 23.6 (9*Z* and 9′*Z* double bonds) and at δ 12.8 (11*E* and 11′*E* double bonds). Pertinent ^{13}C and ^{1}H NMR data are collected on structures **20** and **21** (Fig. 3). Several years after we had published a preliminary communication,[5] Hengartner et al.[6] published an identical synthetic approach to the same 9,9′-*Z* isomer of the bis-acetylene **3**, on large scale.

† The system of numbering carotenoids recommended by I.U.P.A.C. is used throughout this paper, i.e., lycopene.



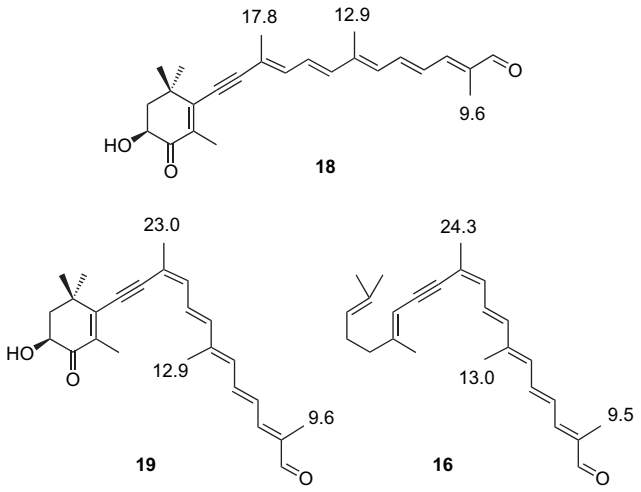


Figure 2. Pertinent ^{13}C NMR chemical shift data for the C-9*Z* polyenal **16** and the related C-9*E*- and C-9*Z*-analogues **18** and **19**, respectively.

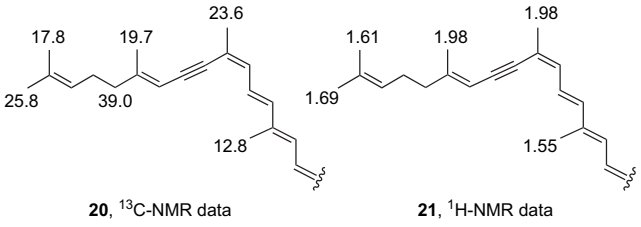


Figure 3. Pertinent ^{13}C and ^{1}H NMR chemical shift data for the 9*Z*- and 9′*Z*-bis-acetylene **3**.

All that now remained to complete our synthesis of natural prolycopene **2** was to carry out a semi-hydrogenation of the bis-acetylene **3** in the presence of Lindlar's catalyst. This was no trivial task especially in view of the limited amounts of **3** that were available. After considerable experimentation, using alternative polyenyne substrates as models, different microhydrogenator designs and various Lindlar catalyst cocktails, we successfully hydrogenated the bis-acetylene **3** on 10 mg scale to produce prolycopene **2**, which was purified by HPLC and obtained in ca. 15% yield. The synthetic material did not separate from naturally derived prolycopene in HPLC analysis and their PMR spectroscopic and mass spectrometric data were closely identical. Hengartner et al.[6] later carried out the same semi-hydrogenation of the bis-acetylene **3** on 10 times the scale we had been able to use, but also in the presence of Lindlar's catalyst, and secured a 44% yield of crystalline prolycopene.

3. Experimental

3.1. General details

Melting points of polyenes were determined in evacuated capillary tubes using a Gallenkamp melting point apparatus.

Infrared spectra were recorded as liquid films, unless stated otherwise, on a Phillips P.U. 9706 spectrophotometer. ^{1}H NMR spectra were determined on a Bruker WM 250 PFT or AM 400 PFT spectrometer and ^{13}C NMR spectra were obtained on the same instruments at 63.0 and 100.0 MHz,

respectively. Samples were dissolved in deuterated chloroform ($CDCl_3$), which provided the deuterium lock for the spectrometers. Tetramethylsilane or residual chloroform was used as an internal standard. Mass measurements were determined on either an AEI MS 902 or a VG 707E spectrometer. Elemental analyses were carried out on a Perkin Elmer 240B elemental analyser.

Due to their sensitivity, strict methods of handling polyenes were adhered to throughout the experimental work. Thus, all solutions of polyenes were handled under a nitrogen blanket in subdued light or in darkness, and all columns and TLC chambers used in chromatographic separations were wrapped in aluminium foil. Transfers of polyenes were carried out rapidly under a blanket of nitrogen and at no time were solutions containing polyenes heated above room temperature; all polyenes were stored under nitrogen at −12 °C. All solvents were distilled before use. Solutions were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure.

Analytical HPLC analyses were carried out on a Zorbax, Silica, 250×4.6 mm column using a Waters 6000 or 6000A pump connected to a Cecil C212 variable wavelength monitor or a Waters Model 440 absorbance detector. Preparative HPLC work was carried out on a C-18 column using a Waters Prep LC/System 500 pump fitted with an integral refractive index detector.

Capillary GLC analysis was performed on a Perkin Elmer Sigma 2 instrument using a Carbowax 50 m column. Preparative GLC was carried out on a 76 cm×8 mm silicone oil (15%) on diatomite column using an Aerograph Auroprep Model A-700 instrument.

3.1.1. (*E*,*Z*)-1-Bromo-2,6-dimethyl-1,5-heptadiene (6). Freshly prepared potassium tertiary butoxide (7.2 g, 6.4 mmol) was added, all at once, to a stirred suspension of bromomethyltriphenylphosphonium bromide (31 g, 7.1 mmol) in dry THF (500 ml) at room temperature under a nitrogen atmosphere, and the resulting mixture was then stirred at room temperature for 30 min. The deep yellow solution of the corresponding ylide was cooled to −60 °C and then 6-methylhept-5-en-2-one (3.0 g, 2.4 mmol) in dry THF (20 ml) was added over 10 min. The mixture was stirred at −60 °C for 1 h, and then allowed to warm to room temperature where it was stirred for a further 24 h. The mixture was evaporated to dryness in vacuo and the residue was then triturated with petrol (bp 40–60 °C) (4×100 ml). The combined organic extracts were evaporated to leave a brown oil. Chromatography followed by distillation gave a 3:1 mixture of *E*- and *Z*-isomers of the vinyl bromide (3.9 g, 81%) as a colourless oil, bp 56 °C at 0.6 mmHg. The *E*- and *Z*-isomers were separated by preparative GC to give: (i) *E*-1-bromo-2,6-dimethyl-1,5-heptadiene,[10] ν_{max} 2920, 1630, 825, 720 cm^{-1}, δ_H 5.9 (1H, q, *J*=1, =C*H*Br), 5.06 (1H, br, =C*H*), 2.08–2.16 (4H, m), 1.8 (3H, d, *J*=1, =CMe), 1.68 (3H, s, Me), 1.60 (3H, s, Me) ppm; δ_C 141.5 (s, =C), 132.2 (s, =C), 123.3 (d, =CH), 101.4 (d, =CH), 38.5 (t, CH_2), 26.4 (t, CH_2), 25.7 (q, CH_3), 19.2 (q, CH_3), 17.7 (q, CH_3) ppm and (ii) *Z*-1-bromo-2,6-dimethyl-1,5-heptadiene, ν_{max} 2980, 2920, 1635, 780, 730 cm^{-1}, δ_H 5.86 (1H, q, *J*=1.5, =C*H*Br), 5.14 (1H, tqn, *J*=7 and 1.5, =C*H*), 2.07–2.37 (4H, m), 1.79 (3H, d, *J*=1.5, =CMe), 1.70 (3H, d, *J*=1.1, =CMe), 1.63 (3H, s, Me) ppm; δ_C 141.5 (s, =C), 132.3 (s, =C), 123.6 (d, =C), 100.9 (d, =C), 34.6 (t, CH_2), 25.6 (q, CH_3), 22.3 (q, CH_3), 17.7 (q, CH_3) ppm.

3.1.2. (2*Z*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-4-yn-1-ol (8). *E*-1-Bromo-2,6-dimethyl-1,5-heptadiene (**6**) (0.54 g, 2.7 mmol) was added to a stirred suspension of tetrakistriphenylphosphine palladium(0) (0.20 g, 0.17 mmol) in freshly distilled, degassed propylamine (5.0 ml) under a nitrogen atmosphere in the dark, and the resulting mixture was then stirred at room temperature for 20 min. *Z*-2-Methylpent-2-en-4-yn-1-ol (0.26 g, 2.7 mmol) followed by freshly purified copper (I) iodide (0.14 g, 0.74 mmol) were added, and the resulting deep brown viscous mixture was then stirred at room temperature for a further 17 h. The mixture was evaporated in vacuo to leave a brown oil, which was dissolved in ether (50 ml). The ether solution was washed with saturated ammonium chloride solution (4×100 ml) and water (2×100 ml), then dried and evaporated in vacuo to leave the crude trienynol as a brown oil. Chromatography [silica G, ether/petrol ether (bp 40–60 °C), 1:2], followed by bulb-to-bulb distillation gave the 2*Z*,6*E* alcohol (0.41 g, 70%) as a colourless liquid, bp 150 °C at 0.3 mmHg. Found: C, 82.5; H, 10.9%. $C_{15}H_{22}O$ requires: C, 82.5; H, 10.2%; λ_{max} (EtOH) 264 inf (14,000), 272 (16,700), 286 inf (11,600) nm; ν_{max} 3320, 2920, 2190, 1615, 835 cm^{-1}, δ_H 5.83 (1H, tq, *J*=6.5 and 1.4, =C*H*CH_2OH), 5.43 (1H, =C*H*C), 5.08 (1H, br, Me_2C=C*H*), 4.35 (2H, d, *J*=6.5, CH_2OH), 3.20 (1H, br, –OH), 2.12–2.17 (4H, m), 1.92 (6H, 2×=CMe), 1.69 (3H, =CMe), 1.61 (3H, =CMe) ppm; δ_C 152.8 (s, =C), 134.2 (d, =CH), 132.4 (s, =C), 123.5 (d, =CH), 121.7 (s, =C), 104.9 (t, =CH), 93.1 (s, ≡C), 90.2 (s, ≡C), 61.7 (t, CH_2), 39.0 (t, CH_2), 26.5 (t, CH_2), 25.9 (q, CH_3), 23.6 (q, CH_3), 19.9 (q, CH_3), 17.9 (q, CH_3) ppm; *m*/*z* 218.1667; $C_{15}H_{22}O$ requires *M* 218.1671.

3.1.3. (2*Z*,6*Z*)-3,7,11-Trimethyldodeca-2,6,10-trien-4-yn-1-ol (13). *Z*-1-Bromo-2,6-dimethyl-1,5-heptadiene (0.19 g, 0.94 mmol) and *Z*-2-methylpent-2-en-4-yn-1-ol (0.09 g, 0.95 mmol) were reacted together in an identical manner to that described for the preparation of the corresponding 2*Z*,6*E* alcohol. The product (0.13 g, 63%) was obtained as a colourless oil. Found: C, 82.6; H, 10.6%. $C_{15}H_{22}O$ requires: C, 82.5; H, 10.2%; λ_{max} (EtOH) 263 inf (11,700), 272 (13,900), 285 inf (9750) nm; ν_{max} 3320, 2920, 2190, 1615, 830 cm^{-1}, δ_H 5.82 (1H, tq, *J*=6.6 and 1.4, =C*H*CH_2OH), 5.42 (1H, d, *J*=1.4 =C*H*C), 5.14 (1H, tqn, *J*=6.3 and 1.4, Me_2C=C*H*), 4.32 (2H, d, *J*=6.6, CH_2OH), 2.73 (1H, br, –OH), 2.33 (2H, t, *J*=7.6, CH_2C=CH_2), 2.13 (2H, q, *J*=7.6, CH_2CH_2), 1.91 (3H, d, *J*=1.2, =CMe), 1.83 (3H, d, *J*=1.4, =CMe), 1.69 (3H, d, *J*=0.8, =CMe), 1.62 (3H, Me) ppm; δ_C 152.9 (s, =C), 134.0 (d, =CH), 132.2 (s, =C), 123.7 (d, =CH), 121.8 (s, =C), 105.4 (d, =CH), 92.8 (s, ≡C), 89.7 (s, ≡C), 61.5 (t, CH_2), 35.2 (t, CH_2), 26.4 (t, CH_2), 25.8 (q, CH_3), 23.4 (q, CH_3), 22.9 (q, CH_3), 17.8 (q, CH_3) ppm; *m*/*z* 218.1673; $C_{15}H_{22}O$ requires *M* 218.1671.

3.1.4. (2*E*,6*Z*)-3,7,11-Trimethyldodeca-2,6,10-trien-4-yn-1-ol (14) and (2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-4-yn-1-ol (15). A 3:1 mixture of *E*- and *Z*- isomers of 1-bromo-2,6-dimethyl-1,5-heptadiene (**6**) (0.54 g,

2.7 mmol) was added to a stirred suspension of tetrakis-triphenylphosphine palladium(0) (0.20 g, 0.17 mmol) in freshly distilled, degassed *n*-propylamine (5.0 ml) under a nitrogen atmosphere in the dark, and the resulting mixture was then stirred at room temperature for 20 min. *E*-2-Methylpent-2-en-4-yn-1-ol (0.26 g, 2.7 mmol) followed by freshly purified copper (I) iodide (0.14 g, 0.74 mmol) were added, and the resulting deep brown viscous mixture was then stirred at room temperature for a further 17 h. The mixture was evaporated in vacuo to leave the crude mixture of trienynol isomers as a brown oil. Chromatography [silica G, ether/petrol ether (bp 40–60 °C), 1:2], followed by bulb-to-bulb distillation gave a 3:1 mixture of the *E*-2 and the *E*-6 isomers (0.27 g, 46%) as a colourless oil (bp 150 °C at 0.3 mmHg). The two isomers were separated by preparative reverse phase HPLC to give: (i) the (2*E*,6*E*)-isomer (**15**), Found: C, 82.5; H, 10.4%. $C_{15}H_{22}O$ requires: C, 82.5; H, 10.2%; λ_{max} (EtOH) 262 inf (13,300), 271 (16,000), 284 inf (11,500) nm; ν_{max} 3320, 2925, 2200, 1615, 740, 700 cm^{-1}, δ_H 5.97 (1H, tq, *J*=6.8 and 1.4, =C*H*CH_2OH), 5.38 (1H, =C*H*C), 5.08 (1H, br, Me_2C=C*H*), 4.21 (2H, d, *J*=6.8, CH_2OH), 2.24 (1H, br, –OH), 2.11–2.12 (4H, m), 1.9 (3H, d, *J*=1, =CMe), 1.86 (3H, d, *J*=0.7, =CMe), 1.68 (3H, =CMe), 1.60 (3H, =CMe) ppm; δ_C 152.1 (s, =C), 134.1 (d, =CH), 132.1 (s, =C), 123.5 (d, =CH), 121.4 (s, =C), 104.8 (d, =CH), 94.3 (s, ≡C), 86.1 (s, ≡C), 59.1 (t, CH_2), 38.8 (t, CH_2), 26.3 (t, CH_2), 25.7 (q, CH_3), 19.4 (q, CH_3), 17.7 (q, CH_3) ppm and (ii) the (2*E*,6*Z*)-isomer (**14**), Found: C, 82.5; H, 10.4%. $C_{15}H_{22}O$ requires: C, 82.5; H, 10.2%; λ_{max} (EtOH) 262 inf (11,000), 271 (13,000), 286 (9820) nm; ν_{max} 3320, 2925, 2195, 1615, 1005, 833 cm^{-1}, δ_H 5.96 (1H, tq, *J*=6.9 and 1.3, =C*H*CH_2OH), 5.38 (1H, =C*H*C), 5.15 (1H, tt, *J*=7.1 and 1.3, Me_2C=C*H*), 4.23 (2H, d, *J*=6.9, CH_2OH), 2.32 (2H, t, *J*=7.5, CH_2CH_2), 2.14 (2H, q, *J*=7.5, CH_2CH_2), 1.86 (3H, d, *J*=0.6, =CMe), 1.86 (3H, d, *J*=1.4, =CMe), 1.70 (3H, Me), 1.63 (3H, Me) ppm; δ_C 152.5 (s, =C), 133.8 (d, =CH), 132.1 (s, =C), 123.8 (d, =CH), 121.6 (s, =C), 105.3 (d, =CH), 93.7 (s, ≡C), 86.0 (s, ≡C), 59.2 (t, CH_2), 35.0 (t, CH_2), 26.4 (t, CH_2), 25.7 (q, CH_3), 17.7 (q, CH_3), 17.4 (q, CH_3) ppm; *m*/*z* 218.1663; $C_{15}H_{22}O$ requires *M* 218.1671.

3.1.5. (2*Z*,6*E*)-1-Bromo-3,7,11-trimethyldodeca-2,6,10-trien-4-yne (9). A solution of 1,2-dibromotetrachlorethane (0.21 g, 0.64 mmol) in dry ether (5 ml) was added over 5 min to a stirred solution of (2*Z*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-4-yn-1-ol (**8**) (0.82 g, 3.8 mmol) and triphenylphosphine (0.17 g, 0.65 mmol) in dry ether (5 ml) at 0 °C under a nitrogen atmosphere in the dark, and the mixture was then stirred at this temperature for 10 min. The mixture was warmed to room temperature and the stirring was then continued for a further 20 min, by which time a colourless precipitate of triphenylphosphine oxide had formed. The precipitate was filtered off, and the ether was then evaporated to leave a brown oil. Chromatography [silica G, ether/petrol ether (bp 40–60 °C), 1:3] gave the 2*Z*,6*E*-alkyl bromide (0.12 g, 98%) as a colourless oil, which showed λ_{max} (EtOH) 283 (13,000) nm; ν_{max} 2920, 2190, 1610, 835, 780, 755 cm^{-1}, δ_H 5.87 (1H, tq, *J*=8.0 and 1.3, =C*H*CH_2Br), 5.47 (1H, =C*H*C=), 5.09 (1H, br, Me_2C=C*H*), 4.25 (2H, d, *J*=8.0, CH_2Br), 2.15 (4H, m), 1.94 (6H, 2×=CMe), 1.69 (3H, Me), 1.61 (3H, Me) ppm; δ_C 153.3 (s, =C), 132.2 (s, =C), 130.12 (d, =CH), 124.7 (s, =C), 123.3 (d, =CH), 104.8 (d, =CH), 95.1 (s, ≡C), 89.3 (s, ≡C), 38.9 (t, CH_2), 30.8 (t, CH_2), 25.7 (q, CH_3), 23.3 (q, CH_3), 19.6 (q, CH_3), 17.7 (q, CH_3) ppm; *m*/*z* 280.0836; $C_{15}H_{21}{}^{79}Br$ requires *M* 280.0826.

3.1.6. (2*Z*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-4-ynyl-triphenylphosphonium bromide (4). Triphenylphosphine (0.26 g, 0.99 mmol) was added to a solution of freshly prepared (2*Z*,6*E*)-1-bromo-3,7,11-trimethyldodeca-2,6,10-trien-4-yne (**9**) (0.279 g, 0.99 mmol) in dry benzene (15 ml) and the mixture was then stirred in the dark, overnight under a nitrogen atmosphere. The benzene was evaporated leaving a sticky pale brown solid, which was triturated with dry ether (6×10 ml). The ether was evaporated leaving the phosphonium salt (0.46 g, 85%) as an extremely hygroscopic, flaky colourless solid, which showed λ_{max} ($CHCl_3$) 230 (36,300), 277 (18,640), 268 (17,830), 303 (13,500) nm; ν_{max} ($CHCl_3$ solution) 3380, 2900, 2480, 2200, 1615, 1010 cm^{-1}, δ_H 7.70–7.90 (15H, m), 5.65 (1H, br, =C*H*CH_2), 5.21 (1H, =C*H*C=), 5.04 (1H, br, Me_2C=C*H*), 4.94 (2H, dd, *J*=6 and 12, CH_2PPh_3), 2.07–2.12 (4H, m), 1.83 (3H, d, *J*=6, =CMe), 1.72 (3H, =CMe), 1.69 (3H, =CMe), 1.60 (3H, =CMe) ppm; δ_C 153.4 (s, =C), 135.0 (d, =CH), 133.8 (d, *J*=9.5, =CH), 132.3 (s, =C), 130.3 (d, *J*=12.2, =CH), 128.9 (d, *J*=13.8, =CH), 123.2 (d, =CH), 118.5 (d, *J*=10.5, =CH), 118.1 (d, *J*=85.5, =CH), 104.3 (d, =CH), 94.8 (s, ≡C), 89.3 (s, ≡C), 38.6 (t, CH_2), 27.0 (t, *J*=50.1, CH_2), 26.1 (t, CH_2), 25.6 (q, CH_3), 23.5 (q, CH_3), 19.5 (q, CH_3), 17.7 (q, CH_3) ppm; FAB mass spectrum *m*/*z* 463 (M−Br) (73%), 262 (53%), 183 (57%), 69 (94%), 55 (100%).

The corresponding 2*E*,6*E*-triphenylphosphonium salt was prepared in a similar manner from the 2*E*,6*E*-alcohol (**15**), and was obtained as a very hygroscopic flaky colourless solid, which showed λ_{max} (EtOH) 200 (31,000), 231 inf (10,800), 267 (5560), 275 (6180), 287 inf (4940), 303 inf (4630) nm; ν_{max} 2920, 2440, 2200, 1610, 920 cm^{-1}, δ_H 7.69–7.91 (15H, m), 5.66 (1H, q, *J*=7.4 =C*H*CH_2), 5.21 (1H, =C*H*C=), 5.04 (1H, br, Me_2C=C*H*), 4.79 (2H, dd, *J*=16 and 8, CH_2PPh_3), 2.10 (4H, m), 1.84 (3H, =CMe), 1.67 (3H, =CMe), 1.59 (3H, =CMe), 1.54 (3H, d, *J*=4.0, =CMe) ppm; δ_C 153.5 (s, =C), 135.2 (d, =CH), 133.9 (d, *J*=9.6, =CH), 132.2 (s, =C), 130.4 (d, *J*=12.4, =CH), 128.9 (d, *J*=13.8, =CH), 123.2 (d, *J*=10.9, =CH), 117.8 (d, *J*=85.5, =CH), 104.3 (d, =CH), 93.2 (s, ≡C), 88.3 (s, ≡C), 38.7 (t, CH_2), 26.2 (t, CH_2), 25.7 (q, CH_3), 25.4 (t, *J*=47.8, CH_2), 19.5 (q, CH_3), 18.7 (q, CH_3), 17.8 (q, CH_3) ppm.

3.1.7. (10*Z*)-2,7,11,15,19-Pentamethyleicosa-2,4,6,8,10,14,18-hepten-12-yn-1-al (16). Aqueous 2 M sodium hydroxide (2 ml, 4 mmol) was added to a stirred solution of (2*Z*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-4-ynyl-triphenylphosphonium bromide (**4**) (0.4 g, 0.74 mmol) in 1,2-dichloroethane (200 ml) and the mixture was then stirred under an argon atmosphere, at room temperature, in the dark for 10 min. A solution of the C_{10}-triene dialdehyde (**5**) (0.483 g, 2.9 mmol) in 1,2-dichloroethane (40 ml) was added to the stirred deep red coloured solution of the ylide over a period of 10 min and stirring was then continued at room temperature for a further 2 h. Glacial acetic acid (2 ml, 35 mmol)

was added, followed by ether (200 ml) and the mixture was then washed with water (4×100 ml). The combined aqueous washings were re-extracted with ether (2×100 ml), and the combined ether solutions were then dried and evaporated leaving a viscous orange oil. Purification by preparative thin layer chromatography [silica G, ether/petrol ether (bp 40–60 °C), 1:2] gave the (*Z*-10) C_{25} aldehyde (0.092 g, 36%) as a bright orange-red oil. λ_{max} (hexane) 391 inf (26,800), 411 (32,000), 434 (24,300) nm; ν_{max} ($CHCl_3$ solution) 2920, 1660, 1560, 985 cm^{-1}, δ_H 9.45 (1H, C*H*O), 6.29–6.72 (7H, m, =CH), 5.53 (1H, =CH), 5.10 (1H, br, =CH), 2.17 (4H, m), 2.02 (3H, Me), 2.00 (3H, Me), 1.99 (3H, Me), 1.70 (3H, Me), 1.62 (3H, Me) ppm; δ_C 39.0 (t, CH_2), 26.6 (t, CH_2), 25.7 (q, CH_3), 23.8 (q, CH_3), 19.7 (q, CH_3), 17.8 (q, CH_3), 13.1 (s), 9.7 (q, CH_3) ppm; *m/z* 348.2454; $C_{25}H_{32}O$ requires *M* 348.2453.

3.1.8. (9*Z*,9′*Z*)-7,8,7′,8′-Tetradehydrolycopene (3). Aqueous 2 M sodium hydroxide (1 ml, 2 mmol) was added to a stirred solution of (2*Z*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-4-ynyl-triphenylphosphonium bromide (**4**) (0.286 g, 0.53 mmol) in 1,2-dichloroethane (20 ml), and the mixture was then stirred under an argon atmosphere, at room temperature, in the dark for 10 min. A solution of the (10*Z*) C_{25}-aldehyde (**16**) (0.092 g, 0.026 mmol) in 1,2-dichloroethane (30 ml) was added and the mixture was stirred at room temperature for a further 2 h. Glacial acetic acid (1 ml, 17.5 mmol) was added, followed by ether (200 ml) and the mixture was then washed with water (4×50 ml). The combined aqueous washings were re-extracted with ether (2×100 ml), and the ether solutions were then dried and evaporated leaving a deep red solid. The solid was purified by preparative thin layer chromatography [silica G, 10% acetone in *n*-hexane] to give the tetradehydro C_{40} compound (0.05 g, 61%) as a deep red solid. HPLC analysis showed that the solid consisted of a 2:1 mixture of the desired 9*Z*,11*E*,9′*Z*,11′*E*-isomer (**3**) and the 9*Z*,11*E*,9′*Z*,11′*Z*-isomer (**17**).

A very dilute solution of iodine in benzene (0.5 ml) was added to a solution of the tetrahydrolycopene isomers (0.08 g, 0.015 mmol) in *n*-hexane (10 ml) under a nitrogen atmosphere. The solution was stirred under a nitrogen atmosphere at room temperature for 3.5 h by which time HPLC analysis showed that only the 9*Z*,11*E*,9′*Z*,11′*E*-isomer of the tetrahydrolycopene was present. The solution was washed thoroughly with 1 M sodium thiosulfate solution (4×3 ml) and water (2×10 ml), then dried and evaporated to leave a deep red solid (0.075 g). Crystallisation from hexane/ethanol gave the (9*Z*,9′*Z*) tetrahydrolycopene as minute orange crystals, mp 100 °C, which showed λ_{max} (*n*-hexane) 419 (37,000), 443 (55,600), 472 (49,100) nm; ν_{max} ($CHCl_3$ solution) 1600, 980, 930, 880 cm^{-1}, δ_H 6.80 (2H, dd, *J*=15 and 11, C11, C11′-*H*), 6.60 (AA′ of AA′BB′ spin system, C15, C15′-*H*), 6.35 (2H, d, *J*=15, C12, C12′-*H*), 6.29 (2H, d, *J*=11, C10, C10′-*H*), 6.25 (BB′ of AA′BB′ spin system, C14, C14′-*H*), 5.52 (2H, C6, C6′-*H*), 5.10 (2H, br, C2, C2′-*H*), 2.16 (8H, m, 4×CH_2), 1.98 (12H, 4×=CMe), 1.95 (6H, 2×=CMe), 1.69 (6H, 2×=CMe), 1.61 (6H, 2×=CMe) ppm; δ_C 152.1 (s, =C), 137.3 (d, =CH), 136.6 (s, =C), 135.7 (d, =CH), 133.1 (d, =CH), 132.3 (s, =C), 130.2 (d, =CH), 127.2 (d, =CH), 123.5 (d, =CH), 119.9 (s, =C), 105.4 (d, =CH), 94.9 (s, ≡C), 92.4 (s, ≡C), 39.0 (t, CH_2), 26.4 (t, CH_2), 25.8 (q, CH_3), 23.6 (q, CH_3), 19.6 (q, CH_3), 17.8 (q, CH_3), 12.8 (q, CH_3) ppm; *m/z* 532.4058; $C_{40}H_{52}$ requires *M* 532.4069.

3.1.9. Prolycopene (2). Lindlar's catalyst (10 mg) was added to freshly distilled, degassed ethyl acetate (5 ml) in a microhydrogenator in the dark, and the system was then flushed out three times with hydrogen. A solution of the tetrahydrolycopene (**3**) (10 mg, 0.019 mmol) in ethyl acetate (2 ml) was added and hydrogen uptake started immediately. After 30 s hydrogen uptake had ceased! The catalyst was filtered off and the ethyl acetate was then evaporated in vacuo to leave a bright orange oil. Analysis by HPLC showed that the oil was a mixture of compounds, the major one of which was isoretentive with authentic prolycopene. The prolycopene was separated by HPLC to give a red solid (<1 mg), which showed λ_{max} (*n*-hexane) 417, 438, 468 nm; δ_H 6.62–5.95 (16H, m), 5.07 (2H, br), 2.05–2.07 (8H, m), 1.98 (6H, 2×CH_3), 1.87 (6H, 2×CH_3), 1.78 (6H, 2×CH_3), 1.65 (6H, 2×CH_3), 1.56 (6H, 2×CH_3) ppm; *m/z* 536.4388; $C_{40}H_{56}$ requires *M* 536.4382. The synthetic prolycopene did not separate from authentic, naturally derived prolycopene in chromatographic analysis and their visible absorption, PMR spectroscopic data, together with mass spectrometry data were closely identical.

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References and notes

1. Zechmeister, L.; LeRosen, A. L.; Went, F. W.; Pauling, L. *Proc. Natl. Acad. Sci. U.S.A.* **1941**, *27*, 468–474.
2. (a) Englert, G.; Brown, B. O.; Moss, G. P.; Weedon, B. C. L.; Britton, G.; Goodwin, T. W.; Simpson, K. L.; Williams, R. J. H. *J. Chem. Soc., Chem. Commun.* **1979**, 545–547; (b) Englert, G. *Helv. Chim. Acta* **1979**, *62*, 1497–1500.
3. Clough, J. M.; Pattenden, G. *J. Chem. Soc., Chem. Commun.* **1979**, 616–619.
4. Clough, J. M.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1983**, 3011–3018.
5. Pattenden, G.; Robson, D. C. *Tetrahedron Lett.* **1987**, *28*, 5751–5754.
6. Hengartner, U.; Bernhard, K.; Meyer, K.; Englert, G.; Glinz, E. *Helv. Chim. Acta* **1992**, *75*, 1848–1865.
7. cf. Pattenden, G. *Carotenoid Chemistry and Biochemistry*; Britton, G., Goodwin, T. W., Eds.; Pergamon: Oxford, 1982; pp 87–96.
8. For a recent review on aspects of stereocontrolled polyene synthesis see: Thirsk, C.; Whiting, A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 999–1023. For some recent examples of methods for the construction of polyenes see: Bouchez, L. C.; Vogel, P. *Chem.—Eur. J.* **2005**, *11*, 4609–4620; Lipshutz, B. H.; Clososki, G. C.; Chrisman, W.; Chung, D. W.; Ball, D. B.; Howell, J. *Org. Lett.* **2005**, *7*, 4501–4504; Coleman, R. S.; Walczak, M. C. *Org. Lett.* **2005**, *7*, 2289–2291 and references therein.
9. Lindlar, H. *Helv. Chim. Acta* **1952**, *35*, 446–450.

10. Matsumoto, M.; Kuroda, K. *Tetrahedron Lett.* **1980**, *21*, 4021–4024.
11. See: Negishi, E.; Anastasia, L. *Chem. Rev.* **2003**, *103*, 1979–2077; Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4442–4489; see also: Wipf, P.; Graham, T. H. *J. Am. Chem. Soc.* **2004**, *126*, 15346–15347.
12. Bringmann, G.; Schneider, S. *Synthesis* **1983**, 139–141.
13. Weedon, B. C. L. *Pure Appl. Chem.* **1969**, *20*, 531–543. We thank the late B. C. L. Weedon for providing intermediates used in the synthesis of (**5**).
14. Carey, L.; Clough, J. M.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1983**, 3005–3009.
15. Bernhard, K.; Kienzle, F.; Mayer, H.; Mueller, R. K. *Helv. Chim. Acta* **1980**, *63*, 1473–1490.

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Efficient synthesis of 2,5-diketopiperazines using microwave assisted heating

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Abstract—In this study a general, efficient and environmentally benign solution phase synthesis of 2,5-diketopiperazines (DKPs) using microwave assisted heating in water is described. A series of 11 structurally different DKPs have been synthesized from dipeptide methyl esters. A range of common laboratory solvents have been tested as well as different reaction times and temperatures. Both classic thermal and microwave assisted heating have been investigated. Microwave assisted heating for 10 min using water as solvent proved, by far, to be the most efficient method of cyclization giving moderate to excellent yields (63–97%) of DKPs. In contrast to other published procedures, this method seems independent of the amino acid sequence.

1. Introduction

2,5-Diketopiperazines (DKPs) are cyclic dipeptide derivatives, which show a multitude of interesting biological activities,[1] e.g., efficient interactions with opioid receptors,[2] potent cytotoxic effects via a variety of mechanisms[3–5] and neuroprotective effects.[6] Recently, they have also been associated with blockade of L-type calcium channels,[7] tryptase inhibition,[8] oxytocin receptor antagonism[9] and plasminogen activator inhibition.[10] For some time we have been working on the use of suitable scaffolds for the development of novel peptidomimetics and in that context we have become interested in DKP derivatives. However, to be able to synthesize and investigate the properties of large numbers of DKPs an efficient, robust and reproducible method for their synthesis is needed.

DKPs can be synthesized from the corresponding dipeptides both in solution and on solid phase. There are many reports in the literature of general methods for solid phase synthesis.[11] They are all in small scale and due to the problems with scaling-up of solid phase reactions, mainly for economic reasons, this is a less useful procedure for large-scale synthesis of DKPs.[12] In contrast, for syntheses in the solution phase there are no general procedures available in the literature which lead to high yields of DKPs independent of the amino acid composition. In solution, the methods are generally based on cyclization of dipeptide methyl esters,[11] or direct cyclization of unprotected dipeptides.[11–13] Several of the reported methods have shortcomings, e.g., the Fischer method,[14] in which the dipeptide methyl esters are subjected to excess ammonia, has been reported to cause epimerization to a varying extent.[15,16a] A method based on the cyclization of the dipeptide methyl ester in toluene/2-butanol (1:4) has been reported[16a] to give generally good yields. However, there have also been reports of low yielding reactions using this method.[15,16b] Unfortunately, when using any of these or similar reaction conditions for the synthesis of DKPs we were not able to reproduce the reported results, even if high temperatures and long reaction times were used. In our hands, only dipeptides containing the conformationally restricted amino acid proline cyclized successfully. We therefore set out to identify the optimal reaction conditions for an efficient and general synthetic procedure for DKPs. To accomplish that we have used a series of 11 dipeptide methyl esters as starting materials and investigated the influence of amino acid composition, solvent, reaction time and reaction temperature, the latter using both thermal and microwave heating. During the last decade microwave assisted heating has proven to be highly successful in speeding up reactions otherwise run for long periods of time, but to the best of our knowledge the use of microwave heating for the formation of DKPs has only been investigated to a limited extent.[17,18]

In the present study it was found that cyclization of any of the tested dipeptide methyl ester hydrochlorides in water using microwave assisted heating reproducibly resulted in high to excellent yields of the corresponding DKPs.

Keywords: Diketopiperazines; Microwave heating; Dipeptide synthesis; Peptide cyclization.
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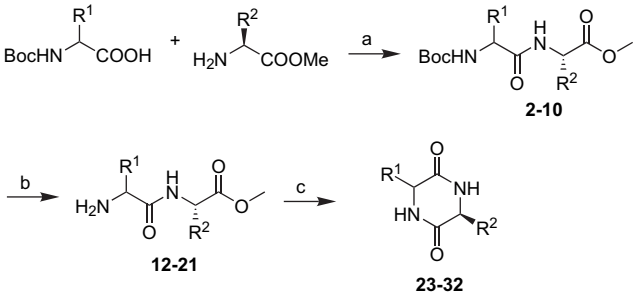
2. Results and discussion

Our synthetic procedure to obtain DKPs involved three steps (Scheme 1). First, the dipeptide methyl ester derivatives were formed via coupling of an *N*-Boc-protected amino acid with an amino acid methyl ester using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). EDC was chosen as it can easily be removed from the crude reaction mixture by extraction with 10% aqueous citric acid. In the second step, the Boc-group was cleaved using HCl–saturated methanol to afford the dipeptide methyl esters as their hydrochloride salts. Both the coupling reactions and the Boc-deprotection reactions proceeded in high yields (84–94% and 80–94% isolated yield, respectively). (For experimental procedures and structural characterization of compounds **1**–**22**, see Supplementary data.) In the final step, the dipeptide was cyclized in the presence of triethylamine as the base. For this step several different reaction conditions were tested and the results are discussed below.



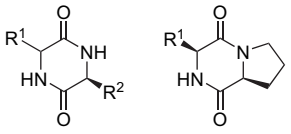
Scheme 1. Reagents: (a) EDC/NMM, CH_2Cl_2; (b) HCl (g)/MeOH; (c) H_2O, 2.5 equiv Et_3N/thermal or microwave heating.

As there are reports in the literature on microwave assisted Boc-removal[17–19] it was first investigated whether the Boc-protected dipeptides could be directly used for DKP formation both in thermally and microwave heated reactions. Dipeptide **2** (Boc-Phe–TrpOMe)[20] was therefore heated to reflux overnight in toluene/2-butanol (1:4), water, toluene and in *tert*-butanol, but unfortunately incomplete removal of the Boc-group was observed in all the reactions. Therefore, to allow comparisons of classical thermal heating and microwave assisted heating, the hydrochloride salts of the dipeptide methyl esters were used as starting materials in all cyclization reactions.

2.1. Optimization of individual reaction parameters

2.1.1. Choice of amino acids. To identify the optimal reaction conditions for the cyclization of dipeptides to DKPs a series of 11 dipeptide methyl ester hydrochlorides (**12**–**22**) were synthesized and cyclized to form the corresponding DKPs (**23**–**33**, Table 1). The amino acids were chosen to cover a range of physico-chemical properties such as polarity, conformational flexibility, steric and electrostatic properties. Thus, DKPs containing two sterically demanding aromatic side chains (**23**) or flexible aliphatic and/or aromatic side chains of varying size (**26**, **27**, **29**–**32**) have been synthesized (Scheme 1). In addition, DKPs containing functionalized amino acids (**24**, **25**, **28** and **32**) have also been investigated.

Table 1. Structures of the synthesized diketopiperazines



Compound	R^1	R^2
23	Benzyl	(3-Indolyl)–CH_2
24	(3-Indolyl)–CH_2	CH_2OH
25	(3-Indolyl)–CH_2	CH_2OBn
26	Benzyl	Butyl
27	Benzyl	Isobutyl
28	(3-Indolyl)–CH_2	CH_2CONH_2
29	L-Isopropyl	Butyl
30	D-Isopropyl	Butyl
31	H	Butyl
32	Isobutyl	4-OH–benzyl
33	Benzyl	—

Compound **30** was synthesized in order to investigate the influence of a D-amino acid on the cyclization efficiency, using the L-diastereomer **29** as comparison (see below). Compound **33** was synthesized as a reference compound as it is well known that proline facilitates the formation of DKPs.[11]

2.1.2. Choice of solvent, heating method and optimization of reaction time and temperature. To investigate the influence of solvent properties on the DKP formation dipeptide **16** (Phe–LeuOMe)[21] was used as a test compound. A range of common laboratory solvents were tested (Table 2) including the solvent mixture toluene/2-butanol (1:4) previously used in DKP synthesis.[16a] The reactions were stirred efficiently as DKPs are known to easily form gels in some solvents.[22] The reaction rates for the thermally heated reactions were slow according to TLC, and the reactions had to be heated to reflux for 12 h. The isolated yields of the cyclized product **27** varied from 5–6% in highly polar solvents such as water, DMF and MeOH to 36–38% in *tert*-butanol and toluene (Table 2).

Using microwave assisted heating for the cyclization of **16** gave low yields of **27** in all solvents except for water

Table 2. Isolated yields of **27** obtained in the cyclization reactions using 50 mg of **16** in the presence of 2.5 equiv of triethylamine

Solvent	Yield (%)	
	Δ	MW
Toluene	38	8
tert-Butanol	36	12
Acetonitrile	33	5
1,2-Dichloroethane	33	6
Toluene/2-butanol (1:4)	33	10
1,2-Dimethoxyethane	25	6
tert-Butanol/H_2O (1:1)	24	9
Benzene	17	5
CCl_4	16	5
1,4-Dioxane	10	7
Methanol	6	5
DMF	5	6
H_2O	5	67

The thermally heated reactions (Δ) were heated to reflux for 12 h and the microwave assisted (MW) reactions were heated for 10 min at a temperature 40 °C above the boiling point of the solvent. Each reaction was performed at least twice.

(Table 2), which resulted in the formation of **27** in 67% yield. The other solvents tested gave only low yields (5–12%) of product and resulted in complex reaction mixtures according to TLC.

For the microwave assisted reactions the yields were highest when run at a temperature of 40 °C above the boiling point of the solvents. This was shown for the cyclization of **16** in water for which the yields of **27** increased from 5% at 120 °C, to 67% at 140 °C, 63% at 150 °C and then decreased to 44% at 170 °C. At 170 °C the reaction became yellowish in colour and proved to be difficult to purify as a complex mixture of products was formed, none of the desired product could be isolated from the reaction mixture.

The optimal reaction time in the microwave heated reactions was also investigated. Already after 5 min at 140 °C in water the cyclization of **16** afforded **27** in 53% yield. The yield improved to 67% when heated for 10 min at the same temperature. Longer reaction times such as 20 or 45 min gave no further increase in yield. Therefore the reaction time for all the microwave reactions was set to 10 min.

2.2. Optimization of combined reaction parameters

Based on the results obtained in the test reactions four solvents were finally chosen in the cyclization reactions on all dipeptide methyl ester hydrochlorides synthesized (**12**–**22**): water as it proved to be the only suitable solvent in the microwave assisted reactions, *tert*-butanol and toluene as they usually gave the highest yields in the thermally heated reactions, and the toluene/2-butanol (1:4) mixture. Although giving moderate yields in this work, it has been previously proved to be suitable for the synthesis of DKPs.[16a] Both classical heating (refluxing temperature, 12 h) and microwave mediated heating (40 °C above the boiling point, 10 min) were used. All reactions were run at least twice to secure reproducibility.

The syntheses of **23**–**33** were accomplished with varying success (for isolated yields of products see Table 3). In most cases the DKPs were easy to isolate as they were not soluble to any great extent in any of the solvents tested and precipitated spontaneously upon formation. Thus the work-up procedure was straightforward; the reaction mixture was concentrated in vacuo, the residue was re-suspended in water and the solid product was filtered off, no further purification procedures were necessary (purity >98% according to NMR spectroscopy). Compounds **29**, **30** and **33**, which were partly soluble in the reaction solvent, were purified by flash chromatography as described in Section 4.

Despite the small volume of solvent used (3 mL), there were no signs of polymerization in any of the reactions nor were there any signs of epimerization at any of the temperatures or in any solvents chosen.[11]

Using classical thermal heating the yields of DKPs were generally low or moderate, with *tert*-butanol usually resulting in higher yields than in the other solvents (see Table 3), the only exception being **25**, which was formed in 57% yield in water but only in 10% yield in *tert*-butanol. Some combinations of solvent and dipeptide gave moderate to good yields, e.g., 62% yield of **23** in *tert*-butanol and 41% in water, or compound **31**, which was formed in 88% yield in *tert*-butanol and 51% in the toluene/2-butanol mixture. As described earlier in the literature diastereomeric dipeptides are generally cyclized in significantly different yields,[11] for **29** and **30** a facilitated cyclization to the D-valine containing derivative (**30**) was shown. In fact, **30** was formed considerably faster than **29**, showing signs of cyclization already after 5 min. Compounds **26**, **28** and **29** could not be obtained in yields higher than 10% independent of solvent used, whereas high yields of the proline containing DKP **33** were obtained in all solvents.[11] In conclusion, no general characteristics of the dipeptide that would result in high yields of DKPs in a given solvent could be observed in the thermally heated reactions.

Table 3. Isolated yields obtained in cyclization reactions (50 mg of dipeptide in the presence of 2.5 equiv of triethylamine) using different solvents and heating procedures, classical thermal heating (Δ) or microwave assisted heating (MW)

	Yield (%)/Δ[a]				Yield (%)/MW[b]			
	H_2O	Toluene/2-BuOH[c]	Toluene	*t*-BuOH	H_2O[d]	Toluene/2-BuOH[e]	Toluene[e]	*t*-BuOH[f]
23	41	8	20	62	73	14	18	10
24	<5	9	5	41	81	<5	10	5
25	57	6	5	10	70	6	<5	12
26	5	5	10	10	97	12	12	15
27	7	32	24	37	68	17	14	24
28	<5	<5	7	10	71	21	7	6
29	7	5	<5	8	63	15	8	8
30	35	7	22	41	84	12	<5	6
31	33	51	29	88	70	10	14	10
32	28	23	12	37	83	5	12	<5
33	89	88	83	93	93	73	70	76

Each reaction has been run at least twice with a difference in yield not higher than ±3%.
[a] The reactions were heated to reflux for 12 h.
[b] The reaction time was 10 min.
[c] The reaction temperature was 110 °C.
[d] The reaction temperature was 140 °C.
[e] The reaction temperature was 150 °C.
[f] The reaction temperature was 125 °C.

For the microwave assisted syntheses of **23**–**32** the only suitable solvent was water, giving moderate to excellent yields of cyclized products (63–97%). Reactions in the other solvents gave low yields, varying from <5 to 24% (Table 3), mainly due to the formation of complex mixtures according to TLC. Water on the other hand often gave a dazzling white reaction matrix from which the products were easily separated from the starting materials with no sign of any byproducts. Interestingly, the synthesis of **26**, **28** and **29**, which only produced traces of product using thermal heating, were formed in 97, 71 and 63% yield, respectively, using microwave heating in water.

It is notable that **27** was formed in higher yields than the structurally related **26** in *tert*-butanol, toluene and toluene/2-butanol (1:4) using thermal heating (37, 24 and 32% compared to 10, 10 and <5%) but the other way round in water when using microwave assisted heating (68 and 97%, respectively). This may be explained by the fact that **27** gave a solid gel instead of crystals in the microwave assisted reactions. The reaction mixture might therefore no longer have

the physical properties of a classic solvent. No formation of gels was detected in the thermally heated reactions.

In general, compound **30** was formed in much higher yields than its diastereomer **29** both in the microwave assisted and the classic thermal heated reactions. Dipeptides with different configuration at the α-carbons probably give less steric hindrance in the *cis*-amide conformation. In addition, the steric hindrance between the two side chains in **30** is lower than that in **29** because of its *trans*-configuration.[11] Interestingly, the solubility of **30** was shown to be higher than that of **29** in both water and MeOH.

As expected for cyclization of dipeptides containing a proline residue and thereby a higher *cis*-amide content[23] the yields of **33** were excellent in all solvents using both heating methods (70–96%). However, using thermal heating the formation of **33** was still quite slow so the reaction had to be heated to reflux for 12 h.

The dipeptides containing serine or benzyl-protected serine residues (**13** and **14**, respectively) were possible to cyclize in acceptable yields using different solvents: unprotected **13** gave 41% yield of **24** in *tert*-butanol whereas **14** preferentially cyclized in water producing **25** in 57% yield. Both dipeptides cyclized efficiently in water using microwave heating, producing **24** and **25** in 81 and 70% yield, respectively. Debenzylation of **25** using catalytic hydrogenation (5% Pd/C in EtOH) proceeded smoothly and **24** could be isolated in high yields (96%) (data not shown).

2.3. Conformational effects observed in 23, 26 and 32

During the structural characterization of the DKPs several derivatives showed extraordinary chemical shifts in ^{1}H NMR spectra. To explain these unexpected results computer assisted molecular modelling was performed using the MacroModel program (v 7.1) and the Amber 94 force field. It is known from the literature that DKPs containing aromatic side chains can adopt extraordinary conformations in solution as the otherwise rather flexible aromatic rings often choose severely restricted conformations.[2b,24] This was also observed for some of the DKPs obtained in this study, e.g., strong shielding effects in ^{1}H NMR spectra of compound **23** indicated that one of the benzylic protons in phenylalanine was affected by the aromatic ring of the tryptophane residue.[25] The chemical shift for this proton was δ 1.40 compared to δ 2.60 for the other β-proton.

Computer assisted conformational searches of compound **23** (performed both in vacuum and in simulated water or chloroform environments) corroborated this finding as the global minimum and other low energy conformers (ΔE<3.7 kJ/mol) showed that either one of the four β-protons could be shielded (Fig. 1, above left).

Also in the ^{1}H NMR spectra of compound **32** a similar shielding effect was observed on the signal from the CH_2-group in the leucine side chain. This resulted in a chemical shift of δ 0.10 compared to δ 1.54 for the same signal in **27** where no shielding effect was observed. Conformational analysis of **32** showed a global minimum conformation and low energy conformations in which the hydrogen atoms of the CH_2-group were directed towards the aromatic ring of tyrosine (Fig. 1, above right). Interestingly, the published X-ray crystal structure of **32**[26] shows the same conformational effects as those observed in solution and in the computer calculations in this study.

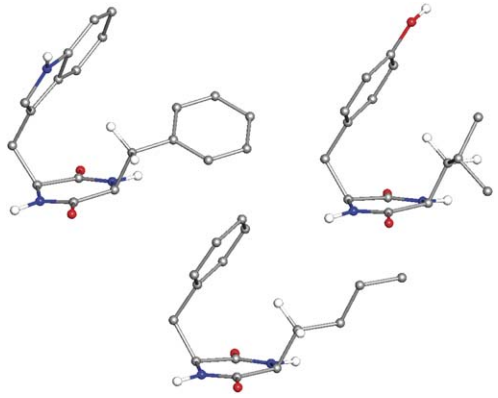


Figure 1. Global minimum conformations (Amber 94 force field, MacroModel v 7.1) of **23** (above left), **32** (above right) and **26** (below). In **23** the shielded benzylic hydrogen atom in phenylalanine is shown. In **32** and **26** the shielded β-hydrogens in leucine and norleucine, respectively, are shown. The other hydrogens in the structures have been omitted for clarity.

A similar shielding effect was also observed in the ^{1}H NMR spectra of **26** in which the β-methylene protons of norleucine were experiencing different magnetic environments. The shielding effect was also observed in the computer modelling (Fig. 1, lower middle). No shielding effects were observed in the NMR spectra of **27**, this is especially interesting as compound **27** is an isomer of **26**.

3. Conclusions

In this study we have developed a general, efficient and environmentally benign synthetic procedure for the formation of DKPs from the corresponding dipeptide methyl ester hydrochlorides. The results show that the highest yielding way to synthesize DKPs is to use microwave assisted heating with water as solvent. The reactions were run only for short times (10 min), and as most products precipitated during the reaction the work-up procedure was simple, and the products isolated in moderate to excellent yields (63–97%). Although classic thermal heating provided good yields for some derivatives, the optimal conditions for a certain dipeptide could not be predicted. The observations of constrained conformations of the DKPs in ^{1}H NMR spectra have been confirmed by computational analyses.

4. Experimental

4.1. General

All reagents and solvents were of analysis or synthesis grade. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL JNM-EX 400-spectrometer at 400 and 100 MHz, respectively. The designations of atoms for interpretation of NMR spectra are given in Figure 2. The reactions were monitored by thin-layer chromatography (TLC), on silica plated aluminium

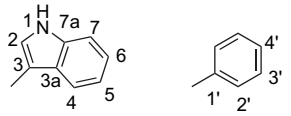


Figure 2. Designation of atoms for interpretation of NMR data.

sheets (silica gel 60 F254, E. Merck), detecting spots by UV and/or 2% ninhydrin in ethanol followed by heating. Column chromatography was performed on wet packed silica (silica gel 60 (0.040–0.063 mm), E. Merck) using flash chromatography. Melting points were measured in a Büchi Melting Point B-540 apparatus and are uncorrected. Optical rotations were measured at room temperature with a Perkin–Elmer 341 LC polarimeter. The microwave reactions were carried out in a Biotage Initiator instrument with a fixed hold time. The IR spectra were obtained on a Perkin–Elmer 16 PC spectrometer. Elemental analyses were performed at Mikrokemi AB, Uppsala, Sweden and at Kolbe Mikroanalytisches Laboratorium, Mülheim and der Ruhr, Germany. Conformational analyses were performed using the Amber 94 force field as implemented in the MacroModel program 7.1 run on a Silicon Graphics Octane workstation.

The synthetic procedure and characterization of compounds **1**–**22** are found in the Supplementary data. Compounds **23**, **27** and **33** are commercially available.

4.2. General procedure for coupling reactions using EDC (Scheme 1)

The methyl ester of the C-terminal amino acid was dissolved in dry solvent (CH_2Cl_2 or DMF) (10 mL), followed by addition of NMM. The reaction mixture was stirred for 40 min at 0 °C whereupon EDC and the Boc-protected N-terminal amino acid were added. The reaction was thereafter stirred for 3 h at 0 °C and then overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and extracted with 10% aqueous citric acid. The organic layer was dried ($MgSO_4$), concentrated in vacuo and the crude product was purified by flash chromatography.

4.3. General procedure for dipeptide cyclization (Scheme 1)

The hydrochloride salt of the deprotected dipeptide was dissolved in the solvent (H_2O, *tert*-butanol, toluene/2-butanol (1:4) or toluene) (3 mL) and 2.5 equiv of triethylamine was added. In each reaction 50 mg of each compound was used. The microwave assisted heated reactions were run for 10 min and the classic thermally heated reactions were heated to reflux overnight. The crude product precipitated spontaneously and the reaction mixture was concentrated in vacuo, suspended in H_2O and filtered. In the microwave assisted reaction the procedure was the same with the exceptions that the reaction temperature was set at 40 °C above the boiling point of the solvent. For choice of solvents and the isolated yields of the reactions see Table 3.

4.3.1. c(L-Phenylalaninyl-L-tryptophanyl) (23). Compound **12** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **23** was isolated as white crystals.

Mp 284–286 °C (lit.[27] mp 284 °C). $[\alpha]_D$ −174.4 (*c* 0.3, CH_3OH) (lit.[28] $[\alpha]_D$ −245.9 (*c* 1, CH_3OH)). IR (KBr) ν_{max} 3420, 3050, 1670, 1456, 1328 cm^{-1}.[28,29] ^1H NMR (CD_3OD) δ 7.59 (d, *J*=7.3 Hz, 1H, indole), 7.34 (d, *J*=7.3 Hz, 1H, indole), 7.19–7.01 (m, 6H, indole and Ph-*H*), 6.61–6.57 (m, 2H, indole and Ph-*H*), 4.19–4.16 (m, 1H, α-C*H*), 3.98–3.88 (m, 1H, α-C*H*), 3.03 (dd, *J*=13.4, 3.5 Hz, 1H, CH_2-indole), 2.85–2.80 (m, 1H, CH_2-Ph), 2.60 (dd, *J*=13.4, 3.5 Hz, 1H, CH_2-indole), 1.44–1.38 (m, 1H, CH_2-Ph). ^{13}C NMR (CD_3OD) δ 165.6, 164.7 (C═O, amides), 136.3, 135.9 (C-1′ and C-7a), 129.5, 128.2 (C-2′ and C-3′), 127.7 (C-3a), 126.6 (C-4′), 124.5 (C-2), 121.4 (C-6), 118.9, 118.7 (C-4 and C-5), 115.0 (C-7), 111.2 (C-3), 56.4, 55.8 (α-*C*H), 40.2 (*C*H_2-Ph), 29.8 (*C*H_2-indole). Anal. Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60; Found C, 72.0; H, 5.9; N, 12.6.

4.3.2. c(L-Tryptophanyl-L-serinyl) (24). Compound **13** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **24** was isolated as white crystals.

Mp 268–269 °C. $[\alpha]_D$ −100 (*c* 0.5, CH_3OH). IR (KBr) ν_{max} 3412, 3349, 3204, 1733, 1635 cm^{-1}. ^1H NMR (CD_3OD) δ 7.60 (d, *J*=8.1 Hz, 1H, H-6-indole), 7.34 (d, *J*=8.1 Hz, 1H, H-3-indole), 7.13-7.05 (m, 2H, indole), 7.04–6.98 (m, 1H, indole), 4.24–4.19 (m, 1H, α-C*H*), 3.83–3.81 (m, 1H, α-C*H*), 3.41–3.33 (m, 2H, CH_2-Ser), 3.30–3.26 (m, 1H, CH_2-indole), 2.91–2.85 (m, 1H, CH_2-indole). ^{13}C NMR (CD_3OD) δ 168.8, 163.5 (C═O, amides), 136.7 (C-7a), 127.6 (C-3a), 124.1 (C-2), 121.2 (C-6), 118.8, 118.4 (C-4 and C-5), 111.0 (C-7), 108.5 (C-3), 63.4 (*C*H_2OH), 57.5, 56.1 (α-*C*H), 30.6 (*C*H_2-indole). Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38; Found C, 61.4; H, 5.6; N, 15.6.

4.3.3. c(L-Tryptophanyl-*O*-benzyl-L-serinyl) (25). Compound **14** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **25** was isolated as white crystals.

Mp 250–251 °C. $[\alpha]_D$ −52.4 (*c* 0.45, DMSO). IR (KBr) ν_{max} 3338, 2947, 1725, 1674 cm^{-1}. ^1H NMR (DMSO-d_6) δ 10.92 (s, 1H, N*H*, indole), 8.05 (dd, *J*=2.6 Hz, 2H, N*H*, amides), 7.36–6.92 (m, 10H, Ar-*H*), 4.18 (d, *J*=12.0 Hz, 1H, CH_2-Ph), 4.10 (d, *J*=12.0 Hz, 1H, CH_2-Ph), 4.08–4.05 (m, 1H, α-C*H*), 3.79 (ddd, *J*=6.0, 2.8, 2.6 Hz, 1H, α-C*H*), 3.24–3.16 (m, 2H, CH_2-Ser), 3.07 (dd, *J*=14.3, 4.4 Hz, 1H, CH_2-indole), 2.54–2.50 (m, 1H, CH_2-indole). ^{13}C NMR (DMSO-d_6) δ 167.7, 165.5 (C═O, amides), 138.4, 136.5, 128.7, 128.2 (C-2′ and C-3′), 128.0, 124.9, 121.4, 119.3, 119.0 (Ar), 111.8 (C-7), 109.4 (C-3), 72.7, 72.2 (*C*H_2-Ser and *C*H_2-Ph), 56.0, 55.7 (α-*C*H), 30.6 (*C*H_2-indole). Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56; Found C, 69.3; H, 5.8; N, 11.5.

4.3.4. c(L-Phenylalaninyl-L-norleucinyl) (26). Compound **15** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **26** was isolated as white crystals.

Mp 269 °C. $[\alpha]_D$ −20 (*c* 0.2, CH_3OH). IR (KBr) ν_{max} 3425, 3057, 1976, 1578, 1425 cm^{-1}. ^1H NMR (CD_3OD) δ 7.30–7.17 (m, 5H, Ph-*H*), 4.32–4.29 (m, 1H, α-C*H*), 3.68–3.64

(m, 1H, α-C*H*), 2.97–2.91 (m, 2H, CH_2-Ph), 1.15–1.04 (m, 3H, CHCH_2-Nle and CH_2CH$_3$-Nle), 0.91–0.82 (m, 2H, CH$_2$CH_2-Nle), 0.78 (dt, *J*=7.3, 1.8 Hz, 3H, CH_3-Nle), 0.53–0.47 (m, 1H, CHCH_2-Nle). ^{13}C NMR (CD$_3$OD) δ 168.8, 167.6 (C=O, amides), 135.4 (C-1′), 130.4, 128.2 (C-2′ and C-3′), 127.1 (C-4′), 56.0, 54.6 (α-*C*H), 38.7 (*C*H$_2$-Ph), 33.8 (CH*C*H$_2$-Nle), 26.4 (CH$_2$*C*H$_2$-Nle), 21.9 (*C*H$_2$CH$_3$-Nle), 12.9 (*C*H$_3$-Nle). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; Found C, 68.9; H, 7.6; N, 10.9.

4.3.5. c(L-Phenylalaninyl-L-leucinyl) (27). Compound **16** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **27** was isolated as white crystals.

Mp 282–284 °C. [α]$_D$ −8.0 (*c* 0.3, CH$_3$OH). IR (KBr) ν_{max} 3430, 3012, 1666, 1570, 1389 cm^{-1}.[21,30] ^{1}H NMR (CDCl$_3$) δ 7.36–7.18 (m, 5H, Ph-*H*), 4.28–4.24 (m, 1H, α-C*H*), 3.90–3.85 (m, 1H, α-C*H*), 3.28–3.22 (m, 1H, CH_2-Ph), 3.09–3.02 (m, 1H, CH_2-Ph), 1.54 (app s, 2H, CH_2-Leu), 1.24 (app s, 1H, C*H*-Leu), 0.86 (app t, *J*=6.2 Hz, 6H, CH_3-Leu). ^{13}C NMR (CDCl$_3$) δ 171.6, 168.0 (C=O, amides), 135.1 (C-1′), 129.9, 129.2 (C-2′ and C-3′), 127.7 (C-4′), 56.3, 53.3 (α-*C*H), 42.9 (*C*H$_2$-Leu), 40.3 (*C*H$_2$-Ph), 24.1 (*C*H-Leu), 23.2, 20.9 (*C*H$_3$-Leu). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; Found C, 69.0; H, 7.8; N, 10.5.

4.3.6. c(L-Tryptophanyl-L-asparginyl) (28). Compound **17** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **28** was isolated as off-white crystals.

Mp 272–274 °C. [α]$_D$ −36 (*c* 0.2, DMSO). IR (KBr) ν_{max} 3450, 3207, 3048, 1672, 1559, 1507 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 10.90 (s, 1H, N*H*, indole), 7.94 (d, *J*=1.8 Hz, 1H, N*H*, amide), 7.67 (d, *J*=1.5 Hz, 1H, N*H*, amide), 7.57 (d, *J*=7.7 Hz, 1H, indole), 7.12 (s, 1H, indole), 7.05 (t, *J*=7.0 Hz, 1H, indole), 6.96 (t, *J*=7.5 Hz, 1H, indole), 6.91 (d, *J*=7.7 Hz, 1H, indole), 4.13 (t, *J*=4.2 Hz, 1H, α-C*H*), 3.99 (dd, *J*=7.0, 3.7 Hz, 1H, α-C*H*), 3.21 (dd, *J*=14.5, 4.6 Hz, 1H, CH_2-indole), 3.10 (dd, *J*=14.5, 4.6 Hz, 1H, CH_2-indole), 2.18 (dd, *J*=15.7, 4.4 Hz, 1H, CH_2-Asn), 1.48 (dd, *J*=15.7, 8.1 Hz, 1H, CH_2-Asn). ^{13}C NMR (DMSO-d_6) δ 172.0 (C=O, Asn), 167.8, 167.5 (C=O, amides), 136.5 (C-7a), 128.2 (C-3a), 125.0 (C-2), 121.4, 119.4, 119.0 (C-4, C-5 and C-6), 111.8, 109.4 (C-3 and C-7), 55.8 (α-*C*H, Trp), 51.9 (α-*C*H, Asn), 38.8 (*C*H$_2$-Asn), 29.2 (*C*H$_2$-indole). Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66; Found C, 59.9; H, 5.6; N, 18.3.

4.3.7. c(L-Valinyl-L-norleucinyl) (29).[31] Compound **18** and triethylamine were reacted as described in the general procedure (Section 4.3). The crude product had to be purified by flash chromatography using CH$_2$Cl$_2$/CH$_3$OH (95:5) as eluent. Pure **29** was isolated as white crystals.

Mp 254–256 °C. [α]$_D$ −87.7 (*c* 0.4, CH$_3$OH). IR (KBr) ν_{max} 3310, 3019, 1800, 1640 cm^{-1}. ^{1}H NMR (CD$_3$OD) δ 3.96–3.93 (m, 1H, α-C*H*), 3.83–3.81 (m, 1H, α-C*H*), 2.30–2.22 (m, 1H, C*H*-Val), 1.87–1.82 (m, 1H, CHCH_2-Nle), 1.79–1.74 (m, 1H, CHCH_2-Nle), 1.43–1.32 (m, 4H, CH_2-Nle), 1.04 (d, *J*=7.3 Hz, 3H, CH_3-Val), 0.94 (d, *J*=7.3 Hz, 3H, CH_3-Val), 0.93 (t, *J*=7.0 Hz, 3H, CH_3-Nle). ^{13}C NMR (CD$_3$OD) δ 169.5, 168.4 (C=O, amides), 60.1, 54.6 (α-*C*H), 34.1, 32.0 (*C*H-Val and CH*C*H$_2$-Nle), 27.1 (CH$_2$*C*H$_2$-Nle), 22.1 (*C*H$_2$CH$_3$-Nle), 17.9, 16.3 (*C*H$_3$-Val), 12.9 (*C*H$_3$-Nle). Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.23; H, 9.50; N, 13.20; Found C, 62.4; H, 9.8; N, 13.5.

4.3.8. c(D-Valinyl-L-norleucinyl) (30).[31] Compound **19** and triethylamine were reacted as described in the general procedure (Section 4.3). The crude product had to be purified by flash chromatography using CH$_2$Cl$_2$/CH$_3$OH (95:5) as eluent. Pure **30** was isolated as white crystals.

Mp 263–265 °C. [α]$_D$ −6.6 (*c* 0.3, CH$_3$OH). IR (KBr) ν_{max} 3324, 3019, 1776, 1589 cm^{-1}. ^{1}H NMR (CD$_3$OD) δ 4.04 (td, *J*=4.8, 1.1 Hz, 1H, α-C*H*), 3.79 (dd, *J*=3.7, 1.1 Hz, 1H, α-C*H*), 2.32–2.24 (m, 1H, C*H*-Val), 1.96–1.86 (m, 1H, CHCH_2-Nle), 1.80–1.71 (m, 1H, CHCH_2-Nle), 1.39–1.27 (m, 4H, CH_2-Nle), 1.03 (d, *J*=7.0 Hz, 3H, CH_3-Val), 0.94 (d, *J*=7.0 Hz, 3H, CH_3-Val), 0.92 (t, *J*=7.0 Hz, 3H, CH_3-Nle). ^{13}C NMR (CD$_3$OD) δ 169.6, 169.0 (C=O, amides), 60.2, 54.0 (α-*C*H), 32.8, 32.2 (*C*H-Val and CH*C*H$_2$-Nle), 25.7 (CH$_2$*C*H$_2$-Nle), 22.2 (*C*H$_2$CH$_3$-Nle), 17.5, 15.7 (*C*H$_3$-Val), 12.9 (*C*H$_3$-Nle). Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.23; H, 9.50; N, 13.20; Found C, 62.0; H, 9.8; N, 13.5.

4.3.9. c(Glycinyl-L-norleucinyl) (31).[32] Compound **20** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **31** was isolated as fluffy white crystals.

Mp 256 °C. [α]$_D$ −1.4 (*c* 1, DMSO). IR (KBr) ν_{max} 3200, 2954, 1682, 1468, 1337 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 8.18 (s, 1H, N*H*-amide), 7.98 (s, 1H, N*H*-amide), 3.81–3.63 (m, 3H, CH_2-Gly and α-C*H*), 1.71–1.61 (m, 2H, CHCH_2-Nle), 1.33–1.23 (m, 4H, CH_2-Nle), 0.87 (t, *J*=7.4 Hz, 3H, CH_3-Nle). ^{13}C NMR (DMSO-d_6) δ 168.6, 166.6 (C=O, amides), 54.7 (α-*C*H), 44.8 (*C*H$_2$-Gly), 33.2 (CH*C*H$_2$-Nle), 26.8 (CH$_2$*C*H$_2$-Nle), 22.5 (*C*H$_2$CH$_3$-Nle), 14.4 (*C*H$_3$-Nle). Anal. Calcd for $C_8H_{14}N_2O_2$: C, 56.45; H, 8.29; N, 16.46; Found C, 56.5; H, 8.3; N, 16.1.

4.3.10. c(L-Leucinyl-L-tyrosinyl) (32). Compound **21** and triethylamine were reacted as described in the general procedure (Section 4.3). Pure **32** was isolated as white crystals.

Mp 301–303 °C (lit.[33] mp 295–296 °C). [α]$_D$ 33.3 (*c* 0.3, CH$_3$OH). IR (KBr) ν_{max} 3306, 3206, 2953, 1667, 1467 cm^{-1}. ^{1}H NMR (CD$_3$OD) δ 6.99 (d, *J*=8.4 Hz, 2H, Ph-*H*), 6.70 (d, *J*=8.4 Hz, 2H, Ph-*H*), 4.22 (t, *J*=4.0 Hz, 1H, α-C*H*), 3.65 (dd, *J*=10.0, 4.2 Hz, 1H, α-C*H*), 3.19 (dd, *J*=13.5, 3.7 Hz, 1H, CH_2-Ph), 2.81 (dd, *J*=13.5, 3.7 Hz, 1H, CH_2-Ph), 1.47–1.36 (m, 1H, C*H*-Leu), 0.87 (ddd, *J*=13.8, 9.6, 4.4 Hz, 1H, CH_2-Leu), 0.73 (app t, *J*=8.1 Hz, 6H, CH_3-Leu), 0.10 (ddd, *J*=13.8, 9.6, 4.4 Hz, 1H, CH_2-Leu). ^{13}C NMR (CD$_3$OD) δ 171.4, 167.6 (C=O, amides), 157.0 (C-4′), 131.4 (C-2′), 125.7 (C-1′), 115.1 (C-3′), 56.3, 52.8 (α-*C*H), 43.9 (*C*H$_2$-Leu), 38.1 (*C*H$_2$-Ph), 23.3 (*C*H-Leu), 22.1, 20.0 (*C*H$_3$-Leu). Anal. Calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14; Found C, 65.1; H, 7.3; N, 9.9.

4.3.11. c(L-Phenylalaninyl-L-prolinyl) (33). Compound **22** and triethylamine were reacted as described in the general procedure (Section 4.3). The crude product had to be purified by flash chromatography using CH_2Cl_2/CH_3OH (95:5) as eluent. Pure **33** was isolated as white crystals. For ^1H and ^{13}C NMR spectral data see Ref. 34.

Mp 130–132 °C (lit.[35] mp 132 °C). $[\alpha]_D$ −184 (*c* 0.3, CH_2Cl_2). IR (KBr) ν_{max} 3250, 3054, 1679, 1580, 1487 cm^{-1}.[36] ^1H NMR ($CDCl_3$) δ 7.33–7.25 (m, 5H, Ph-*H*), 5.80 (br s, 1H, N*H*, amide), 4.26 (dd, *J*=10.4, 3.5 Hz, 1H, α-C*H*, Phe), 4.05 (t, *J*=7.9 Hz, 1H, α-C*H*), 3.66–3.51 (m, 3H, CH_2-Ph, dd, *J*=14.3, 10.3 Hz, δ-CH_2), 2.78 (dd, *J*=14.3, 10.3 Hz, 1H, CH_2-Ph), 2.33–2.27 (m, 1H, β-CH_2), 2.03–1.83 (m, 3H, β-CH_2 and γ-CH_2). ^{13}C NMR (CD_3OD) δ 169.5, 165.1 (C=O, amides), 136.1 (C-1′), 129.3, 129.2 (C-2′ and C-3′), 127.6 (C-4′), 59.2, 56.3 (α-*C*H), 45.5 (δ-*C*H_2), 36.9 (*C*H_2-Ph), 28.4 (β-*C*H_2), 22.6 (γ-*C*H_2). Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; Found C, 68.7; H, 6.7; N, 11.2.

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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.2006.05.010.

References and notes

1. (a) Prasad, C. *Peptides* **1995**, *16*, 151–164; (b) Prasad, C. *Endocr. Nutr. Control Basic Biol. Funct.* **1993**, 47–57.
2. (a) Balboni, G.; Guerrini, R.; Salvadori, S.; Tomatis, R.; Bryant, S. D.; Bianchi, C.; Attila, M.; Lazarus, L. H. *Biol. Chem.* **1997**, *378*, 19–29; (b) Bryant, S. D.; Balboni, G.; Guerrini, R.; Salvadori, S.; Tomatis, R.; Lazarus, L. H. *Biol. Chem.* **1997**, *378*, 107–114; (c) Crescenzi, O.; Fraternali, F.; Picone, D.; Tancredi, T.; Balboni, G.; Guerrini, R.; Lazarus, L. H.; Salvadori, S.; Temussi, P. A. *Eur. J. Biochem.* **1997**, *247*, 66–73.
3. Cell cycle inhibitors (a) Kanoh, K.; Kohno, S.; Asari, T.; Harada, T.; Katada, J.; Muramatsu, M.; Kawashima, H.; Sekiya, H.; Uno, I. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2847–2852; (b) Wang, H.; Usui, T.; Osada, H.; Ganesan, A. *J. Med. Chem.* **2000**, *43*, 1577–1585; (c) Usui, T.; Kondoh, M.; Cui, C.-B.; Mayumi, T.; Osada, H. *Biochem. J.* **1998**, *333*, 543–548; (d) Wang, H.; Ganesan, A. *Tetrahedron Lett.* **1997**, *38*, 4327–4328; (e) Hayashi, Y.; Orikasa, S.; Tanaka, K.; Kanoh, K.; Kiso, Y. *J. Org. Chem.* **2000**, *65*, 8402–8405; (f) Caballero, E.; Avendano, C.; Menendez, J. C. *J. Org. Chem.* **2003**, *68*, 6944–6951; (g) Kanoh, K.; Kohno, S.; Katada, J.; Takahashi, J.; Uno, I.; Hayashi, Y. *Bioorg. Med. Chem.* **1999**, *7*, 1451–1457; (h) Edmondson, S.; Danishefsky, S. J.; Sepp-Lorenzino, L.; Rosen, N. *J. Am. Chem. Soc.* **1999**, *121*, 2147–2155; (i) Sanz-Cervera, J. F.; Stocking, E. M.; Usui, T.; Osada, H.; Williams, R. M. *Bioorg. Med. Chem.* **2000**, *8*, 2407–2415; (j) Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, *52*, 12651–12666.
4. Induction of maturation: Graz, C. J. M.; Grant, G. D.; Brauns, S. C.; Hunt, A.; Jamie, H.; Milne, P. J. *J. Pharm. Pharmacol.* **2000**, *52*, 75–82.
5. DNA minor groove binders: Boger, D. L.; Fink, B. E.; Hedrick, M. P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1019–1020.
6. Prakash, K. R. C.; Tang, Y.; Kozikowski, A. P.; Flippen-Anderson, J. L.; Knoblach, S. M.; Faden, A. I. *Bioorg. Med. Chem.* **2002**, *10*, 3043–3048.
7. Kilian, G.; Jamie, H.; Brauns, S. C. A.; Dyason, K.; Milne, P. J. *Pharmazie* **2005**, *60*, 305–309.
8. del Fresno, M.; Fernandez-Forner, D.; Miralpeix, M.; Segarra, V.; Ryder, H.; Royo, M.; Albericio, F. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1659–1664.
9. Wyatt, P. G.; Allen, M. J.; Borthwick, A. D.; Davies, D. E.; Exall, A. M.; Hatley, R. J. D.; Irving, W. R.; Livermore, D. G.; Miller, N. D.; Nerozzi, F.; Sollis, S. L.; Szardenings, A. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2579–2582.
10. (a) Folkes, A.; Roe, M. B.; Sohal, S.; Golec, J.; Faint, R.; Brooks, T.; Charlton, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2589–2592; (b) Einholm, A. P.; Pedersen, K. E.; Wind, T.; Kulig, P.; Overgaard, M. T.; Jensen, J. K.; Bodker, J. S.; Christensen, A.; Charlton, P.; Andreasen, P. A. *Biochem. J.* **2003**, *373*, 723–732.
11. Fischer, P. M. *J. Pept. Sci.* **2003**, *9*, 9–35.
12. Dinsmore, C. J.; Beshore, D. C. *Tetrahedron* **2002**, *58*, 3297–3312.
13. (a) Rodionov, I. L.; Rodionova, L. N.; Baidakova, L. K.; Romashko, A. M.; Balashova, T. A.; Ivanov, V. T. *Tetrahedron* **2002**, *58*, 8515–8523; (b) Milne, P. J.; Oliver, D. W.; Roos, H. M. *J. Cryst. Spectrosc. Res.* **1992**, *22*, 643–649; (c) Jeedigunta, S.; Krenisky, J. M.; Kerr, R. G. *Tetrahedron* **2000**, *56*, 3303–3307; (d) Akerlund, J.; Harmeier, S.; Pumphrey, J.; Timm, D. C.; Brand, J. I. *J. Appl. Polym. Sci.* **2000**, *78*, 2213–2218.
14. Fischer, E. *Chem. Ber.* **1906**, *39*, 2893–2931.
15. Lee, S.; Kanmera, T.; Aoyagi, H.; Izumiya, N. *Int. J. Pept. Protein Res.* **1979**, *13*, 207–217.
16. (a) Nitecki, D. E.; Halpern, B.; Westley, J. W. *J. Org. Chem.* **1968**, *33*, 864–866; (b) Eriksson, J.; Arvidsson, P. I.; Davidsson, O. *Chem.—Eur. J.* **1999**, *5*, 2356–2361.
17. Pandey, S. K.; Awasthi, K. K.; Saxena, A. K. *Tetrahedron* **2001**, *57*, 4437–4442.
18. Lopez-Cobenas, A.; Cledera, P.; Sanchez, J. D.; Perez-Contreras, R.; Lopez-Alvarado, P.; Ramos, M. T.; Avendano, C.; Menendez, J. C. *Synlett* **2005**, 1158–1160.
19. Siro, J. G.; Martin, J.; Garcia-Navio, J. L.; Remuinan, M. J.; Vaquero, J. J. *Synlett* **1998**, 147–148.
20. Hartrodt, B.; Neubert, K.; Jakubke, H. D.; Balaspiri, L.; Telegdy, G. *Pharmazie* **1982**, *37*, 403–407.
21. Blaha, K.; Smolikova, J.; Vitek, A. *Collect. Czech. Chem. Commun.* **1966**, *31*, 4296–4311.
22. Hanabusa, K.; Matsumoto, M.; Kimura, M.; Kakehi, A.; Shirai, H. *J. Colloid Interface Sci.* **2000**, *224*, 231–244.
23. Capasso, S.; Vergara, A.; Mazzarella, L. *J. Am. Chem. Soc.* **1998**, *120*, 1990–1995.
24. (a) Kopple, K. D.; Marr, D. H. *J. Am. Chem. Soc.* **1967**, *89*, 6193–6200; (b) Grant, G. D.; Hunt, A. L.; Milne, P. J.; Roos, H. M.; Joubert, J. A. *J. Chem. Crystallogr.* **1999**, *29*, 435–447.
25. (a) Umezawa, Y.; Tsuboyama, S.; Takahashi, H.; Uzawa, J.; Nishio, M. *Tetrahedron* **1999**, *55*, 10047–10056; (b)

Umezawa, Y.; Tsuboyama, S.; Takahashi, H.; Uzawa, J.; Nishio, M. *Bioorg. Med. Chem.* **1999**, *7*, 2021–2026.
26. Suguna, K.; Ramakumar, S.; Kopple, K. D. *Acta Crystallogr.* **1984**, *C40*, 2053–2056.
27. Zahn, H.; Brandenburg, D. *Liebigs Ann. Chem.* **1966**, *692*, 220–300.
28. Kimura, Y.; Tani, K.; Kojima, A.; Sotoma, G.; Okada, K.; Shimada, A. *Phytochemistry* **1996**, *41*, 665–669.
29. Maes, C. M.; Potgieter, M.; Steyn, P. S. *J. Chem. Soc., Perkin Trans. 1* **1986**, 861–866.
30. (a) Pines, H.; Sih, N. C. *J. Org. Chem.* **1965**, *30*, 280–284; (b) Kanzaki, H.; Imura, D.; Nitoda, T.; Kawazu, K. *J. Antibiot.* **2000**, *53*, 58–62.
31. Davis, J. W. *Ann. Chim. (Paris)* **1954**, *9*, 399–430.
32. Siemion, I. Z.; Picur, B. *Org. Magn. Reson.* **1984**, *22*, 171–175.
33. Kopple, K. D.; Ghazarian, H. G. *J. Org. Chem.* **1968**, *33*, 862–864.
34. (a) Takeda, Y.; Fujita, T.; Shingu, T.; Ogimi, C. *Chem. Pharm. Bull.* **1987**, *35*, 2569–2573; (b) Basso, A.; Bradley, M. *Tetrahedron Lett.* **2003**, *44*, 2699–2702.
35. Johne, S.; Groeger, D. *Pharmazie* **1974**, *29*, 181–186.
36. (a) Bobylev, M. M.; Bobyleva, L. I.; Strobel, G. A. *J. Agric. Food Chem.* **1996**, *44*, 3960–3964; (b) Gautschi, M.; Schmid, J. P.; Peppard, T. L.; Ryan, T. P.; Tuorto, R. M.; Yang, X. *J. Agric. Food Chem.* **1997**, *45*, 3183–3189.