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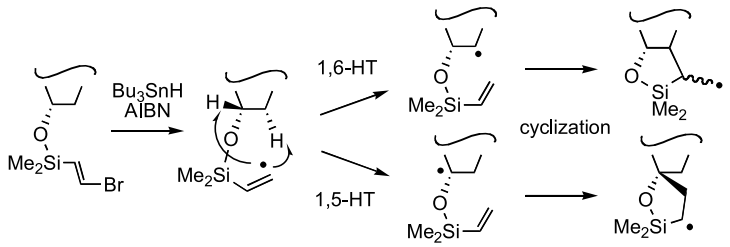
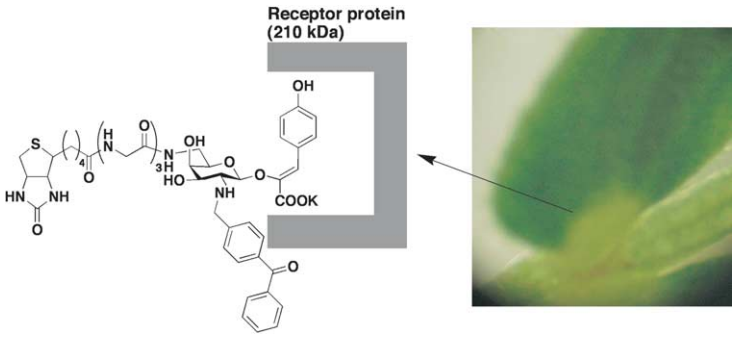
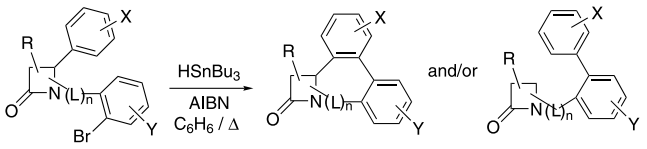
Contents

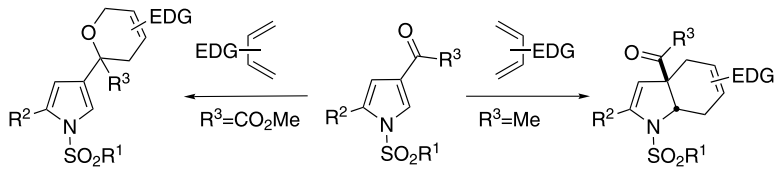
REPORT

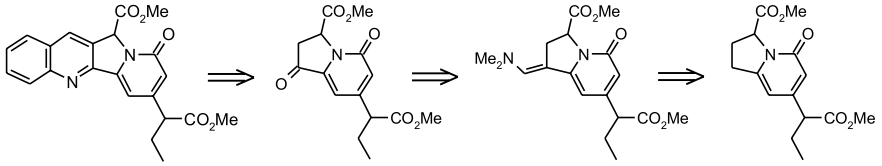
ARTICLES

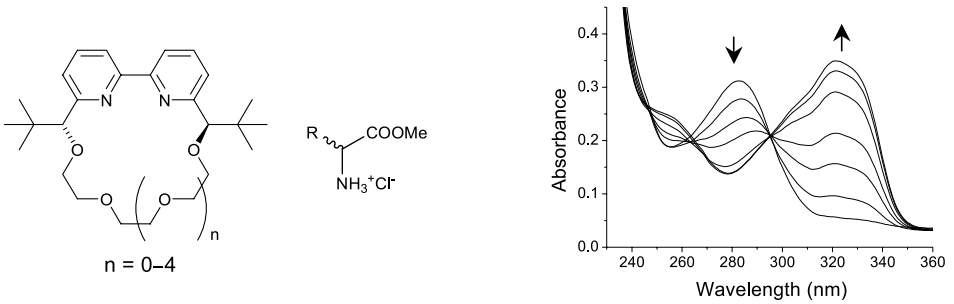


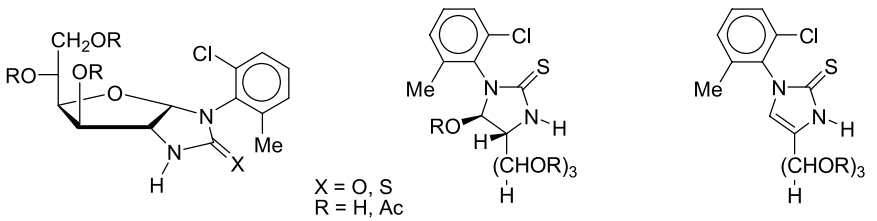




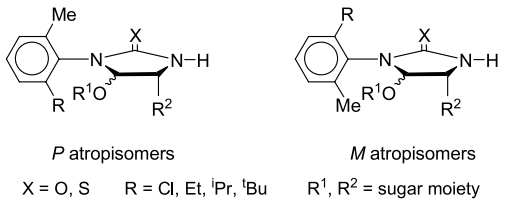




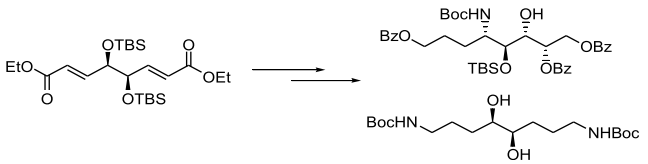


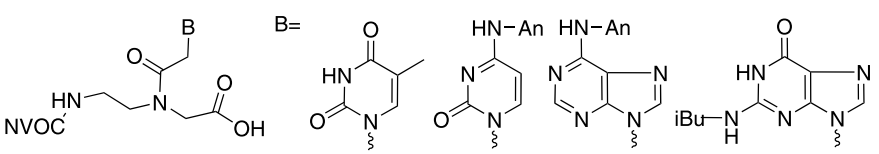


Stable atropisomers of these compounds have been prepared and separated.

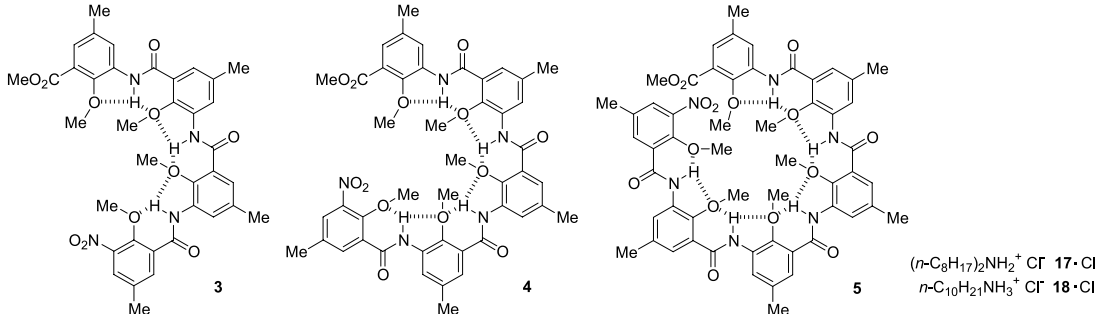


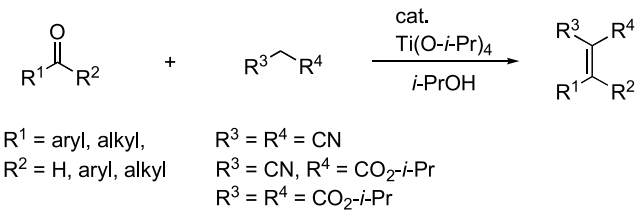
The axial stereochemistry of these atropisomers has been determined by both NMR and X-ray analyses.

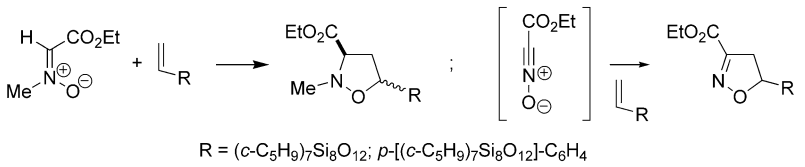


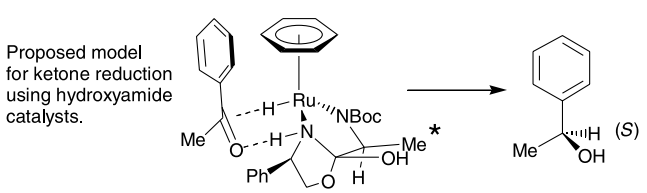


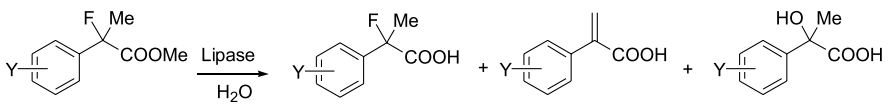
The synthesis of NVOC-protected peptide nucleic acid monomers is described.

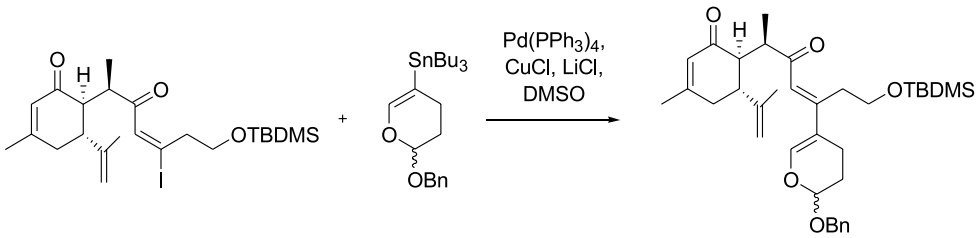


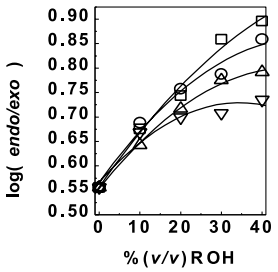




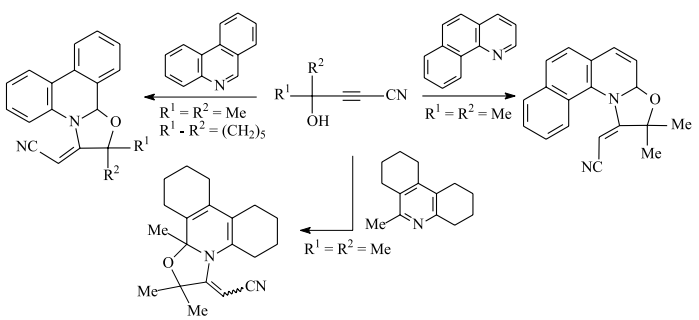


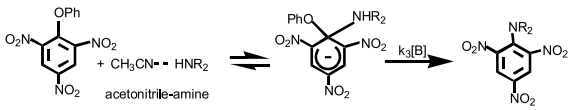


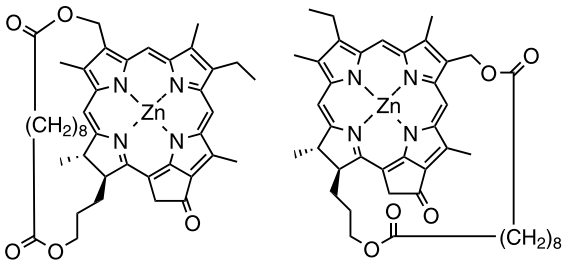


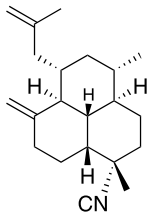


The *endo*/*exo* ratios for the reaction of cyclopentadiene with methyl acrylate have been examined in the aqueous solutions of alcohols containing various salts.

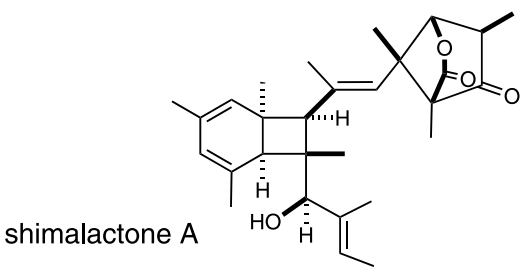


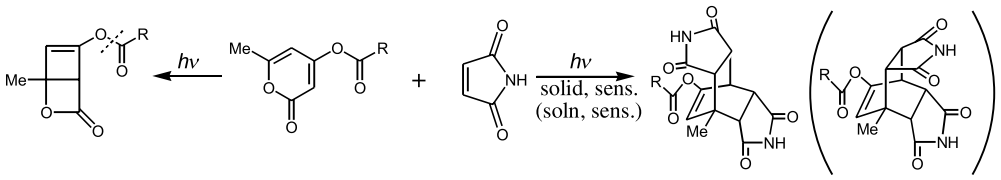






The relative stereochemistry of amphilectene dterpenes previously reported from *Cribochalina* sp. has been reassigned by X-ray analysis.





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Recent advances in ether dealkylation

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Contents

Keywords: *O*-Dealkylation; Deprotection; Debenzylation; Review.

Abbreviations: Ac, acetyl; Alloc/Aloc, allyloxycarbonyl; BMIM, 1-*n*-butyl-3-imidazolium; Bn, benzyl; Boc, *t*-butoxycarbonyl; BOM, benzyloxymethyl; Bz, benzoyl; CAN, ceric ammonium nitrate; Cbz, benzyloxycarbonyl; CSI, chlorosulfonyl isocyanate; DAST, diethylaminosulfur trifluoride; DCM, dichloromethane; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DIB, (diacetoxyiodo)benzene; DMAC, dimethylacetamide; DMEU, *N*,*N*-dimethylethylene urea; DMPU, *N*,*N*-dimethylpropylene urea; DMT, dimethoxytrityl; Dppp, 1,3-bis(diphenylphosphino) propane; EDG, electron-donating group; EWG, electron-withdrawing group; HMIMBr, 3-methylimidazolium bromo-hydrogenate; IL, ionic liquid; LAH, lithium aluminum hydride; LN, lithium naphthalide; LVT, low-valent titanium; MEM, 2-methoxyethoxymethyl; Mes, mesitylene; MMB, *m*-methoxybenzyl; MOM, methoxymethyl; MPB, *m*-methyoxybenzyl; MXM, *m*-xylylmethyl; NAP, 2-naphthyl-methyl; NMO, *N*-methylmorpholine *N*-oxide; PBB, *p*-bromobenzyl; PCB, *p*-chlorobenzyl; PDPM, *p*-phenyldiphenylmethanol; PMB, *p*-methoxybenzyl; PMBOM, *p*-(methoxybenzyloxy)methyl; PNB, *p*-nitrobenzyl; PPB, *p*-phenylbenzyl; PPDPM, *p*-phenyl phenyldiphenylmethanol; PTSA, *p*-toluenesulfonic acid; Pv, pivaloyl; SET, single electron transfer; TAM, *t*-amyl; TBDMS, *t*-butyldimethylsilyl; TBDPS, *t*-butyldiphenylsilyl; TBDS, *t*-butyldimethylsilyl; TBDSOTf, *t*-butyldimethylsilyl triflate; TBS, *t*-butyldimethylsilyl; TES, triethylsilyl; THP, tetrahydropyranyl; TIPS, tri-*i*-propylsilyl; TMAH, trimethylammonium hydrogenate; TMSI, trimethylsilyl iodide; Tr, trityl (triphenylmethyl); Ts, *p*-tolylsulfonyl.

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1. Introduction

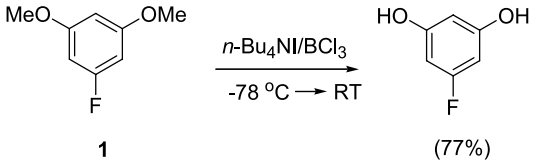
The *O*-dealkylation of ethers, or ether cleavage, remains an integral functional group transformation, primarily as a deprotection step to unmask a hydroxyl group. The utility of this reaction extends to both academic and commercial pursuits including natural product, pharmaceutical and fine chemical syntheses.

This topic has most recently been reviewed in 1983 by Bhatt and Kulkarni[1] and in 1996 by Ranu and Bhar.[2] The former review attempted to cover all reagents of synthetic value through 1981 while the latter focused primarily on developments since the prior review. A review in 1997 by Guibé[3] on allylic protecting groups included a subsection on removal of this specific group. This review will cover the recent developments in the field from 1995 through the end of 2004, focusing on those reagents that are of practical, synthetic value and display some level of generality. Subject overlap with the prior reviews was kept to a minimum. The ethers covered are those in which the oxygen-bearing carbon atom being removed is only attached to other carbon or hydrogen atoms. Thus, such species as acetals, ketals, silyl ethers and tetrahydropyranyl ethers are excluded, as are methods that further functionalize the deprotected alcohols (acylation, silylation, oxidation for example). The extent to which these excluded groups are affected (or not) by the reagents cited in the review, will be mentioned. In addition, in a few cases, we have included well-known reagents that have been utilized in large-scale syntheses. For the more practical methodologies, examples where the reagent was subsequently used in the synthesis of complex molecules has been included periodically. Whenever applicable, the reagent selectivity relative to other types of hydroxyl protecting groups will be highlighted, as this remains a key factor in the choice of reagent, especially in poly-functional molecules. The review has been organized by functional group then by reagent type. The groups are: (1) aryl and alkyl ethers (including propargylic), (2) allyl ethers (including isoprenyl), (3) benzylic ethers (including trityl), and (4) cyclic ethers. In some Figures, an arrow has been placed to denote the dealkylation site, indicative of regio- or chemo-selectivity.

2. Deprotection of aryl and alkyl ethers

2.1. Methyl/ethyl ethers

2.1.1. Lewis acids. A Pfizer group[4] demonstrated the utility of BCl_3 as a dealkylating reagent can be greatly enhanced by the addition of tetrabutylammonium iodide. The reactions are run with 2.5 equiv of each reagent in DCM. This reagent combination displays enhanced reactivity over BBr_3 as shown in the bis demethylation of 3,5-dimethoxy-fluorobenzene (**1**) (Scheme 1). Methyl and ethyl aryl ethers are readily cleaved, but an isopropyl group is not.



Scheme 1.

The removal of a benzyl group was achieved in the presence of a methyl ether; also, an electron-withdrawing group (CN) can influence the removal of a *meta*-positioned methyl ether over an *ortho*-positioned methyl ether (Fig. 1). The reaction does not proceed in the absence of the iodide. The yields generally ranged from 70 to 98%. This methodology was recently employed in the synthesis of *rac*-Juglomycin A[5] and some selective glucocorticoid receptor antagonists.[6]

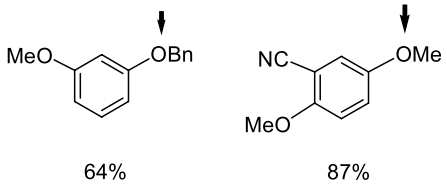
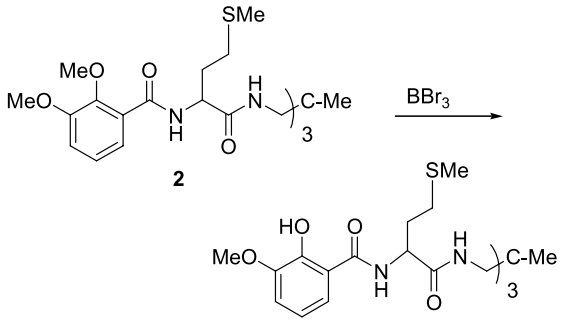


Figure 1.

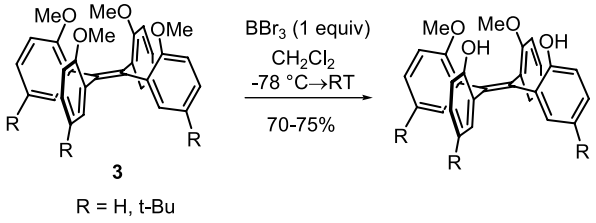
A unique intramolecular attack of a divalent sulphur atom was demonstrated to be the source of a selective *O*-demethylation of enterobactin analogue **2** in the presence of BBr_3 (Scheme 2).[7] The mechanism was proposed to proceed via a simultaneous attack of the S atom on the



Scheme 2.

oxygen bearing methyl group and the Br atom upon the sulphur bearing methyl group. The former having been activated by the boron halide creating an oxonium intermediate species.

An interesting series of selective *O*-demethylations was observed with tetrakis(2-hydroxyphenyl)ethene derivatives.[8] Reacting **3** with BBr_3 (1 equiv) gave the doubly deprotected (*Z*)-isomer (Scheme 3). In contrast, reaction with TMSI (1 equiv) gave the singly deprotected species in 50% yield. Higher yields could be attained by careful monitoring of the reaction with additional reagent.



Scheme 3.

Finn[9] has established an order of reactivity for the deprotection of aryloxy ethers with BBr_3 such that benzyl, propargyl and methyl ethers can be sequentially removed (Fig. 2). Allyl ethers undergo Claisen rearrangements under the reaction conditions (DCM, −20 °C to rt).

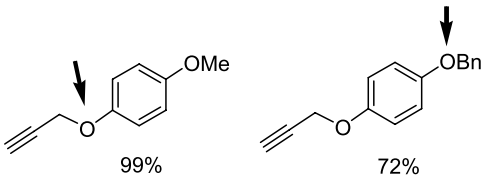


Figure 2.

The novel use of $BeCl_2$ for the demethylation of a series of aryl methyl ethers derived from benzophenones, xanthones, anthraquinones and substituted arenes (**4**–**6**) has been demonstrated in high yields using 3 equiv of reagent.[10] The reactions proceed to completion within 8 h in refluxing toluene. In the case of carbonyl-containing aryl methyl ethers in which the ether resides in the *ortho* position, the enhanced selectivity and reaction rate is attributed to coordination of the reagent to the carbonyl (Fig. 3).

The need for a scaleable process to dealkylate a nitrocatechol methyl ether led Learmonth[11] to re-investigate the

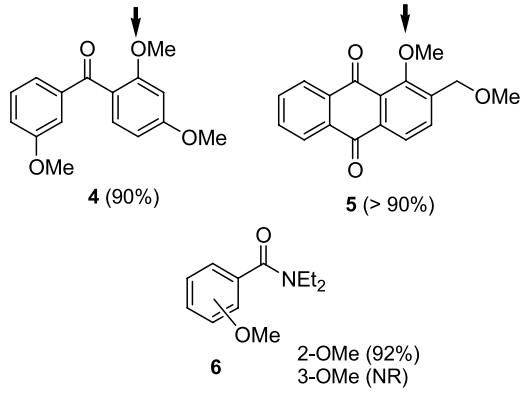


Figure 3.

aluminum chloride/pyridine combination (1:3 molar ratio) in environmentally-benign solvents. While typically performed in refluxing methylene chloride, this particular reaction gave better results in ethyl acetate (99% yield; 1.5 h-reflux) to provide drug candidate **7**, a selective inhibitor of catechol *O*-methyl transferase. Complex mixtures were obtained with typical demethylating reagents including BBr_3, thiophenolate anion and pyridinium hydrochloride. Other demethylation examples with similar compounds (e.g., **8**) were reported in 70–96% yield (Fig. 4).

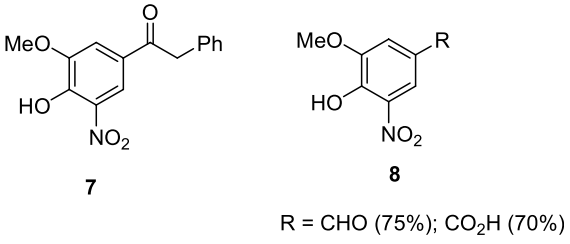


Figure 4.

The synthesis of a series of 3,5,7-trihydroxy-6-methoxy flavones was predicated on the selective dealkylation of differentially protected intermediates.[12] The reaction of fully protected acetophenone **9** with $AlCl_3$ selectively removed only the isopropyl group in quantitative yield, whereas $AlBr_3$ showed less selectivity and removed the 6-methoxy in addition to the isopropyl. Selectivity for the 6-methoxy group was achieved using the combination of $AlBr_3$/NaI in 94% yield. The selective removal of the isopropyl group in **10** was facilitated by converting the 3-position into a tosyloxy functionality in 90% yield (Fig. 5).

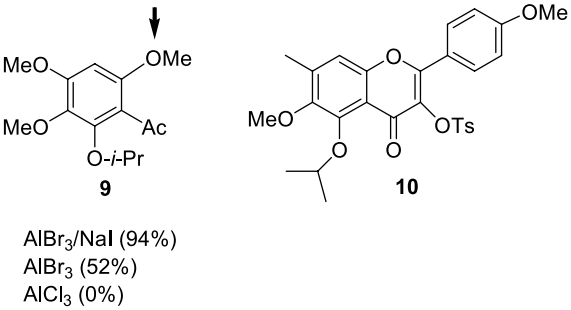


Figure 5.

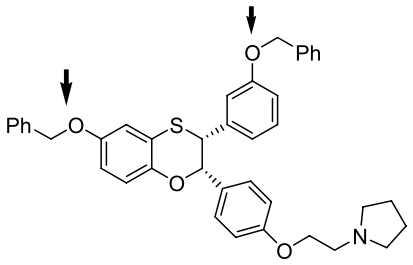


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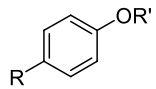
Figure 6.

An improvement to the existing $SiCl_4$/NaI methodology was reported from this laboratory by the addition of catalytic boron trifluoride.[13] While initially developed for the difficult double debenzylation of dihydrobenzoxathiin derivative **11** (81% yield) (Fig. 6) under investigation as a selective estrogen receptor modulator, the protocol was expanded to a variety of *O*-dealkylations. These included the removal of allyl and methyl groups from the corresponding aryl ethers (**12**) in MeCN at 70 °C (Fig. 7). Enhanced reaction rates were observed for all the examples with the catalyst present. Yields ranged from 82 to 98%.



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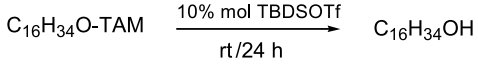
R' = Me;R = *i*-Pr (89%);
R' = Me; R = Ph (82%)
R' = allyl; R = Me (90%)

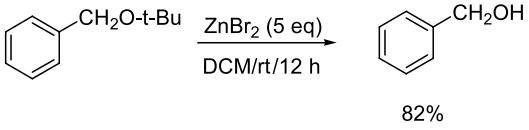
Figure 7.

Removal of the novel *t*-amyl (TAM) group from alkyl ethers with *t*-butyldimethylsilyl triflate (TBDSOTf) has been described.[14] When 20 mol% of the reagent is used in dichloromethane, the corresponding alcohol is obtained in good to excellent yield (Scheme 4) but when 2,6-lutidine is employed with stoichiometric TBDSOTf, the corresponding silyl ether is obtained. Methyl and allylic ethers are immune to this reagent system. Trimethylsilyl triflate also effects the transformation to the alcohol.



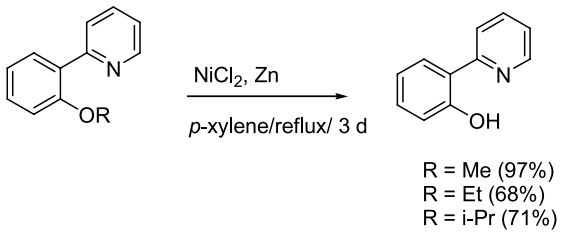
Scheme 4.

Zinc bromide (3–5 equiv) in methylene chloride cleaves the t-butyl group from aliphatic, phenyl and benzyl ethers in yields from 78 to 82% (Scheme 5).[15]



Scheme 5.

A mixture of $NiCl_2$ (1 equiv) and zinc powder (3 equiv) in refluxing *p*-xylene was shown to *O*-dealkylate (Me, Et, *i*-Pr) anisole derivatives that are *o*-substituted with a nitrogen-containing functionality which serves to chelate the metal and facilitate ether cleavage.[16] The reaction fails in the absence of such a nitrogen atom. Lengthy reaction times (3 days) are required for complete conversion (Scheme 6).



Scheme 6.

In 2003, the utility of ionic liquids (IL) was extended to include the ability to cleave alkyl ethers. Pioneering work by Driver and Johnson[17] showed that 3-methylimidazolium bromohydrogenate (HmimBr–HBr) could cleave anisole in modest yields at rt (62–65%)(Fig. 8).

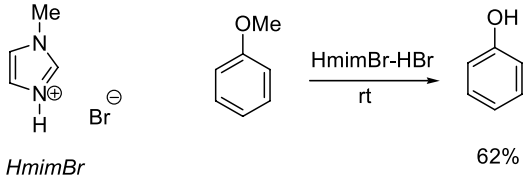
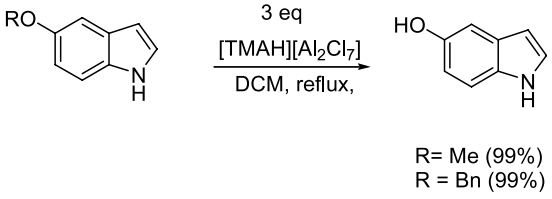


Figure 8.

The scope of this methodology was expanded by Kemperman and co-workers[18] who investigated the ability of chloroaluminate ionic liquids, namely $[TMAH][Al_2Cl_7]$ to cleave aryl methyl, allyl and benzyl ethers at 40 °C in >97% yield (Scheme 7).



Scheme 7.

They also studied the comparative dealkylative abilities of three chloroaluminate ionic liquids (TMAH, BMIM, EMIM) in the selective demethylation of 4,5-dimethoxyindanone **13** (Fig. 9). All three showed improved reaction rates and selectivity to remove the 4-methyl group as compared to $AlCl_3$ (96%—24 h vs 70%—42 h). The TMAH IL was the preferred reagent as it is less costly to prepare (one step).

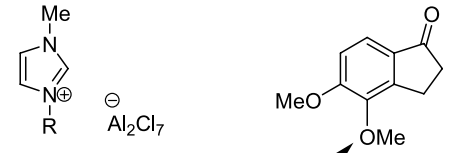


Figure 9.

The enhanced rate is explained by the presence of a high concentration of chloride ions that accelerates the rate determining step, namely the attack on the methyl C atom.

Another IL system, 1-*n*-butyl-3-imidazolium tetrafluoroborate [Bmim][BF_4] in combination with 1 equiv aq HBr, cleaved aryl methyl and aryl benzyl ethers, such as **14**, at 115 °C in excellent yields (85–95%) (Fig. 10).[19] The IL presence allows fewer HBr equivalents to be used than usual. PTSA can also be used as the proton source. The yields in the absence of the IL were significantly lower even at extended age periods. These IL systems are touted as conforming to the principles of green chemistry due to their recyclability, low cost, and safety profile.

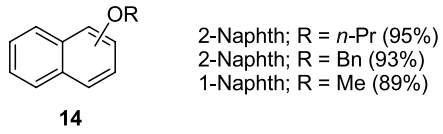


Figure 10.

The pyridine–hydrochloride system for the *O*-demethylation reaction has been modified by applying microwave irradiation under solvent free conditions.[20] The reactions of variously substituted anisoles (**15**) are complete within 16 minutes and provide the corresponding phenols in good to excellent yields (65–95%) (Fig. 11).

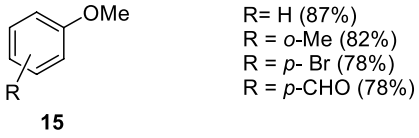
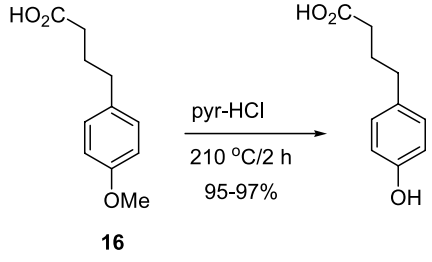


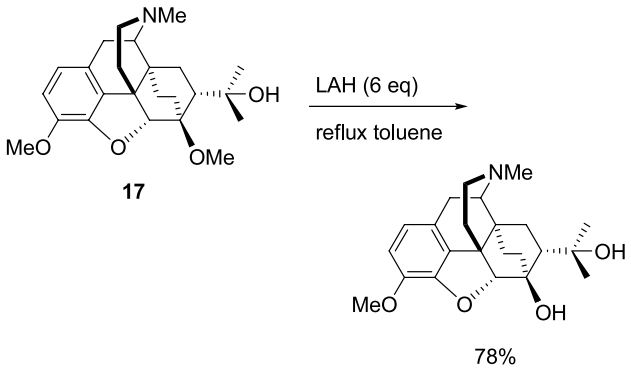
Figure 11.

The conventional pyridine–hydrochloride system was demonstrated by Schmid[21] on a pilot-plant scale (190 L glassware) on methoxyphenylbutyric acid (**16**). The reaction was run at 200 °C and was complete after 2 h to give des-methyl product in 96% yield (Scheme 8). The authors cite the undesirable features of the standard selections of methods available for their choice of this approach.



Scheme 8.

2.1.2. Hydrides. A selective *O*-demethylation of **17** was observed in the presence of an aryl methyl ether utilizing LAH (6 equiv) in refluxing toluene (Scheme 9).[22] Thus a series of ring-constrained analogues of buprenorphine were *O*-demethylated in the 6-position via assistance by the neighboring oxygen atom that presumably forms an aluminum hydride species that attacks the lithium-activated methyl ether moiety.



Scheme 9.

2.1.3. Oxidative. The mild deprotection of oligosaccharide propargylic ethers **18**, via isomerization to the allenyl ether, followed by treatment with 5 mol% OsO_4 with NMO in aq acetone at rt has been described by Mereyala (Fig. 12).[23] High yields (88–97%) of the corresponding alcohol were obtained (10–18 h). Acid-sensitive groups such as isopropylidene and cyclic ketals were unreactive under these conditions. Other ethers deprotected similarly are allenyl, allyl and enol ethers (i.e., compound **19**) (Fig. 12). In the latter two cases, both aliphatic and aromatic examples were given.

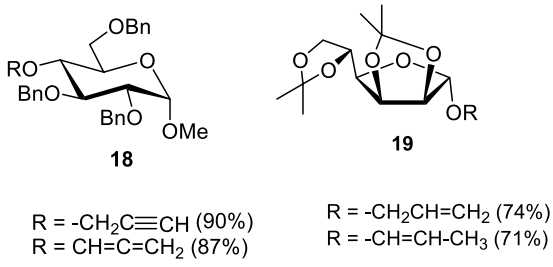
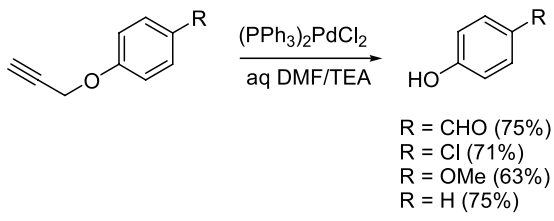


Figure 12.

A new protocol for the deprotection of aryl propargylic ethers using 4 mol% $(Ph_3P)_2PdCl_2$ with triethylamine (8 equiv) in aq DMF at 80 °C has been described (Scheme 10).[24] Adjacent methyl aryl ethers are unaffected under the reaction conditions. Isolated yields generally range from 55 to 75%. Compatibility with several aryl substituents such as aldehydes, ketones, and halides was demonstrated.



Scheme 10.

Sulphur transfer agent, tetrathiomolybdate ($(BnNEt_3)_2MoS_4$) has been shown to deprotect propargyl ethers of aliphatic alcohols (**20**) and phenols (**21**) in MeCN at 28 °C (Fig. 13).[25] This system is selective for the propargyl ether

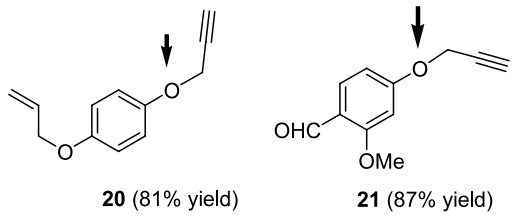
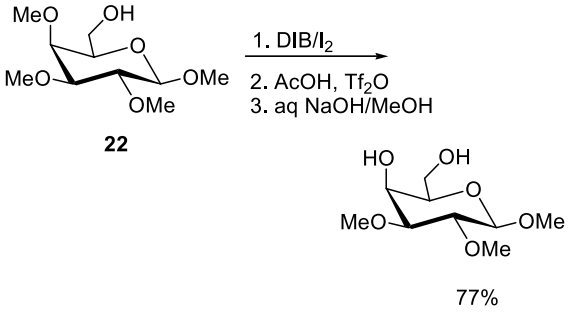


Figure 13.

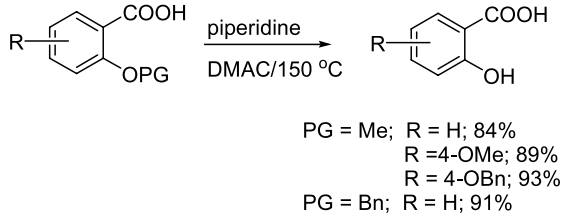
in the presence of reducible functionalities, such as NO_2 and Ac, as well as methyl and allyl aryl ethers. Yields range from 75 to 95% with 1.0 equiv of the reagent. The reagent is readily prepared from ammonium molybdate, hydrogen sulfide and tetrabutylammonium chloride.

The selective *O*-demethylation of an ether adjacent to a hydroxyl group in carbohydrate substrates (i.e., **22**) was accomplished with (diacetoxyiodo)benzene (DIB) and I_2 under irradiative conditions (tungsten lamp) (Scheme 11).[26] In this tandem radical hydrogen abstraction–oxidation approach the abstraction from the methyl group yields a C-radical that is stabilized by the nearby (2.3–2.8 Å) oxygen atom. Oxidation of the C-radical provides an oxycarbenium ion that is trapped by acetate from the reagent to form a mixture of acetals (*O*-acetoxymethyl and methylenedioxy) that upon basic hydrolysis provides the diol in 77% overall yield in one-pot.



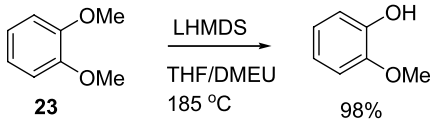
Scheme 11.

2.1.4. Base. The previous report of nucleophilic attack of iodide on the methyl group of *o*-anisic acid as a dealkylation method, led Nishioka to study other nucleophiles, namely amines, for this dealkylation.[27] A study of various solvent and amine combinations led to the optimized system in which substituted derivatives of *o*-anisic acid were reacted with 3 equiv of piperidine in DMAC (Scheme 12). This approach is *o*-selective (relative to the benzoic acid moiety) as *m*- and *p*-methoxy susbstituents were unaffected.

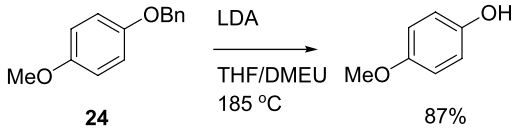


Scheme 12.

The hindered bases NaHMDS and LDA (1.5 equiv) were both shown to dealkylate aryl and heteroaryl methyl ethers in 81–94% yield in THF/DMEU at 185 °C in a sealed tube.[28] The selective mono *O*-demethylation of *o*-dimethoxybenzenes (e.g., **23**) can be achieved with the former base (Scheme 13) while selective *O*-debenzylation of benzyloxy anisoles (e.g., **24**) can be attained with the latter (2.5 equiv) (Scheme 14).

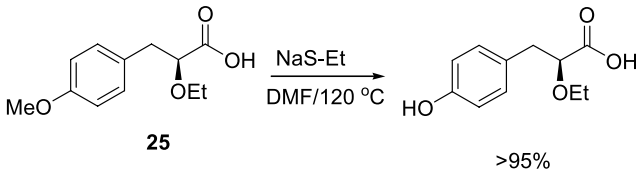


Scheme 13.



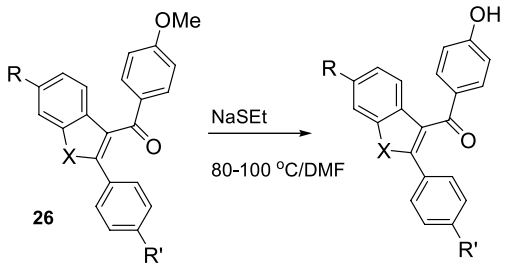
Scheme 14.

Another well-studied class of basic reagents are the sodium thiolates. An AstraZeneca group showed the improved selectivity for the demethylation of a differentially protected substrate en route to the synthesis of key chiral intermediate **25** (Scheme 15).[29] Initially, BBr_3 was used but showed selectivity for removing the ethyl group, not the desired methyl group. Aq HI was only partially selective for the methyl group but satisfactory results were obtained with sodium ethanethiolate in DMF (>20 h).



Scheme 15.

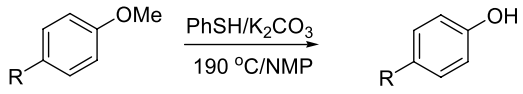
While sodium ethanethiolate is an often-used methodology for the demethylation of aryl ethers, little was known about its regioselectivity. A systematic study of this reagent in DMF was undertaken and revealed notable trends.[30] For benzophenone derivatives (e.g., **26**), the methyl ether *para* to the ketone is selectively removed in the presence of other methyl ethers even when they are situated on another aromatic ring (Scheme 16). Even a modest degree of chemoselectivity (2:1) was observed in the presence of a *para* benzyl ether. The role of electronic factors was studied with a series of simple anisole derivatives. A clear pattern emerged whereby EWG in the *para* (CN, NO_2, Ac) gave improved results (77–89% yield) in comparison to electron neutral and EDG (H, halides, alkyl, alkoxy) that gave poor results (5–10% yield). The position of the EWG also had an effect as the *m*-acetyl example gave a reduced yield (18% vs 77%) compared to the *para* case. *para*-Substituted halides (Br, Cl) gave anomalous results (27–47% yield) perhaps due to thiol formation.



X	R	R'	yield
CH_2CH_2	OMe	H	87%
CH_2CH_2	H	OMe	83%
CH_2CH_2	OMe	OMe	77%
O	OMe	OMe	75%
NEt	OMe	OMe	54%

Scheme 16.

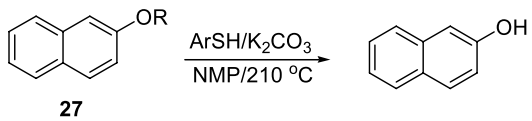
The catalytic use of in situ generated phenylthiolate anion in NMP for the rapid (<30 min) removal of methyl and benzyl group from aryl ethers (Scheme 17) was reported.[31] Potassium carbonate (2–5 mol%) was combined with thiophenol to prepare the reagent that shows the usual favorable reactivity towards aromatic ethers containing EWG.



R	yield
2-NH_2	80
4-Cl	70
3-CHO	85
4-Ac	90

Scheme 17.

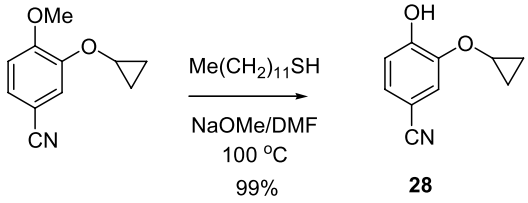
A systematic study of the dealkylating capabilities of in situ formed phenylthiolate anion was investigated by Chakraborti.[32] NMP was the solvent of choice based on their standard reaction of the dealkylation of 2-methoxynaphthalene, while DMEU and DPMU also gave high yields albeit in vacuo (146 and 106 °C, respectively). Several bases in NMP (5 mol%) gave >90% yield for the standard reaction including potassium carbonate, sodium bicarbonate, sodium hydroxide and lithium amide. Aside from demethylation, this reagent also removed propargyl, allyl, and benzyl groups from their respective 2-naphthyl ethers (**27**) (Scheme 18). A similar electronic effect was observed (vida supra).



R	Yield
OMe	97%
OBn	90%
O-All	75%
O-Propargyl	72%

Scheme 18.

The odor associated with the use of sodium ethanethiolate and its reaction by-product, ethyl methyl sulfide, led Frey to employ longer chain thiols to avoid this environmental issue.[33] The combination of dodecanethiol and sodium methoxide (1.7 equiv each) in DMF gave a 99% yield of phenol **28** (Scheme 19). This protocol was extended to other anisole derivatives in excellent yield. Modest regioselectivity for the mono-demethylation of the *meta* position of 3,4-dimethoxybenzonitrile (5:1) was reported.



Scheme 19.

The use of metal thiolates continues to attract attention as a viable method to dealkylate aromatic ethers. The first example of *tris O*-demethylation with this protocol was described by Tanaka and co-workers[34] en route to the total synthesis of (−)-Macrocarpal C, a biologically active compound. For the last step, 10 equiv of lithium *p*-thiocresolate in HMPA–toluene under refluxing conditions gave **29** in 58% yield (Fig. 14).

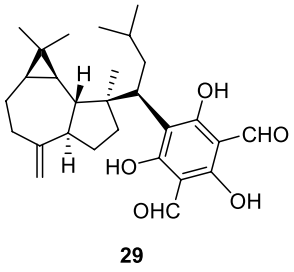
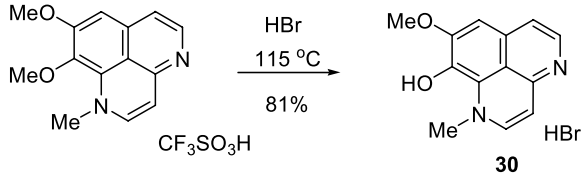


Figure 14. Structure of (−)-Macrocarpal C.

2.1.5. Acid. The key step in the synthesis of marine natural product isoaaptamine (**30**) was a selective *O*-demethylation using 48% HBr.[35] This served to remove the methyl group from the C-9 position in 81% yield (Scheme 20). Increasing the reaction temperature to 145 °C led to removal of both methyl groups. This compound is under investigation for broad-spectrum antimicrobial activity.



Scheme 20.

2.1.6. Other. The scope of the deprotection of aryl methyl ethers under Birch conditions (Li metal–ethylenediamine (EDA)) was studied by Sugai.[36] The formation of the over-reduced species was suppressed by using an optimized amount of reagent. Demethylation of **31** with Li (5 equiv) and EDA (7 equiv) in THF at −10 °C/3 h gave an 81% yield of the phenol with only 6% of the over-reduced cyclohexene. In dimethyl ether **32**, selective mono-demethylation could be achieved in 90% when the reaction was run at −10 °C and the second could be removed in 57% overall yield if run at 0–22 °C. Sterically hindered anisole derivatives, such as **33** could be demethylated in 82–83% yield (Fig. 15). Silyl ethers and esters do not survive these strongly basic conditions.

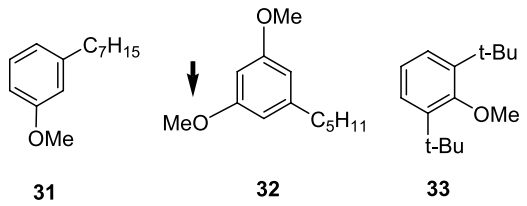


Figure 15.

The total synthesis of (−)-cylindrocyclophane A by Hoye included the novel perdemethylation of tetra-*O*-methyl ether **34** with MeMgI under solvent-free conditions at 160 °C (1 h/60% yield) (Fig. 16).[37] The $AlBr_3$/EtSH reagent system that was successful for Evans' vancomycin synthesis did not work.

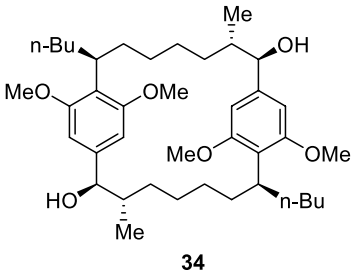
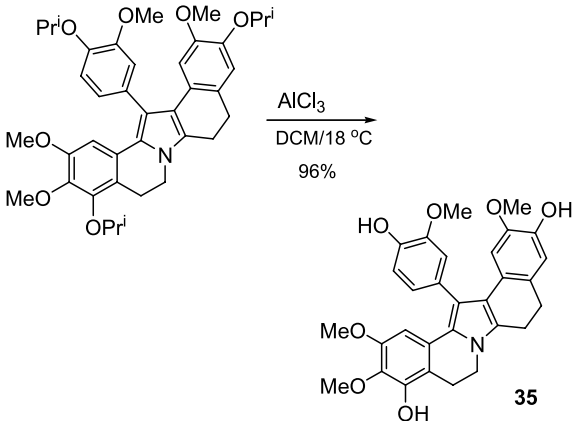


Figure 16. Structure of (−)-Cylindrocyclophane A.

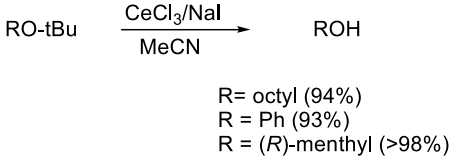
2.2. Branched alkyl ethers

The utility of aluminum chloride as an ether cleaving reagent was extended by Banwell who demonstrated that it could selectively cleave isopropyl aryl ethers in the presence of methyl aryl ethers under mild conditions.[38] The methodology was initially applied to the synthesis of complex marine natural product **35** (Scheme 21) but works equally well for simpler, differentially-protected arenes. Aluminum chloride showed superior selectivity in comparison to boron trichloride. While functional groups such as halides, aldehdyes and acetates were well tolerated, the presence of alkynes led to complex mixtures. The concurrent removal of a TIPS group was also observed in one example.



Scheme 21.

Bartoli extended the utility of cerium chloride/NaI to include the dealkylation of alkyl (1° and 2°) and aromatic *t*-butyl ethers. High yields of the alcohols were obtained (>93%) in MeCN using 1 equiv of reagent (Scheme 22).[39]



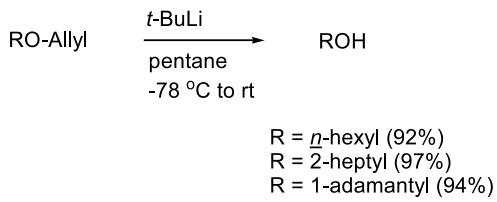
Scheme 22.

3. Allyl and related ethers

The protection of alcohols with allyl and related (prenyl, methyallyl, cinnamyl, homoallyl) groups is predominantly confined to carbohydrate synthesis due to their stability under the conditions required for glycoside formation. These groups are moderately stable to acids and bases, and offer the potential for selective dealkylation of differentially protected sites. Initially, the deprotection schemes involved a metal- or base-induced (potassium *tert*-butoxide in DMSO) isomerization to the 1-propenyl analog then hydrogenolysis or oxidative cleavage. More recently though, direct methods have been added to the arsenal of deprotection methodologies.

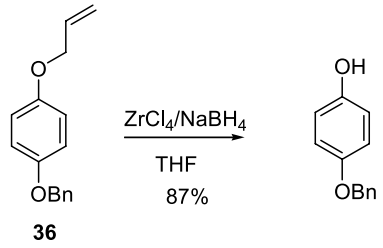
3.1. Allyl ethers

3.1.1. Bases. Bailey has described the *O*-deallylation of primary, secondary and tertiary allyl ethers with pyrophoric *t*-butyllithium (1 equiv/−78 °C) in pentane (Scheme 23).[40] The corresponding alcohols were obtained in >89% yield after warming to rt (1 h). Selectivity for the allyl group in the presence of benzyl, acetonide and TBDS protecting groups was demonstrated. The reaction works less well in EE and THF, the result of poorer aggregation in these solvents. The authors propose an S_N2' mechanism for the reaction.



Scheme 23.

3.1.2. Sodium borohydride. The combination of sodium borohydride and Lewis acids provides the basis for a series of new deallylation methodologies. This combination generally produces diborane in situ. Use of zirconium (IV) chloride (1 equiv) with $NaBH_4$ in THF was shown to deprotect a series of *O*-allyl aromatic (i.e., **36**) and aliphatic ethers in 80–95% yield at rt (Scheme 24).[41] Selectivity in the presence of an aromatic methyl and benzyl ether was shown.



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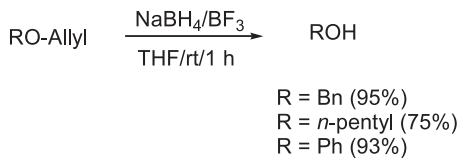
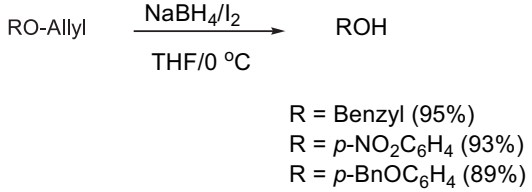
Scheme 24.

The $NaBH_4/BF_3$ system deallylated both aliphatic and aryl ethers in yields ranging from 75 to 95% at rt (Scheme 25).[42]



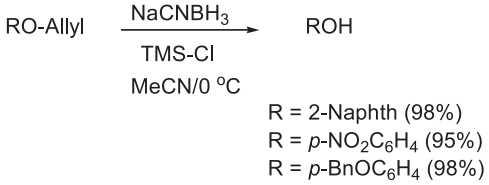
Scheme 25.

The iodine–borohydride combination also is an efficient deallylation system for both aliphatic and aromatic ethers (Scheme 26) and was unreactive towards neighboring methyl and benzyl ethers, as well as a THP group.[43] Similar results were observed with borane–dimethyl sulfide solution.



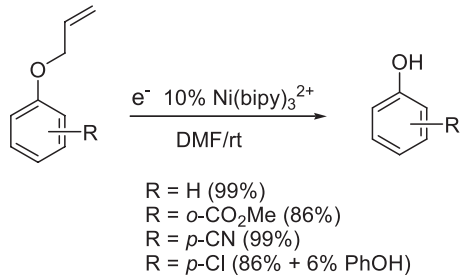
Scheme 26.

Sodium cyanoborohydride (1 equiv) with TMS-Cl (1 equiv) in MeCN (15 min) is another reagent combination that converts allyl ethers to the alcohol in yields up to 98% (Scheme 27).[44] Similar chemoselectivity was observed (vida supra).



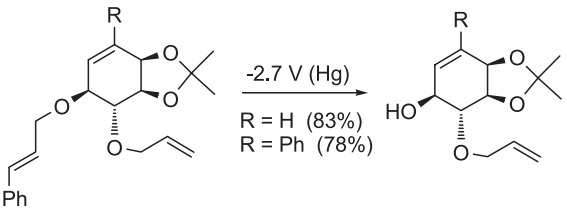
Scheme 27.

3.1.3. Electrochemical reduction. The reductive deprotection of allyl ethers via electrochemically generated nickel has been reported by Duñach. The reaction employs 10 mol% Ni(II) complexes, typically with 2,2′-bipyridyl ligands, in DMF at rt (Scheme 28).[45] Aryl, aliphatic and benzylic allyl ethers can be cleaved with this method while demonstrating selectivity in the presence of enol and homoallyl ethers. Some reducible groups (esters, nitriles) were unaffected by the reaction conditions but an *o*-bromo group was removed.

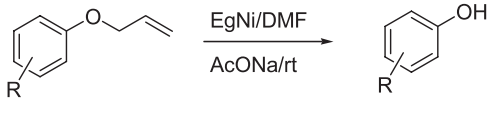


Scheme 28.

The two electron reduction of the starting complex to Ni(0) is followed by oxidative insertion to the C–O bond to provide a Ni(II) π-allyl complex, a subsequent $1e^-$ reduction forms a Ni(I) π-allyl intermediate.[46] The addition of Mg^{2+} ions to facilitates the reaction by undergoing a metal exchange reaction to form a magnesium phenate that is hydrolyzed to the phenol, thus enhancing the catalytic cycle. A similar result was obtained by replacing the Ni with 10 mol% $PdCl_2$, again in DMF at rt.[47] The reduction of both allyl and cinnamyl groups in the presence of reducible groups were achieved. Hudlicky[48] selectively removed a cinnamyl group in the presence of an allyl group in a series of conduritol substrates while retaining the stereochemical integrity of the alcohol (Scheme 29). These results are not achievable with conventional reagents according to the authors. In another case, a benzyl group was left intact under the same conditions.[49]



Scheme 29.

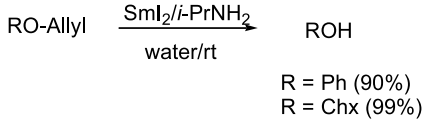


R	yield
4-CO_2Me	94
4-CHO	91
4-Br	72
4-OMe	76
4-OBn	73

Scheme 30.

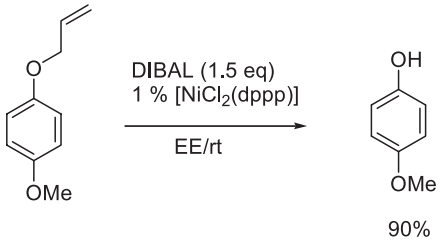
Electrochemically generated nickel ('EgNi'; 4 equiv) from a nickel anode in DMF deallylates aryl ethers in the presence of sodium acetate and Et_4NBF_4.[50] Again, neighboring ester or nitrile groups were unaffected, as were neighboring methyl or benzyl groups (Scheme 30).

3.1.4. Other reductions. A chemical electron-transfer approach for this transformation was described by Hilmersson utilizing SmI_2 (5 equiv) in aq THF in the presence of an amine (Scheme 31).[51] Aryl, primary and anomeric ethers are rapidly cleaved with this reagent in high yields while methyl, thioethyl and benzylic ethers are unaffected.



Scheme 31.

A more practical deallylation procedure by Ogasawara[52] used DIBAL (1.5 equiv) with 1 mol% [$NiCl_2$(dppp)] in ethereal solvents at rt (Scheme 32). Selectivity towards an allyl ether in the presence of a methyl ether was shown for the aryl allyl ether substrates (82–90% yield) while aliphatic allyl ethers with benzyl, prenyl, or THP protected ethers present were selectively removed (80–95%). When ester groups were present, the replacement of DIBAL with 3–4 equiv sodium borohydride gave the alcohols in 73–85% yield. The reduction is thought to proceed via the known hydroalumination–elimination pathway. This methodology was applied to the total/formal synthesis of khafrefungin (87% yield; Fig. 17),[53] *rac*-guanacastepene (71% yield),[54] and desmethoxymitomycin A (Et_3Al used in place of DIBAL; 86%).[55]



Scheme 32.

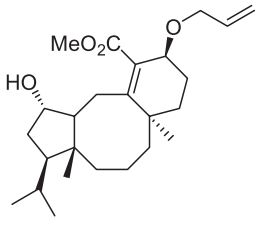
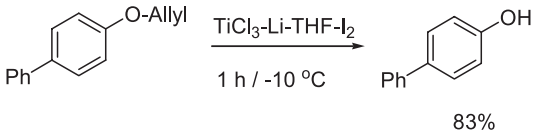


Figure 17. Structure of khafrefungin.

While the electron-transfer induced demethylation of aryl ethers using low valent titanium was described in 1991, its application towards allyl ethers was only recently reported by Banerji.[56] The reagent, generated by the Rieke method ($TiCl_3$–Li–THF) can be activated by the addition of 1 equiv



Scheme 33.

iodine to allow the deprotection of a phenol (Scheme 33) and cholesterol (6 h/rt) in 83 and 79% yield, respectively. Higher temperatures, longer reaction times and lower yields were observed in the absence of iodine.

3.1.5. Palladium-based reagents. The palladium-based reagents continue to attract attention based on their catalytic nature and ability to operate under mild conditions, although mostly in acidic media (allyl scavenger) or in the presence of a reducing agent. Thayumanavan[57] developed an elegant methodology using merely 1 mol% $Pd(PPh_3)_4$ in MeOH at rt with potassium carbonate (3 equiv). This system was highly effective for aryl allyl ethers with either EWG or EDG present (82–96%). Compatibility with reducible functional groups (CN, NO_2, CHO) was observed, as was high chemoselectivity for removal of an aryl allyl ether in the presence of an alkyl allyl ether. The latter can be deprotected at higher temperatures. The author applied this methodology to the synthesis of dendrons and others to the synthesis of 7,7′-disubstituted binols (90% yield),[58] and chiral 1,4-butanediols (89% yield),[59] both of which involved a double deprotection (Fig. 18).

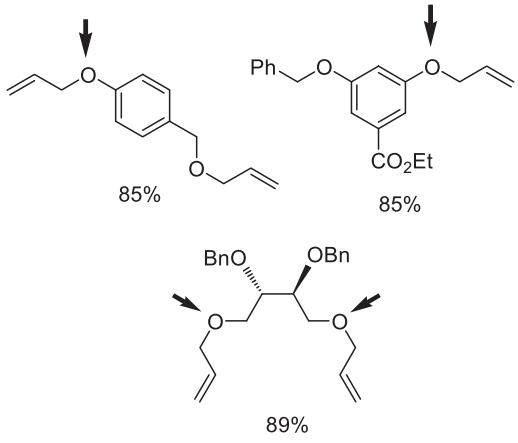
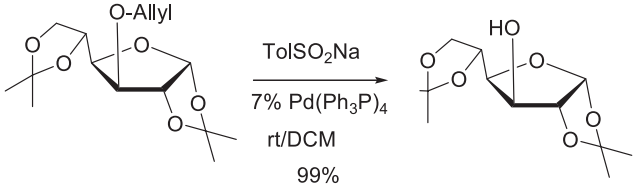


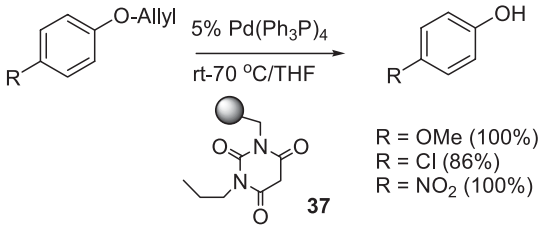
Figure 18. Conditions: 1-5 mole% $Pd(PPh_3)_4$, 6 equiv K_2CO_3, EtOH (6-16 h).

Nagakura[60] reported a single example whereby sodium toluenesulfinate performed better than other standard acidic allyl scavengers in the deprotection of a glucofuranose derivative with 7 mol% $Pd(PPh_3)_4$ (25 min/99% yield) (Scheme 34).



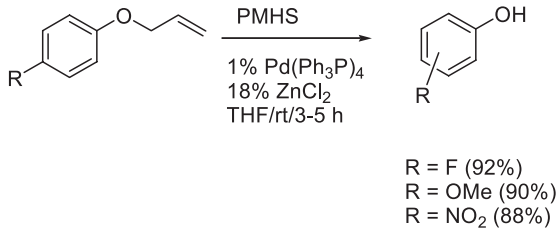
Scheme 34.

Deprotection of allyl ethers employing 5 mol% $Pd(PPh_3)_4$ in conjunction with solid-supported barbituric acid (**37**) in THF at 90 °C/24 h gives the product alcohols in 80–100% yield for a series of aryl and carbohydrate (*sec*-alcohol) systems (Scheme 35).[61] Similar chemoselectivity and functional group compatibility was described (vida supra).



Scheme 35.

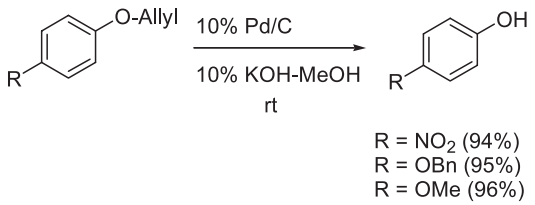
Another selective, yet mild set of deallylation conditions with $Pd(PPh_3)_4$ was presented by Chandrasekhar.[62] In concert with polymethylhydrosiloxane (PMHS; 2 equiv) and zinc (II) chloride (18 mol%), this system is able to deprotect a variety of allyl ethers including aryl, benzylic, acyclic secondary, aliphatic and carbohydrate substrates at rt in yields from 85 to 94% (Scheme 36). Chemoselectivity was demonstrated in the aliphatic series as prenyl, Bn, THP, MOM and TBS protecting groups were not removed from doubly-protected 1,5-pentanediol (85–92%).



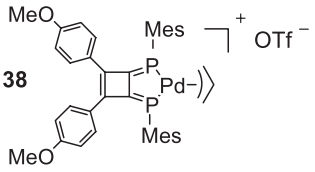
Scheme 36.

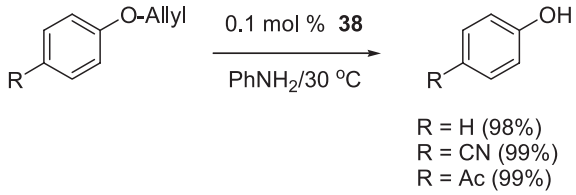
Hara[63] reported the deprotection of *O*-allylphenols with catalytic 10% Pd/C in 10% KOH in MeOH. The reaction time was highly dependant on the aryl ring substituents; electron donating substituents required longer periods (24–96 h) while electron withdrawing groups proceeded faster (9 h). Related protecting groups (methallyl, isoprenyl and 1,1-diemthyl-2-propenyl) were also cleaved in the *p*-nitrophenyl ether series in >95% yield (Scheme 37). Chemoselectivity for the allyl group in the presence of benzyl, methyl and alkyl THP ethers was seen. Compelling evidence that the reaction proceeds via the SET mechanism was presented.

Aliphatic and aryl ethers are readily cleaved in air with novel (π-allyl) palladium complex **38** in aniline.[64] The aryl



Scheme 37.

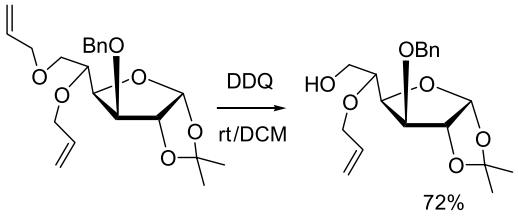




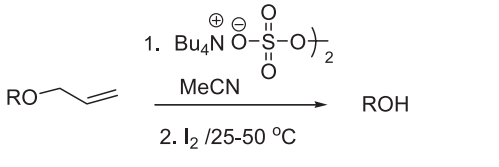
Scheme 38.

ethers required only 0.1 mol% catalyst and reactions were complete, generally, in less than an hour at 30 °C (Scheme 38) whereas the aliphatic systems needed 2 mol% and 2–8 h for complete reaction at 50 °C. The reagent is compatible with aryl functionalities like CN, CHO, ester, ketone and Br. Hydroxyl protecting groups such as Ac, MOM, acetonide, THP and TBDMS were unreactive towards these conditions. Enhanced selectivity for this complex vs $Pd(PPh_3)_4$ was recorded for allyl allyloxybenzoates, whereby the former reagent shows little affinity for the ester and the latter deallylates both sites. Ozawa proposed a mechanism whereby the typical oxidative addition to the C–O bond is not involved.

3.1.6. Oxidative. Stoichiometric DDQ (1.2 equiv) removes allyl groups from primary alcohols under mild conditions in DCM in 85–92% yield but is unreactive towards anomeric and secondary alcohols (Scheme 39).[65] Selectivity for the allyl groups of a primary alcohol in presence of a benzyl group was observed but reverse selectivity was seen for a benzyl group in the presence of an anomeric allylic ether. Removal of the hydroquinone by-product can often hinder product isolation with this reagent though.



Scheme 39.

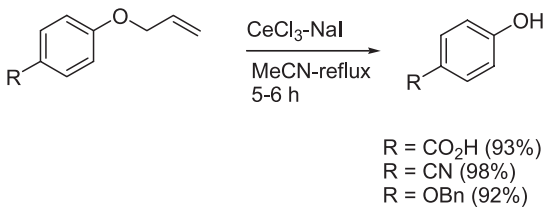


R = menthyl (93%)
R = Ph $(CH_2)_2$ (90%)
R = $PhCH_2C(CH_3)_2CH_2$ (95%)

Scheme 40.

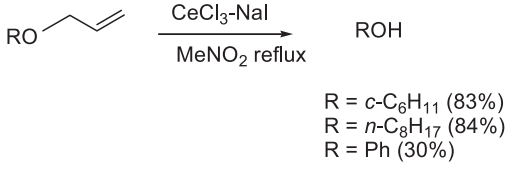
The oxidative deprotection of allyl ethers utilizing tetrabutylammonium peroxydisulfate, readily prepared from tetrabutylammonium hydrogensulfate and potassium peroxydisulfate, was reported by Kim.[66] The one-pot procedure with 1 equiv iodine served to hydrolyze the proposed vinyl hemiacetal intermediate to the product alcohol (Scheme 40). Removal of the allyl group from 1°, 2° and 3° ethers was achieved, as was the typical compatibility with other hydroxyl protecting groups. Another report used sodium methoxide instead of I_2 to hydrolyze the reaction intermediates.[67]

3.1.7. Lewis acids. Two groups have reported on the application of cerium (III) chloride heptahydrate/sodium iodide to the deprotection of allyl ethers. In one instance the use of refluxing MeCN was advocated (69–98% yield) for a series of aryl and aliphatic systems (Scheme 41).[68]



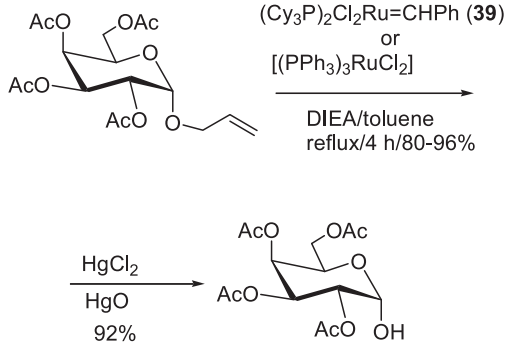
Scheme 41.

In the other report,[69] MeCN gave marginal results but success was achieved in nitromethane, but only for primary and secondary aliphatic moieties (Scheme 42). Use of 1,3-propanethiol as an allyl iodide scavenger improved the reaction efficiency. In both cases, selectivity in the presence of Bn and THP protecting groups was observed.



Scheme 42.

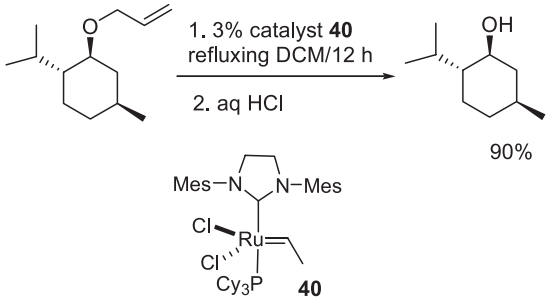
3.1.8. Metal-catalyzed isomerization. The deallylation of glycosides via isomerization with 10 mol% $[Ph_3P]_3RuCl_2$ in refluxing toluene (4 h) with DIEA, followed by hydrolysis of the enol ether with $HgCl_2$–HgO was reported by Roy (Scheme 43).[70] The advantage over other metals is the



Scheme 43.

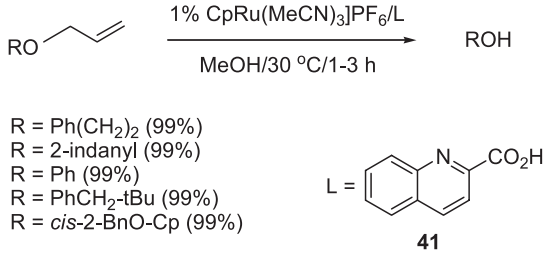
availability, lower cost, and better selectivity. Unaffected protecting groups include *O*-isopropylidine, Ac, and Bn. The reaction can be run in one-pot and gave the products in excellent yield (84–96%). The authors note the first generation Grubb's catalyst (**39**) also is effective in this regard, albeit in lower yields.

Meanwhile, Cossy[71] found that the second generation Grubbs' catalyst (**40**; 3–8 mol%) is an effective catalyst for this purpose. In a limited number of examples, deprotection of allyl ethers derived from secondary and tertiary alcohols was effected in refluxing DCM (12 h), followed by acidification (75–95% yield) (Scheme 44). A methallyl example also worked whereas an isoprenyl group was unaffected.



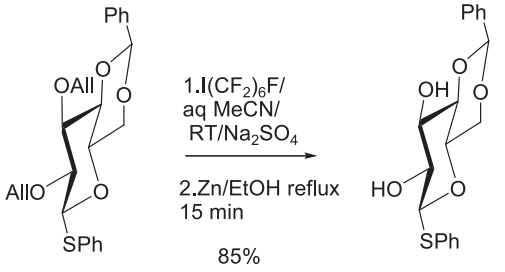
Scheme 44.

Kitamura[72] screened a variety of ligands in combination with $[(CpRu(II)(MeCN)_3]PF_6$ at 30 °C in trying to develop an efficient deprotection protocol for allyl ethers. The optimized conditions used quinaldic acid (**41**) (1:1 mole ratio with catalyst) to cleave allyl ethers with turnover numbers (TON) of up to 1000 (0.5–3 h) (Scheme 45). Compatible solvents include MeOH and mixed systems (1:1) with water, MeCN, DMF and THF. The scope of substrates included the allyl ethers of primary, secondary and tertiary alcohols, as well as phenols. Compatibility with neighboring alkenes and alkynes in the aliphatic series was also reported, without signs of isomerization. The allyl group of a multi-functional dipeptide was chemoselectively removed in >99% yield. An improved synthesis of this catalyst was recently reported.[73]



Scheme 45.

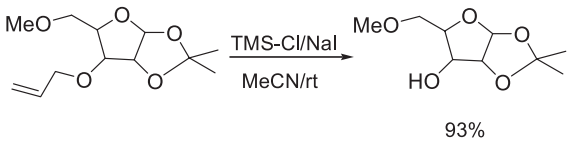
3.1.9. Miscellaneous. The advent of fluorous chemistry has led to the development of an *O*-allyl removal process whereby initial reaction of the sugar substrates with $I(CF_2)_6X$ (X=Cl, F) gives the perfluoroalkylated species which is removed with Zn powder in refluxing EtOH to



Scheme 46.

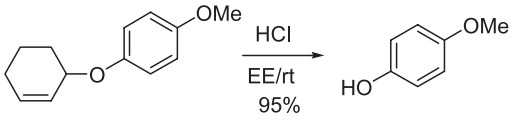
provide the alcohol in 72–93% yield for the two-step procedure (Scheme 46).[74]

The in situ generation of trimethylsilyl iodide (from TMS-Cl and NaI), a well-known reagent for the demethylation of ethers, has been applied to deallylation as well.[75] Phenolic benzylic and aliphatic ethers were rapidly deprotected using 1.5 equiv of reagent (>90% yields) (Scheme 47). The selective deprotection of an allyl ether in the presence of an aliphatic methyl ether in a sugar substrate was reported.

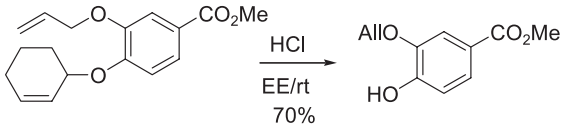


Scheme 47.

A new protecting group for phenols, cyclohex-2-en-1-yl ether, has been described by Depreux.[76] Ether formation is achieved by reacting 3-bromocyclohexene with the phenol and potassium carbonate in acetone at rt/24 h. The deprotection of a collection of protected phenols was accomplished with anhydrous HCl in ether at rt in yields mostly >85% (Scheme 48). Aryl substituents such as nitro, methyl ester, bromide and acetate were not affected. The selective deprotection in the presence of methyl or allyl ethers was most notable (Scheme 49).



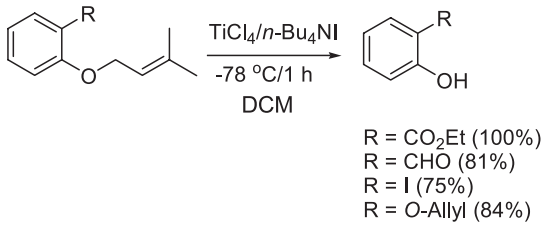
Scheme 48.



Scheme 49.

3.2. Branched allyl ethers (prenyl ethers and others)

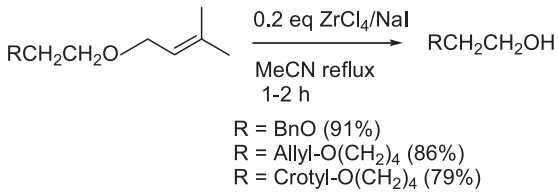
The selective cleavage of a prenyl ether in the presence of an allyl or crotyl ethers was performed by Oshima using $TiCl_4$/*n*-Bu_4NI (1.1 and 1.0 equiv, respectively).[77] Aryl prenyl ethers with a directing group in the *o*-position were easily cleaved at −78 °C over 10–60 min (Scheme 50). In the absence of such a neighboring group, no reaction was



Scheme 50.

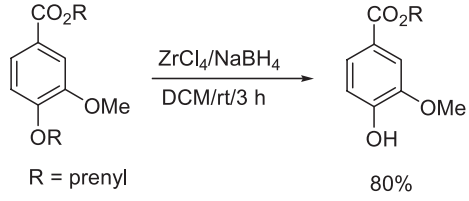
observed; such that an *o*-prenyl ether can be removed in the presence of a *p*-prenyl ether for the related benzaldehyde derivative. Aliphatic (1° and 2°) prenyl ethers are also reactive with this reagent at 0 °C. The author supposes the neighboring atom coordinates the iodo-titanium ate species, thus activating the iodide toward nucleophilic attack of the oxygen-bearing carbon atom of the ether.

Another Lewis acid based system, catalytic zirconium (IV) chloride/sodium iodide, deprenylates both aryl and aliphatic ethers in refluxing MeCN over 1–2 h (Scheme 51).[78] The product alcohols were obtained in 78–92% yield with selectivity demonstrated in the presence of allyl or crotyl ethers in a differentially protected aliphatic diol.



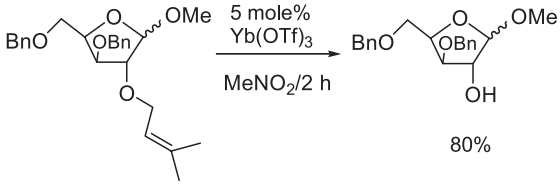
Scheme 51.

The zirconium (IV) chloride/sodium borohydride combination (1, 4 equiv, respectively) also cleaves prenyl ethers at rt (Scheme 52).[79] Yields in the range of 70–96% were obtained selectively for aryl prenyl ethers in the presence of OBn, OMe and prenyl esters.



Scheme 52.

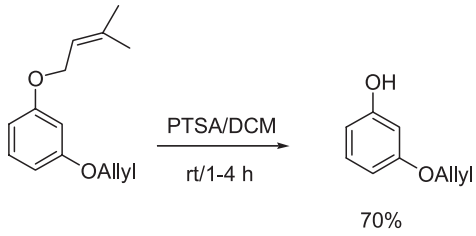
Ytterbium triflate (5 mol%) is another reagent that selectively catalyzes the removal of a prenyl group in the presence of allyl or crotyl ethers under mild conditions (Scheme 53).[80] Yields of 74–90% were observed for aryl ethers with various electronic substituents and one example



Scheme 53.

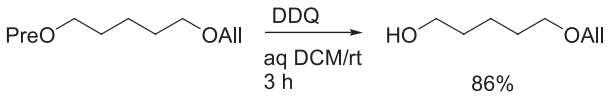
with a differentially-protected furanose (OBn, OMe) that was selectively deprotected in 80% yield.

p-Toluenesulfonic acid (PTSA) efficiently removes prenyl aryl ethers selectively under mild conditions in DCM over 1–4 h (70–98% yield) (Scheme 54).[81] Other hydroxyl protecting groups not affected by these conditions include methyl, benzyl and allyl ethers.



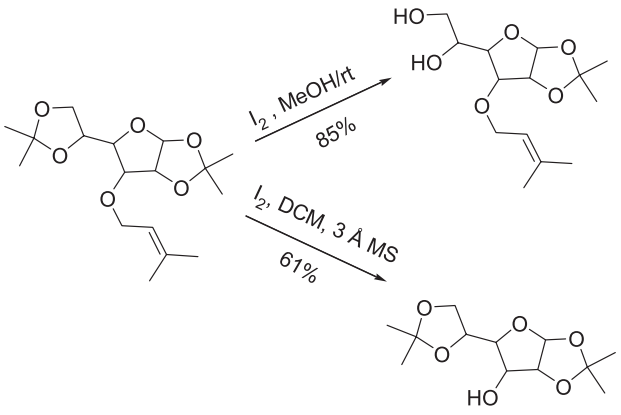
Scheme 54.

DDQ (1.2 equiv) can also deprotect prenyl ethers of 1°, 2° and 3° aliphatic ethers in aq DCM at rt, even selectively in the presence of an allyl protected alcohol (86% yield), as in the case of differentially-protected 1,5-pentane-diol (Scheme 55).[82] $Mn(OAc)_3$ can be used as a re-oxidant allowing the DDQ equiv to be reduced to 0.1 but leads to prolonged reaction time (18 h vs 90 min). The reaction is thought to proceed via DDQ hydride abstraction from the activated methylene carbon followed by quenching of the resulting carbocation with water and subsequent decomposition of the hemiacetal.



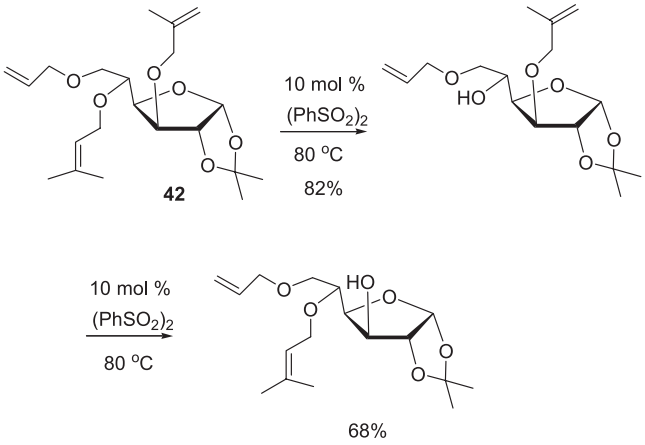
Scheme 55.

Similar reactivity was described using iodine (1.5 equiv) in DCM under mild conditions (Scheme 56).[83] Addition of molecular sieves is critical to the success of the reaction in order to trap the HI formed in the reaction to prevent reaction with acid-labile groups, such as isopropylidene. Substoichiometric amounts of iodine (0.4 equiv) can be used but requires a longer reaction period (4.5 h vs 15 min with 1.5 equiv for the menthol example). A side-by-side comparison of these two reagents, as well as mechanistic insight into the iodine system is provided in a full paper by Vatèle.[84]



Scheme 56.

Remarkable selectivity in the order methylprenyl > prenyl > methallyl ≫ allyl was observed by Vogel using 10 mol% diphenyldisulfone ($(PhSO_2)_2$) in a sealed tube at 80 °C (61–93% yield) (Scheme 57).[85] Such that triple-differentially protected glucofuranoside **42** can be deprotected step wise in the order given above leaving an allyl protected site unaffected. The authors explain this reactivity order by noting the energy barrier of a direct hydrogen abstraction mechanism, depends on the ionization energy of the alkene. The more highly substituted alkenes have lower energy barriers as it can better stabilize charge-transfer configurations of the transition states.



Scheme 57.

Similar reactivity has been observed using catalytic amounts of the polysulfone derived from methylidene–cyclopentane and sulphur dioxide.[86]

Hara[63] reported on the deprotection of branched *O*-allylphenols with catalytic 10% Pd/C in 10% KOH in MeOH. Methallyl, isoprenyl and 1,1-dimethyl-2-propenyl groups were cleaved in the *p*-nitrophenyl ether series in > 95% yield (Fig. 19).

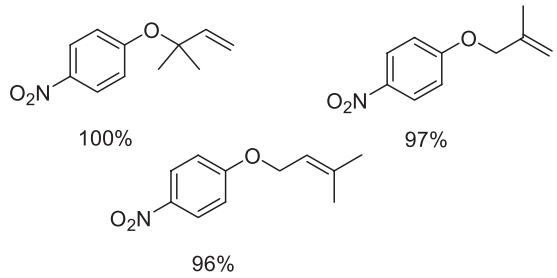
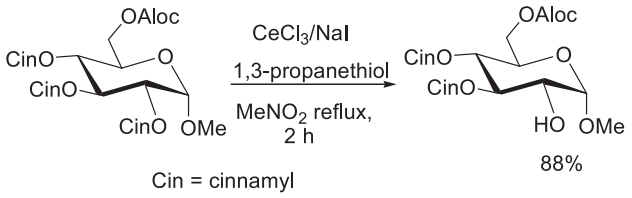


Figure 19. Conditions: 10% Pd/C, 10% KOH-MeOH, rt 24–30 h.

Bartoli's cerium(III) chloride heptahydrate/sodium iodide reagent more easily removes the branched allyl protecting groups than the parent allyl group itself.[68] Crotyl, cinnamyl and β-methallyl octyl ethers were deprotected in 2–10 h versus 30 h for the allyl octyl ether. The prenyl example



Scheme 58.

gave a low yield though. A differentially protected monosaccaride was triply de-cinnamylated in the presence of Aloc (Scheme 58), and TBDPS groups in 52–88% yield.

4. Benzylic and related ethers

4.1. Benzylic ethers

4.1.1. Lewis acids. Yamamoto[87] reported a novel debenzylation of aryl ethers such as **43** using catalytic amounts (1–3 mol%) of rare earth metals including scandium(III) triflyl methide $Sc(CTf_3)_3$ (Fig. 20). Reactions are run in anisole over 0.5–2.5 h at 100 °C and the product obtained in 87–97% yield. Cleavage of secondary benzyl ethers resulted in poor yields due to competitive dehydroxylation and/or debenzyloxylation, however activated benzyl ether **44** gave a near quantitative yield of the corresponding *sec*-alcohol with several reagents including free triflimide.

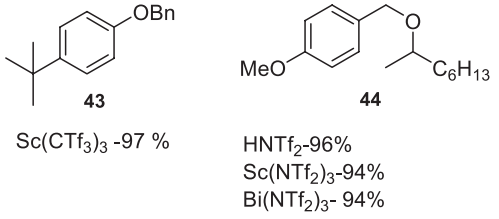


Figure 20.

Falck et al.[88] devised a novel approach to the selective cleavage of benzyl ethers using a combination of $CrCl_2$ (3 equiv) and LiI (4 equiv) in wet ethyl acetate at 75 °C. $CrCl_2$ or LiI alone resulted in little or no cleavage, however a combination of $CrCl_2$/LiBr or $CrCl_2$/*n*-Bu_4NI was quite effective. Functional groups like esters, THP and silyl groups are tolerated. Selective cleavage of a secondary benzyl ether in a glycerol derivative depicted excellent selectivity. An allylic benzyl ether, which was resistant to standard dealkylating conditions, was deprotected in high yield with this method (Fig. 21).

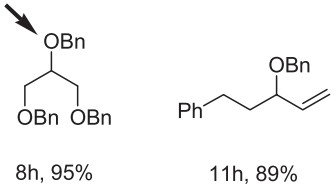


Figure 21.

Debenzylation of D-glucuronolactone derivative **45** was accomplished without compromising the anomeric center, acetonide, or lactone functional groups. In a subsequent study, the group expanded the scope of this technology for the regioselective deprotection of polybenzylated carbohydrates.[89] Yields ranged from 79 to 95%. Inositol derivative **46** was selectively cleaved at the C_2 position resulting in 81% yield of the parent alcohol. Three-point coordination between Cr and the carbohydrate is critical for optimal regioselectivity (Fig. 22).

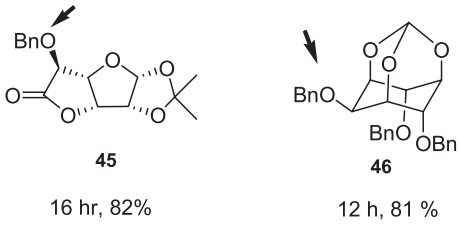
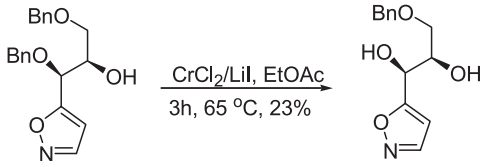


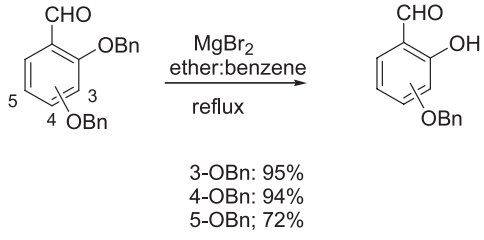
Figure 22.

When preparing isoxazole containing natural products, Piancatelli[90] used $CrCl_2$/LiI for the selective cleavage of a secondary benzyl ether in the presence of a primary benzyl ether and a free hydroxyl group albeit in lower yield (23%) (Scheme 59).



Scheme 59.

Benzyl ethers *ortho* to a carbonyl group were selectively deprotected with $MgBr_2$ in ether–benzene solution.[91] De-*O*-benzylation of various benzene and naphthalene aldehyde derivatives gave yields ranging from 63 to 95% (Scheme 60). A six-membered chelation ring generated via coordination of the carbonyl and the *ortho* ether groups is believed to facilitate the bromide anion mediated debenzylation. The role of Et_2O as a coordinating solvent is also considered critical. Generally, high yields were reported for benzylic derivatives while moderate yields were obtained for naphthalene derivatives.



Scheme 60.

Iodotrimethylsilane (TMSI) mediated bis-debenzylation of **47** provided a selective estrogen receptor modulator (SERM) candidate in our laboratory (Fig. 23).[92] The combination of thiourea and *N*-methylimidazole was effectively used to scavenge the benzyl iodide by-product,

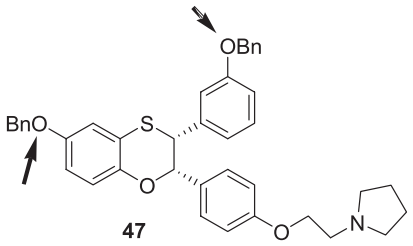
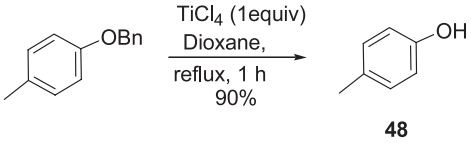


Figure 23. Conditions: TMSI (6.9 eq), thiourea (2.5 eq), *N*-methylimidazole (1.3 eq), CH_3CN, −10 °C to rt, 82%.

which would otherwise result in significant amounts of ring and *N*-benzylated impurities. The reaction ran at −10 °C to rt over 12 h, and the scavengers were completely removed during an aqueous work up. Catalytic hydrogenolysis was not effective due to the presence of sulphur in the benzoxathiin ring, which resulted in catalyst poisoning.

Rajakumar and Murali emphasized the need for dioxane as the solvent in the deprotection of phenolic ethers using $TiCl_4$.[93] A nucleophilic cleavage of an intermediate *O*-$TiCl_3$ complex by solvent molecules is believed to facilitate the deprotection. Tetrahydrofuran was not feasible as it was cleaved by $TiCl_4$ resulting in the formation of *p*-chlorobutanol. Use of catalytic $TiCl_4$ was not effective. The deprotection of a series of benzyl and allyl ethers was described (yields: 78–90%). The synthesis of cresol (**48**) is typical. (Scheme 61).



Scheme 61.

4.1.2. Reductive cleavage. Clerodane diterpenoids are potential medicinal and insecticidal agents. In the course of preparing an advanced intermediate **49** for the synthesis of clerodanes, a mild and efficient reductive cleavage of benzyl ethers was developed by Liu using lithium naphthalenide (LN) (Fig. 24).[94,95] Hydrogenolysis was incompatible with the disubstituted double bond, while acidic reagents such as ferric chloride gave exclusively cyclic products. Alcohols, C=C bonds, and protecting groups including THP, silyl, and methoxy methyl ethers are compatible with the reaction conditions. For ketone substrates like **50**, prior enolization with LDA was advised before deprotection.

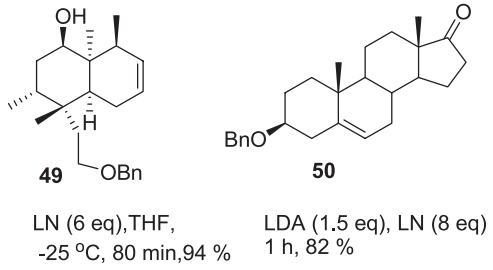
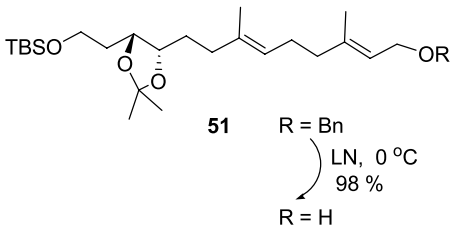


Figure 24.

An excellent application of this methodology was reported by Xu and co-workers[96] during the total synthesis of a novel tetraterpenoid, methyl isosartortuoate. Near quantitative cleavage of benzyl ether **51** in the presence of a TBS and isopropylidene acetal groups was observed at 0 °C (Scheme 62).



Scheme 62.

Early biological studies charge Brefeldin A (BFA) with antifungal, antitumor, antiviral and nematocidal activities. In the total synthesis of BFA and 7-*epi*-BFA, respectively, bis debenzylations of intermediates **52** and **53** were successfully achieved with LN (Fig. 25).[97] Reductive cleavage of **52** using sodium in liquid ammonia was not selective.

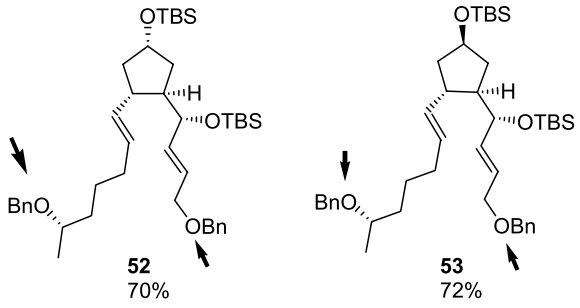


Figure 25.

The reductive cleavage technique was modified by Yus and co-workers[98] who used catalytic naphthalene (8 mol%) with excess lithium for the cleavage of benzyl ethers, such as **54**, **55** and expanded its use for the cleavage of allyl ethers (Fig. 26). Substrates were added to the reagent at temperatures ranging from −78 °C to rt. In general, benzyl ethers gave better yields. The same protocol was used for the deprotection of *N*-substituted tosylamides, carboxamides and *N*,*N*-disubstituted amides.

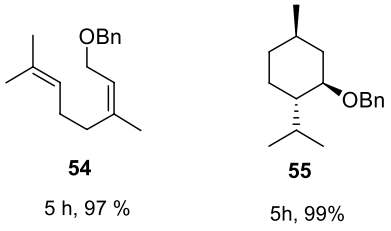


Figure 26. Conditions: Li (excess), $C_{10}H_8$ (8 mol%), THF, −78 to −10 °C.

Sinaÿ used triisobutylaluminium (TIBAL) for a regioselective de-*O*-benzylation of monosaccharidic benzylated phenylsulfonylethylidene (PSE) acetals (Fig. 27).[99] The reaction was run at 50 °C in toluene. The presence of two

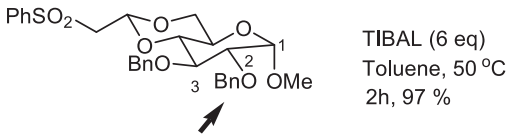


Figure 27.

contiguous *cis*-oriented alkoxy groups appears to be crucial for selective mono de-*O*-benzylation. While such substrates give quantitative yield of a mono debenzylated product, substrates with no *cis*-oriented alkoxy groups have resulted in decomposition. In a case where the substituents at positions 1–3 are all *cis* oriented, a mixture of products was obtained, including a ring opening product that resulted from reduction at the anomeric center (C_1–O bond cleavage).

A mild and novel potassium-induced electron transfer process resulted in selective cleavage of benzyl ethers.[100] The reaction proceeded at rt in 92–99% yield using K–*t*-$BuNH_2$/*t*-BuOH/18-crown-6 (Fig. 28). The method is compatible with TBDMS, THP, epoxy ethers and conjugated C=C bonds. The compatibility of TBDMS and THP groups under basic medium makes this method particularly advantageous. The same protocol was used for cleavage of benzylidene acetals giving the corresponding diols in 73–94% yield. The linking of K^+ with 18-crown-6 is believed to promote the electron transfer from K to the substrate, facilitating formation of an alkoxide anion. Proton transfer from *t*-BuOH or *t*-$BuNH_2$ to the alkoxide is the final step in the proposed mechanism.

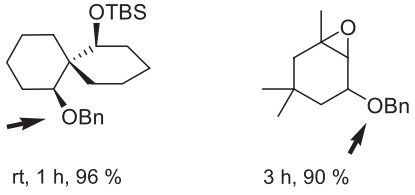
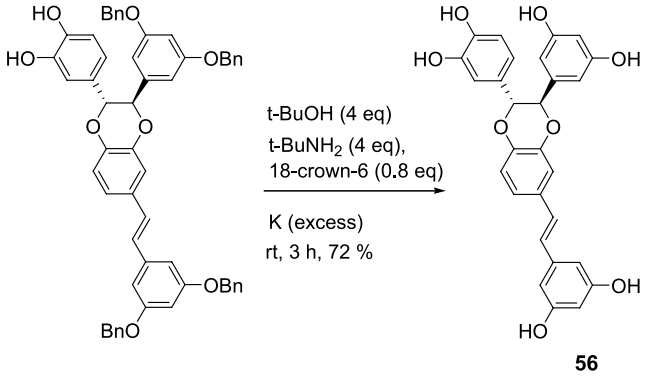
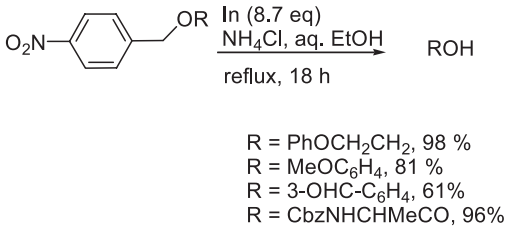


Figure 28. Conditions: K (10 eq), t-$BuNH_2$ (2 eq) t-BuOH (2 eq), 18-crown-6 (0.1 eq).

Pan et al.[101] used the same method for an efficient final step quadruple debenzylation in the total synthesis of a possible cytotoxic and hepatoprotective agent. The final product (±), maackin (**56**), was obtained in 72% yield (Scheme 63).



Scheme 63.



Scheme 64.

Indium-mediated reductive cleavage of *p*-nitrobenzyl (PNB) ethers was accomplished in aqueous ammonium chloride (Scheme 64).[102] On treatment with indium metal the nitro group was reduced and the ether bond cleaved, liberating the free alcohol along with a *p*-toluidine by-product that was removed during aqueous workup. Other groups like methoxy, Ac, aldehyde, and Cbz groups were unaffected. The same reagent can also be used for deprotection of *p*-nitrobenzyl esters.

A Japanese group[103] demonstrated this indium-based methodology in the total synthesis of anti-inflammatory flavonoids. Selective removal of the PNB ether of a poly-protected intermediate **57** gave a 73% yield of the corresponding alcohol (Fig. 29).

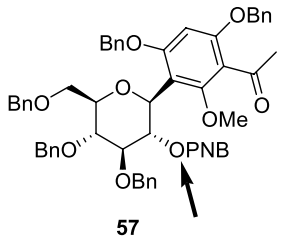


Figure 29. Conditions: ln/NH_4Cl (aq), MeOH/i-PrOH 85 °C, 73%.

Cleavage of *p*-cyanobenzyl ethers (OBnCN) was observed using triethylgermyl sodium (Et_3GeNa) in dioxane (Fig. 30).[104] The reagent, prepared from Et_6Ge and Na in HMPA, was also effective for the cleavage of amines and thiols. An electron-transfer mechanism was proposed. Thus, reduction of *p*-cyanobenzyl ether by Et_3GeNa generates a radical anion that is cleaved to form an alkoxyl anion, which is then protonated by water to give the desired alcohol.

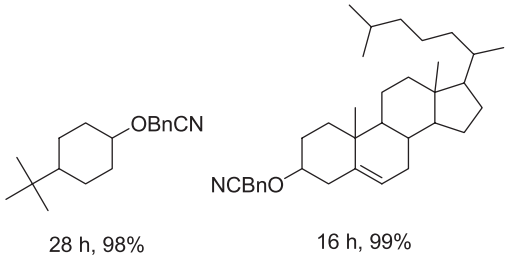


Figure 30. Conditions: Et_3GeNa (2.4 eq)/1,4-dioxane/HMPA/50 °C.

A combination of excess lithium and ethylenediamine in oxygen-free THF was effective in deprotecting benzyl and aryl methyl ethers (Fig. 31).[105] Formation of a radical anion via coordination of Li with substrate, diamine and THF is considered crucial for the demethylation reaction. When

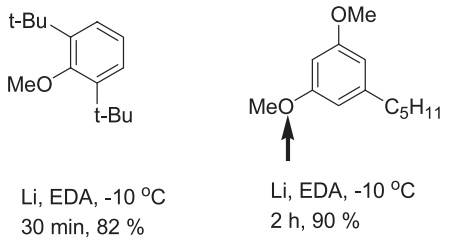


Figure 31.

both *ortho* positions are occupied by an alkyl group, accelerated rates and high yields were recorded. *para*-Allyl and *ortho* halogen groups displayed a retarding effect. Demethylation of aryl ethers with *para* electron-withdrawing substituents resulted in decomposition.

Application of this methodology to geranyl benzyl ether (**58a**) gave geraniol in 92% yield (Fig. 32). Allylic and propargylic ethers are not compatible as the former is isomerized and the later is reduced. The group also developed *m*-xylylmethyl (MXM) as an alternative alcohol protecting group that is cleaved faster under reductive conditions but is immune to hydrogenation conditions using Pd/C at atmospheric pressure.

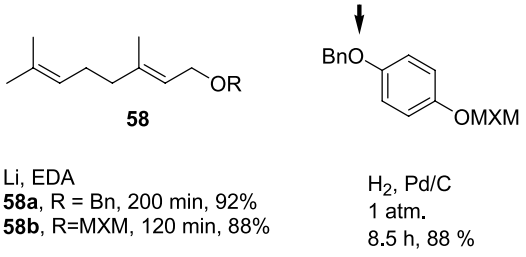
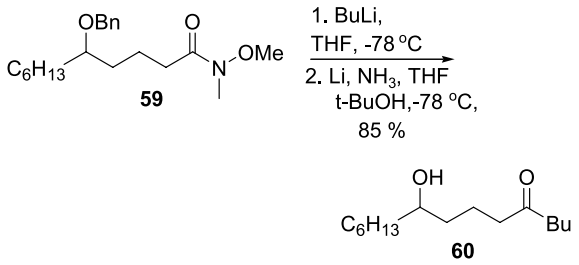
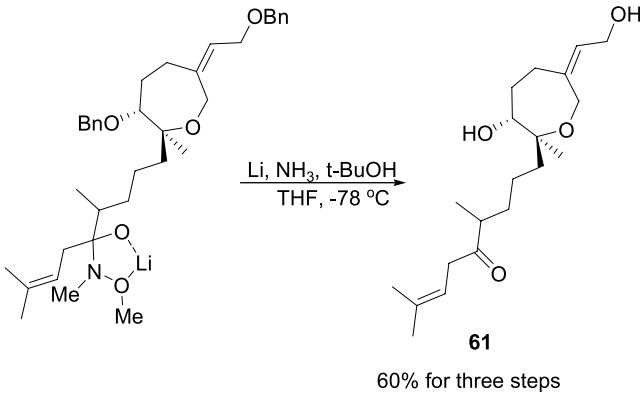


Figure 32.

Cossy et al.[106] disclosed the use of Weinreb amides as latent carbonyl protecting groups for a combined nucleophilic addition/Birch reduction process to generate ω-hydroxy ketones. Once a stable tetrahedral intermediate was generated by the addition of an organometallic reagent to the Weinreb amide, Birch reduction led to rapid cleavage of Bn, PMB and Tr protecting groups. Generally yields of the hydroxy ketone ranged from 58 to 92%. The transformation of **59** to **60**, is typical (Scheme 65). Alkynes are not reduced at lower temperatures, however, if the reduction step is carried out at higher temperatures for extended period of time, partial and over-reduction of ketones was observed.



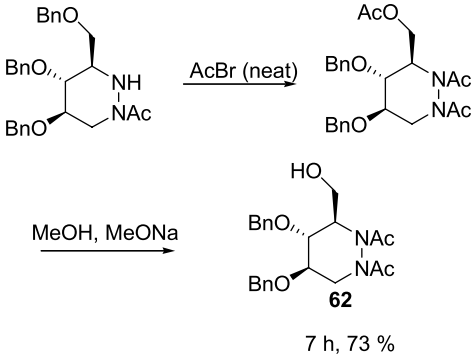
Scheme 65.



Scheme 66.

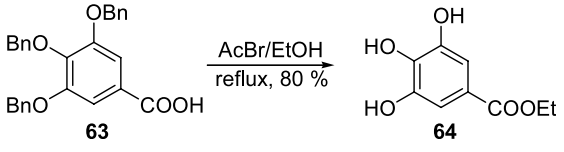
The group relied on the same technology[107] for the final step in the total synthesis of (+)-(2′*S*,3′*R*)-zoapatanol **61**. A 60% yield was reported for the three steps namely: Weinreb amide formation, prenyl group installation and bis-debenzylation (Scheme 66).

4.1.3. Acidic reagents. During the synthesis of an azafagomine derivative with glycosidase inhibitory activities, Bols and co-workers[108] selectively debenzylated a primary benzyl ether. The desired transformation was accomplished in neat acetyl bromide. Subsequent *O*-deacylation gave the target precursor **62** in 73% overall yield (Scheme 67).



Scheme 67.

Benzyl ethers were cleaved with in situ generated HBr, prepared from a reaction between acetyl bromide and an alcoholic solvent.[109] *Tris*-debenzylation of **63** gave ester **64**, in 80% yield (Scheme 68). Deprotection of *N*-*t*-Boc, *N*-Cbz and *N*-Ac groups was also effective under these conditions.



Scheme 68.

Sterically hindered benzyl ethers that resisted hydrogenolysis with a variety of catalysts including Pd/C and $Pd(OH)_2$, were readily removed by reaction with *N*-bromosuccinimide and light in the presence of aqueous

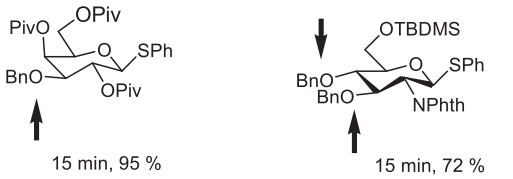


Figure 33. Conditions: NBS (2.5 eq), $CaCO_3$ (4 eq) white light (375 W), CCl_4:H_2O (2:1).

calcium carbonate (Fig. 33).[110] This mild in situ HBr generating tactic was used for the debenzylation of several galactopyranoside derivatives in 72–95% yield. The reaction conditions are compatible with the presence of glycosyl, thiophenyl, phthalimide, fluoride, and ester groups.

Aqueous HBr in the presence of tetrabutylammonium bromide cleaves benzyl ethers[111] in 53–87% yield. The highest yield was reported for deprotection of 4-benzyloxy-3,5-dimethylbenzoic acid (**65**) (Fig. 34), however 4-benzyloxybenzoate subjected to the same reaction conditions resulted in no debenzylation.

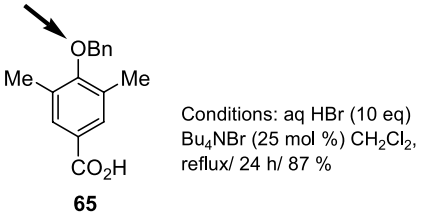
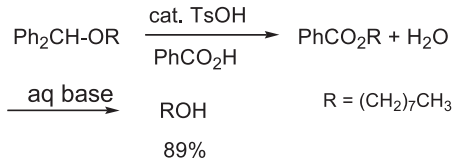


Figure 34.

Cleavage of diphenylmethyl ethers was accomplished in refluxing benzene in the presence of excess benzoic acid and catalytic amounts of TsOH, the intermediate ester was then hydrolyzed to the alcohol (Scheme 69). The process was carried out with removal of water via a Dean–Stark trap.[112]



Scheme 69.

4.1.4. Hydrogenolysis. Titanium loaded hexagonal mesporous silica (Ti-HMS) accelerates the deprotection of benzyl ethers in the presence of acid sensitive functional groups

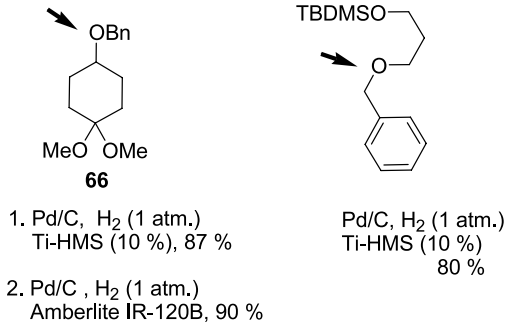


Figure 35.

(Fig. 35).[113] The reaction was run in the presence of 10 mol% of Ti-HMS using 5% Pd/C at 1 atm of H_2. TBDMS, THP and acetal groups are tolerated. Among other strongly acidic cation-exchange resins screened Amberlite IR-120B demonstrated a similar selectivity for benzyl ether **66**.

Raney-Ni demonstrated improved catalytic activity in a multiphase system (aqueous KOH–isocotane-Aliquat® 336) (Fig. 36).[114] The modifier, Aliquat® 336, is believed to promote catalytic activity and chemoselectivity by coating the catalyst particles. This process does not discriminate aldehydes and carbon–carbon double bonds. A chemoselective debenzylation of Boc-*O*-benzylserine (**67**) resulted in the recovery of quantitative amount of Boc-serine.

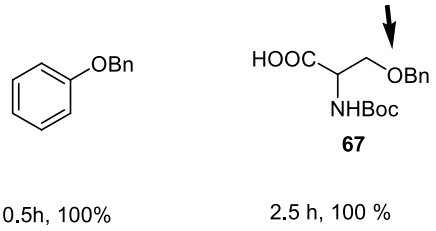


Figure 36. Conditions: Aliquat 336 (0.35 equiv), Raney Ni (5%) isooctane, KOH (2%aq.), H_2, 50 °C.

A one-pot deprotection of benzyl and PMB ethers with excess chlorosulfonyl isocyanate (CSI)/Na_2CO_3 followed by treatment with NaOH/MeOH was reported (Fig. 37).[115] In the case of PMB ethers, reaction with CSI was done at −78 °C in DCM while Bn ethers required refluxing conditions. CSI is believed to activate the ether bond via formation of the corresponding *N*-chlorosulfonyl-*N*-benzylcarbamoyl derivative, which is easily hydrolyzed at rt using NaOH. Generally good yields were obtained, however lower yields were indicated for benzophenone and benzonaphthol, 33 and 16%, respectively. Selective deprotection of Bn ethers was achieved in the presence of allylic groups, an unprotected alcohol, cyclic/TBDPS ethers, and esters.

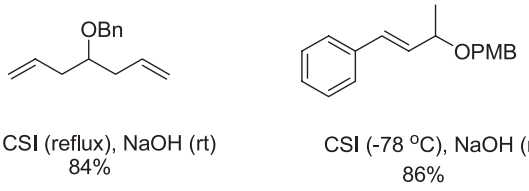
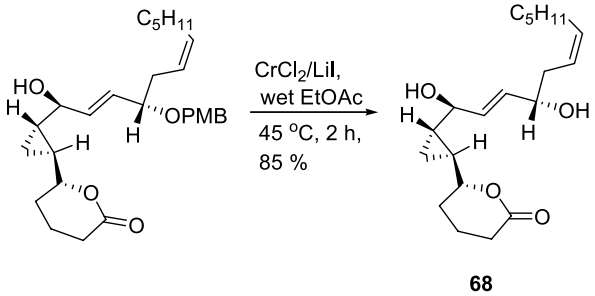


Figure 37.

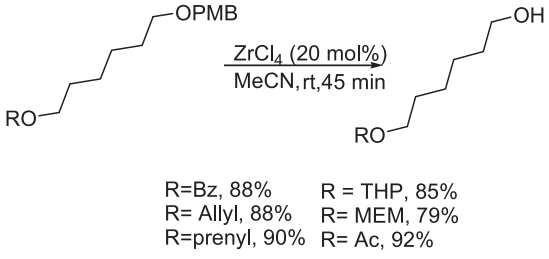


Scheme 70.

4.2. PMB ethers

4.2.1. Lewis acids. Falck et al.[116] employed the $CrCl_2$/LiI methodology to PMB ethers as seen in the final step in the asymmetric synthesis of a marine eicosanoid, constanolactone **68** (Scheme 70).

Sharma[117] has recently reported a fast and mild process for the cleavage of PMB ethers using catalytic amount of $ZrCl_4$ in MeCN (Scheme 71) in 72–92% yield within 30–90 min. PMB ethers were cleaved in the presence of acid sensitive Boc, isopropylidene, glycosidic groups THP/MEM ethers, and base sensitive Ac, Bz groups. Substrates with *O*-allyl and *O*-prenyl ethers were cleaved efficiently. Trityl ethers are not immune to this reagent, as a substrate with both trityl and PMB ethers underwent double deprotection resulting in 76% yield of a diol. In a comparative study, the group demonstrated that $ZrCl_4$ was superior to other Lewis acids like $AlCl_3$, $BiCl_3$, $TiCl_4$ and $FeCl_3$.



Scheme 71.

A combination of $CeCl_3 \cdot 7H_2O$ and NaI in refluxing acetonitrile selectively cleaves PMB ethers to the corresponding alcohols (Fig. 38).[118] Solvents known to strongly coordinate with cerium, including DMF and ethyl acetate, were avoided. PMB ethers are cleaved in the presence of esters and protecting groups like THP, Ac, benzyl and methoxy groups, however the method is not selective towards TBDMS ethers. Lower chemical yields were reported when using catalytic amounts of reagent. An additional electron donating group on the ring accelerates the rate of cleavage as seen in the deprotection of 2,4-dimethoxybenzyl ether **69**. A reverse effect was observed with electron withdrawing substituents.

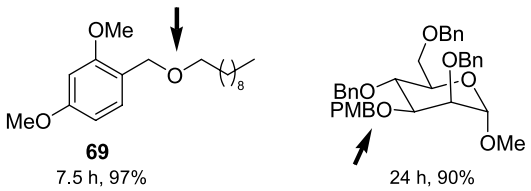


Figure 38.

Complexation of a strong Lewis acid, $SnCl_4$, with polyhydroxylated carbohydrates resulted in unusual regioselectivity and partial deprotection of PMB ethers. (Fig. 39).[119] Preferential mono or bis cleavage of PMB ethers was achieved with careful control of reaction conditions (amount of $SnCl_4$ and temperature). The formation of a tri-oxo tin complex involving the 6-*O*-PMB

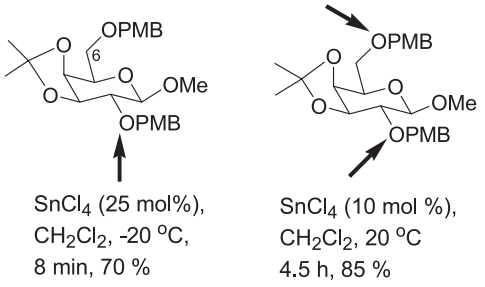


Figure 39.

is proposed to account for the mono-selectivity. The reaction conditions are compatible with benzyl, TMS, and methoxy protecting groups, also, a substrate bearing an extremely acid sensitive isopropylidene acetal gave a high yield (90%) of the corresponding alcohol.

Bouzide et al.[120] described the combination of catalytic $AlCl_3$ or $SnCl_2 \cdot 2H_2O$ with EtSH as an efficient and selective deprotecting agent for PMB ethers (Fig. 40). The mild reaction conditions tolerate functional groups like methoxy, TBDPS, benzyl, acetyl and *p*-nitrobenzoyl esters. Deprotection of PMB protected *p*-cresol, using $AlCl_3$ resulted in a mixture of *p*-cresol (32%) and *o*-alkylated by-product (62%), however *o*-alkylation was significantly reduced (12%) when using $SnCl_2 \cdot 2H_2O$. *o*-Substituted aryl PMB ethers, and those with electron withdrawing groups generally afford high yield of the corresponding alcohols. In a subsequent report, the group has expanded the application of this protocol to the regioselective cleavage of PMB ethers of furanose derivatives.[121]

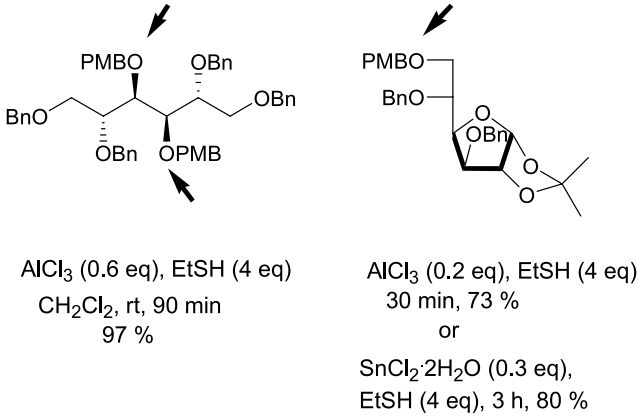
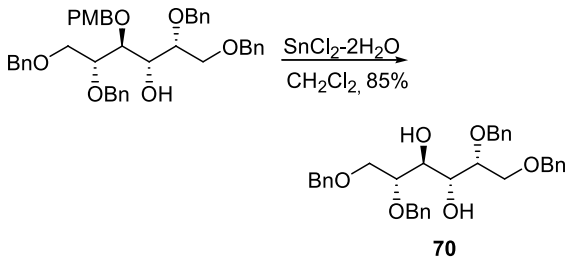


Figure 40.



Scheme 72.

Mannitol derivatives are known to have HIV protease inhibitory activities. A Pharmacor group[122] recently utilized a similar tactic in the preparation of one of these derivatives (**70**) in 85% yield (Scheme 72).

4.2.2. Oxidative cleavage. The use of stoichiometric DDQ for the oxidative cleavage of PMB and related ethers remains a popular protocol.[123] However, two distinct drawbacks remain: its cost and the difficulty of removing the HDDQ by-product. Chandrasekhar and Yadav devised a novel DDQ regeneration technique via oxidative recycling using excess ferric chloride (Fig. 41).[124] The reaction is run in aq DCM using 10 mol% DDQ and 3 equiv $FeCl_3$.

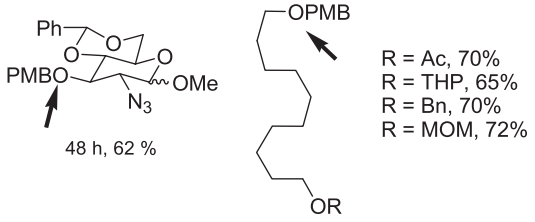
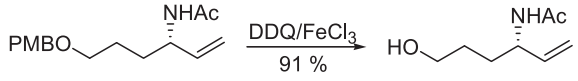


Figure 41. Conditions: DDQ (10 mol%), $FeCl_3$ (3 eq), CH_2Cl_2:H_2O (10:1).

The same strategy was later employed by the group for the cleavage of a PMB ether in the asymmetric synthesis of an anti-convulsive drug, (*S*)-vigabatrin (Scheme 73).[125]



Scheme 73.

Researchers from the same laboratory have also reported using $Mn(OAc)_3$ for the recycling of DDQ.[126] Presumably, the quinone is regenerated by an electron-transfer mechanism which results in a simultaneous reduction of Mn(III) to Mn(II). Acid sensitive groups like TBS and THP are tolerated; base sensitive benzoyl groups are also compatible with this reagent system. A homoallylic PMB ether and a PMB sugar ether gave good yields, while a propargylic ether gave moderate yield (61%) (Fig. 42). The reaction was run (10–24 h) in DCM using 10 mol% of DDQ and 3 equiv $Mn(OAc)_3$.

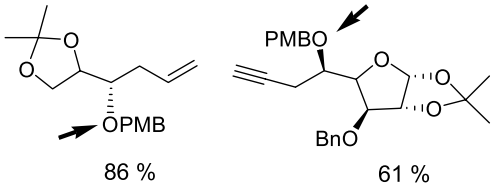
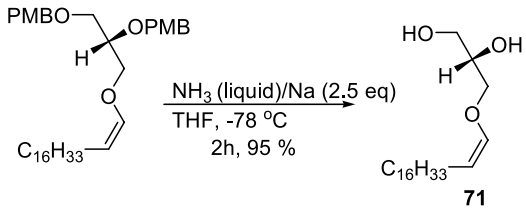


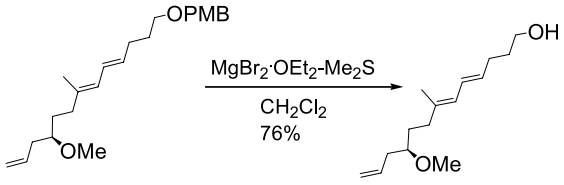
Figure 42.

4.2.3. Reductive cleavage. During the synthesis of *O*-vinyl ether phospholipid plasmalogen, Bittman et al.[127] were unable to use DDQ and CAN as the oxidative removal of PMB resulted in destruction of the core structure. However, a Birch reduction was successfully applied and the final product **71** isolated in 95% yield (Scheme 74). Interestingly, the use of Li was not suitable as it isomerized the double bond.



Scheme 74.

In the asymmetric synthesis of Curacin A, a novel antimitotic agent, Onoda et al.[128] encountered product decomposition during oxidative cleavage with DDQ to remove a PMB group from advanced intermediate **72**. Alternatively, the combination of $MgBr_2 \cdot OEt_2$ and Me_2S was effective, however five repeated reactions were required to isolate 76% of **73** (Scheme 75). Deprotection of aliphatic PMB ethers, proceeded in modest to good yields (35–90%) and PMB ethers were selectively cleaved in the presence of benzyl, TBDMS ethers, and acetonides. Cleavage of MOM and BOM ethers was not successful.



Scheme 75.

Unexpected PMB ether cleavage during a glycosylation lead Hinklin and co-workers to discover the use of primary and secondary sulfonamides with catalytic amounts of TfOH or silver triflate as effective deprotecting agents for PMB ethers (Fig. 43).[129] The group has further developed the use of sulfonamide-functionalized resins (safety-catch resin) for solid phase organic synthesis, using dioxane as a solvent. Competitive sulfonimine formation which results in lower yields for some substrates was avoided by using secondary sulfonamides.

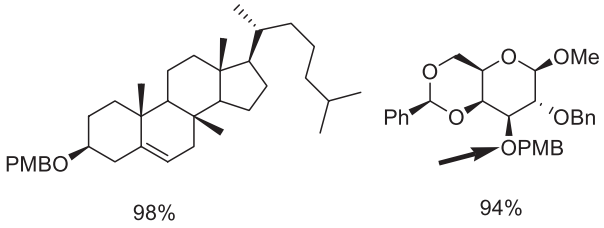
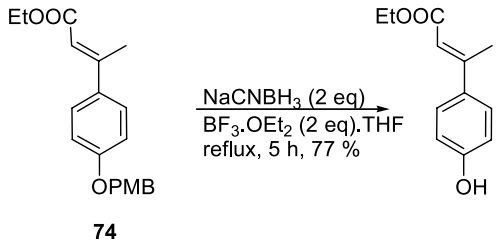


Figure 43. Conditions: 0.55 eq $TsNH_2$, 0.1 eq TfOH, Et_2O.

A mixture of sodium cyanoborohydride and boron trifluoride etherate in refluxing THF cleaved PMB ethers.[130] The reaction is rather efficient and clean with aliphatic PMB ethers. *p*-Nitrophenol was deprotected with only $BF_3 \cdot OEt_2$, as competing reduction of the nitro group results in the presence of $NaCNBH_3$. Deprotection of cinnamate **74**, gave the corresponding phenol in 77% yield without affecting the cinnamate moiety (Scheme 76). While a carbonyl group is deoxygenated with this procedure, double bonds and ester groups are tolerated. Amine and amide functional groups



Scheme 76.

have an inhibitory effect due to possible complex formation with BF_3.

4.3. Removal of new protecting groups

Sharma and Rakesh[131] developed the *p*-phenyl benzyl (PPB) group as a new protecting group for alcohols. Protection is done under acidic conditions by reacting the parent alcohol with *p*-phenylbenzyl trichloroacetamidate in the presence of TfOH or under basic conditions by reacting the alcohol with PPBBr in the presence of NaH. Unlike the PMB group, the PPB group is compatible under acidic conditions, and its deprotection accomplished under known oxidative cleavage techniques (DDQ/$Mn(OAc)_3$). Selective cleavage was obtained in the presence of benzyl and diphenylmethyl groups (Fig. 44).

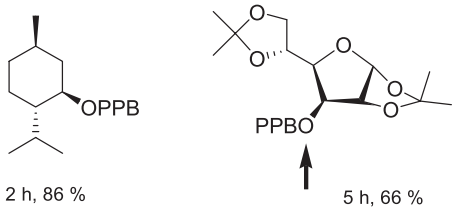


Figure 44. Conditions: DDQ (10 mol%), $Mn(OAc)_3$ (3 eq).

Continuing to develop new masking/unmasking techniques for alcohols, Sharma[132] recently reported two acid-tolerant protecting groups; namely *p*-phenyldiphenyl methanol (PDPM) and *p*-phenylphenyl diphenylmethanol (PPDPM). The alcohols were protected by reacting the substrates with PPDM-OH and PPDPM-OH in the presence of catalytic amount of $Yb(OTf)_3$ in DCM at rt. The *p*-phenyl group facilitated the cleavage under oxidative conditions and enhanced the rate of acid catalyzed hydrolysis. Eight examples were given for installation and removal of these protecting groups along with *p*-methoxydiphenyl methanol (MDPM). Generally MDPM and PDPM groups were cleaved with DDQ and PPDPM ethers were cleaved with catalytic TFA (10 mol%) (Fig. 45).

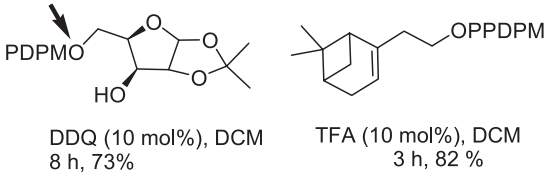


Figure 45.

p-Halobenzyl ethers (PBB = *p*-bromobenzyl; PCB = *p*-chlorobenzyl) were successfully used as protecting groups by Buchwald.[133] These ethers are then converted to labile arylamines via Pd-catalyzed amination. Rapid deprotection of the amine benzyl ethers was observed with Lewis acids ($TiCl_4$, $SnCl_4$). Alternatively, dichloroacetic acid (DCA), cerium (IV) ammonium nitrate and $ZnCl_2$ can be used. Selective cleavage was achieved in the presence of silyl ethers (TIPS), PMB groups and glycal double bonds (Fig. 46).

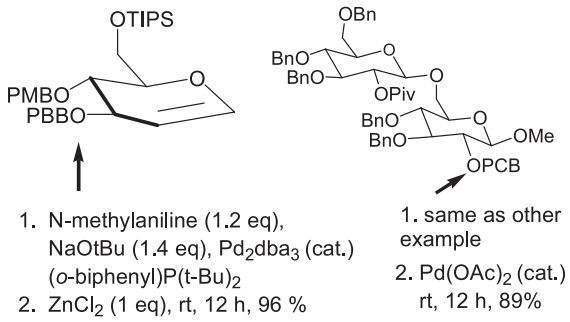
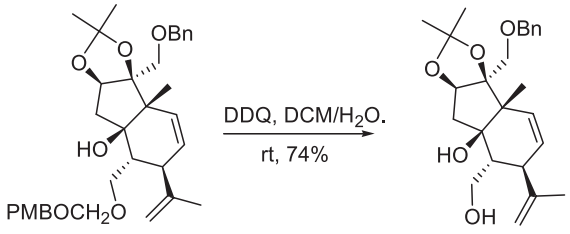


Figure 46.

The PMB-like protecting group *p*-(methoxybenzyloxy)-methyl (PMBOM), devised by Trost et al.[134] was critical in the total synthesis of Corianin, a possible therapeutic agent for schizophrenia. The best diastereoselecivity was obtained using this protecting group during the addition of lithiated acetonitrile in the preparation of an early intermediate, presumably due to the ability of Li to coordinate with the π-cloud of the aromatic ring. The PMBOM group is installed in almost quantitative yield by reacting the alcohol with PMBOM-Cl in the presence of DIEA at rt. Selective cleavage of this protecting group was accomplished at the latter stage of the synthesis using DDQ (Scheme 77).



Scheme 77.

Burke and co-workers[135] chose the PMB (*p*-methoxybenzyl) and *m*-methoxybenzyl (MMB) groups to protect two –OH groups during the synthesis of (+)-breynolide (Fig. 47),

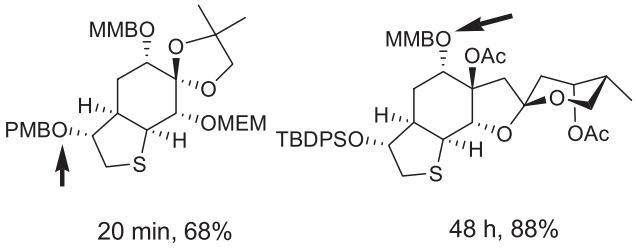


Figure 47. Conditions: DDQ, DCM:H_2O (10:1), rt.

a compound that displayed oral hypocholesterolemic activity in rats. While PMB was oxidatively removed at an intermediate stage using DDQ at rt with in 20 min, MMB survived acidic conditions that were required for the spiroketalization step that was used to set the correct stereochemical configuration of the final product. The MMB ether was cleaved using DDQ over 48 h, before the TBDPS ether and two acetate moieties were hydrolyzed.

Spencer and co-workers[136] developed 2-naphthylmethyl (NAP) as a protecting group for alcohols. NAP is more labile to catalytic hydrogenolysis than the benzyl group and selective removal could be achieved in the presence of Bn, free hydroxyl, ketone and MeO groups, giving 86–96% yields (Fig. 48).

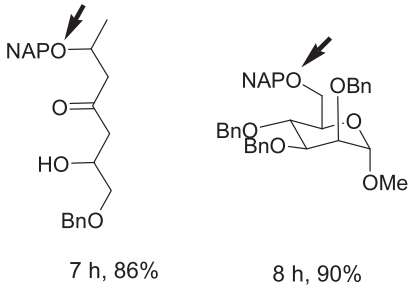
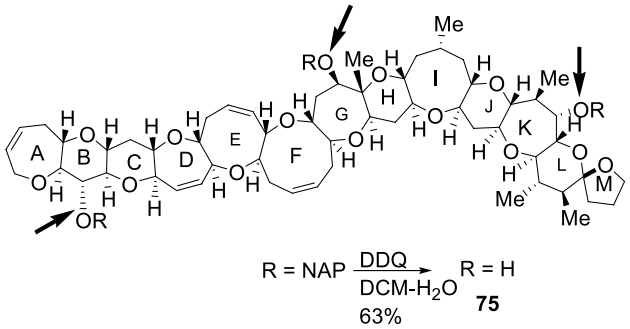


Figure 48. Conditions: H_2, Pd/C, EtOH, rt.

In a subsequent study,[137] the group explored the sequential removal of PMB and NAP protecting groups by oxidative cleavage. Their study showed CAN was superior to DDQ in the selective removal of PMB, and DDQ is more efficient for the cleavage of NAP in the presence of benzyl protecting groups. The first total synthesis of GlyCAM-1 oligosaccharide structures by Matta et al.[55b] was credited to the stability of NAP under acidic as well as basic conditions.

Ciguatoxin CTX3C (**75**) is one of the principal agents for seafood poisoning, and its total synthesis depended on liberating three hydroxyl group in the final step. Reductive cleavage of the Bn ethers was complicated due to the allylic ether in ring A, and use of DDQ resulted in decomposition. Hirama and his co-workers[138] were able to improve the total synthesis using a more acid-stable NAP protecting group, which survived acidic reaction conditions. DDQ assisted *tris*-deprotection of NAP at the final stage resulted in a 63% yield of **75** (Scheme 78).



Scheme 78.

4.4. Trityl ethers

4.4.1. Lewis acids. Sabitha et al.[139] demonstrated that bismuth chloride is an efficient catalyst for the rapid cleavage of trityl ethers. The detritylation proceeded within minutes in the presence of a variety of acid and base sensitive functional groups as well as carbohydrates, terpenes and amino acids (Fig. 49).

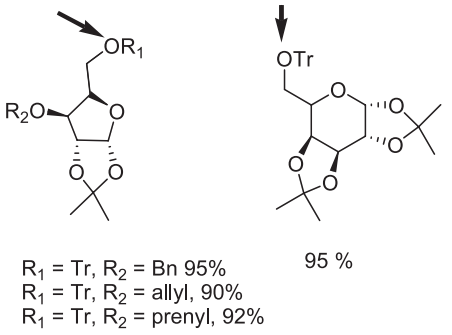


Figure 49. Conditions: $BiCl_3$, (5 mol%), MeCN, rt, 10 min.

The novel catalytic detritylation with ceric triflate $Ce(OTf)_4$ (Fig. 50) at rt was reported.[140] The reaction, run in wet acetonitrile under mild conditions, cleaved Tr and DMT protecting groups in 82–95% yield. Generally, the rate of cleavage was faster for DMT groups as cleavage of DMT-protected anisyl alcohol was instantaneous giving the parent alcohol in 95% yield. Primary and secondary aliphatic, benzylic, trityl and DMT ethers were easily converted to their corresponding alcohols. An effective detritylation of nucleosides was demonstrated in the transformation of **76** to the parent alcohol.

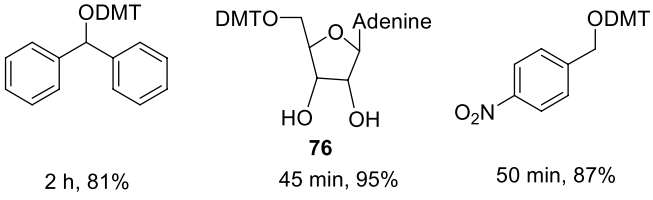


Figure 50.

Another group[141] used BCl_3 in DCM at −30 °C for the selective removal of primary and secondary trityl ethers in the presence of TBDMS, TBDPS, TES, Bn, PMB, and Pv groups. Removal of trityl-protected 5-hydroxypentanal resulted in an in situ cyclization, giving a lactol. Cleavage was complete within 15 min resulting in 80–99% yield of the corresponding alcohols (Fig. 51). Commercially

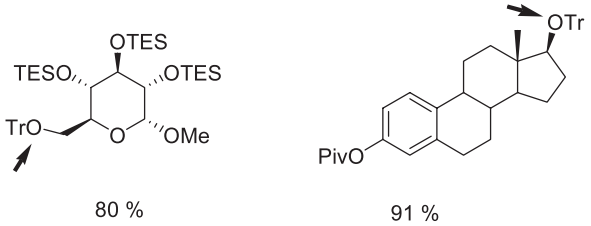


Figure 51. Conditions: 1. BCl_3 (0.6 eq), CH_2Cl_2, 30 min. 2. MeOH.

available solutions of BCl_3 in DCM, hexanes, heptanes and xylenes were equally effective. $BF_3 \cdot OEt_2$ was less effective, and BBr_3 resulted in rapid deprotection and loss of selectivity.

Catalytic indium tribromide in aq MeCN was used for a chemoselective cleavage of trityl ethers by Yadav and co-workers.[142] Trityl ethers were deprotected in high yields (80–95%) (Fig. 52). The reaction can also be carried out in water at 60 °C in the presence of 5 mol% $InCl_3$ or $InBr_3$. Olefins, esters, acetonides, acetates, benzoates, *t*-Boc, Cbz groups and Bn, Me, PMB and TBDPS ethers are not affected. The process is environmentally benign as the catalyst could be recovered during workup and recycled.

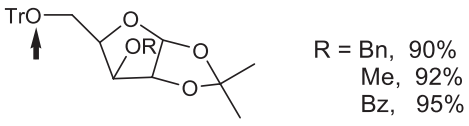
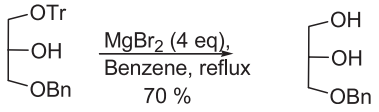


Figure 52. Conditions: $lnBr_3$ (5 mol%), heat, MeCN, 2–3 h.

Trityl protecting groups were selectively removed in the presence of TBDMS and TIPS, using $MgBr_2$ in refluxing benzene (Scheme 79).[143] Diminished activity of the reagent was observed in the presence of coordinating solvents like Et_2O. The same conditions work for removal of isopropylidene protecting groups.



Scheme 79.

4.4.2. Acidic reagents. A facile cleavage of Tr and DMT ethers using catalytic CBr_4 in refluxing MeOH gave 85–93% yields of products (Fig. 53).[144] The more acid sensitive DMT group is cleaved more rapidly and at lower temperatures than the Tr ether. Other protecting groups like Bn, Me, Ac, Ts, allyl, phenyl, propargyl, PMB and TBDPS are not cleaved. Acid sensitive protecting groups such as Boc and Cbz are also unaffected. The deprotection is attributed to an in situ generation of HBr from CBr_4 and MeOH.

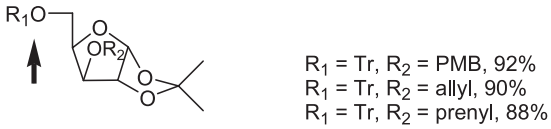


Figure 53. Conditions: CBr_4 (10 mol%), MeOH/reflux, 1.5–3.5 h.

Chen and co-workers[145] further improved this protocol whereby the in situ generation of HBr is achieved under mild conditions using photo-irradiation. They have provided several examples of the chemoselective deprotection of Tr-protected saccharides in high yield (86–95%).

The combination of I_2 in alcoholic solvents was effectively used for deprotection of Tr and DMT ethers (Fig. 54).[146] Traces of in situ generated HI are believed to be the reactive species.

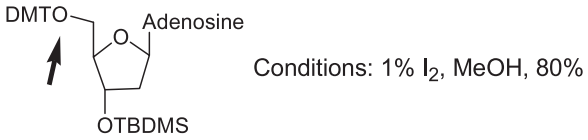


Figure 54.

Keith[147] recently used the same tactic for selective *o*-cleavage of PMB and MOM ethers. Three examples of PMB ethers were cited in moderate yields (50–71%) (Fig. 55). Higher yields were obtained for cleavage of MOM ethers (36–99%).

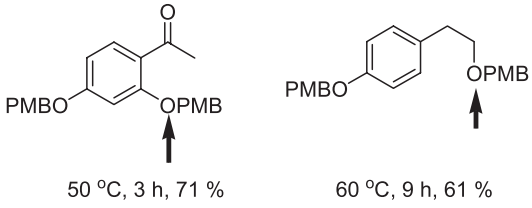
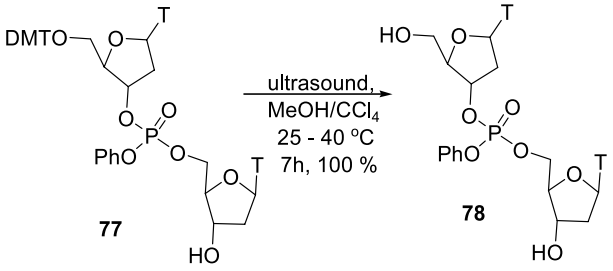


Figure 55.

The deprotection of primary DMT ethers was accomplished using ultrasound in MeOH–CCl_4 (1:1) at ambient temperatures.[148] This technique was used for the cleavage of DMT ethers in the presence of C=C bonds, esters, TBDMS, and Ac, groups. Nine examples were provided with yields ranging from 69 to 100%. Dinucleotide **77** gave quantitative yield of alcohol **78** (Scheme 80).



Scheme 80.

Das[149] developed economical, silica-supported sodium hydrogen sulfate ($NaHSO_4$–SiO_2) as a novel heterogeneous catalyst for deprotection of Tr ethers. Excellent yields (90–100%) were obtained within 3 h (Fig. 56). Other protecting groups including Bn, MOM, MEM, Allyl, Ac, Bz and Ts were not removed. Tr-protected amines are cleaved. The reaction was run in DCM–MeOH (9:1) using a catalytic amount of reagent (exact amount not specified).

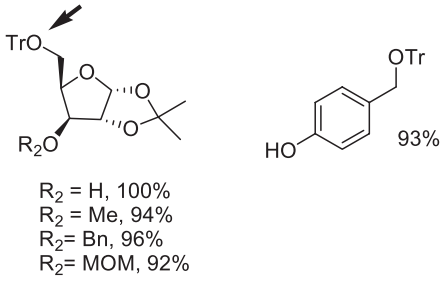


Figure 56. Conditions: $NaHSO_4$-SiO_2, CH_2Cl_2-MeOH (9:1), rt/2.5 h.

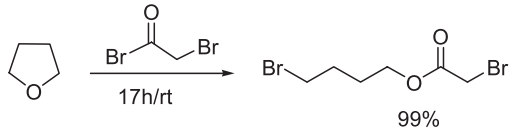
A low-valent titanium (LVT) reagent was developed as a single electron reductant for detritylation.[150] The reaction gave 15–92% yields of free alcohols. The rate of cleavage of protected phenols with LVT reagents is in the order *O*-allyl > *O*-trityl > *O*-benzyl. The technique is also applicable for the cleavage of *N*-trityl bond in trityl amines.

5. Cyclic ethers

The ring-opening reactions of cyclic ethers differs dramatically from the dealkylation of alkyl ethers. Whereas the former is mainly intended to further functionalize the substrate, the latter is primarily utilized to deprotect an alcohol. The emerging trend in cleavage of cyclic ethers is the asymmetric ring opening of epoxides. This topic has just recently been reviewed (>100 references).[151] Accordingly, only a few examples will be covered herein, representing the 'best in class' for a particular transformation.

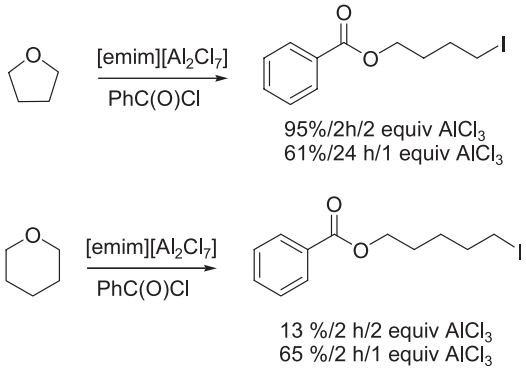
5.1. Acylative cleavage

Bromoacetyl bromide can readily cleave THF to give the dibromo ester in high yield (Scheme 81).[152]

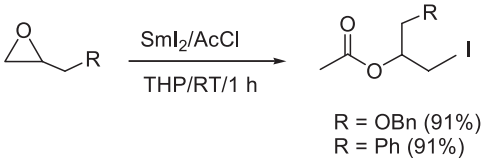


Scheme 81.

Ionic liquids have been applied to the cleavage of cyclic ethers as well. The combination of 1-ethyl-3-methylimidazolium heptachlorodialuminate [emim] [Al_2Cl_7] with benzoyl chloride generates the iodobenzoate adducts in variable yields depending on the mole fraction of $AlCl_3$ used and the substrate (Scheme 82).[153]



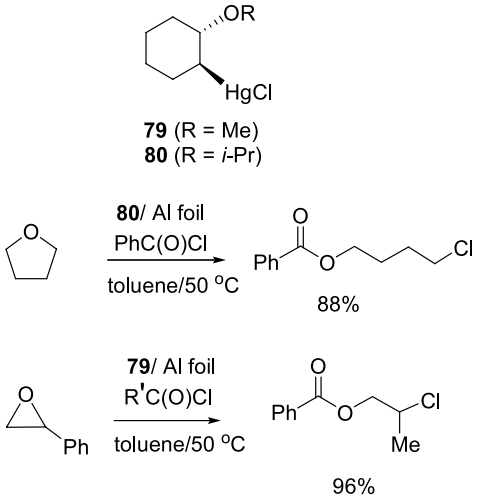
Scheme 82.



Scheme 83.

The regioselective acylative cleavage of monosubstituted epoxides, most notably, with samarium iodide in the presence of acetyl chloride was described by Kim (Scheme 83).[154] The corresponding iodo-esters derived from attack of the iodide at the less substituted carbon atom of the ring were obtained in excellent yield under mild conditions.

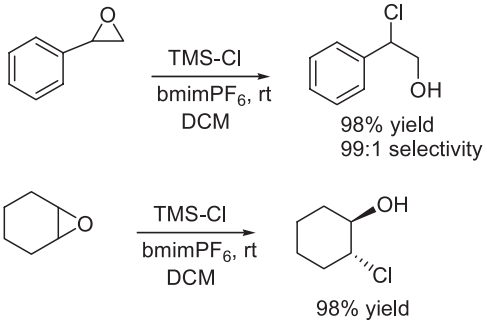
The acylative cleavage of epoxides and THF with organomercury compounds (**79**, **80**) in the presence of aluminum and an acid chloride provides the corresponding chloroesters in good-excellent yields (Scheme 84).[155]



Scheme 84.

5.2. Synthesis of halohydrins

A recent development in the synthesis of chlorohydrins is the first use of an ionic liquid in this regard. A series of terminal and bicyclic epoxides were converted into the chlorohydrin with high stereo- and regioselectivity with catalytic amounts of $bmimPF_6$ with TMS-Cl (Scheme 85).[156]



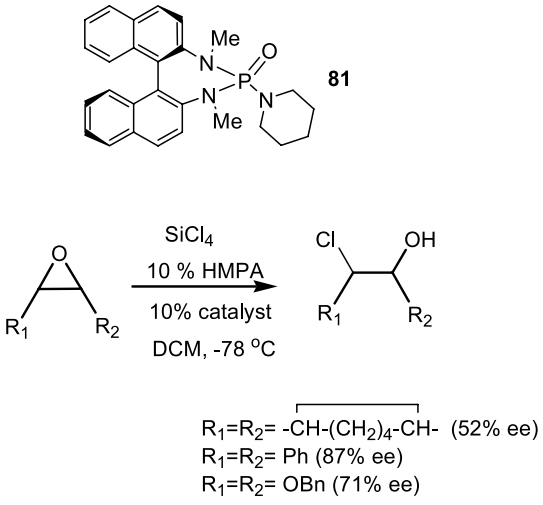
Scheme 85.

The chemoselectivity of triphenylphosphonium bromide over HBr for the ring opening of epoxides in the presence of acid-sensitive functionalities has been reported (Scheme

Scheme 86.

86).[157] The corresponding bromohydrins were synthesized in high yield in the presence of an ethylene ketal, benzyloxymethoxy ether and a trimethylsilyl ether functionality via competition experiments.

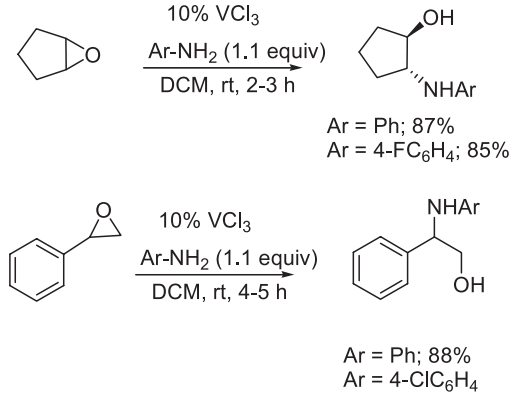
Denmark reported the first enantioselective ring opening of *meso* epoxides to prepare enantio-enriched chlorohydrins using $SiCl_4$ and catalytic HMPA. The reaction is promoted with catalytic phosphoramide **81** (Scheme 87). Selectivity was highly substrate dependent, as the acyclic substrates gave better results than the cyclic ones.



Scheme 87.

5.3. Synthesis of functionalized *sec*-alcohols

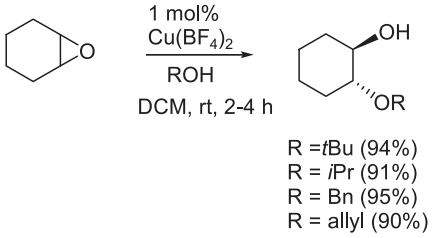
The discovery of catalysts for the asymmetric ring-opening of epoxides via hydrolytic kinetic resolution (HKR) is an emerging trend in the synthesis of the highly-valued enantiopure alcohols. Jacobsen's chiral salen Co(III) complexes are able to generate these products in high ee and yield via the HKR protocol. The reaction of phenols with mono-substituted epoxides provide the corresponding α-aryloxy alcohol,[158] whereas with water as the nucleophile, chiral 1,2-diols are generated. Cyclic oligomeric analogues of the first-generation catalyst provide enhanced reactivity and stereoselectivity.[159] Some of the phenol–epoxide combinations that were unreactive towards the monomeric catalyst gave impressive results with the oligomer.



Scheme 88.

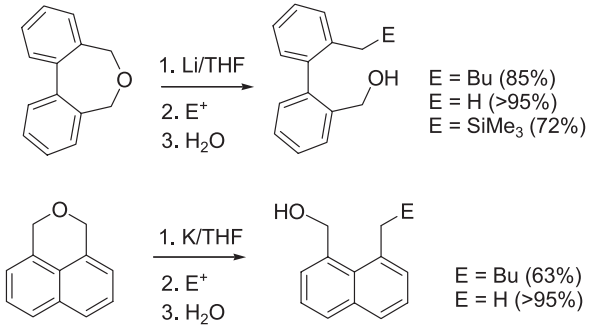
The synthesis of β-amino alcohols by means of a vanadium (III)chloride-catalyzed epoxide ring opening, in the presence of an aromatic amine under mild conditions (Scheme 88).[160]

The epoxide ring opening catalyzed with 1 mol% copper(II) tetrafluoroborate with various alcohols gives the corresponding hydroxy ethers in excellent yield under mild conditions (Scheme 89).[161]



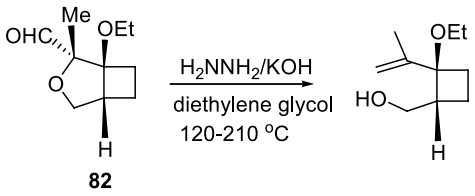
Scheme 89.

The reductive cleavage of benzannulated ethers with alkali metals, followed by treatment with electrophiles gave the unsymmetrical disubstituted biaryls or naphthalenes in moderate–high yield (Scheme 90).[162]



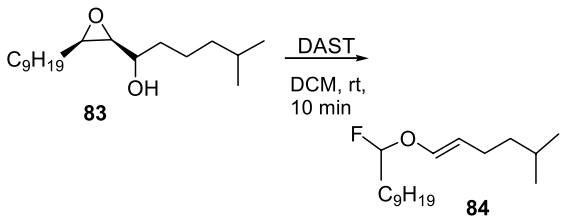
Scheme 90.

En route to *cis*-1,2-disubstituted cyclobutanes, Ghosh implemented a novel ring opening of ether **82** under Wolff–Kishner conditions (Scheme 91).[163]

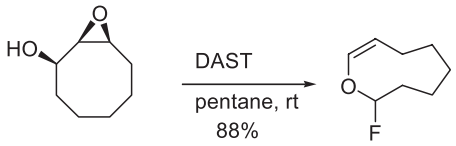


Scheme 91.

The reaction of epoxy alcohol **83** with diethylaminosulfur trifluoride (DAST) gave exclusive formation of fluorinated vinyl ether **84** (Scheme 92).[164] This methodology was applied to the synthesis of fluorinated cyclic vinyl ethers via ring expansion of *syn* bicyclic epoxy alcohols (Scheme 93), the *anti* isomers led to mixtures of products.[165]

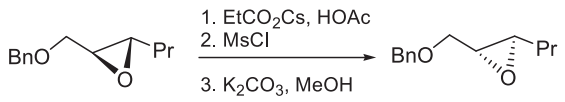


Scheme 92.



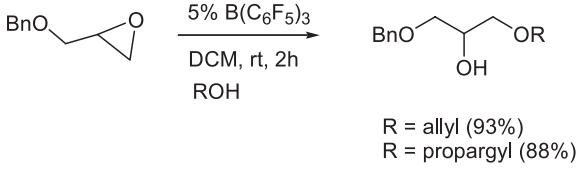
Scheme 93.

The inversion of configuration of sterically hindered epoxides has been accomplished by Prieto.[166] A two-step procedure via cleavage of the epoxide with cesium propionate followed by activation of the resulting regioisomeric hydroxy-esters as the mesylate and ring closure with potassium carbonate (Scheme 94).



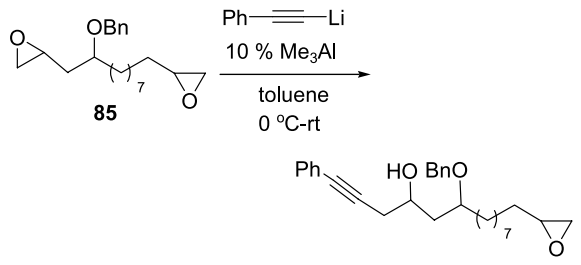
Scheme 94.

A series of epoxides were cleaved with 5 mol% $B(C_6F_5)_3$ with propargyl and allyl alcohol to give the corresponding hydroxy ethers in yields from 78 to 95% yield (Scheme 95).[167] Neighboring silyl and benzyl ethers were unaffected.



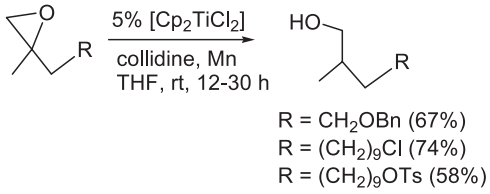
Scheme 95.

Trimethyl aluminum catalyzes the addition of alkynyl lithium reagents to alkoxy-substituted epoxides to give the hydroxy alkynes. The catalyst efficiency was directly related to the proximity of the alkoxy group to the epoxide as evidenced by the regioselectivity observed in bis epoxide **85**. The reaction is proposed to proceed via a penta-coordinate organoaluminum complex (Scheme 96).[168]



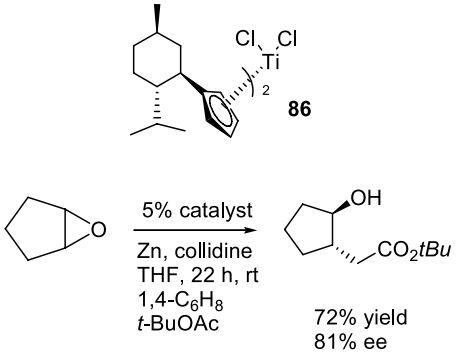
Scheme 96.

A catalytic version of the titanium-mediated epoxide ring opening reaction was developed by Gansäuer using a stoichiometric reductant (manganese) to regenerate the catalyst in the presence of collidine.[169] The protocol can tolerate functionalities, such as chlorides, ketones and benzyl ethers that are usually reactive towards typical electron transfer reagents (Scheme 97).



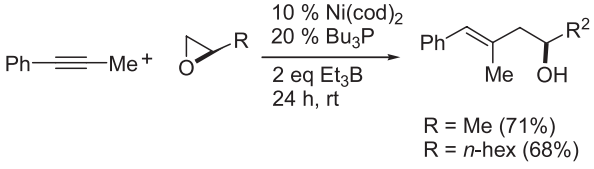
Scheme 97.

By modifying the titanium catalyst with (−)-menthol (**86**), this same group applied this methodology to generate *sec*-alcohols from 80 to 91% ee (Scheme 98).[170]



Scheme 98.

An unprecedented nickel-catalyzed reductive coupling of alkynes with alkyl-substituted epoxides provides the corresponding enol ethers with high regioselectivity (>95:5) for both the epoxide and the alkyne (Scheme 99).[171] Notable is the unexpected *endo* epoxide-opening product.



Scheme 99.

Intramolecular examples are also described.

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Radical hydrogen abstraction–cyclization with a 2-bromovinylsilyl group as a bifunctional tether

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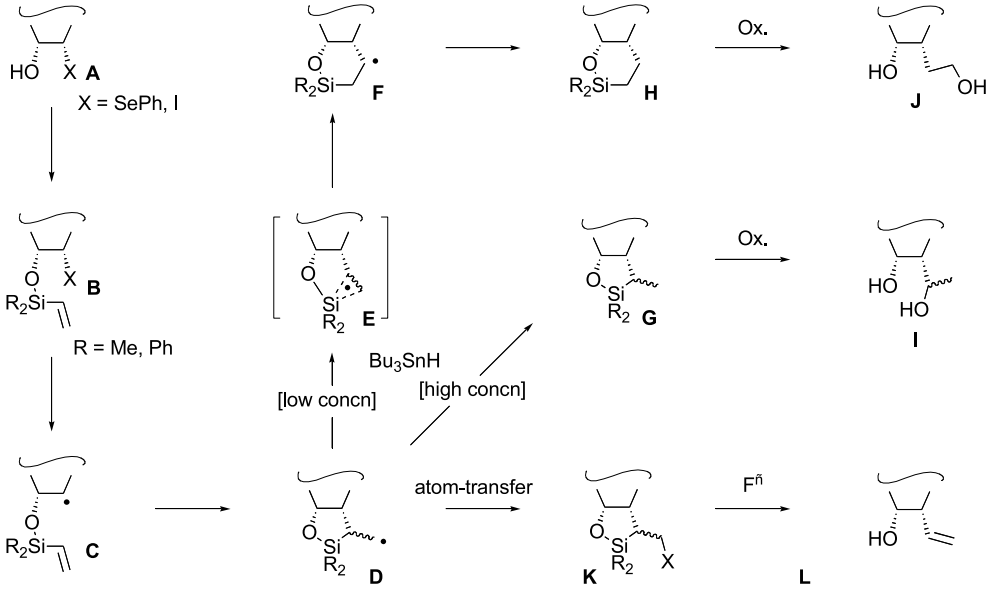
Abstract—In order to develop an efficient method for the regio- and stereoselective introduction of a carbon substituent, a radical hydrogen abstraction–cyclization using 1- and 2-bromovinylsilyl groups as bifunctional tethers was studied. Although, the selective introduction of a carbon substituent at the position β to the hydroxyl was unsuccessful, a carbon substituent was introduced with the 2-bromovinylsilyl ethers via a hydrogen abstraction–cyclization. These reactions were analyzed based on the bond dissociation energies obtained by theoretical calculations.

1. Introduction

Intramolecular radical cyclizations employing the temporary silicon connection approach are very useful for the introduction of a carbon substituent.[1] We previously, developed a regio- and stereoselective method for introducing a 1-hydroxyethyl or 2-hydroxyethyl group at the position β to the hydroxyl in halohydrins or α-phenylselenoalkanols by using an intramolecular radical cyclization reaction with a dimethyl- or diphenylvinylsilyl group as the radical acceptor (Scheme 1).[2] Thus, the selective introduction of both the 1-hydroxyethyl and the



Scheme 1.

Keywords: Radical reaction; Hydrogen abstraction; Silicon tether; Cyclization.
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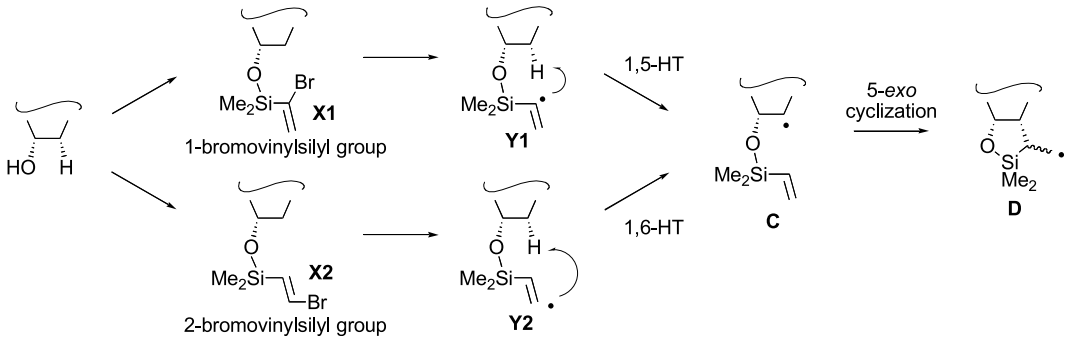
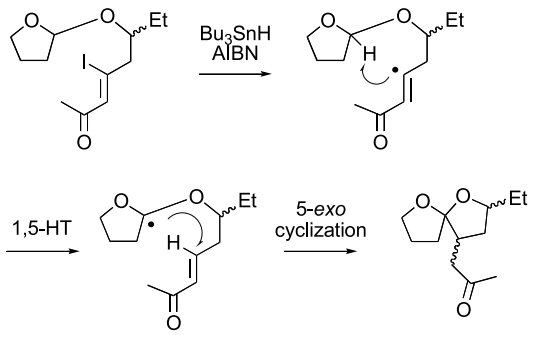


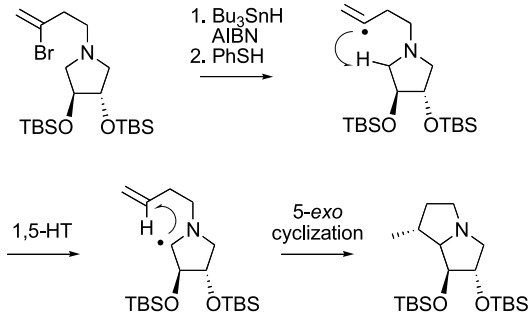
Scheme 2.

2-hydroxyethyl groups can be achieved, depending on the concentration of Bu_3SnH in the reaction system, via the 5-*exo*-cyclization intermediate **G** or the 6-*endo*-cyclization intermediate **H**, respectively, after oxidative cleavage by treatment of the cyclization products under Tamao oxidation conditions. We also have developed an efficient method for the stereoselective introduction of a vinyl and an ethynyl group via a radical atom-transfer reaction followed by treatment with fluoride ion,[3] and have applied them to the synthesis of various branched nucleosides and *C*-glycosides.[2,4]

Although these radical reactions are actually effective for the regio- and stereoselective introduction of a carbon substituent, the substrates are limited to halohydrins or α-phenylselenoalkanols, and the preparation of these substrates can be troublesome.[2b,5] Therefore, we decided to seek another approach to produce radical **C** via a hydrogen abstraction by vinylsilyl radicals, employing either 1-bromovinylsilylether **X1** or 2-bromovinylsilylether **X2** (Scheme 2). Radical hydrogen abstractions have been studied extensively and many useful reactions have been reported.[6] Brown et al.[6b] and Robertson et al.[6d] reported efficient hydrogen abstraction–cyclizations, namely hydrogen abstraction by vinyl radicals and subsequent addition of the transferred radical to the vinyl group (Schemes 3 and 4). These results, obtained via effective hydrogen abstraction by vinyl radicals, suggested that **Y1** or **Y2** generated from **X1** or **X2**, respectively, might abstract the hydrogen at the position β to the silyloxy group to form radical **C** as shown in Scheme 2. Compared with the previous procedures in Scheme 1, this process would make it much easier to introduce a carbon substituent at the position β to a hydroxyl group.



Scheme 3.



Scheme 4.

We report here, radical hydrogen abstraction–cyclizations employing either the 1- or 2-bromovinylsilyl group. Studies on the difference between α- and β-silylvinyl radicals in their reactivity investigated by theoretical calculations are also described.

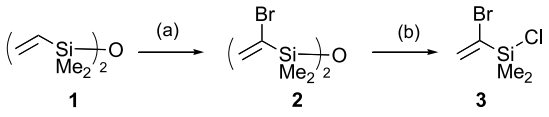
2. Results and discussion

2.1. Working hypothesis

Our strategy, using 1-bromovinyldimethylsilyl (1-BVDS) ethers **X1** or 2-bromovinyldimethylsilyl (2-BVDS) ethers **X2** as the reaction substrates, is shown in Scheme 2. Treatment of the substrate **X1** with a radical initiator would produce the α-silylvinyl radical **Y1**. We expected that the 1,5-hydrogen transfer (HT) reaction of **Y1** would preferentially proceed to give the desired radical **C**. We also planned to investigate the reaction with 2-BVDS ethers **X2**. If the 1,6-HT reaction of the β-vinylsilyl radical **Y2** generated from the substrate **X2** occurred, the radical **C** would be formed. Radical **C** would then cyclize via a 5-*exo*-mode to produce the radical **D**, which corresponds to the key intermediate in the reaction pathways shown in Scheme 1.

2.2. Synthesis of 1- and 2-bromovinylsilylating reagents

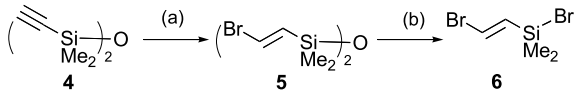
Initially, we prepared 1-bromovinyldimethylsilyl chloride **3** and 2-bromovinyldimethylsilyl bromide **6** as reagents to introduce the 1- or 2-bromovinylsilyl group into alcohols. The chlorosilane **3** was synthesized according to the method reported by Tamao et al. (Scheme 5).[7] The 2-bromovinylsilylating reagent **6** was prepared from 1,1,3,3-tetramethyl-1,3-diethynyldisiloxane (**4**). Hydrostannylation of **4** and the subsequent stannane–bromine exchange gave **5**, which was



Conditions: (a) 1) Br_2, –60 °C to rt, 2) Et_2NH, reflux (68%), (b) $MeSiCl_3$, HMPA, cat. H_2O, 60 °C (56%)

Scheme 5.

treated with $SiBr_4$, HMPA and a catalytic amount of water, followed by distillation to provide the desired bromide **6** (Scheme 6).

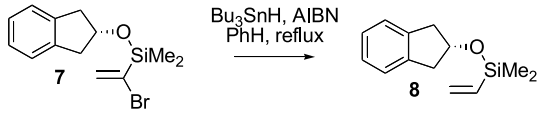


Conditions: (a) 1) Bu_3SnH, AIBN, 80 °C, 2) NBS, CH_2Cl_2, –40 °C, (b) Br_4Si, HMPA, cat. H_2O, 60 °C (19% from **4**)

Scheme 6.

2.3. Radical hydrogen abstraction–cyclization with 2-indanol derivatives

We first, tried an approach based on 1,5-HT reactions with 2-indanol derivatives **7**, which was prepared by treating 2-indanol with **3** in THF containing pyridine. The substrate **7** was subjected to various reductive radical reaction conditions with Bu_3SnH (0.01 M or lower). However, none of the desired products, derived from C–C bond formation at the position β to the hydroxyl, like **9** or **10** (Scheme 7, Fig. 1), was obtained. In these reactions, the simply reduced product **8**[8] was predominantly formed, implying that the 1,5-HT step had failed. This result was consistent with Curran's report.[9]



Scheme 7.

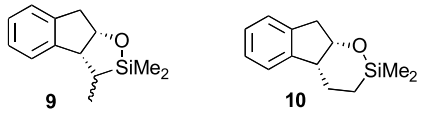


Figure 1. Potential products in the reaction of **7** and **11**.

We next prepared the 2-BVDS ethers **11** by treating 2-indanol with **6** in THF containing pyridine, and examined whether the hydrogen abstraction–cyclization through 1,6-hydrogen transfer occurred by using **11** as the substrate (Scheme 8, Table 1). A solution of 1.2 equiv of Bu_3SnH and 0.6 equiv of AIBN in benzene was added slowly over 4 h, using a syringe pump, to a solution of **11** in refluxing benzene, and the resulting products, without purification, were treated under Tamao oxidation conditions. At this stage, the mixture was partially purified using Edelson's procedure,[10] where the 2-indanol (**14**) was separated out. The remaining mixture was acetylated to give a mixture of **12** via 1,5-HT and **13** via 1,6-HT in 20% yield (**12/13**= 1.2:1, run 1). Similar reactions at higher temperature in refluxing toluene (run 2) or octane (run 3) increased the yield of the branched 2-indanols **12** and **13** (33%, run 2; 45%, run 3). When $(TMS)_3SiH$ was used as a reductant in refluxing octane, compounds **12** and **13** were likewise obtained (run 4) as in the case of Bu_3SnH. We examined various radical reaction conditions; however, the yield of the desired compound **13**, which had carbon substituents at the position β to the hydroxyl, was not further improved.

Table 1. Radical hydrogen abstraction–cyclization reaction with the 2-BVDS ether **11**

Run	Conditions	Yield (%)	
		12+**13** (**12**:**13**)	**14**
1	Bu_3SnH, AIBN, PhH, reflux (80 °C)	20 (1.2:1)	64
2	Bu_3SnH, AIBN, PhMe, reflux (111 °C)	33 (2.2:1)	51
3	Bu_3SnH, AIBN, octane, reflux (125 °C)	45 (3.3:1)	33
4	$(TMS)_3SiH$, AIBN, octane, reflux (125 °C)	39 (3.3:1)	33

2.4. Radical hydrogen abstraction–cyclization with *t*-butylcyclohexanol derivatives

Curran and co-workers also reported that the 1,5-HT step[9] failed in the 1-BVDS ether of the mannose derivative **15** as it did in our case with the substrate **7**. However, they achieved a 1,5-HT reaction with the *o*-bromophenylsilyl ethers of the mannose derivative **16** and the *trans*-*t*-butylcyclohexanol **17**[11] (Fig. 2).

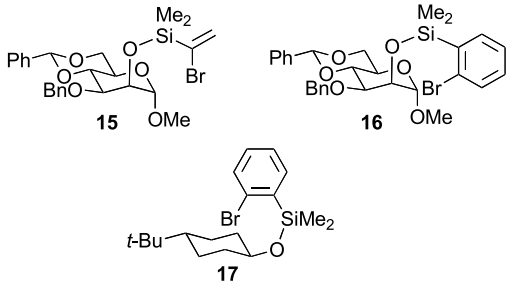
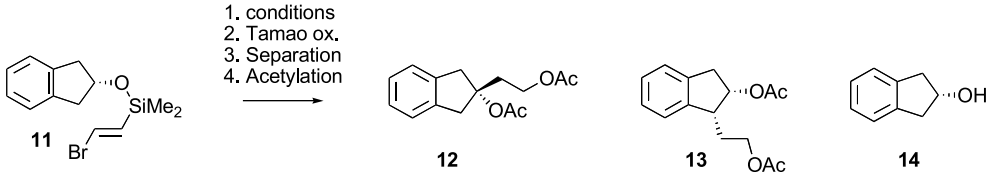
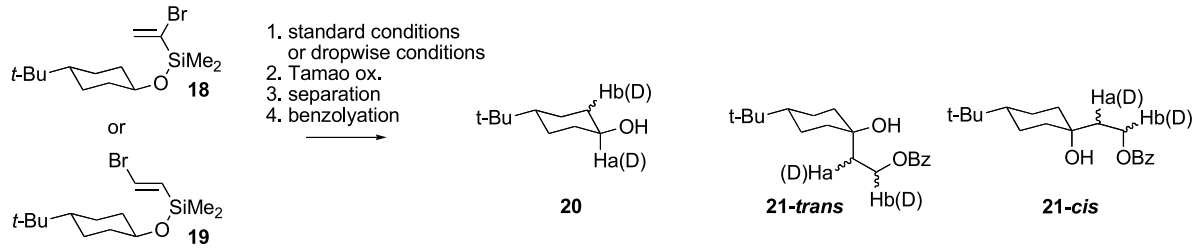


Figure 2. Substrates for the radical hydrogen abstraction reaction.

In order to make clear the differences in the reactivity of 2-BVDS, 2-BVDS and *o*-bromophenylsilyl ethers, we examined the deuterium-labeling experiments using the 1- and 2-BVDS ethers of *trans*-*t*-butylcyclohexanol as substrates under two kinds of reaction conditions with Bu_3SnD.



Scheme 8.



Scheme 9.

Table 2. Radical hydrogen abstraction–cyclization reaction with 1- or 2-BVDS ethers of *trans-t*-butylcyclohexanol

Run	Substrates	Conditions	Yield% (D ratio)[a]		
			20	**21**-*trans*	**21**-*cis*
1	**18**	Standard	96 (Ha, <1%; Hb, <1%)	—	—
2	**18**	Dropwise	74 (Ha, <1%; Hb, <1%)	—	—
3	**19**	Standard	85 (Ha, 28%; Hb, <1%)	5 (Ha, 25%; Hb, 75%)	1 (Ha, 75%; Hb, 25%)
4	**19**	Dropwise	22 (Ha, 3%; Hb, <1%)	50 (Ha, 13%; Hb, 28%)	16 (Ha, 22%; Hb, 10%)

[a] The ratio of deuterium incorporation was determined as % by ^{2}H NMR experiment.

In one, the standard conditions were those used in the previous studies by Curran et al.,[11] that is, a mixture of the substrate, Bu_3SnD (1.5 equiv) and AIBN (0.3 equiv), was stirred in refluxing benzene. In the other, the dropwise conditions of run 3 in Table 1 were followed, except for using Bu_3SnD instead of Bu_3SnH (Scheme 9, Table 2).

We first, examined the 1-BVDS ether **18** of *trans-t*-butylcyclohexanol. A mixture of **18**, Bu_3SnD and AIBN in benzene, was heated under reflux for 4 h (standard conditions) and the product was purified after Tamao oxidation. We recovered *trans-t*-butylcyclohexanol (**20**) in 96% yield, which, by ^{2}H NMR experiments, was hardly deuterated (run 1). We also recovered *trans-t*-butylcyclohexanol (**20**) under dropwise conditions in 74% yield and it was also hardly deuterated (run 2). Thus, we could not isolate the branched *t*-butylcyclohexanols like **21**-*trans* or **21**-*cis*[12] in either of the reaction conditions. These results indicate that the hydrogen abstraction did not proceed in the reaction with the 1-BVDS ether of *trans-t*-butylcyclohexanol.

We next, examined the 2-BVDS ether **19** of *trans-t*-butylcyclohexanol. The substrate **19** was treated under the standard conditions, and the resulting products, without purification, were treated under Tamao oxidation conditions. At this stage, the mixture was purified by column chromatography to give the partly deuterated *trans-t*-butylcyclohexanol (**20**) in 85% yield (run 3). These results indicate that the hydrogen abstraction occurred to form the radical at the position α to the hydroxyl, at least to some extent, which was reduced by Bu_3SnD prior to the cyclization. The remaining mixture was benzoylated to give the branched *trans-t*-butylcyclohexanol **21**-*trans* and its *cis* isomer **21**-*cis* in low yield. Under the dropwise conditions, the slightly deuterated *t*-butylcyclohexanols were obtained in 22% yield, where the yield of the branched *t*-butylcyclohexanols (**21**-*trans*, **21**-*cis*) was dramatically increased (run 4) compared with the standard conditions. However, none of the products with a carbon substituent at the position β to the hydroxyl, such as **13**, were detected in the reaction.[13]

Thus, it was clear that the β-silylvinyl radical derived from the 2-BVDS ethers was able to abstract the hydrogen, while the reactivity seemed to be lower than that of β-silylphenyl radicals derived from the *o*-bromophenylsilyl ethers.[11] Furthermore, the α-silylvinyl radical derived from 1-BVDS ethers was almost incapable of abstracting the hydrogen atom at any of the positions in this system.

2.5. Computational chemistry

Although, the 1,5-hydrogen transfer (HT) is the most favorable pathway in intramolecular hydrogen transfer reactions, the 1,5-HT reaction with 1-bromovinylsilyl ethers (**7**, **18**) did not occur. In hydrogen abstractions, it is essential that the initially generated radical can be significantly unstable (reactive) to abstract a hydrogen atom. We wondered if the silicon atom in the tether moiety might have stabilized the vinyl radical at the position α, making the radical rather inactive. Experimental and computational studies have demonstrated that the α- or β-substituent on carbon-center radicals often affects the stability of the radicals.[14] The effect of a silicon atom at the position α or β to aliphatic radicals has been investigated by ab initio calculations to show that the silicon effectively stabilizes these radicals.[15]

Therefore, we decided to investigate theoretically the α- or β-silicon effects on non-aliphatic, that is, vinyl and phenyl radicals. Bond dissociation energies (BDE) of various vinyl and phenyl radicals were calculated and were compared with that of the secondary C–H in propane. BDEs are relevant to the stability of the radical generated by dissociation, which is useful for understanding hydrogen

Table 3. Theoretical bond dissociation energies (BDEs) and relative to the secondary C–H bond dissociation energy in propane (ΔBDEs)

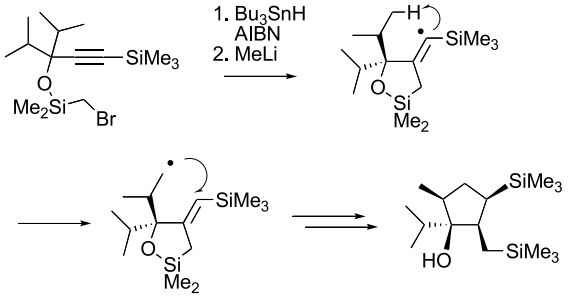
Species[a]	ZPE (a.u.)[b]	E_0 (a.u.)[c]	BDE (kJ/mol)	ΔBDE (kJ/mol)[d]
$CH_3CH(–H)CH_3$ (**22**)	—	—	409.1	0.0
$H_2C{=}C(–H)CMe_2OMe$ (**23**)	0.166034	−310.880777	444.8	35.7
$H_2C{=}C{\cdot}CMe_2OMe$ (**23′**)	0.152144	−310.213389	—	—
$H_2C{=}C(–H)SiMe_2OMe$ (**24**)	0.157112	−562.511049	409.6	0.5
$H_2C{=}C{\cdot}SiMe_2OMe$ (**24′**)	0.143105	−561.699963	—	—
$(H–)HC{=}CHCMe_2OMe$ (**25**)	0.166034	−310.880777	458.7	49.6
$HC{\cdot}{=}CHCMe_2OMe$ (**25′**)	0.152449	−310.208106	—	—
$(H–)HC{=}CHSiMe_2OMe$ (**26**)	0.157112	−562.511049	442.5	33.4
$HC{\cdot}{=}CHSiMe_2OMe$ (**26′**)	0.143684	−561.687426	—	—
$(o\text{-}H–)C_6H_4CMe_2OMe$ (**27**)	0.213960	−464.492189	462.7	53.6
$\cdot C_6H_4CMe_2OMe$ (**27′**)	0.200754	−463.817990	—	—
$(o\text{-}H–)C_6H_4SiMe_2OMe$ (**28**)	0.204631	−715.963861	451.4	42.3
$\cdot C_6H_4SiMe_2OMe$ (**28′**)	0.191771	−715.293962	—	—

[a] The dissociation H atom is shown in parentheses.
[b] Zero-point energy scaled by 0.9806.
[c] Energy including ZPE at B3LYP/6-31G(d) level.
[d] ΔBDE = BDE (**23**–**28**) − BDE (**22**).

abstraction and free radical reactions.[16] Geometry optimization and single-point energy calculations were performed using the density functional theory with the B3LYP functional[17] and 6-31G(d) basis set. The zero-point energies (ZPE) were calculated using the scale factor 0.9806 reported by Scott et al.[18] The values of BDE were determined by the isodesmic reaction reported to by Marshall et al.[19a] and DiLabio et al.[19b] to eliminate systematic errors. These results are summarized in Table 3.

B3LYP calculations showed that the BDE of the vinyl C–H in allyl ether **23** is ca. 35.7 kJ/mol larger than that of the secondary C–H in propane (**22**), while the BDE of the vinyl C–H in vinylsilyl ether **24** is almost the same as that of the secondary C–H in propane (**22**). Therefore, it is likely that the α-silylvinyl radicals **23′** are unable to abstract the hydrogen atom from a secondary C–H in alkanes. On the other hand, the BDE of vinylsilyl ether **26** for generating the β-silylvinyl radical **26′** is ca. 30 kJ/mol larger than that of the secondary C–H in propane (**22**). Although, the BDE of **26** is smaller than that of the corresponding carbon congener **25**, the β-silylvinyl radical **26′** might be reactive enough to abstract a hydrogen atom from the secondary C–H in alkanes. The BDE of the C–H (*ortho*) in the phenyl group in **28** is much larger (ΔBDE = 42.3 kJ/mol) even if silicon substituents are attached to the phenyl group. Therefore, this highly reactive property of *o*-silylphenyl radical would make the hydrogen abstraction possible even without the dropwise conditions.[11]

Another reason that the hydrogen abstraction with 1-bromovinylsilyl ethers (**7**, **18**) did not occur might be the following. Malacria et al. reported the efficient radical hydrogen abstraction by α-silylvinyl radical in the radical cascade reaction (Scheme 10).[20] In their substrates, the three-dimensional positioning of the radical and the abstracted hydrogen atom is strictly limited to proceed effective abstraction. However, our substrates (**7**, **18**), having a 1-bromovinylsilyl group on the hydroxyl, are conformationally flexible and therefore, have no steric demand for promoting the intramolecular hydrogen abstraction. Accordingly, the radical might be reduced by the reductant in preference to the hydrogen abstraction.



Scheme 10.

3. Conclusion

We investigated the radical hydrogen abstraction–cyclization with 1- or 2-bromovinylsilyl tether as a bifunctional tether. Although, the selective introduction of a carbon substituent at the position β to the hydroxyl was unsuccessful, a carbon substituent was introduced with the 2-bromovinylsilyl ethers in moderate yield, via a hydrogen abstraction–cyclization. We also investigated theoretically the cause of the failure of the reaction with 1-bromovinylsilyl ethers by comparing the BDE of the vinyl C–H of the vinylsilyl ether with the BDE of the secondary C–H in propane, which suggested that the silicon-substituted vinyl radical, generated from 1-bromovinylsilyl ethers, is too stable to abstract the hydrogen.

4. Experimental

4.1. General procedure

Melting points are uncorrected. NMR spectra were recorded at 270 and 400 MHz (^{1}H), at 61 MHz (^{2}H), and at 100 MHz (^{13}C), and are reported in ppm downfield from Me_4Si. Mass spectra were obtained by electron ionization (EI) or the fast atom bombardment (FAB) method. Thin-layer chromatography was performed on Merck coated plate $60F_{254}$. Silica gel chromatography was performed with Merck silica gel 5715 or 9385 (neutral). Reactions were carried out under an argon atmosphere.

4.1.1. 2-Bromovinyldimethylbromosilane (6). A mixture of 1,1,3,3-tetramethyl-1,3-diethynyldisiloxane (**4**, 10 g, 55 mmol), Bu_3SnH (32.5 mL, 121 mmol), AIBN (903 mg, 5.5 mmol) was stirred at 80 °C for 2 h. After cooling the solution to −40 °C, NBS (21.5 g, 121 mmol) and CH_2Cl_2 (60 mL) were added, and the mixture was stirred at the same temperature for 2 h and then at 0 °C for 2 h. The resulting mixture was evaporated, and the insoluble material was filtered off. The filtrate was purified by distillation (bp 53–115 °C/3 mmHg) to give crude **5** including some impurities (10.6 g, ca. 40%). The mixture of crude **5** (10.6 g), $SiBr_4$ (1.37 mL, 11 mmol), HMPA (332 μL, 3.0 wt%), and H_2O (29 μL, 0.25 wt%) was stirred at 60 °C for 18 h. Distillation of the resulting mixture gave **6** (bp 55–65 °C/13 mmHg, 5.0 g, 19% from **4**) as a pale brown liquid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.84 (d, 1H, J=15.5 Hz), 6.63 (d, 1H, J=15.5 Hz), 0.67 (s, 6H); HRMS (EI) calcd for $C_4H_8^{79}Br_2Si$ 241.8750, found 241.8762 (M^+).

4.1.2. (±)-2-(1-Bromovinyldimethylsiloxy)indane (7). A mixture of 2-indanol (**14**) (403 mg, 3.0 mmol), 1-bromovinyldimethylchlorosilane (**3**, 540 μL, 3.6 mmol), and pyridine (315 μL, 3.9 mmol) in THF (30 mL) was stirred at room temperature for 1 h, and then evaporated. The residue was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (neutral SiO_2, 0–2% AcOEt in hexane) to give **7** (851 mg, 95%) as a colorless liquid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.21–7.12 (m, 4H), 6.37 (d, 1H, J=2.0 Hz), 6.33 (d, 1H, J=2.0 Hz), 4.74 (m, 1H), 3.16 (dd, 2H, J=6.6, 15.9 Hz), 2.94 (dd, 2H, J=5.3, 15.9 Hz), 0.33 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.45, 135.47, 130.73, 126.33, 126.19, 124.68, 124.28, 74.16, 73.03, 42.59, 42.24, −2.27; HRMS (EI) calcd for $C_{13}H_{17}^{79}BrOSi$ 296.0232, found 296.0246 (M^+).

4.1.3. (±)-2-Vinyldimethylsiloxyindane (8). A mixture of 2-indanol (**14**) (81 mg, 0.6 mmol), vinyldimethylchlorosilane (99 μL, 0.72 mmol), and pyridine (61 μL, 0.75 mmol) in THF (6 mL) was stirred at room temperature for 1.5 h, and then evaporated. The residue was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (neutral SiO_2, 0–3% AcOEt in hexane) to give **8** (105 mg, 80%) as a colorless liquid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.19–7.11 (m, 4H), 6.18 (dd, 1H, J=14.5, 19.8 Hz), 6.02 (dd, 1H, J=4.6, 14.5 Hz), 5.80 (dd, 1H, J=4.6, 19.8 Hz), 4.66 (m, 1H), 3.12 (dd, 2H, J=6.6, 15.2 Hz), 2.89 (dd, 2H, J=5.9, 15.2 Hz), 0.22 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.63, 137.46, 132.79, 126.10, 124.25, 73.61, 42.29, −1.58; HRMS (EI) calcd for $C_{13}H_{18}OSi$ 218.1127, found 218.1123 (M^+).

4.1.4. (±)-2-(2-Bromovinyldimethylsiloxy)indane (11). A mixture of 2-indanol (**14**) (201 mg, 1.5 mmol), 2-bromovinyldimethylbromosilane (**6**, 282 μL, 1.8 mmol), and pyridine (158 μL, 1.95 mmol) in THF (15 mL) was stirred at room temperature for 2 h, and then evaporated. The residue was partitioned between AcOEt and aqueous HCl (1 M), and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (neutral SiO_2, 0–2% AcOEt in hexane) to give **11** (435 mg, 98%) as a colorless liquid: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.21–7.13 (m, 4H), 6.67 (d, 1H, J=15.8 Hz), 6.55 (d, 1H, J=15.8 Hz), 4.66 (m, 1H), 3.13 (dd, 2H, J=6.6, 15.8 Hz), 2.88 (dd, 2H, J=6.6, 15.8 Hz), 0.24 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.54, 136.99, 126.41, 124.48, 119.59, 73.97, 42.44, −1.04; LRMS (EI) m/z 296 (^{79}Br, M^+), 298 (^{81}Br, M^+). Anal. Calcd for $C_{13}H_{17}BrOSi$: C, 52.53; H, 5.76. Found: C, 52.33; H, 5.71.

4.1.5. General procedure for radical hydrogen abstraction–cyclization reaction with indane derivatives 7 and 11. To a refluxing solution of **7** or **11** (59 mg, 0.20 mmol) in a solvent (40 mL) was added dropwise a mixture of Bu_3SnH or $(TMS)_3SiH$ (0.26–0.30 mmol) and AIBN (0.04–0.06 mmol) in a solvent [5 mL, when octane was used as a solvent (Table 1, runs 3 and 4), 10% benzene in octane was used to dissolve AIBN] over 4 h, and then the resulting mixture was evaporated. A mixture of the residue, KF (0.4 mmol), $KHCO_3$ (0.2 mmol), 30% aqueous H_2O_2 (227 μL, 2.0 mmol) in THF/MeOH (2:1, 3 mL) was stirred at room temperature for 15–20 h. Saturated aqueous $Na_2S_2O_3$ was added, and the resulting mixture was filtered through Celite. The filtrate was dried (Na_2SO_4) and evaporated. A mixture of the residue, CsF (122 mg, 0.8 mmol), CsOH (0.4 mmol), and silica gel (60 mg) in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min and was applied to a short (2 cm) plug of silica gel. Elution with 50% hexane in AcOEt afforded the mixture, by which tin compounds were removed to some extent. 2-Indanol (**14**) was separated from the mixture by column chromatography (SiO_2, 5–50% AcOEt in hexane). A mixture of the resulting mixture, Ac_2O (0.2–0.4 mmol), and DMAP (0.2–0.4 mmol) in MeCN (1 mL) was stirred at room temperature for 15–25 h. To the mixture was added MeOH (0.5 mL), and evaporated. The residue was partitioned between AcOEt and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 8% AcOEt in hexane) to give a mixture of **12** and **13**. The ratio of **12** and **13** was determined by ^{1}H NMR analysis. Compounds **12** and **13** were separable by preparative TLC (17% AcOEt in hexane). 2-(2-*Acetoxyethyl*)-2-*acetoxyindane* (**12**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (m, 4H), 4.16 (t, 2H, J=6.6 Hz), 3.43 (d, 2H, J=16.8 Hz), 3.21 (d, 2H, J=16.8 Hz), 2.46 (t, 2H, J=6.6 Hz), 2.03 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.43, 170.23, 139.66, 126.47, 124.14, 89.12, 60.67, 44.57, 35.11, 22.02, 20.94; HRMS (EI) calcd for $C_{15}H_{18}NaO_4$ 285.1096, found 285.1103 (MNa^+). 1-(2-*Acetoxyethyl*)-2-*acetoxyindane* (**13**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.20 (m, 4H), 5.57 (m, 1H), 4.22 (t, 2H, J=6.6 Hz), 3.36 (dt, 1H, J=6.0, 8.3 Hz), 3.21 (dd, 1H, J=5.8, 16.6 Hz), 2.99 (dd, 1H, J=3.2, 16.6 Hz), 2.13–2.03 (m, 2H), 2.06 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.62, 170.41, 142.92, 139.63, 126.85, 126.54, 124.49, 123.30, 76.16, 62.77, 44.66, 38.43, 26.63, 21.08, 20.94; HRMS (FAB, positive) calcd for $C_{15}H_{19}O_4$ 263.1297, found 263.1283 (MH^+).

4.1.6. *trans*-1-(1-Bromovinyldimethylsilyl)-4-*t*-butylcyclohexanol (18). Compound **18** (620 mg, 97%) was

obtained as a colorless liquid from *trans-t*-butylcyclohexanol (313 mg, 2.0 mmol) as described above for the synthesis of **7**, after purification by column chromatography (neutral SiO_2, 0–3% AcOEt in hexane): ^1H NMR (270 MHz, $CDCl_3$) δ 6.34 (d, 1H, $J=2.0$ Hz), 6.33 (d, 1H, $J=2.0$ Hz), 3.58 (m, 1H), 1.94 (m, 2H), 1.84 (m, 2H), 1.29 (m, 2H), 1.09–0.93 (m, 3H), 0.84 (s, 9H), 0.30 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.09, 130.36, 72.79, 46.95, 36.02, 32.20, 27.57, 25.68, −2.04; LRMS (EI) *m/z* 318 (^{79}Br, M^+), 320 (^{81}Br, M^+). Anal. Calcd for $C_{14}H_{27}BrOSi$: C, 52.65; H, 8.52. Found: C, 52.43; H, 8.34.

4.1.7. *trans*-1-(2-Bromovinyldimethylsilyl)-4-*t*-butylcyclohexanol (19). Compound **19** (464 mg, 73%) was obtained as a colorless liquid from *trans-t*-butylcyclohexanol (313 mg, 2.0 mmol) as described above for the synthesis of **11**, after purification by column chromatography (neutral SiO_2, 0–4% AcOEt in hexane): ^1H NMR (400 MHz, $CDCl_3$) δ 6.65 (d, 1H, $J=15.4$ Hz), 6.53 (d, 1H, $J=15.4$ Hz), 3.50 (m, 1H), 1.87 (m, 2H), 1.75 (m, 2H), 1.27 (m, 2H), 1.06–0.90 (m, 3H), 0.84 (s, 9H), 0.20 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.28, 118.95, 72.27, 46.89, 36.11, 32.18, 27.55, 25.66, −1.03; LRMS (EI) *m/z* 318 (^{79}Br, M^+), 320 (^{81}Br, M^+). Anal. Calcd for $C_{14}H_{27}BrOSi$: C, 52.65; H, 8.52. Found: C, 52.60; H, 8.44.

4.2. General procedure for radical hydrogen abstraction–cyclization reaction with 1- or 2-bromovinylsilyl ethers of *trans-t*-butylcyclohexanol 18 and 19

By the standard conditions. A mixture of **18** (64 mg, 0.2 mmol) or **19** (64 mg, 0.2 mmol), Bu_3SnD (65 μL, 0.24 mmol), and AIBN (3.3 mg, 0.02 mmol) was heated under reflux for 4 h. The resulting mixture was evaporated, and a mixture of the residue, KF (23 mg, 0.4 mmol), $KHCO_3$ (20 mg, 0.2 mmol), and aqueous H_2O_2 (35%, 194 μL, 2.0 mmol) in THF/MeOH (2:1, 3 mL) was stirred at room temperature for 15–20 h. To the mixture was added saturated aqueous $Na_2S_2O_3$ (1 mL), and the resulting mixture was filtered through Celite. The filtrate was dried (Na_2SO_4) and evaporated.

By the dropwise conditions. To a refluxing solution of **18** or **19** (64 mg, 0.2 mmol) in octane (40 mL) was added dropwise a mixture of Bu_3SnH (81 μL, 0.30 mmol) or Bu_3SnD (81 μL, 0.30 mmol) and AIBN (10 mg, 0.06 mmol) in octane/benzene (1:9 10 mL) over 4 h, and the resulting mixture was stirred for further 1 h, then evaporated. A mixture of the residue, KF (23 mg, 0.4 mmol), $KHCO_3$ (20 mg, 0.2 mmol), 35% aqueous H_2O_2 (194 μL, 2.0 mmol) in THF/MeOH (2:1, 3 mL) was stirred at room temperature for 15–20 h. Saturated aqueous $Na_2S_2O_3$ (1 mL) was added, and the resulting mixture was filtered through Celite. The filtrate was dried (Na_2SO_4) and evaporated.

4.2.1. D-label experiment with *trans*-1-(1-bromovinyldimethylsilyl)-4-*t*-butylcyclohexanol (18). Slightly deuterated *trans*-4-*t*-butylcyclohexanol (20). *By the standard conditions*. After the treatment of **18** (64 mg, 0.2 mmol) according to the above general procedure (standard conditions), the resulting residue was purified by column chromatography (SiO_2, 5–15% AcOEt in hexane) to give **20** (30 mg, 96%) slightly deuterated at the 1 and 2 positions as a white solid: ^2H NMR (61 MHz, $CHCl_3$) δ 3.50 (br s, 0.005 ^2H), 1.22 (br s, 0.001 ^2H).

By the dropwise conditions. After the treatment of **18** (64 mg, 0.2 mmol) according to the above general procedure (dropwise conditions), the resulting residue was purified by column chromatography (SiO_2, 5–15% AcOEt in hexane) to give **20** (23 mg, 74%) slightly deuterated at 1 and 2 position as a white solid: ^2H NMR (61 MHz, $CHCl_3$) δ 3.50 (br s, 0.009 ^2H), 2.00 (br s, 0.001 ^2H), 1.21 (br s, 0.002 ^2H).

4.2.2. Synthesis of the non-deuterated branched *t*-butylcyclohexanols (21-*trans* and 21-*cis*) as reference compounds. After the treatment of **19** (64 mg, 0.2 mmol) according to the above general procedure (dropwise conditions) with Bu_3SnH instead of Bu_3SnD, *trans-t*-butylcyclohexanol **20** was separated from the mixture by column chromatography (SiO_2, 5–50% AcOEt in hexane). A solution of the resulting mixture, BzCl (46 μL, 0.4 mmol), and pyridine (32 μL, 0.4 mmol) in MeCN (1 mL) was stirred at room temperature for 20 h and then evaporated. The residue was partitioned between AcOEt and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash column chromatography (SiO_2, 5–12% AcOEt in hexane) to give **21**-*trans* (25 mg, 42% from **19**) as a white solid and **21**-*cis* (9 mg, 14% from **19**) as a white solid.

trans-1-(2-*Benzoyloxy*)-4-*t*-*butylcyclohexanol* (**21**-*trans*): mp 122–123 °C (white needles from hexane/AcOEt); ^1H NMR (400 MHz, $CDCl_3$) δ 8.04–7.42 (m, 5H), 4.52 (t, 2H, $J=6.8$ Hz), 2.03 (t, 2H, $J=6.8$ Hz), 1.91 (m, 2H), 1.74 (m, 2H), 1.62 (br s, 1H), 1.42 (m, 2H), 1.11 (m, 3H), 0.86 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.19, 132.58, 129.96, 129.15, 128.05, 71.57, 61.44, 47.40, 39.16, 34.70, 32.18, 27.57, 24.45; LRMS (EI) *m/z* 304 (M^+). Anal. Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.90; H, 9.27.

cis-1-(2-*Benzoyloxy*)-4-*t*-*butylcyclohexanol* (**21**-*cis*): mp 93–94 °C (white plates from hexane/AcOEt); ^1H NMR (400 MHz, $CDCl_3$) δ 8.03–7.42 (m, 5H), 4.52 (t, 2H, $J=6.6$ Hz), 1.92 (t, 2H, $J=6.6$ Hz), 1.80 (m, 2H), 1.62 (m, 2H), 1.51 (br s, 1H), 1.44–1.28 (m, 4H), 0.97 (m, 1H), 0.87 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.18, 132.56, 129.99, 129.18, 128.05, 69.88, 61.31, 47.64, 42.12, 37.69, 32.33, 27.48, 22.30; LRMS (EI) *m/z* 304 (M^+). Anal. Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.82; H, 9.31.

4.2.3. D-label experiment with *trans*-1-(2-bromovinyldimethylsilyl)-4-*t*-butylcyclohexanol (19). *By the standard conditions*. After the treatment of **19** (64 mg, 0.2 mmol) according to the above general procedure (normal conditions), *trans-t*-butylcyclohexanol **20** (26.5 mg, 85%) partly deuterated at the α or β position of the benzoyloxy group was separated from the mixture by column chromatography (SiO_2, 5–50% AcOEt in hexane). A mixture of the resulting mixture, BzCl (12 μL, 0.1 mmol), and pyridine (8 μL, 0.1 mmol) in MeCN (0.5 mL) was stirred at room temperature for 20 h. The mixture was evaporated, and the residue was partitioned between AcOEt and H_2O, washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash column chromatography (SiO_2,

5–12% AcOEt in hexane) to give **21**-*trans* (5 mg, 5% from **19**) as a white solid and **21**-*cis* (<1 mg, <1% from **19**) as a white solid.

Partly deuterated trans-t-butylcyclohexanol (**20**): ^{2}H NMR (61 MHz, $CHCl_3$) δ 3.50 (br s, 0.28 ^{2}H), 2.01 (br s, 0.01 ^{2}H), 1.21 (br s, 0.001 ^{2}H).

Deuterated trans-1-(2-*benzoyloxy*)-4-*t*-*butylcyclohexanol* (**21**-*trans*): ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.04–7.41 (m, 5H), 4.52 (d, 0.5H, J=6.6 Hz), 4.51 (t, 0.75H, J=6.6 Hz), 2.02 (d, 1.5H, J=6.6 Hz), 2.01 (t, 0.25H, J=6.6 Hz), 1.91 (m, 2H), 1.73 (m, 2H), 1.60 (br s, 1H), 1.42 (m, 2H), 1.13 (m, 3H), 0.86 (s, 9H).

Deuterated cis-1-(2-*benzoyloxy*)-4-*t*-*butylcyclohexanol* (**21**-*cis*): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–7.41 (m, 5H), 4.52 (d, 1.5H, J=6.6 Hz), 4.51 (t, 0.25H, J=6.6 Hz), 1.92 (d, 0.75H, J=6.6 Hz), 1.90 (t, 0.5H, J=6.6 Hz), 1.80 (m, 2H), 1.57 (m, 3H), 1.44–1.28 (m, 4H), 0.97 (m, 1H), 0.87 (s, 9H).

4.2.4. D-label experiment with *trans*-1-(2-bromovinyldimethylsilyl)-4-*t*-butylcyclohexanol (19). *By the dropwise conditions*. After the treatment of **19** (64 mg, 0.2 mmol) according to the above general procedure (dropwise conditions), *trans*-*t*-butylcyclohexanol **20** (7.0 mg, 22%) was separated from the mixture by column chromatography (SiO_2, 5–50% AcOEt in hexane). A mixture of the resulting mixture, BzCl (62 μL, 0.54 mmol), and pyridine (43 μL, 0.54 mmol) in MeCN (1 mL) was stirred at room temperature for 15 h. The mixture was evaporated, and the residue was partitioned between AcOEt and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash column chromatography (SiO_2, 5–12% AcOEt in hexane) to give **21**-*trans* (30 mg, 50% from **19**) as a white solid and **21**-*cis* (10 mg, 16% from **19**) as a white solid. *Partly deuterated trans-t-butylcyclohexanol* (**20**): ^{2}H NMR (61 MHz, $CHCl_3$) δ 4.52 (br s, 0.28 ^{2}H), 2.02 (br s, 0.13 ^{2}H).

Partly deuterated trans-1-(2-*benzoyloxy*)-4-*t*-*butylcyclohexanol* (**21**-*trans*): ^{2}H NMR (61 MHz, $CHCl_3$) δ 4.53 (br s, 0.10 ^{2}H), 1.93 (br s, 0.22 ^{2}H).

Partly deuterated cis-1-(2-*benzoyloxy*)-4-*t*-*butylcyclohexanol* (**21**-*cis*): ^{2}H NMR (61 MHz, $CHCl_3$) δ 3.50 (br s, 0.03 ^{2}H).

4.3. Computations

Geometry optimizations and single-point energy calculations were performed using B3LYP/6-31G*. The ZPE was calculated with scale factor 0.9806.

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13. The reason why 1,6-HT did not occur in the reaction with *t*-butylcyclohexanol derivative **19** but occurred in the reaction with indane derivatives **11** might be due to differences of the conformation and/or the BDE between the two substrates. The substrate **11** has a benzylic hydrogen, the BDE(C–H), of which is considerably lower than that of usual secondary C–H, and therefore, the 1,6-HT might have occurred.

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Detection of 210 kDa receptor protein for a leaf-movement factor by using novel photoaffinity probes

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Abstract—Circadian rhythmic plant leaf-movement, called nyctinasty, is controlled by a time-course change in the internal concentration of the leaf-movement factor in the plant body. We revealed that specific binding proteins (210 and 180 kDa) for the leaf-movement factor, potassium lespedezate (**1**), are contained in the plasma membrane of the plant motor cell by using novel synthetic photoaffinity probes. These proteins are localized on the motor cell in the plant body, and would be potential receptors for the leaf-movement factor to control the leaf-movement. Our study is a rare successful result of the detection of membrane receptors by using a synthetic photoaffinity probe designed on a biologically active natural product. And these results also advance a guideline for probe design towards successful photoaffinity labeling.

1. Introduction

In recent years, the importance of post-genomic studies is highly emphasized. The existence of many receptors are predicted and found in the sequence of genomic DNA. However, many of them are orphan receptors whose ligand is unknown. They have some types of trans-membrane domain and are thought to be potential receptors. Then, detection of the ligand of the orphan receptor is one of the most important issues of the post-genomic era, and the detection of a receptor protein for a biologically active natural product is a central issue in bioorganic chemistry or chemical genetics.[1]

Our study focuses on the bioorganic chemistry of biologically intriguing phenomena, such as nyctinasty: 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 the biological clock (Fig. 1).

Figure 1. *Cassia mimosoides* L: in the daytime (left), and at night (right).

Charles Darwin, well-known for his theory of evolution, was the first to establish the basis of this field in the 19th century.[2] 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. 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.[3] This also suggests that mechanism of nyctinasty is concerned with the water control in the plant body of legumes, which is an important food resource of human race. An issue of great interest is the regulation of the opening and closing of the potassium channels involved in nyctinastic leaf movement. We have revealed that nyctinasty is controlled by a pair of leaf-movement factors: leaf-opening and leaf-closing substances.[4] Recently, we revealed that the target cell of the leaf-opening substance is the motor cell,[5] which plays a key role in nyctinastic leaf-movement.[3] Thus, the next important issue is the detection and isolation of a receptor molecule for the leaf-movement factor, that will be located on the plasma membrane of the motor cell.

Photoaffinity labeling is a powerful method for the detection of receptors from the viewpoint of bioactive substances.[6] However, in general, it is difficult to get a successful result in photoaffinity labeling experiments. One reason for this difficulty in photoaffinity labeling would be attributable to

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the unsuitable molecular design of the probe. Molecular design of the probe molecule incurs many difficulties: the most important one is that the molecular design of the probe requires the cost of either high binding affinity or high biological activity because these two factors are never compatible. The arrangement of a large photoaffinity group against the binding site of a bioactive substance presents a serious dilemma. The shorter the distance between a large photoaffinity group and the binding site of the molecule is, the higher the ability to capture its receptor becomes, but the weaker its bioactivity becomes, and vice versa. The location of the photoaffinity labeling group in the probe should be discussed systematically for solving this issue. However, no guidelines have been reported for the discussion of the location of photoaffinity probes because of the difficulty of synthesizing systematic structural variation of the probes without important loss of the bioactivity. In this paper, we report the detection of a receptor molecule for the leaf-movement factor using novel synthetic probes, and advance a guideline for the probe design toward successful photoaffinity labeling.

2. Results

2.1. Molecular design of photoaffinity probes

We developed novel synthetic probes for photoaffinity labeling to look for the native factor receptor based on the structure of potassium isolespedezate (**1**),[7] a bioactive substance effective for leaf-opening of a leguminous plant, *Cassia mimosoides* L (Fig. 1). Structure–activity relationship studies on **1** revealed that the *p*-hydroxyphenylpyruvate moiety of **1** is indispensable for the leaf-opening activity of **1**, and is expected to be a binding site to its receptor.[8] On the other hand, structural modification in the glycon moiety of **1** did not cause any drop in bioactivity. For example, β-D-galactopyranoside, α-D-mannopyranoside, and β-L-glucopyranoside analogs of **1** were as effective as **1** in the bioassay using *Cassia* leaves.[9] Thus, we planned to introduce a large photoaffinity group to the glycon moiety of **1**. The photoaffinity group should be connected through an amide bond, which is expected to be stable against the hydrolysis by esterase in the plant body.

However, molecular design of the probe molecule incurs many difficulties: the most important one is that the molecular design of the probe requires the cost of either high photoaffinity labeling yield or high biological activity because these two factors are never compatible. The arrangement of a large photoaffinity group against the binding site of a bioactive substance presents a serious dilemma (Fig. 2). The shorter the distance between a large photoaffinity group and the binding site of the molecule is, the higher the ability to capture its receptor becomes, but the weaker its bioactivity becomes, and vice versa.

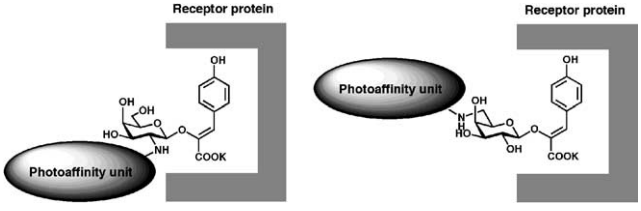
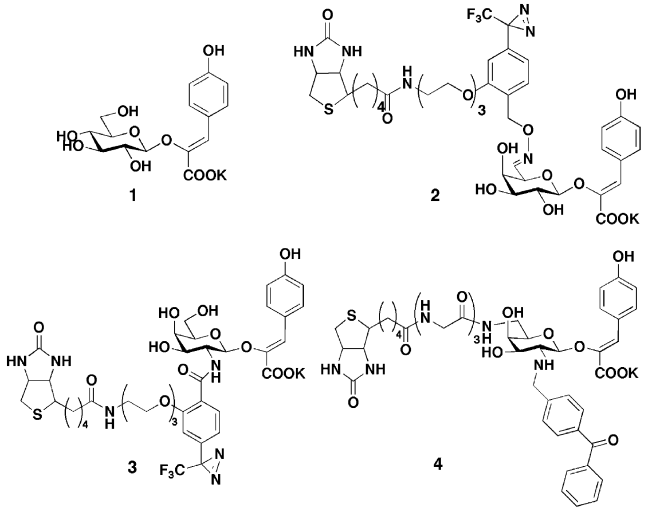


Figure 2. Molecular design of probe compound based on **1**.

We assumed that the 6′-and 2′-positions of the glycone moiety are suitable for the photoaffinity group. A 2′-modified probe is expected to give higher labeling yield than a 6′-modified one because of the short distance between the photoaffinity group and the potential binding site to the receptor. On the other hand, the 6′-modified probe is expected to show higher bioactivity than the 2′-modified one because of the long distance between the photoaffinity group and a potential binding site. Thus, we synthesized both 2′- and 6′-modified probes to compare the efficiency of the photoaffinity labeling.

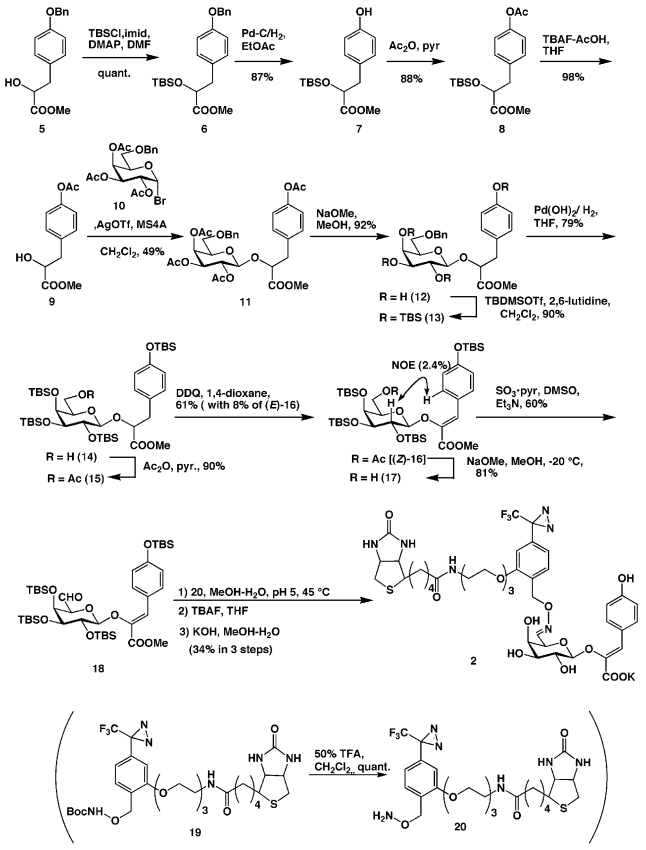
We compared the results of labeling experiments using these probes designed based on different concepts.

We synthesized biologically active photoaffinity probes (**2**, **3** and **4**) based on the structure of **1**. These probes are efficient tools for the biotinylation of the receptor proteins for **1**. Novel probes **2** and **3** have trifluoromethydiazirine[10] as photoaffinity groups, and **4** has a benzophenone group.[11] Probe **2**, which bears a photoaffinity group on the 6′-position of the sugar moiety, was designed for higher bioactivity than **3** and **4** by reducing the steric hindrance caused by a large photolabeling group. On the other hand, probes **3** and **4** bear a photolabeling group on the 2′-position of the sugar moiety, which is adjacent to the *p*-hydroxyphenylpyruvate group. These probes were designed for high labeling yield with the receptor molecule. We compared the results of labeling experiments using these probes that were designed on different concepts.



2.2. Synthesis of probe 2

Synthesis of photoaffinity probe **2** was carried out according to the synthetic route in Scheme 1.[12] A photoaffinity group was introduced using commercially available Affilight-CHO™ (**19**), which has a trifluoromethydiazirine and biotin group.[13] Introduction of **19** requires an aldehyde or a ketone group. Thus, we prepared the 6′-aldehyde analog of **1**. For



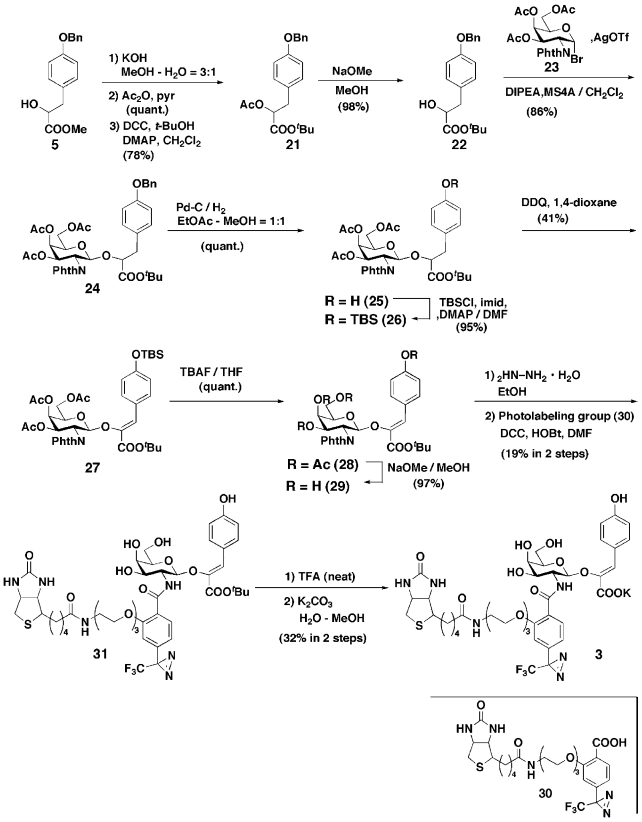
Scheme 1. Synthesis of photoaffinity probe **2**.

the sake of later oxidation, the primary hydroxyl group of D-galactose should be protected by a different protective group from secondary hydroxyl groups. Thus, **10**, which has different protective groups on primary and secondary hydroxyl groups was synthesized via 6-*O*-benzyl diacetonegalactose according to the method by Xia et al.[14] Selective acetonide formation between secondary hydroxyl groups using copper sulfate with catalytic sulfuric acid and following protection of the residual primary hydroxyl group with a benzyl group gave **10**.

Compound **9** was synthesized as shown in Scheme 1. Coupling of **9** and **10** gave **11**, and then the product was subjected to deacetylation, and the resulting secondary hydroxyl groups were protected with TBSOTf to give **13**. After conversion of the protective group on the 6′-position of the galactose moiety in **13** from the benzyl to an acetyl group, resulting **15** was treated with excess amount of DDQ (7 equiv) for 2 days to give **16**. This oxidation gave an 8:1 mixture of (*Z*)- and (*E*)-**16**. The stereochemistry was assigned by NOE correlation observed in (*Z*)-**16** (Scheme 1). These stereoisomers were separable by column chromatography. The selective deacetylation of **16** was achieved by carrying out the reaction at −20 °C. When the reaction was carried out at higher temperature, TBS on the phenolic hydroxyl group was deprotected simultaneously. Then, the resulting primary hydroxyl group was oxidized to give aldehyde (**18**). Coupling reaction of **18** with **20**, which was prepared from **19** was carried out in aq methanol, and the following deprotection and purification by HPLC using Develosil C8 UG-5 column gave the two isomers on the oxime moiety of photoaffinity probe **2** (34% yield of (*E*)-**2** and 25% yield of (*Z*)-**2**, respectively).

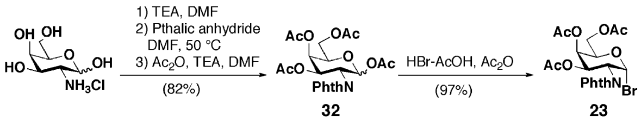
2.3. Synthesis of probe 3

Probe **3** was synthesized according to Scheme 2. We used galactosamine as a starting material to introduce the photolabeling unit on an amino group of the 2′-position. The photolabeling unit was connected with the aglycon moiety via an amide linkage. This molecular design would be effective for the improvement of labeling yield This change in molecular design requires revision of the synthetic route that was used in **2**.



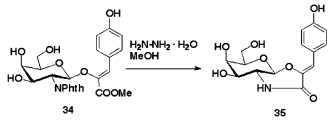
Scheme 2. Synthesis of photoaffinity probe **3**.

Aglycon **22** was synthesized from **5**, which was prepared according to Scheme 1. On the other hand, we synthesized 3, 4, 6-tri-*O*-acetyl-2-deoxy-2-phtalimido- α, β-D-galactopyranosyl bromide (**23**) from D-galactosamine hydrochloride. We modified previously reported synthetic conditions of **24**[15] from D-galactosamine hydrochloride because of the low yield (17%). In Ref. 15, we synthesized **23** from **32** prepared by Lee's method.[16] However, Lee's method gave **32** in a very low yield (32% from D-galactosamine hydrochloride) because of the difficulty in a protection of amine by phtalimide. We modified Lee's method and a one-pot protection of D-galactosamine by phtalimide and then acetic anhydride was carried out using DMF as a solvent to give **32** in a quantitative yield (Scheme 3). Compound **23** prepared from **32** was coupled with **22** by modified Königs-Knorr's method to give **24** (Scheme 3). When we used normal Königs-Knorr's condition, only 56% of coupling product **24** was obtained together with



Scheme 3. Synthesis of **24**.

carboxylate without *tert*-butyl group. However, addition of diisopropylethylamine (DIPEA) prevented the deprotection of *tert*-butyl group to give **24** in 86% yield. Thus, total yield of **24** from galactosamine hydrochloride was improved from 17%[15] to 67%. After conversion of the benzyl group in **24** to TBS, DDQ oxidation was carried out to give **27**. Deprotection of **27** with TBAF and then sodium methoxide gave **29**. Resulting **29** was further deprotected with hydrazine monohydrate, and then coupled with the photolabeling unit (**30**) bearing trifluoromethydiazirine and biotin, which is separately prepared according to the method by Hatanaka.[13] Low yield in coupling reaction was due to the low solubility of biotin unit **30** in any solvent. Free amine, which was obtained in the deprotection of **31**, was used in the coupling reaction without purification. *t*-Butyl ester in **29** was essential for the synthesis of **31**. When we used the methyl ester of **29** (**34**)[9] instead of *t*-butyl ester (**29**), lactam (**35**) [$\delta_{H\text{-}2'}$ 3.63 ppm] was obtained quantitatively in the following deprotection of the amino group on the 2′-position (Scheme 4). The *t*-butyl group would prevent the formation of lactam by its steric hindrance.

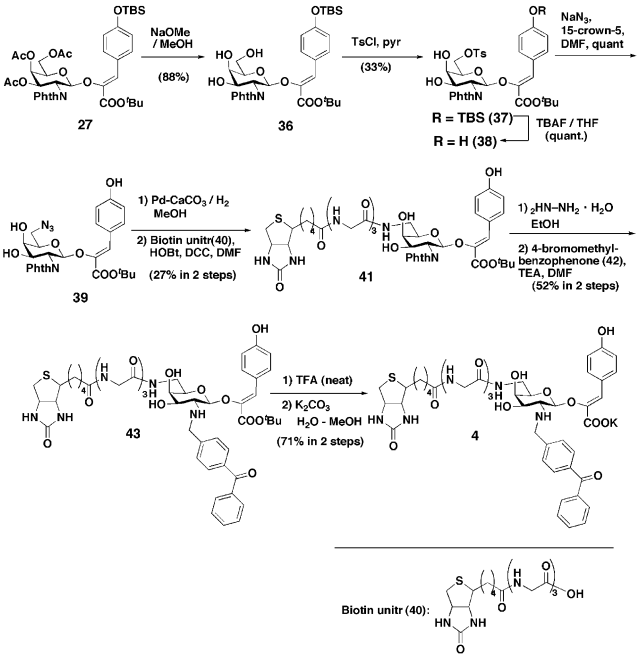


Scheme 4. Lacton formation from **34**.

However, deprotection of the *t*-butyl ester in **31** raises a serious problem. We examined the reaction conditions on the deprotection of **31** thoroughly. But the use of several weak acids, such as formic acid, acetic acid, and benzoic acid gave a complex mixture of decomposed products or ended in no reaction. The resulting complex mixture mainly contained the fragments obtained by the dissociation of the glycosidic bond or the amide bond. After many trials, we found that the treatment of **31** by neat TFA within 30 s at rt provided good results. Under this condition, deprotection proceeded in 82% yield. Following neutralization and purification gave probe **3**. In probe **3**, the photolabeling group on the 2′-position is extruded to the direction of aglycon, which is expected to be a binding site with the receptor.

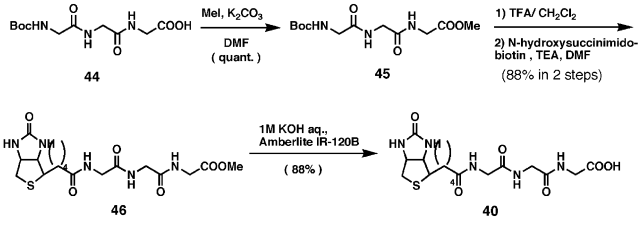
2.4. Synthesis of probe 4

Synthesis of novel probe **4** was carried out according to the route in Scheme 5.[17] In this probe, a large benzophenone group and a large biotin unit were separately introduced on the 2′- and 6′-position of the sugar moiety, respectively. For this purpose, we synthesized 2′, 6′-diamino galactose skeleton from D-galactosamine. We synthesized properly protected diamine as intermediate, and then, biotin coupled with a triglycine linker was introduced on the 6′ position of the glycon moiety, and then 4-bromomethylbenzophenone was introduced on its 2′ position.



Scheme 5. Synthesis of photoaffinity probe **4**.

Synthetic intermediate **27** was prepared according to the same method as Scheme 2. Similar to the case of probe **3**, *t*-butyl ester in **27** was essential for avoiding the lactam formation in the following deprotection of the amino group on the 2′-position. Compound **27** was deprotected with sodium methoxide to give **36**. Compound **36** was then treated with *p*-toluenesulfonyl chloride to give **37** with 47% of recovered **36**. After deprotection with TBAF, the resulting **38** was treated with sodium azide to give **39**. After catalytic hydrogenation with Pd–$CaCO_3$, the resulting crude amine was coupled without purification with biotinyl glycylglycylglycine (**40**), which is prepared separately (Scheme 6). Low yield in coupling reaction was due to the low solubility of biotin unit **40** in any solvent. Coupling product **41** was deprotected with hydrazine monohydrate, and the resulting crude amine was used for coupling reaction with 4-bromomethyl benzophenone (**42**) without purification in the presence of TEA. Coupling product **43** was then deprotected by neat TFA to give probe **4**. Deprotection was not achieved by 30 s treatment with TFA at rt, which is the condition used in the synthesis of probe **3**. Deprotection of **43** required much longer reaction time (25 min at rt) than in case of probe **3**.



Scheme 6. Synthesis of biotin unit (**40**).

2.5. Bioactivity of the probes

The resulting probes were tested in a bioassay using the leaves of *C. mimosoides*. When the leaves of *C. mimosoides* were separated from the stem, they continued leaf movement according to the circadian rhythm: open in the daytime and closed at night. The leaf-opening activity of the probe was judged by its bioactivity based on the leaves opening after 7:00 PM.

Photoaffinity probe **2**, which bears a photoaffinity group and a biotin group on the 6′-position of the galactose unit, showed leaf-opening activity at 5×10^{-5} M for *C. mimosoides*. This is one-fiftieth as active as the natural product. On the other hand, the bioactivity of probe **3** (8×10^{-5} M) was one-eightieth that of the natural product. Similarly, probe **4** was effective at 1×10^{-4} M, which is one-hundredth as effective as the natural product. Thus, all three synthetic probes were bioactive in the bioassay using the leaves of *C. mimosoides*. Their bioactivity changed according to the distance between the aglycone moiety and the large photoaffinity group. The molecular size of the photoaffinity group also affected bioactivity. These results showed that the nearer the large photoaffinity group was arranged to the aglycone unit, the weaker the bioactivity of the probe became.

We then compared the results of photolabeling examinations using these synthetic probes.

2.6. Photoaffinity labeling with synthetic probes[17]

Photoaffinity probes **3** and **4**, which bear a photolabeling group close to the potential binding site, are expected to label their receptor more effectively than probe **2**. On the other hand, probe **2**, which bears a photolabeling group far from the potential binding site showed stronger bioactivity than **3** and **4** in the bioassay, and is estimated to be easier to bind with its receptor molecule than probe **3** and **4**. Which type of probe gives a better result in the photolabeling examination? This is a very important problem because the result would give a general guideline for the molecular design of photoaffinity probes. Both **2** and **3** have a trifluoromethyldiaziridine group as a photoaffinity group. We then compared the results of photolabeling examinations using these probes **2** and **3**.

From previous experiments using fluorescence-labeled **1**, the target cell of **1** is revealed to be a motor cell, which is contained in the pulvini of *C. mimosoides*. Thus, photolabeling examination needed a large amount of the plasma membrane fraction of the motor cell, which is expected to contain a receptor of the leaf-opening substance **1**. We collected a large amount of plant motor cells by cutting off a large number of sections of plant pulvini (size; ca. 0.5 mm×0.5 mm) containing the motor cell one by one from plant leaves under a stereoscopic microscope. One cross-linking experiment needed about nine hundred plant sections.

Successive homogenization in extraction buffer (0.25 M sucrose, 3 mM EDTA, 2.5 mM DTT, 25 mM Tris–MES, pH 7.8) at 4 °C, filtration with nylon mesh, and twice ultracentrifugation (1st; 3000×g, 15 min, 4 °C, 2nd; 100,000×g, 60 min, 4 °C) gave a pellet of the crude membrane fraction. The content of protein in that fraction was determined to be 134 μg by the Bradford method with BSA as a reference. The membrane ATPase activity, which is a reference for the purity of the plasma membrane was determined to be 0.67 μmol/mg protein min by Sandstom's method.[18] The crude membrane fractions were suspended and incubated with 3 μM aq solutions of probe **2** or **3** for 60 min at rt, respectively. After cross-linking, by irradiation of UV-light (365 nm) for 10 min, the suspended membrane fraction was solubilized by the addition of an electrophoresis buffer containing SDS. The membrane fraction was analyzed by SDS-PAGE (7.5% T). After western blotting, detection of the bands of the potential receptor for leaf-opening substance was carried out by chemiluminescence detection with ECL Advance Western Blotting Detection Kit (Amersham Bioscience Co. Ltd), which is a method for the detection of bands of biotinylated proteins. Photolabeling experiment with probe **3** gave two bands corresponding to binding proteins for **1**. One is due to a protein of 210 kDa molecular weight, and the other to a protein of 180 kDa (lane 2 in Fig. 3). The molecular weight was estimated from comparison with a biotinylated molecular weight marker (Amersham Bioscience Co. Ltd).

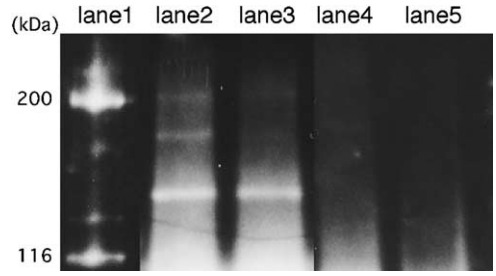


Figure 3. SDS PAGE analyses (10–20% *T* gradient gel) of potential membrane receptor using probe **2** and **3** by the chemiluminescence detection (Lane 1: molecular weight marker, lane 2: membrane protein of motor cell incubated with **3**, lane 3: membrane protein of motor cell incubated with **3** in the presence of 5000-fold molar excess of **1**, lane 4: membrane protein of leaf cell incubated with **2**, and lane 5: membrane protein of motor cell incubated with **2** in the presence of 5000-fold molar excess of **1**).

Specific bindings of the probe **3** were confirmed by the disappearance of the corresponding bands in the photolabeling examination in the presence of 5000-fold molar excess of non-labeled leaf-opening substance **1** (lane 3 in Fig. 3). Binding of probe **3** with binding proteins of 210 and 180 kDa would be inhibited competitively under this condition.

The bands for proteins smaller than 100 kDa were also observed in both lane 2 and lane 3 in Figure 3. However, these bands were concluded to be non-specific bands due to the biotin unit, because they were also detected in the cross-linking examination using photolableing unit **20** without the structure of **1**. The reproducibility was checked by labeling experiments repeated 10 times.

On the other hand, no specific band was detected in the labeling experiments with probe **2** under the same conditions (lane 4 and 5 in Fig. 3). And no band was

detected even in the experiment using 1.8-fold concentration of probe **2** (5 μM). These results suggested that the close arrangement of the photolabeling group and the binding site in a photoaffinity probe is most important for a successful result of a photolabeling examination. Molecular design of a photoaffinity probe should be carried out by evaluating the close arrangement of photoaffinity group to the binding site even at the cost of strong bioactivity due to its steric hindrance.

Next, we examined the difference between probe **3** and **4** in the efficiency of photoaffinity labeling for these potential receptor proteins. Photoaffinity labeling experiment using probe **4** was carried out under the same conditions as in the case of probe **3**. As shown in Figure 4, probe **4** with benzophenone as a photolabeling group seems to give clearer band corresponding to 210 and 180 kDa (lane 2 in Fig. 4) than probe **3** with trifluoromethyldiazirine (lane 2 in Fig. 3). Specific bindings of the probe **4** were also confirmed by the disappearance of the corresponding bands in the photolabeling examination in the presence of 5000-fold molar excess of non-labeled leaf-opening substance **1** (lane 3 in Fig. 4).

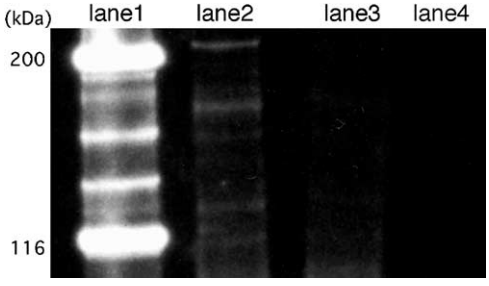


Figure 4. SDS PAGE analyses of potential membrane receptor for **1** by the chemiluminescence detection (Lane 1: molecular weight marker, lane 2: membrane protein of motor cell incubated with **4**, lane 3: membrane protein of motor cell incubated with **4** in the presence of 5000-fold molar excess of **1**, and lane 4: membrane protein of leaf cell incubated with **4**).

From these results, probe **4** which bears a benzophenone group near the binding site gave the best result in photoaffinity labeling examination. And it must be emphasized that probe with strongest bioactivity is not always the best one in photoaffinity labelling experiments. This result suggest the important information for the design of photoaffinity probe for the successful photoaffinity labeling.

2.7. Localization of the potential receptor protein

We also examined the localization of these binding proteins. In the fluorescence study with a fluorescent probe,[5] it was revealed that leaf-opening substance **1** exclusively binds to the motor cell, and not with other parts of the plant body at all. If these binding proteins were the genuine receptor for the leaf-movement factor, they would be localized in the motor cell. Photolabeling experiment with a crude membrane fraction prepared from the section of plant leaves, which contain no motor cell gave no specific band on the chemiluminescence detection for biotinylated proteins (lane 4 in Fig. 4). From these results, we proposed that potential receptor proteins for **4** (210 and 180 kDa) that have

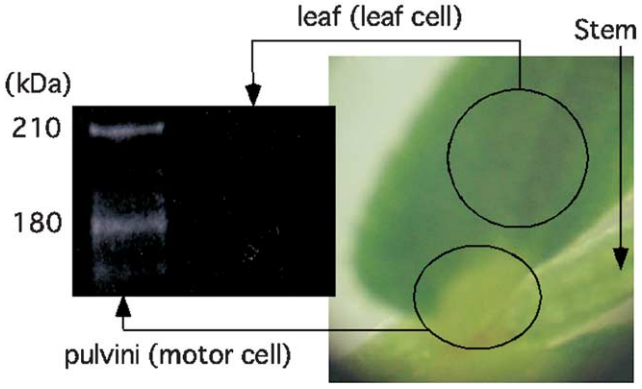


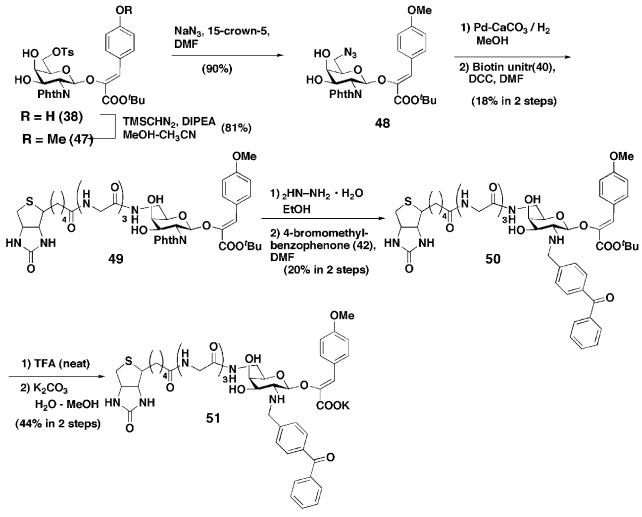
Figure 5. The localization of potential receptor protein for **1** in the plant.

specific binding activity for **1** are located in the plasma membrane of the motor cell (Fig. 5). And no other part of the plant contain these potential receptors. This result was consistent with the result from fluorescence study.

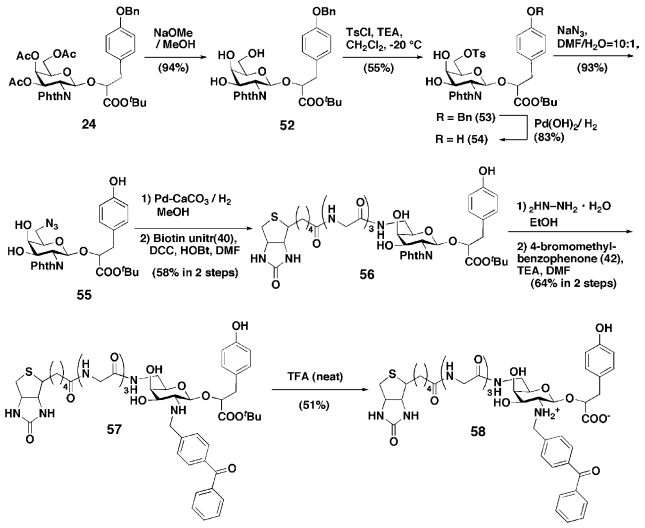
2.8. Photoaffinity labeling using biologically inactive probes based on analogs of 1

Next, we carried out a photoaffinity labeling examination using probes based on a biologically inactive analog of **1**. If this potential receptor concerns leaf-opening of this plant, biologically inactive probe should not give the band corresponding to the potential receptor protein. Based on the result of structure–activity relationship study of **1**,[8,9] we synthesized a biologically inactive probe **51** and **58** according to a synthetic route in Schemes 7 and 8, respectively. Compound **38**, which is a synthetic intermediate of probe **4**, was methylated by $TMSCHN_2$ to give **47**. Biologically inactive probe **51** was synthesized from **47** according to the same procedure as in the case of probe **4** (Scheme 7). And **24** was used for the synthesis of probe **58** as shown in Scheme 8.

Resulting **51** whose phenolic hydroxyl group was protected as a methyl ether and **58** whose olefin was reduced to single bond, did not show leaf-opening activity even at 1×10^{-4} M. Probe **51** gave a band at 200 kDa, a different band



Scheme 7. Synthesis of biologically inactive probe (**51**).



Scheme 8. Synthesis of biologically inactive probe (**58**).

from the 210 and 180 kDa ones, which were detected using a biologically active probe (**4**) (Fig. 6). And probe **58** gave no band in SDS-PAGE analysis (Fig. 6). These results showed that the bands of 210 and 180 kDa were strongly correlated with the biological activity of the probe, which strongly suggests that these proteins are the genuine receptors of the leaf-opening substance.

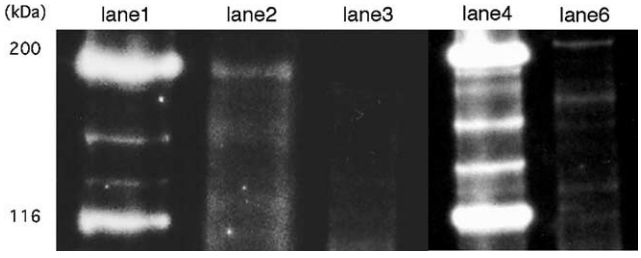


Figure 6. SDS PAGE analyses using biologically inactive probe **51** (detected by the chemiluminescence detection, Lane 1: molecular weight marker, lane 2: membrane protein of motor cell incubated with **51**, lane 3: membrane protein of motor cell incubated with **58**, lane 4: molecular weight marker, lane 5: membrane protein of motor cell incubated with **4**).

3. Discussion

These results are an important advance in the bioorganic studies of nyctinasty, and would be an important clue for the molecular mechanism of nyctinasty that has been a historical mystery since the era of Darwin.

Potential receptor proteins reported in this paper, are key molecules connecting the bioorganic study using low-molecular weight natural products with molecular biology within the cell. Because nyctinastic leaf-movement is induced by opening and closing of potassium channels, it is generally considered that potential receptor proteins would be a subunit of the potassium channels or H^+-ATPase, which is known to be involved in the regulation of channel movements. However, no subunit of potassium channels and no H^+-ATPase of a plant have been reported to have such large molecular weight as 200 kDa.[19] Thus, these potential receptors might be a new type of protein concerning the control of potassium channels. Trials for the cloning of this receptor are now in progress.

Photoaffinity labeling is known to provide important information on the binding site of some bioactive substance to the known receptor proteins. However, photoaffinity labeling has provided very few successful results in the search for unknown receptors of some bioactive substances, especially, in the case of membrane receptor.[20] Our study showed that careful molecular design is necessary for success in each unknown receptor by photoaffinity labeling.

Our study also showed that discovery of a new biologically active substance led to the discovery of an unknown membrane receptor.

The chemical approach could be compatible with the molecular biological approach in the study of cell biology, and it will become more important in this field.

4. Experimental

4.1. General experimental

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_3OH (^{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 FAB-MS and HR FAB-MS spectra were recorded on a Jeol JMS-700 or JMS-SX102 spectrometer, using glycerol or *m*-nitrobenzylalcohol as a matrix. The HR ESI-MS spectra were recorded on a Bruker APEX-III. The IR spectra were recorded on a JASCO FT/IR-410. 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 ($\phi 20 \times 250$ 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 μm pore size, 47 mm. dia.) 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 75C18-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. Methyl 3-*p*-benzyloxyphenyl-2-*t*-butyldimethylsilyloxy propionate (6). Compound **5** (1.00 g, 3.50 mmol) was dissolved in DMF (35 mL). To this solution, TBSCl (845 mg, 5.25 mmol), imidazole (714 mg, 10.5 mmol), and a catalytic amount of DMAP (10 mg, 82.0 μmol) were added, and then this reaction mixture was stirred overnight under argon atmosphere. This reaction mixture was then mixed with water, and extracted by *n*-hexane/ethyl acetate 1:1, and the organic layer was washed with brine, dried over abs Na_2SO_4, concentrated in vacuo, and purified by silica

gel column chromatography (*n*-hexane/ethyl acetate 3:1) to give **6** (1.53 g, quant.).

Compound **6**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.26–7.43 (5H, m), 7.11 (2H, d, J=8.3 Hz), 6.87 (2H, d, J=8.3 Hz), 5.03 (2H, s) 4.27 (1H, dd, J=3.9, 8.8 Hz), 3.70 (3H, s), 2.99 (1H, dd, J=3.9, 13.7 Hz), 2.81 (1H, dd, J=8.8, 13.7 Hz), 0.77 (9H, s), −0.15 (3H, s), −0.22 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 173.5, 157.4, 136.9, 130.6, 129.6, 128.4, 127.7, 127.3, 114.6, 73.9, 69.9, 51.9, 40.8, 25.7, 18.3, −5.4, −5.5 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 423.1986, $C_{23}H_{32}O_4NaSi$ requires *m/z* 423.1968; IR (film) ν: 1756, 1612, 1512 cm^{-1}.

4.1.2. Methyl 3-*p*-hydroxyphenyl-2-*t*-butyldimethylsilyloxy propionate (7). Compound **6** (1.53 g, 3.82 mmol) was dissolved in ethyl acetate (1.5 mL). To this solution, a catalytic amount of Pd–C (200 mg) was added. The reaction mixture was stirred overnight under argon atmosphere, filtered with Celite, concentrated in vacuo, and purified by silica gel TLC (*n*-hexane/ethyl acetate 1:1) to give **7** (1.03 g, 87%).

Compound **7**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.06 (2H, d, J=8.3 Hz), 6.73 (2H, d, J=8.3 Hz), 4.27 (1H, dd, J=3.9, 9.3 Hz), 3.70 (3H, s), 2.97 (1H, dd, J=3.9, 13.7 Hz), 2.80 (1H, dd, J=9.3, 13.7 Hz), 0.79 (9H, s), −0.14 (3H, s), −0.21 (3H, s) ppm; ^{13}C NMR (67.8 MHz, $CDCl_3$, rt): 173.7, 154.3, 130.8, 129.2, 115.0, 73.9, 51.9, 40.8, 25.7, 18.3, −5.37, −5.43 ppm; HR FAB MS (positive): $[M+H]^+$ Found *m/z* 311.1653, $C_{16}H_{27}O_4Si$ requires *m/z* 311.1679; IR (film) ν: 3419, 1736, 1614, 1597, 1516 cm^{-1}.

4.1.3. Methyl 3-*p*-acetoxyphenyl-2-*t*-butyldimethylsilyloxy propionate (8). Compound **7** (1.03 g, 3.32 mmol) was dissolved in pyridine (20 mL). To this solution acetic anhydride (0.94 mL, 9.95 mmol) was added at 0 °C. The reaction mixture was then stirred overnight under argon atmosphere, and evaporated with toluene to remove pyridine as an azeotropic mixture. The residue was then purified by silica gel column chromatography (*n*-hexane/ethyl acetate 4:1) to give **8** (1.03 g, 88%).

Compound **8**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.20 (2H, d, J=8.8 Hz), 6.98 (2H, d, J=8.8 Hz), 4.29 (1H, dd, J=3.9, 9.3 Hz), 3.70 (3H, s), 3.04 (1H, dd, J=3.9, 13.7 Hz), 2.85 (1H, dd, J=9.3, 13.7 Hz), 2.26 (3H, s), 0.76 (9H, s), −0.14 (3H, s), −0.23 (3H, s) ppm; ^{13}C NMR (67.8 MHz, $CDCl_3$, rt): 173.2, 169.3, 149.3, 134.9, 130.6, 121.2, 73.6, 51.9, 40.9, 25.6, 21.2, 18.2, −5.4, −5.6 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 375.1608, $C_{18}H_{28}O_5$ NaSi requires *m/z* 375.1604; IR (film) ν: 1760, 1508 cm^{-1}.

4.1.4. Methyl 3-*p*-acetoxyphenyl-2-hydroxy propionate (9). Compound **9** (83.1 mg, 0.238 mmol) was dissolved in THF (1.2 mL). To this solution, THF solution (1.2 mL) of TBAF (1 M THF solution, 0.47 mL, 0.470 mmol) and acetic acid (27 μL, 0.473 mmol) was added. The rection mixture was stirred overnight under argon atmosphere, mixed with water, and extracted with ethyl acetate. The organic layer was then washed with brine, dried over abs Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 1:1) to give **9** (55.2 mg, 98%).

Compound **9**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.21 (2H, d, J=8.3 Hz), 7.00 (2H, d, J=8.3 Hz), 4.43 (1H, dd, J=4.4, 6.8 Hz), 3.76 (3H, s), 3.10 (1H, dd, J=4.4, 14.2 Hz), 2.94 (1H, dd, J=6.8, 14.2 Hz), 2.27 (3H, s) ppm; ^{13}C NMR (67.8 MHz, $CDCl_3$, rt): 174.2, 169.4, 149.3, 133.9, 130.3, 121.3, 71.1, 52.4, 39.7, 21.1 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 261.0716, $C_{12}H_{14}O_5Na$ requires *m/z* 261.0739; IR (film) ν: 3482, 1743, 1508 cm^{-1}.

4.1.5. Methyl 2-(2′,3′,4′-tri-*O*-acetyl-6′-benzyl-β-D-galactopyranosyl-oxy)-3-*p*-acetoxyphenyl propionate (11). Compound **9** (25.0 mg, 0.105 mmol) and **10** (48.3 mg, 0.105 mmol) were dissolved in dry CH_2Cl_2 (1.0 mL). To this solution, MS4A (50 mg), and then AgOTf (30 mg, 0.117 mmol) were added at −30 °C. The reaction mixture was stirred for 2 h warming slowly to rt. The reaction mixture was filtered with Celite, and washed with chloroform. Then, the organic layer was washed with satd $NaHCO_3$ and then brine, dried over abs Na_2SO_4, and evaporated in vacuo. The residue was then purified by silica gel column chromatography (toluene/acetone 4:1) to give **11a** (18.3 mg, 28%) and **11b** (13.6 mg, 21%) with recovered **10** (9.2 mg, 37%).

Compound **11a**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.25–7.35 (5H, m), 7.16 (2H, d, J=8.8 Hz), 6.94 (2H, d, J=8.8 Hz), 5.42 (1H, d, J=3.4 Hz), 5.21 (1H, dd, J=7.8, 10.3 Hz), 4.98 (1H, dd, J=3.4, 10.3 Hz), 4.59 (1H, t, J=5.9 Hz), 4.50 (1H, d, J=12.2 Hz), 4.48 (1H, d, J=7.8 Hz), 4.38 (1H, d, J=12.2 Hz), 3.77 (1H, t, J=6.3 Hz), 3.60 (3H, s), 3.49 (1H, dd, J=6.3, 9.8 Hz), 3.44 (1H, dd, J=6.3, 9.8 Hz), 3.10 (1H, dd, J=5.9, 14.2 Hz), 3.04 (1H, dd, J=5.9, 14.2 Hz), 2.26 (3H, s), 2.06 (3H, s), 1.96 (3H, s), 1.95 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.7, 170.1, 169.9, 169.7, 169.3, 149.4, 137.4, 133.3, 130.7, 128.4, 127.81, 127.78, 121.1, 99.6, 75.9, 73.5, 72.2, 70.7, 68.6, 67.43, 67.41, 51.8, 38.2, 21.2, 20.8, 20.74, 20.66 ppm; HR FAB MS (positive): $[M+H]^+$ Found *m/z* 617.2250, $C_{31}H_{37}O_{13}$ requires *m/z* 617.2234 IR (film) ν: 1749, 1508 cm^{-1}; $[\alpha]_D^{25}$ −38.1° (*c* 1.0, $CHCl_3$).

Compound **11b**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.18–7.30 (5H, m), 7.13 (2H, d, J=8.8 Hz), 6.92 (2H, d, J=8.8 Hz), 5.34 (1H, d, J=3.4 Hz), 5.16 (1H, dd, J=7.8, 10.3 Hz), 4.84 (1H, dd, J=3.4, 10.3 Hz), 4.44 (1H, d, J=11.7 Hz), 4.41 (1H, d, J=7.8 Hz), 4.36 (1H, d, J=11.7 Hz), 4.16 (1H, dd, J=3.9, 9.3 Hz), 3.76 (1H, t, J=6.4 Hz), 3.64 (3H, s), 3.45 (1H, dd, J=6.4, 9.3 Hz), 3.40 (1H, dd, J=6.4, 9.3 Hz), 3.02 (1H, dd, J=9.3, 14.1 Hz), 2.92 (1H, dd, J=3.9, 14.1 Hz), 2.21 (3H, s), 1.98 (3H, s), 1.88 (3H, s), 1.71 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 171.6, 170.2, 170.0, 169.4, 169.2, 149.5, 137.3, 133.9, 130.3, 128.4, 127.80, 127.79, 121.5, 101.8, 80.9, 73.4, 72.1, 71.0, 68.5, 67.3, 67.2, 52.1, 38.0, 21.1, 20.6, 20.5 ppm; HR FAB MS (positive): $[M+H]^+$ Found *m/z* 617.2250, $C_{31}H_{37}O_{13}$ requires *m/z* 617.2234 IR (film) ν: 1751, 1510 cm^{-1}; $[\alpha]_D^{25}$ −14.3° (*c* 1.0, $CHCl_3$).

4.1.6. Methyl 2-(6′-benzyl-β-D-galactopyranosyloxy)-3-*p*-hydroxy-phenyl propionate (12). Compound **11a**

(71.0 mg, 0.115 mmol) was dissolved in MeOH (1.5 mL), and sodium methoxide (6.0 mg, 0.111 mmol) was added to this solution. After 7 h stirring at rt, Amberlite IR-120B (H^+) was added to this solution for neutralization. After filtration, the filtrate was concentrated in vacuo and purified by silica gel TLC ($CHCl_3$/MeOH 10:1) to give **12a** (47.6 mg, 92%).

Similarly, **11b** (68.6 mg, 0.111 mmol) was dissolved in MeOH (1.5 mL), and sodium methoxide (6.0 mg, 0.111 mmol) was added to this solution. After 7 h stirring at rt, Amberlite IR-120B (H^+) was added to this solution for neutralization. After filtration, the filtrate was concentrated in vacuo and purified by silica gel TLC ($CHCl_3$/MeOH 10:1) to give **12b** (41.0 mg, 82%).

Compound **12a**. ^{1}H NMR (400 MHz, CD_3OD, rt): 7.22–7.34 (5H, m), 7.02 (2H, d, J=8.3 Hz), 6.65 (2H, d, J=8.3 Hz), 4.61 (1H, t, J=6.3 Hz), 4.55 (2H, s), 4.29 (1H, d, J=7.8 Hz), 3.78 (1H, d, J=3.4 Hz), 3.65–3.74 (3H, m), 3.64 (3H, s), 3.55 (1H, dd, J=7.8, 9.8 Hz), 3.45 (1H, dd, J=3.4, 9.8 Hz), 3.02 (2H, d, J=6.3 Hz) ppm; ^{13}C NMR (100 MHz, CD_3OD, rt): 174.0, 157.1, 139.5, 131.5, 129.3, 128.8, 128.6, 128.2, 115.9, 104.1, 79.2, 75.4, 74.7, 74.4, 72.4, 70.9, 70.5, 52.4, 39.5 ppm; HR FAB MS (positive): $[M+H]^+$ Found m/z 449.1786, $C_{23}H_{29}O_9$ requires m/z 449.1812 IR (film) ν: 3398, 1736, 1614, 1516 cm^{-1}; $[\alpha]_D^{25}$ −1.9° (*c* 1.0, MeOH).

Compound **12b**. ^{1}H NMR (400 MHz, CD_3OD, rt): 7.21−7/37 (5H, m), 7.04 (2H, d, J=8.3 Hz), 6.67 (2H, d, J=8.3 Hz), 4.52 (2H, s), 4.30 (1H, t, J=6.8 Hz), 4.27 (1H, d, J=7.8 Hz), 3.76 (1H, d, J=3.4 Hz), 3.53–3.72 (4H, m), 3.52 (3H, s), 3.42 (1H, dd, J=3.4, 10.3 Hz), 3.01 (1H, dd, J=6.8, 13.7 Hz), 2.91 (1H, dd, J=6.8, 13.7 Hz) ppm; ^{13}C NMR (100 MHz, CD_3OD, rt): 174.4, 157.2, 139.5, 131.5, 129.3, 128.7, 128.6, 128.0, 116.0, 104.7, 80.8, 75.2, 74.6, 74.3, 72.2, 70.6, 52.3, 38.8 ppm; HR FAB MS (positive): $[M+H]^+$ Found m/z 449.1841, $C_{23}H_{29}O_9$ requires m/z 449.1812 IR (film) ν: 3585, 1738, 1614, 1518 cm^{-1}; $[\alpha]_D^{25}$ −22.0° (*c* 1.0, MeOH).

4.1.7. Methyl 2-(6′-benzyl-2′,3′,4′-tri-*t*-butyldimethylsilyloxy-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl propionate (13). Compound **12a** (47.6 mg, 0.106 mmol) was dissolved in CH_2Cl_2 (1.0 mL). To this solution, 2,6-lutidine (0.18 mL, 1.55 mmol) and TBDMSOTf (0.24 mL, 1.05 mmol) were added at 0 °C. the reaction mixture was stirred for an hour, washed with water and then brine, dried over abs Na_2SO_4, and concentrated in vacuo. The residue was then purified by silica gel column chromatography (*n*-hexane/ethyl acetate 10:1) to give **13a** (86.3 mg, 90%).

Similarly, **12b** (41.0 mg, 91.5 μmol) was dissolved in CH_2Cl_2 (1.0 mL). To this solution, 2,6-lutidine (0.18 mL, 1.55 mmol) and TBDMSOTf (0.24 mL, 1.05 mmol) were added at 0 °C. the reaction mixture was stirred for an hour, washed with water and then brine, dried over abs Na_2SO_4, and concentrated in vacuo. The residue was then purified by silica gel column chromatography (*n*-hexane/ethyl acetate 10:1) to give **13b** (23.5 mg, 28%).

Compound **13a**. ^{1}H NMR (400 MHz, acetone-d_6, 40 °C): 7.19–7.36 (5H, m), 7.03 (2H, d, J=8.3 Hz), 6.66 (2H, d, J=8.3 Hz), 4.70 (1H, br s), 4.53 (3H, m), 4.17 (1H, br s), 3.82–3.94 (2H, m), 3.75 (1H, br s), 3.66 (2H, m), 3.50 (3H, s), 3.01 (1H, dd, J=5.9, 13.7 Hz), 2.93 (1H, m), 0.94 (9H, s), 0.93 (9H, s), 0.90 (9H, s), 0.89 (9H, s), 0.15 (6H, s), 0.14 (3H, s), 0.12 (3H, s), 0.09 (3H, s), 0.08 (3H, s), 0.06 (3H, s), 0.01 (3H, s) ppm; ^{13}C NMR (100 MHz, acetone-d_6, 40 °C): 171.9, 155.0, 139.7, 131.1, 130.3, 128.9, 128.2, 128.0, 120.4, 101.7, 79.1, 77.0, 75.6, 73.7, 73.6, 73.4, 70.7, 51.5, 39.0, 27.1, 26.9, 26.6, 26.1, 19.1, 18.8, 18.7, −3.8, −3.9, −4.2, −4.4 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 927.5061, $C_{47}H_{84}O_9Si_4Na$ requires m/z 927.5090 IR (film) ν: 1751, 1510 cm^{-1}; $[\alpha]_D^{25}$ −15.6° ç(c 1.0, $CHCl_3$).

Compound **13b**. ^{1}H NMR (400 MHz, acetone-d_6, 40 °C): 7.17–7.33 (5H, m), 7.03 (2H, d, J=8.3 Hz), 6.72 (2H, d, J=8.3 Hz), 4.26–4.55 (4H, m), 4.11 (1H, s), 3.88 (1H, dd, J=5.4, 7.4 Hz), 3.54–3.77 (4H, m), 3.44 (3H, s), 3.06 (1H, dd, J=5.4, 13.2 Hz), 2.92 (1H, dd, J=7.4, 13.2 Hz), 0.75–0.95 (36H, m), 0.00–0.16 (24H, m) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 171.9, 155.2, 131.2, 129.02, 128.97, 128.3, 128.1, 120.7, 120.5, 103.8, 80.2, 76.8, 74.4, 73.4, 72.9, 69.5, 51.6, 51.5, 38.2, 27.1, 26.8, 26.5, 26.0, 19.4, 19.3, 19.1, 18.7, −2.0–−4.5 (br) ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 927.5095, $C_{47}H_{84}O_9NaSi_4$ requires m/z 927.5090 IR (film) ν: 1751, 1510 cm^{-1}; $[\alpha]_D^{25}$ −25.4° (*c* 1.0, $CHCl_3$).

4.1.8. Methyl 2-(2′,3′,4′-tri-*t*-butyldimethylsilyloxy-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl propionate (14). Compound **13a** (86.3 mg, 95.3 μmol) was dissolved in THF (1.0 mL). To this solution, a catalytic amount of $Pd(OH)_2$ (10 mg) was added, and the reaction mixture was stirred overnight under hydrogen atmosphere. Then the reaction mixture was filtered with Celite, concentrated in vacuo, and purified by silica gel column chromatography (*n*-hexane/ethyl acetate 5:1) to give **14a** (61.3 mg, 79%).

Similarly, **13b** (23.5 mg, 26.0 μmol) was dissolved in THF (0.25 mL). To this solution, a catalytic amount of $Pd(OH)_2$ (2.5 mg) was added, and the reaction mixture was stirred overnight under hydrogen atmosphere. Then the reaction mixture was filtered with Celite, concentrated in vacuo, and purified by silica gel column chromatography (*n*-hexane/ethyl acetate 5:1) to give **14b** (21.9 mg, quant.).

Compound **14a**. ^{1}H NMR (400 MHz, acetone-d_6, 40 °C): 7.14 (2H, d, J=8.8 Hz), 6.75 (2H, d, J=8.8 Hz), 4.74 (1H, br s), 4.51 (1H, d, J=4.4 Hz), 4.20 (1H, br s), 3.96 (1H, dd, J=4.9, 7.4 Hz) 3.64–3.79 (3H, m), 3.56 (3H, s), 3.42 (1H, br s), 3.06 (1H, dd, J=6.4, 14.2 Hz), 2.99 (1H, dd, J=7.3, 14.2 Hz), 0.99 (9H, s), 0.974 (9H, s), 0.965 (9H, s), 0.93 (9H, s), 0.18–0.19 (15H, m), 0.14 (3H, s), 0.12 (3H, s), 0.11 (3H, s) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 171.9, 154.8, 131.0, 130.4, 120.3, 102.3, 76.8, 76.6, 73.3, 72.8, 61.7, 51.5, 38.8, 30.6, 27.1, 26.5, 26.0, 19.3, 19.1, 18.72, 18.68, −3.2, −3.7, −.3.9, −4.3, −4.4 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 837.4606, $C_{40}H_{78}O_9NaSi_4$ requires m/z 837.4621 IR (film) ν: 3585, 1751, 1610, 1510 cm^{-1}; $[\alpha]_D^{25}$ −18.8° (*c* 1.0, $CHCl_3$).

Compound **14b**. ^{1}H NMR (400 MHz, acetone-d_6, 40 °C): 7.09 (2H, d, J=8.8 Hz), 6.77 (2H, d, J=8.8 Hz), 4.49 (1H, br s), 4.29 (1H, m), 4.14 (1H, br s), 3.92 (1H, m), 3.68–3.79 (2H, m), 3.50–3.63 (5H, m), 3.10 (1H, m), 2.95 (1H, dd, J= 8.8, 14.2 Hz), 0.98 (9H, s), 0.97 (9H, s), 0.96 (9H, s), 0.95 (9H, s), 0.11–0.21 (24H, m) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 172.8, 155.2, 131.1, 120.7, 120.4, 104.0, 80.8, 77.0, 76.7, 76.5, 72.8, 61.4, 51.6, 38.3, 27.1, 26.8, 26.5, 26.0, 19.5, 19.1, 18.74, 18.69, −2.3, −3.3, −3.6, −3.9, −4.3, −4.35, −4.37 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 837.4604, $C_{40}H_{78}O_9Si_4Na$ requires m/z 837.4621 IR (film) ν: 3585, 1738, 1610, 1510 cm^{-1}; $[\alpha]_D^{25}$ −25.1° (c 1.0, $CHCl_3$).

4.1.9. Methyl 2-(6′-*O*-acetyl-2′,3′,4′-tri-*t*-butyldimethylsilyloxy-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl propionate (15). Compound **14a** (61.3 mg, 75.2 μmol) was dissolved in pyridine (0.5 mL). To this solution, acetic anhydride (21 μL, 0.222 mmol) was added, and the reaction mixture was stirred overnight under argon atmosphere. Then, the reaction mixture was mixed with toluene and evaporated to remove pyridine as an azeotropic mixture to give **15a** (58.2 mg, 90%).

Similarly, **14b** (21.9 mg, 26.9 μmol) was dissolved in pyridine (0.2 mL). To this solution, acetic anhydride (7 μL, 0.72 mmol) was added, and the reaction mixture was stirred overnight under argon atmosphere. Then, the reaction mixture was mixed with toluene and evaporated to remove pyridine as an azeotropic mixture to give **15b** (23.8 mg, quant.).

Compound **15a**. ^{1}H NMR (400 MHz, acetone-d_6, 40 °C): 7.06 (2H, d, J=8.8 Hz), 6.73 (2H, d, J=8.8 Hz), 4.70 (1H, br s), 4.60 (1H, dd, J=5.4, 7.8 Hz), 4.52 (1H, m), 4.39 (1H, br s), 4.23 (1H, br s), 3.94 (2H, m), 3.80 (1H, br s), 3.51 (3H, s), 3.01 (1H, dd, J=5.9, 13.7 Hz),4.14 (1H, br s), 3.92 (1H, m), 3.79–3.68 (2H, m), 3.63–3.50 (5H, m), 3.10 (1H, m), 2.91 (1H, m), 2.01 (3H, s), 0.85–0.96 (36H, m), 0.04–0.18 (24H, m) ppm; ^{13}C NMR (100 MHz, acetone-d_6, 40 °C): 172.2, 170.9, 155.3, 131.2, 130.2, 120.6, 100.7, 77.3, 75.0, 73.7, 68.3, 65.8, 51.6, 39.0, 26.6, 26.5, 26.3, 26.1, 21.0, 19.0, 18.8, 18.6, −4.0, −4.2, −4.6 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 879.4697, $C_{42}H_{80}O_{10}Si_4$-Na requires m/z 879.4726 IR (film) ν: 1747, 1610, 1512 cm^{-1}; $[\alpha]_D^{20}$ −12.6° (c 1.0, $CHCl_3$).

Compound **15b**. ^{1}H NMR (400 MHz, acetone-d_6, 40 °C): 7.04 (2H, d, J=8.8 Hz), 6.73 (2H, d, J=8.8 Hz), 4.62–4.07 (5H, m), 3.96–3.69 (3H, m), 3.49 (3H, s), 3.01 (1H, m), 2.93 (1H, dd, J=7.8, 13.7 Hz), 1.93 (3H, s), 0.94 (9H, s), 0.93 (9H, s), 0.91 (9H, s), 0.90 (9H, s), 0.17–0.07 (24H, m) ppm; ^{13}C NMR (100 MHz, acetone-d_6, 40 °C): 171.7, 170.7, 155.3, 131.2, 120.7, 120.6, 101.6, 80.8, 79.2, 77.3, 75.0, 73.6, 65.2, 52.0, 38.5, 26.8, 26.6, 26.5, 26.1, 20.8, 19.0, 18.8, 18.7, −4.1, −4.2, −4.6 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 879.4697, $C_{42}H_{80}O_{10}Si_4$-Na requires m/z 879.4726 IR (film) ν: 1743, 1610, 1510 cm^{-1}; $[\alpha]_D^{25}$ −13.8 °C (c 1.0, $CHCl_3$).

4.1.10. Methyl (*Z*)-2-(6′-*O*-acetyl-2′,3′,4′-tri-*t*-butyldimethylsilyloxy-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl acrylate (16a). A diastereomixture of **15a** and **15b** (124 mg, 0.145 mmol) was dissolved in 1,4-dioxane (1.5 mL), and DDQ (165 mg, 0.727 mmol) was added to this solution. After additional DDQ (65.8 mg, 0.290 mmol) was added to this solution, the reaction mixture was refluxed for 24 h under argon atmosphere. After filtration, the filtrate was mixed with Et_2O, washed with satd $NaHCO_3$ aq, dried over abs Na_2SO_4, and concentrated in vacuo. The residue was separated by silica gel column chromatography (*n*-hexane/ethyl acetate 10:1), and then by silica gel TLC (*n*-hexane/ethyl acetate 5:1) to give (*Z*)-**16** (76.0 mg, 61%) and (*E*)-**16** (9.6 mg, 8%) with recovered **15a** and **15b** as a mixture (9.2 mg, 7%).

Compound (*Z*)-**16**. ^{1}H NMR (400 MHz, acetone-d_6, rt): 7.77 (2H, d, J=8.3 Hz), 6.95 (1H, s), 6.79 (2H, d, J=8.3 Hz), 5.32 (1H, d, J=7.3 Hz), 4.18 (1H, dd, J=7.3, 9.7 Hz), 4.00–4.09 (2H, m), 3.96 (1H, m), 3.76–3.85 (2H, m), 3.72 (3H, s), 1.90 (3H, s), 1.00 (9H, s), 0.96 (18H, s), 0.85 (9H, s), 0.25 (3H, s), 0.19 (9H, s), 0.17 (3H, s), 0.10 (3H, s), 0.07 (3H, s), 0.02 (3H, s) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 170.4, 164.4, 157.2, 139.9, 133.0, 127.8, 126.0, 120.6, 101.8, 79.2, 76.5, 73.6, 73.3, 63.5, 52.1, 27.3, 26.8, 26.7, 26.0, 20.6, 19.6, 19.3, 19.0, 18.8, −2.3, −3.0, −3.46, −3.51, −3.8, −4.21, −4.23, −4.6 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 877.4579, $C_{42}H_{78}O_{10}$-$NaSi_4$ requires m/z 877.4570 IR (film) ν: 1720, 1603, 1508 cm^{-1}; $[\alpha]_D^{26}$ +79.5° (c 1.0, $CHCl_3$).

4.1.11. Methyl (*Z*)-2-(2′,3′,4′-tri-*t*-butyldimethylsilyloxy-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl acrylate (17). Compound (*Z*)-**16** (22.1 mg, 25.8 μmol) was dissolved in MeOH (0.5 mL). To this solution was added sodium methoxide (2.8 mg, 51.9 μmol) at −35 °C, and this reaction mixture was stirred for an hour at −20 °C. After neutralized with Amberlite IR-120B (H^+), the reaction mixture was filtered and separated by silica gel TLC (*n*-hexane/ethyl acetate 3:1) to give **17** (17.0 mg, 81%).

Compound **17**. ^{1}H NMR (400 MHz, acetone-d_6, rt): 7.79 (2H, d, J=8.3 Hz), 6.94 (1H, s), 6.79 (2H, d, J=8.3 Hz), 5.29 (1H, d, J=7.3 Hz), 4.20 (1H, dd, J=7.3, 9.3 Hz), 4.14 (1H, d, J=2.0 Hz), 3.77 (1H, dd, J=2.0, 9.3 Hz), 3.72 (3H, s), 3.46–3.57 (3H, m), 1.01 (9H, s), 0.96 (18H, s), 0.85 (9H, s), 0.23 (3H, s), 0.19 (9H, s), 0.16 (3H, s), 0.11 (3H, s), 0.06 (3H, s), 0.01 (3H, s) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 164.2, 156.9, 139.8, 132.9, 127.7, 125.9, 120.4, 101.9, 76.8, 76.5, 73.3, 72.6, 60.9, 52.0, 27.3, 26.83, 26.76, 26.0, 19.6, 19.4, 19.0, 18.8, −2.2, −3.1, −3.40, −3.42, −3.7, −4.18, −4.21, −4.3 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 835.4450, $C_{40}H_{76}O_9Si_4Na$ requires m/z 835.4464; IR (film) ν: 3448, 1722, 1637, 1603, 1508 cm^{-1}; $[\alpha]_D^{25}$ +57.0° (c 1.0, $CHCl_3$).

4.1.12. Methyl (*Z*)-2-(6′-formyl-3′,4′,5′-tri-*t*-butyldimethylsilyloxy-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl acrylate (18). Compound **17** (73.0 mg, 89.8 μmol) was dissolved in DMSO (1.0 mL), and TEA (1.0 mL), SO_3.pyr (71.0 mg, 447 μmol) were added to this solution. The reaction mixture was stirred for an hour under argon atmosphere, mixed with water, extracted with Et_2O. After washed with satd NH_4Cl aq, satd $NaHCO_3$, and brine, the organic layer was dried over

abs Na_2SO_4, and concentrated in vacuo. The residue was then separated by silica gel column chromatography (*n*-hexane/ethyl acetate 5:1) to give **18** (43.9 mg, 60%).

Compound **18**. ^{1}H NMR (400 MHz, acetone-d_6, rt): 9.43 (1H, br s), 7.85 (2H, d, J=8.8 Hz), 7.04 (1H, s), 6.81 (2H, d, J=8.8 Hz), 5.41 (1H, m), 4.37 (1H, br s), 4.26 (1H, dd, J=6.8, 8.8 Hz), 4.10 (1H, d, J=2.0 Hz), 3.89 (1H, dd, J=2.0, 8.8 Hz), 3.73 (3H, s), 0.95 (18H, s), 0.93 (9H, s), 0.86 (9H, s), 0.194 (6H, s), 0.186 (6H, s), 0.17 (3H, s), 0.08 (3H, s), 0.04 (3H, s), −0.01 (3H, s) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 201.4, 164.2, 157.2, 139.7, 133.0, 127.5, 126.5, 120.5, 101.8, 80.4, 79.0, 75.8, 73.1, 52.1, 27.0, 26.6, 26.4, 25.8, 19.3, 19.1, 18.8, 18.7, −2.6, −3.5, −3.6, −3.7, −4.0, −4.35, −4.38, −4.8 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 833.4303, $C_{40}H_{74}O_9NaSi_4$ requires *m/z* 833.4308; IR (film) ν: 1739, 1720, 1637, 1602, 1508 cm^{-1}; $[\alpha]_D^{25}$ +56.2° (*c* 1.0, $CHCl_3$).

4.1.13. Potassium (*Z*)-2-[6′-[2-[2-[2-(2-biotinylamino-ethoxy)-ethoxy]-ethoxy]-4-(3-tri-fluoromethyl)-3*H*-diazirin-3-yl]benzyloxyimino]-β-D-galactopyranosyloxy]-3-*p*-hydroxyphenyl acrylate (2). Compound **18** (43.9 mg, 54.1 μmol) was dissolved in MeOH (0.5 mL). Affilight-CHO™ (**19**, 15 mg, 21.7 μmol) was deprotected by TFA in CH_2Cl_2, and resulting **20** was added to methanolic solution of **18**. After adjusting pH at 5 by acetate buffer (10 μL), the reaction mixture was stirred overnight at 35 °C, and concentrated in vacuo. The residue was purified by silica gel TLC ($CHCl_3$/MeOH 10:1) to give coupling product (26.3 mg, 87%). 5.7 mg (4.7 μmol) of coupling product was dissolved in THF (0.5 mL), and deprotected by 3 h treatment with TBAF (1 M THF solution, 30 μmol) under argon atmosphere. The reaction mixture was concentrated and purified by silica gel TLC ($CHCl_3$/MeOH 5:1) to give a free acid form of **2** (3.5 mg, 95%). Free acid form of 2 (14.8 mg, 16.3 μmol) was dissolved in MeOH–H_2O (1:1, 1.4 mL). This solution was mixed with 1 M KOH aq (32.6 μL, 32.6 μmol), and stirred for 2 days at rt.

After neutralization with Amberlite IR-120B (H^+), the reaction mixture was filtered, mixed with 0.1 M K_2CO_3 (81.3 μL, 8.13 μmol), and purified by HPLC[Develosil C8-UG-5 (ϕ20×250 mm), 40% MeCN aq] to give (*E*)-**2** (5.4 mg, 34%) and (*Z*)-**2** (4.0 mg, 25%), respectively.

Compound (*E*)-**2**. ^{1}H NMR (400 MHz, CD_3OD, rt): 7.76 (2H, d, J=8.8 Hz), 7.33 (1H, d, J=7.8 Hz), 6.85 (1H, s), 6.82 (1H, d, J=7.8 Hz), 6.72 (2H, d, J=8.8 Hz), 6.71 (1H, d, J=13.2 Hz), 5.13 (2H, s), 4.65 (1H, dd, J=1.4, 4.9 Hz), 4.44 (1H, dd, J=4.4, 7.3 Hz), 4.25 (1H, dd, J=4.4, 7.8 Hz), 4.10–4.17 (3H, m), 3.78–3.87 (3H, m), 3.54–3.70 (3H, m), 3.51 (2H, t, J=5.8 Hz), 3.15 (1H, m), 2.88 (1H, dd, J=4.9, 12.7 Hz), 2.66 (1H, d, J=12.7 Hz), 2.18 (2H, t, J=7.3 Hz), 1.48–1.75 (4H, m), 1.31–1.44 (2H, m) ppm; ^{13}C NMR (100 MHz, CD_3OD, rt): 176.2, 175.5, 158.8, 158.1, 149.7, 133.2, 131.0, 130.7, 130.5, 130.1, 127.0, 123.6 (J=275 Hz), 122.8, 120.1, 115.9, 110.6, 105.0, 75.2, 74.7, 72.6, 71.9, 71.5, 71.3, 70.6, 69.6, 63.3, 61.6, 57.0, 41.1, 40.3, 36.7, 29.7, 29.5, 26.8 ppm; HR FAB MS (negative): $[M-K]^-$ Found *m/z* 925.2872, $C_{40}H_{48}O_{14}F_3N_6S$ requires *m/z* 925.2901; IR (film) ν: 3309, 1685, 1650, 1608, 1578 cm^{-1}; $[\alpha]_D^{22}$ +66.1° (*c* 0.62, MeOH).

4.1.14. *t*-Butyl 3-*p*-benzyloxyphenyl-2-acetyloxypropionate (21). Compound **5** (1.2 g, 4.2 mmol) was dissolved in 33 mL of H_2O–MeOH (2: 31), and 8.4 mL of 1 M KOH aq was added to this solution. After 1 h stirring, the solution was neutralized with Amberlite IR-120B (H^+) and filtered. Evaporation of the filtrate gave a white crystal. This crystal was dissolved in pyridine (20 mL), and acetic anhydride (10 mL) was added to this solution. After 4 h stirring, the reaction mixture was mixed with 20 mL of toluene and concentrated in vacuo. The residue was washed with saturated $NaHCO_3$ aq, 1 M HCl aq and then extracted with chloroform. The organic layer was evaporated and the residue was dissolved in abs CH_2Cl_2 (10 mL). *t*-Butanol (0.62 mL, 6.54 mmol), dimethylaminopyridine (DMAP; 53 mg, 0.44 mmol), and then dicyclohexylcarbodiimide (DCC; 990 mg, 4.80 mmol) were added to this solution. After 2 days stirring, the reaction mixture was mixed with water, and extracted with chloroform, dried over abs Na_2SO_4, and evaporated to dryness. Then the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 5:1) to give **21** (1.26 mg, 78%).

21: ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.43–7.28 (5H, m), 7.13 (2H, d, J=8.6 Hz), 6.98 (2H, d, J=8.6 Hz), 5.03 (2H, s), 5.03 (1H, m), 3.09 (1H, dd, J=5.0, 13.9 Hz), 3.09 (1H, dd, J=8.2, 13.9 Hz), 2.07 (3H, s), 1.39 (9H, s) ppm; ^{13}C NMR (68 MHz, $CDCl_3$ rt): 170.1, 168.5, 157.5, 136.8, 130.2, 128.3, 128.2, 127.7, 127.2, 114.5, 82.0, 73.4, 69.8, 36.4, 27.8, 20.7 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 393.1706, $C_{22}H_{26}O_5Na$ requires *m/z* 393.1678; IR (film) ν: 1741, 1612, 1512, 1454 cm^{-1}.

4.1.15. *t*-Butyl 3-*p*-benzyloxyphenyl-2-hydroxypropionate (22). Compound **21** (1.26 g, 3.40 mmol) was dissolved in methanol (30 mL), and sodium methoxide (220 mg, 4.1 mmol) was added to this solution at 0 °C. After 1 h stirring, the reaction mixture was neutralized by Amberlite IR-120B (H^+) and filtered. The filtrate was evaporated and purified by silica gel column chromatography (*n*-hexane/ethyl acetate 5:1) to give **22** (1.07 g, 98%).

22: ^{1}H NMR (270 MHz, CD_3OD, rt): 7.42–7.24 (5H, m), 7.14 (2H, d, J=8.7 Hz), 6.88 (2H, d, J=8.7 Hz), 5.04 (2H, s), 4.17 (1H, dd, J=5.6, 7.1 Hz), 2.92 (1H, dd, J=5.6, 13.9 Hz), 2.82 (1H,) ppm; ^{13}C NMR (68 MHz, $CDCl_3$ rt): 170.1, 168.5, 157.5, 136.8, 130.2, 128.3, 128.2, 127.7, 127.2, 114.5, 82.0, 73.4, 69.8, 36.4, 27.8, 20.7 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 393.1706, $C_{22}H_{26}O_5Na$ requires *m/z* 393.1678; IR (film) ν: 3502, 1741, 1612, 1512, 1454 cm^{-1}.

4.1.16. 3′,4′,6′-Tri-*O*-acetyl-2′-deoxy-2′-phthalimide-α-D-galactopyranosyl bromide (23). D-Galactosamine hydrochloride (1 g, 4.65 mmol, Sigma-Aldrich Co., Ltd) was suspended in DMF (30 mL), and to this solution, was added triethylamine (1.7 mL, 11.6 mmol). After 20 min-stirring at rt, phtalic anhydride (688 mg, 4.65 mmol) was added to this solution, and the mixture was stirred for 2 h at 50 °C under argon atmosphere. After the reaction mixture was allowed to stand until its temperature dropped to rt, excess amounts of triethylamine (5 mL) and acetic anhydride (5 mL) were added. After 2-day stirring at rt under argon atmosphere, the reaction mixture was concentrated in vacuo, mixed with

water (150 mL), extracted three times with *n*-hexane/ethyl acetate1:1, and dried over abs Na_2SO_4. The organic layer was separated by silica gel column chromatography (*n*-hexane/ethyl acetate 1:1) to give **32** (1.82 g, 82%). Resulting **32** (1.395 g, 2.92 mmol) was mixed with acetic anhydride (0.69 mL, 7.31 mmol), and then treated with HBr–AcOH (30%, 16 mL, excess) under ice-cooling. After stirred for an hour at rt under argon atmosphere, the mixture was concentrated in vacuo. The reaction mixture was mixed with saturated $NaHCO_3$ aq, extracted three times with $CHCl_3$, and dried over abs Na_2SO_4 to give **23** (1.41 g, 97%, Crude).

4.1.17. *t*-Butyl (3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimide-β-D-galactopyranosyloxy)-3-*p*-benzyloxyphenyl propionate (24). Crude 3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimide-β-D-galacto-pyranosyloxy bromide **23** (215 mg, 655 μmol), **22** (379 mg, 762 μmol), dried MS 4A (200 mg), and abs CH_2Cl_2 (6 mL) was mixed. To this solution, was added diisopropylethylamine (DIPEA) (0.11 mL, 657 μmol), and then AgOTf (207 mg, 788 μmol) with stirring at 0 °C under argon atmosphere. After 2 h stirring, reaction mixture was filtered with Celite, and insoluble was washed with $CHCl_3$. The filtrate was washed with satd $NaHCO_3$ aq, and dried over abs Na_2SO_4. After concentration, the residue was separated with silica gel column chromatography (toluene/acetone 10:1) to give **24** (420 mg, 86%) as a mixture of diastereomers. A part of the mixture was then separated by silica gel TLC to give **24a** (79 mg) and **24b** (72 mg).

Compound **24a**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.84–7.68 (4H, m,), 7.38–7.33 (5H, m), 7.03 (2H, d, J=8.3 Hz), 6.74 (2H, d, J=8.3 Hz), 5.90 (1H, dd, J=3.0, 11.2 Hz), 5.47 (1H, d, J=3.0 Hz), 5.23 (1H, d, J=8.2 Hz), 4.98 (2H, s), 4.56 (1H, dd, J=8.2, 11.2 Hz), 4.37 (1H, dd J=8.6 11.5 Hz), 4.20–4.02 (3H, m), 2.92 (2H, d, J=5.8 Hz), 2.21 (3H, s), 2.05 (3H, s), 1.86 (3H, s), 1.11 (9H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.3, 170.3, 169.7, 169.4, 168.1,167.7, 157.3, 137.0, 133.9, 133.7, 132.0, 131.5, 130.6, 128.4, 128.2, 127.8, 127.3, 123.3, 123.1, 114.2, 97.2, 81.5, 70.6, 69.8, 67.5, 66.4, 61.2, 51.0, 37.9, 27.6, 20.7, 20.6, 20.5 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 768.2625, $C_{40}H_{43}O_{13}NNa$ requires *m/z* 768.2632; IR (film) ν: 1749, 1718, 1512, 1389 cm^{-1}; $[\alpha]_D^{24}$ 29.0° (*c* 0.5, $CHCl_3$).

Compound **24b**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.81–7.64 (4H, m), 7.44–7.37 (5H, m), 6.84 (2H, d, J=8.6 Hz), 6.34 (2H, d, J=8.6 Hz), 5.62 (1H, dd, J=3.3, 11.5 Hz), 5.41 (1H, d, J=3.3 Hz), 5.31 (1H, d, J=8.6 Hz), 4.79 (2H, s), 4.58 (1H, dd, J=8.6, 11.5 Hz), 4.23–3.97 (4H, m), 2.75 (2H, d, J=6.6 Hz), 2.19 (3H, s), 2.05 (3H, s), 1.81 (3H, s), 1.44 (9H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.4, 170.3, 170.3, 169.7, 167.8, 167.1, 156.8, 136.9, 134.0, 133.9, 131.1, 129.7, 128.6, 128.5, 127.8, 127.1, 123.3, 114.1, 99.2, 81.7, 81.3, 70.8, 69.3, 67.9, 66.4, 61.2, 60.3, 51.0, 37.8, 27.8, 20.7, 20.6, 20.4 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 768.2603, $C_{40}H_{43}O_{13}NNa$ requires *m/z* 768.2632: IR (film) ν: 1751, 1718, 1511 1389 cm^{-1}; $[\alpha]_D^{24}$ −22.5° (*c* 0.5, $CHCl_3$).

4.1.18. *t*-Butyl (3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimide-β-D-galactopyranosyloxy)-3-*p*-hydroxyphenyl propionate (25). Diastereomixture of **24** (1.13 g, 1.52 mmol) was dissolved in ethyl acetate (15 mL) and 10% Pd–C was added to this solution as catalyst. This solution was stirred overnight under hydrogen atmosphere and filtered with Celite. After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 1:1) to give **25** (1.08 g, quant.) as a mixture of diastereomers.

Similarly, **24a** (79 mg, 0.1 mmol) was dissolved in ethyl acetate (1 mL) and 10% Pd–C was added to this solution as catalyst. This solution was stirred overnight under hydrogen atmosphere and filtered with Celite. After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 1:1) to give **25a** (69 mg, quant.).

Compound **24b** (72 mg, 0.1 mmol) was dissolved in ethyl acetate (1 mL) and 10% Pd–C was added to this solution as catalyst. This solution was stirred overnight under hydrogen atmosphere and filtered with Celite. After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 1:1) to give **25b** (68 mg, quant.).

Compound **25a**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.85–7.67 (4H, m), 6.77 (2H, d, J=8.4 Hz), 6.17 (2H, d, J=8.4 Hz), 5.64 (1H, m), 5.64 (1H, dd, J=3.4, 11.4 Hz), 5.41 (1H, d, J=3.1 Hz), 5.30 (1H, d, J=8.4 Hz), 4.58 (1H, dd, J=8.4, 11.4 Hz), 4.23–3.95 (4H, m), 2.75 (2H, d, J=7.1 Hz), 2.17 (3H, s), 2.05 (3H, s), 1.81 (3H, s), 1.45 (9H, s) ppm; ^{13}C NMR (68 MHz, $CDCl_3$, rt): 170.5, 170.3, 170.3, 169.7, 167.7, 167.1, 154.0, 134.1, 134.0, 131.0, 129.7, 127.8, 123.4, 123.3, 114.7, 99.2, 81.8, 81.5, 70.8, 68.0, 66.5, 61.2, 51.1, 37.9, 27.9, 27.8, 20.7, 20.5 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 678.2148, $C_{33}H_{37}O_{13}NNa$ requires *m/z* 678.2163: IR (film) ν: 3460, 1751, 1718, 1614, 1517, 1390, 1369 cm^{-1}; $[\alpha]_D^{26}$ 5.0° (*c* 1.0, $CHCl_3$).

Compound **25b**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.85–7.68 (4H, m), 6.96 (2H, d, J=8.4 Hz), 6.60 (2H, d, J=8.4 Hz), 6.11 (1H, m), 5.92 (1H, dd, J=3.4, 11.4 Hz), 5.48 (1H, d, J=3.4 Hz), 5.24 (1H, d, J=8.4 Hz), 4.57 (1H, dd, J=8.4, 11.4 Hz), 4.38 (1H, t, J=6.1 Hz), 4.25–4.02 (3H, m), 2.90 (2H, d, J=6.3 Hz), 2.20 (3H, s), 2.06 (3H, s), 1.87 (3H, s), 1.14 (9H, s) ppm; ^{13}C NMR (68 MHz, $CDCl_3$, rt): 170.4, 170.3, 169.7, 169.5, 168.1, 167.7, 133.8, 131.8, 131.3, 130.6, 127.4, 123.3, 123.1, 114.7, 97.2, 81.7, 77.4, 70.6, 67.6, 66.5, 61.2, 51.1, 38.0, 27.9, 27.7, 20.8, 20.8, 20.6 ppm; HR FAB MS (positive): $[M+Na]^+$ Found *m/z* 678.2150, $C_{33}H_{37}O_{13}NNa$ requires *m/z* 678.2163: IR (film) ν: 3460, 1749, 1718, 1614, 1516, 1390, 1371 cm^{-1}; $[\alpha]_D^{26}$ −25.3° (*c* 1.0, $CHCl_3$).

4.1.19. *t*-Butyl (3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimide-β-D-galactopyranosyloxy)-3-*p*-*t*-butyldimethylsilyl-oxyphenyl-propionate (26). Diastereomixture of **25** (1.08 g, 1.65 mmol) was dissolved in dimethylformamide (DMF; 15 mL). To this solution, imidazole (450 mg, 6.62 mmol), *t*-butyldimethylsilyl chloride (625 mg, 4.13 mmol), and a catalytic amount of DMAP were added to this solution. This solution was stirred overnight under argon atmosphere, mixed with water (20 mL), extracted with ethyl acetate. The organic layer was then dried over abs Na_2SO_4 and evaporated to dryness. The residue was then

purified by silica gel column chromatography (*n*-hexane/ethyl acetate 2:1) to give **26** (1.20 g, 95%).

Compound **25a** (69 mg, 0.1 mmol) was dissolved in dimethylformamide (DMF; 1 mL). To this solution, imidazole (26 mg, 377 μmol), *t*-butyldimethylsilyl chloride (36 mg, 235 μmol) and catalytic amount of DMAP were added. This solution was stirred overnight under argon atmosphere, mixed with water (20 mL), extracted with ethyl acetate. The organic layer was then dried over abs Na_2SO_4 and evaporated to dryness. The residue was then purified by silica gel column chromatography (*n*-hexane/ethyl acetate 2:1) to give **26a** (64 mg, 83%).

Compound **25b** (68 mg, 0.1 mmol) was dissolved in dimethylformamide (DMF; 1 mL). To this solution, imidazole (28 mg, 408 μmol), *t*-butyldimethylsilyl chloride (38 mg, 255 μmol) and catalytic amount of DMAP were added. This solution was stirred overnight under argon atmosphere, mixed with water (20 mL), extracted with ethyl acetate. The organic layer was then dried over abs Na_2SO_4 and evaporated to dryness. The residue was then purified by silica gel column chromatography (*n*-hexane/ethyl acetate 2:1) to give **26b** (67 mg, 87%).

Compound **26a**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.81–7.65 (4H, m), 6.76 (2H, d, J=8.4 Hz), 6.19 (2H, d, J=8.4 Hz), 5.64 (1H, dd, J=3.3, 11.4 Hz), 5.42 (1H, d, J=3.3 Hz), 5.31 (1H, d, J=8.6 Hz), 4.59 (1H, dd, J=8.6, 11.4 Hz), 4.20–3.94 (4H, m), 2.74 (2H, d, J=6.7 Hz), 2.19 (3H, s), 2.05 (3H, s), 1.81 (3H, s), 1.42 (9H, s) 0.95 (9H, s), 0.09 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.3, 170.2, 170.2, 169.6, 167.7, 167.0, 153.6, 134.0, 133.9, 131.2, 131.0, 129.6, 128.9, 123.4, 123.3, 119.5, 99.1, 81.5, 81.2, 70.8, 67.9, 66.4, 61.2, 51.1, 37.9, 27.9, 27.8, 25.7, 20.8, 20.7, 20.5, 18.1, −4.5 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 792.3040, $C_{39}H_{51}O_{13}NSiNa$ requires m/z 792.3027: IR (film) ν: 1753, 1718, 1610 1510, 1389, 1369 cm^{-1}; $[\alpha]_D^{24}$ 11.4° (c 1.0, $CHCl_3$).

Compound **26b**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.86–7.69 (4H, m), 6.96 (2H, d, J=8.4 Hz), 6.59 (2H, d, J=8.4 Hz), 5.93 (1H, dd, J=3.1, 11.5 Hz), 5.48 (1H, d, J=3.1 Hz), 5.24 (1H, d, J=8.4 Hz), 4.58 (1H, dd, J=8.4, 11.5 Hz), 4.39 (1H, t, J=6.0 Hz), 4.26–4.03 (3H, m), 2.92 (2H, d, J=6.7 Hz), 2.22 (3H, s), 2.07 (3H, s), 1.87 (3H, s), 1.11 (9H, s), 0.95 (9H, s), 0.11 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.2, 170.2, 169.6, 169.3, 168.1, 167.6, 154.0, 133.8, 133.7, 131.9, 131.4, 130.5, 128.5, 123.3, 123.1, 119.4, 97.2, 81.5, 77.3, 70.6, 67.6, 66.5, 61.2, 51.1, 38.1, 27.7, 25.7, 20.8, 20.7, 20.6, 18.2, −4.4 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 792.3053, $C_{39}H_{51}O_{13}NSiNa$ requires m/z 792.3027: IR (film) ν: 1753, 1720, 1610, 1510, 1389, 1369 cm^{-1}; $[\alpha]_D^{24}$ −27.3° (c 1.0, $CHCl_3$).

4.1.20. *t*-Butyl (*Z*)-2-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimide-β-D-galactopyranosyl-oxy)-3-*p*-*t*-butyldimethylsilyloxyphenyl-2-acrylate *t*-butylester (27). Diastereomixture of **26** (1.20 g, 1.60 mmol) was dissolved in dioxane (35 mL), and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 1.42 g, 6.20 mmol) was added to this solution. This reaction mixture was refluxed under argon atmosphere for 2 days, and then filtered by Celite. The filtrate was concentrated in vacuo, and the residue was dissolved in diethyl ether (50 mL). The organic layer was washed with saturated $NaHCO_3$ aq and then brine, dried over abs Na_2SO_4, and concentrated in vacuo. The residue was then separated by silica gel column chromatography (*n*-hexane/ethyl acetate 3:1) to give **27** (490 mg, 41%).

Compound **27**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.84–7.62 (4H, m), 7.56 (2H, d, J=8.6 Hz), 6.75 (1H, s), 6.70 (2H, d, J=8.6 Hz), 5.93 (2H, m), 5.45 (1H, d, J=3.6 Hz), 4.72 (1H, dd, J=8.6, 11.5 Hz), 4.03 (3H, m), 2.19 (3H, s), 1.97 (3H, s), 1.84 (3H, s), 1.41 (9H, s), 0.97 (9H, s), 0.20 (6H, s) ppm; ^{13}C NMR (68 MHz, $CDCl_3$, rt): 170.0, 169.9, 169.4, 167.8, 167.3, 162.2, 156.1, 139.4, 133.8, 133.6, 131.9, 131.6, 131.2, 125.9, 124.4, 123.2, 123.1, 119.5, 97.0, 81.5, 70.8, 67.6, 66.3, 60.9, 51.4, 28.0, 25.6, 25.5, 20.7, 20.5, 20.5, 18.2, −4.4 ppm; HR FAB MS (positive): $[M+Na]^+$ Found m/z 792.2875, $C_{39}H_{49}O_{13}NSiNa$ requires m/z 792.2871: IR (film) ν: 1753, 1720, 1601, 1508, 1389, 1369 cm^{-1}; $[\alpha]_D^{25}$ −88.2° (c 1.0, $CHCl_3$).

4.1.21. *t*-Butyl (*Z*)-2-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimide-β-D-galactopyranosyloxy)-3-*p*-hydroxyphenyl-2-acrylate *t*-butylester (28). Compound **27** (250 mg, 0.33 mmol) was dissolved in THF (3 mL), and tetrabutyl ammonium fluoride (1.0 M in THF, 0.39 mL, 0.39 mmol) was added to this solution at 0 °C. After 5 min stirring, this solution was mixed with water (5 mL) and extracted by ethyl acetate. This organic layer was washed with brine, dried over abs Na_2SO_4, and evaporated in vacuo. The residue was then separated by silica gel column chromatography (*n*-hexane/ethyl acetate 1:1) to give **28** (215 mg, quant.).

Compound **28**. ^{1}H NMR (270 MHz, $CDCl_3$, rt): 7.87–7.68 (4H, m), 7.58 (2H, d, J=8.7 Hz), 6.80 (1H, s), 6.75 (2H, d, J=8.7 Hz), 5.95 (2H, m), 5.48 (1H, d, J=3.1 Hz), 4.75 (1H, dd, J=8.4, 11.4 Hz), 4.08 (3H, m), 2.21 (3H, s), 2.00 (3H, s), 1.88 (3H, s), 1.44 (9H, s) ppm; ^{13}C NMR (68 MHz, $CDCl_3$, rt): 170.4, 170.3, 169.7, 168.0, 167.7, 162.5, 156.8, 139.2, 134.1, 134.0, 133.8, 132.2, 131.6, 131.1, 124.9, 123.4, 123.2, 115.0, 97.3, 81.8, 70.9, 67.8, 66.5, 61.1, 51.6, 28.0, 28.0, 20.7, 20.6, 20.6 ppm; HR FAB MS (negative): $[M-H]^-$ Found m/z 654.2214, $C_{33}H_{36}O_{13}N$ requires m/z 654.2187: IR (film) ν: 3413, 1751, 1718, 1606, 1514, 1389, 1369 cm^{-1}; $[\alpha]_D^{24}$ −101.5° (c 1.0, $CHCl_3$).

4.1.22. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-β-D-galactopyranosyl-oxy)-3-*p*-hydroxyphenyl-2-acrylate *t*-butylester (29). Compound **28** (215 mg, 0.33 mmol) was dissolved in methanol (3.3 mL) and sodium methoxide (57 mg, 1.06 mmol) was added to this solution at 0 °C. After overnight stirring, sodium methoxide (57 mg, 1.06 mmol) was added to this solution. This reaction mixture was then neutralized with Amberlite IR-120B (H^+), filtered, and evaporated to dryness. The residue was then separated by silica gel column chromatography ($CHCl_3$–MeOH=5:1) to give **29** (168 mg, 97%).

Compound **29**. ^{1}H NMR (270 MHz, CD_3OD, rt): 7.92–7.75 (4H, m), 7.58 (2H, d, J=8.6 Hz), 6.67 (1H, s), 6.67 (2H, d, J=8.6 Hz), 5.78 (1H, d, J=8.0 Hz), 4.62 (1H, dd, J=8.0, 11.0 Hz), 4.58 (1H, dd, J=2.9, 11.0 Hz), 3.98 (1H, d, J=

2.9 Hz), 3.81–3.62 (3H, m), 1.46 (9H, s) ppm; ^{13}C NMR (68 MHz, CD_3OD, rt): 169.9, 169.5, 164.4, 159.1, 140.7, 135.1, 134.9, 133.2, 133.0, 132.6, 125.5, 124.7, 124.0, 123.7, 116.0, 98.6, 82.8, 77.2, 69.4, 69.1, 61.8, 55.5, 28.3, 28.2 ppm; HR FAB MS (negative): $M-H]^-$ Found *m/z* 526.1714, $C_{27}H_{28}O_{10}N$ requires *m/z* 526.1713: IR (film) *ν*: 3402, 1774, 1710, 1606, 1512, 1390 cm^{-1}; $[\alpha]_D^{23}$ −152.7° (*c* 1.0, MeOH).

4.1.23. *t*-Butyl (*Z*)-2-[2′-[2-[2-[2-(2-biotinylamino-ethoxy)-ethoxy]-ethoxy]-4-(3-trifluoro-methyl)-3*H*-diazirin-3-yl]benzyloxyimino]-amino-β-D-galactopyranosyl-oxy)-3-*p*-hydroxyphenyl-2-acrylate (31). Compound **29** (21.3 mg, 53.7 μmol) was dissolved in ethanol (1 mL), and hydrazine monohydrate (10 μL) was added to this solution. After overnight stirring, the reaction mixture was evaporated to dryness. The residue was then separated repeatedly by ODS TLC (H_2O/CH_3CN 2:1 containing 1% acetic acid) to give crude amine (21 mg). Crude amine was dissolved in dimethylformacmide (1.5 mL), and **30** (20.0 mg, 33.2 μmol), hydroxybenzotriazole (HOBt; 8.7 mg, 64.3 μmol), and dicyclohexylcarbodiimide (DCC; 13 mg, 64.3 μmol) were added to this solution. After stirring for 2 days under light-shading conditions, the reaction mixture was evaporated to dryness. The residue was then separated by ODS TLC (H_2O/CH_3CNM 1:1) to give **31** (10.2 mg, 19%).

Compound **31**. ^{1}H NMR (270 MHz, CD_3OD, rt): 7.96 (1H, d, *J*=8.0 Hz), 7.64 (2H, d, *J*=8.5 Hz), 6.99 (1H, d, *J*=8.0 Hz), 6.81 (1H, s), 6.73 (1H, s), 6.67 (2H, d, *J*=8.5 Hz), 5.36 (1H, d, *J*=8.1 Hz), 4.42 (2H, m), 4.25 (3H, m), 3.92–3.85 (5H, m), 3.78–3.46 (11H, m), 2.91 (1H, dd, *J*=3.6, 12.7 Hz), 2.67 (1H, d, *J*=12.7 Hz), 2.15 (2H, t, *J*=7.2 Hz), 1.72–1.57 (6H, m), 1.51 (9H, s) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, rt): 172.3, 164.2, 162.8, 162.6, 157.9, 156.6, 139.5, 132.2, 131.8, 131.3, 125.4, 124.4, 124.1, 123.1, 121.1, 120.4, 119.1, 117.7 ($^1J_{CH}$=273 Hz), 115.2, 111.4, 98.7, 80.9, 76.0, 71.4, 69.8, 69.7, 69.6, 69.2, 68.6, 67.1, 61.1, 59.8, 59.3, 55.5, 52.8, 48.6, 38.4, 35.1, 28.2, 28.1 ($^2J_{CH}$=16 Hz), 27.9, 27.8, 25.3 ppm; HR FAB MS (negative): $[M-H]^-$ Found *m/z* 981.3524, $C_{44}H_{56}O_{14}N_6F_3S$ requires *m/z* 981.3527; IR (film) *ν*: 3340, 1697, 1649, 1608, 1549, 1512, 1458 cm^{-1}; $[\alpha]_D^{24}$ −43.0° (*c* 1.0, MeOH).

4.1.24. Potassium (*Z*)-2-[2′-[2-[2-[2-(2-biotinylamino-ethoxy)-ethoxy]-ethoxy]-4-(3-trifluoro-methyl)-3*H*-diazirin-3-yl]benzyloxyimino]-amino-β-D-galactopyranosyl-oxy)-3-*p*-hydroxyphenyl-2-acrylate (3). TFA (purity> 98%, 40 μL) was added to the 5 mL round-bottom flask in which compound **31** (2.2 mg, 2.24 μmol) was dried. This flask was set to the vacuum line immediately, and dried in vacuo to remove TFA. After drying in vacuo for 1 h under light-shading conditions, the residue was then separated by ODS TLC (H_2O–CH_3CN=3:2) to **3** as free carboxylic acid form, which was then dissolved in H_2O–MeOH (3:2) and neutralized by 0.1 M K_2CO_3 aq. The solution was evaporated to dryness, and the residue was separated by ODS TLC (H_2O/CH_3CN 3:2) to give **3** (0.7 mg, 32%).

Compound **3**. ^{1}H NMR (270 MHz, CD_3OD, rt): 7.88 (1H, d, *J*=8.2 Hz), 7.65 (2H, d, *J*=8.6 Hz), 6.96 (1H, d, *J*=8.2 Hz), 6.80 (2H, s), 6.66 (2H, d, *J*=8.6 Hz), 5.32 (1H, d, *J*=8.6 Hz), 4.43 (2H, m), 4.25 (3H, m), 3.92–3.78 (5H, m), 3.73–3.45 (11H, m), 2.90 (1H, dd, *J*=4.9, 12.9 Hz), 2.67 (1H, d, *J*=12.9 Hz), 2.18 (2H, t, *J*=7.5 Hz), 1.66–1.41 (6H, m) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, rt): 172.3, 165.8, 165.8, 162.8, 157.2, 156.5, 131.6, 131.4, 131.0, 128.4, 125.9, 125.4, 123.1, 120.4 ($^1J_{CF}$=273 Hz), 118.9, 115.0, 111.3, 99.5, 77.6, 75.8, 75.5, 73.2, 69.8, 69.6, 69.2, 68.6, 67.2, 61.1, 60.1, 59.2, 55.4, 53.8, 38.4, 35.1, 29.0, 28.2, 28.0 ($^2J_{CF}$=16 Hz), 25.3 ppm; HR FAB MS (negative): $[M-K]^-$ Found *m/z* 925.2899, $C_{40}H_{48}O_{14}N_6F_3S$ requires *m/z* 925.2901; IR (film) *ν*: 3329, 1693, 1651, 1608, 1549, 1512 cm^{-1}; $[\alpha]_D^{24}$ −21.2° (*c* 0.5, MeOH).

4.1.25. 2-(6′-Amino-β-D-galactopyranosyloxy)-3-hydroxyphenyl-2-acrylolactam (35). Methyl ester of **29** (**34**) (3 mg, 5 μmol) was dissolved in MeOH (0.5 mL). Hydrazine monohydrate (80% solution, 5 μL) was added to this solution. After overnight stirring, the reaction mixture was concentrated in vacuo, and separated by ODS-TLC (RP-18W, H_2O/CH_3CN/AcOH 66:33:1) to give **32** (1.6 mg, 5 μmol).

Compound **35**. ^{1}H NMR (400 MHz, CD_3OD, rt): 7.64 (2H, d, *J*=8.8 Hz), 6.74 (2H, d, *J*=8.8 Hz), 6.71 (1H, s), 4.86 (1H, d, *J*=7.1 Hz), 3.91 (1H, dd, *J*=8.3, 12.5 Hz), 3.87 (1H, dd, *J*=0.7, 2.7 Hz), 3.81 (1H, dd, *J*=4.6, 12.5 Hz), 3.80 (1H, dd, *J*=0.7, 4.6 Hz), 3.66 (1H, dd, *J*=2.9, 10.7 Hz), 3.63 (1H, dd, *J*=7.1, 10.7 Hz) ppm; FAB MS (positive) *m/z* 324 $[M+H]^+$: IR (film) *ν*: 1670, 1606, 1512, 1441 cm^{-1}.

4.1.26. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-β-D-galactopyranosyl-oxy)-3-*p*-*t*-butyldimethysilyloxyphenyl-2-acrylate(36). Compound **27** (1.01 g, 1.32 mmol) in methanol (13 mL) was mixed with sodium methoxide (142 mg, 2.64 mmol) at −30 °C, and the mixture was stirred under argon atmosphere. After 1 h stirring, Amberlite IR-120 B was added to this solution to adjust pH at 7.0. After filtration, the filtrate was concentrated in vacuo, and purified by silica gel column chromatography ($CHCl_3$/MeOH 20:1) to give **36** (719 mg, 88%).

Compound **36**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.83–7.79 (2H, m), 7.69–7.67 (2H, m), 7.53 (2H, d, *J*=8.8 Hz), 6.83 (1H, s), 6.65 (2H, d, *J*=8.8 Hz), 5.71 (1H, d, *J*=8.3 Hz), 4.71 (1H, dd, *J*=8.3, 10.7 Hz), 4.52 (1H, dd, *J*=2.5, 10.7 Hz), 4.12 (1H, d, *J*=2.5 Hz), 3.84 (1H, dd, *J*=4.4, 5.3 Hz), 3.83 (1H, dd, *J*=4.8, 5.3 Hz), 3.66 (1H, dd, *J*=4.4, 4.8 Hz), 1.45 (9H, s), 0.97 (9H, s), 0.19 (6H, s) ppm;^{13}C NMR (68 MHz, $CDCl_3$, rt): 168.1, 163.3, 155.9, 140.1, 133.5, 131.6, 129.8, 123.8, 123.2, 122.6, 119.6, 97.5, 81.8, 74.8, 68.5, 68.1, 61.3, 54.3, 27.9, 25.5, 18.0, −4.5 ppm; HR FAB MS (negative): $[M-H]^-$ Found *m/z* 640.2570, $C_{33}H_{42}O_{10}NSi$ requires 640.2578: IR (film) *ν*: 3419, 1774, 1716, 1637, 1600, 1508, 1471, 1394 cm^{-1}; $[\alpha]_D^{20}$ −4.2° (*c* 1.0, $CHCl_3$).

4.1.27. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-*O*-*p*-toluenesulfonyl-β-D-galactopyranosyloxy)-3-*p*-*t*-butyl-dimetylsilyloxyphenyl-2-acrylate (37). *p*-Toluene sulfonyl chloride (12.3 mg 66 μmol) was added to the solution of **36** (35.3 mg, 55 μmol) in pyridine (0.5 mL) at 0 °C, and the

mixture was stirred for 25 h under argon atmosphere. To remove pyridine, the mixture was evaporated with toluene, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc 1:1) to give **37** (14.3 mg, 33%) with recovered **36** (15.9 mg, 45%).

Compound **37**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.81–7.79 (2H, m), 7.74 (2H, d, J=8.3 Hz), 7.69–7.67 (2H, m), 7.51 (2H, d, J=8.5 Hz), 7.29 (2H, d, J=8.3 Hz), 6.75 (1H, s), 6.69 (2H, d, J=8.5 Hz), 5.79 (1H, d, J=7.1 Hz), 4.57 (2H, m), 4.20 (1H, dd, J=7.1, 10.4 Hz), 4.05 (1H, d, J=6.0 Hz), 3.97 (1H, dd, J=6.0, 10.4 Hz), 3.87 (1H, t, J=6.6 Hz), 2.41 (3H, s), 1.45 (9H, s), 0.99 (9H, s), 0.21 (6H, s) ppm; ^{13}C NMR (68 MHz, $CDCl_3$, rt): 168.2, 163.0, 156.1, 144.8, 140.0, 133.7, 131.8, 129.8, 129.5, 127.7, 127.3, 126.0, 124.0, 123.2, 119.8, 97.0, 82.0, 72.6, 68.0, 67.7, 54.2, 28.1, 25.7, 21.6, 18.2, −4.3 ppm; HR FAB MS (positive) $[M+Na]^+$ Found m/z 818.2672, $C_{40}H_{49}NO_{12}SSiNa$ requires 818.2642: IR (film) ν: 3484, 1776, 1716, 1600, 1508, 1389, 1368 cm^{-1}; $[\alpha]_D^{20}$ −7.9° (c 1.0, $CHCl_3$).

4.1.28. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-*O*-*p*-toluenesulfonyl-β-D-galactopyranosyloxy)-3-*p*-hydroxyphenyl-2-acrylate (38). TBAF (1.0 M in THF, 0.13 mL, 0.13 mmol) was added to the solution of **37** (85.8 mg, 0.11 mmol) in THF (1.0 mL) at 0 °C, and the mixture was stirred for 5 min under argon atmosphere. The reaction mixture was mixed with water, and extracted with ethyl acetate, and the organic layer was washed with brine, dried over abs Na_2SO_4. After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc 2:3) to give **38** (49.0 mg, quant.).

Compound **38**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.74–7.58 (6H, m), 7.41 (2H, d, J=7.8 Hz), 7.21 (2H, d, J=8.4 Hz), 6.78 (1H, s), 6.61 (2H, d, J=8.4 Hz), 5.77 (1H, d, J=5.8 Hz), 4.61 (1H, dd, J=8.4, 10.5 Hz), 4.55 (1H, d, J=5.4 Hz), 4.22 (1H, dd, J=5.4, 10.5 Hz), 4.05–4.01 (2H, m), 3.83 (1H, t, J=6.3 Hz), 2.33 (3H, s), 1.46 (9H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 168.8, 163.1, 156.8, 145.0, 139.5, 133.9, 132.0, 131.5, 129.9, 127.8, 124.8, 124.4, 123.5, 115.3, 96.9, 82.1, 72.7, 68.1, 68.0, 67.9, 54.3, 28.1, 21.6 ppm; HR FAB MS (negative) $[M-H]^-$ Found m/z 680.1780, $C_{34}H_{34}O_{12}NS$ requires 680.1802; IR (film) ν: 3458, 1774, 1714, 1606, 1511, 1392 cm^{-1}; $[\alpha]_D^{22}$ −8.4° (c 1.0, $CHCl_3$).

4.1.29. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-azido-β-D-galactopyranosyloxy)-3-*p*-hydroxyphenyl-2-acrylate (39). 15-crown-5 (0.15 mL, 0.72 mmol) and sodium azide (47 mg, 0.72 mmol) was added to the solution of **38** (49.0 mg, 72 μmol) in DMF (0.7 mL) and stirred for a day under argon atmosphere. The reaction mixture was mixed with water, extracted with ethyl acetate. And the organic layer was washed with brine, dried over abs Na_2SO_4. After evaporation, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH 10:1) to give **39** (40.5 mg, quant.).

Compound **39**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.75–7.60 (4H, m), 7.44 (2H, d, J=8.4 Hz), 6.78 (1H, s), 6.64 (2H, d, J=8.4 Hz), 5.85 (1H, d, J=8.0 Hz), 4.68–4.50 (3H, m), 3.92 (1H, d, J=5.6 Hz), 3.65 (1H, dd, J=5.6 Hz), 3.52 (1H, dd, J=8.0, 11.6 Hz), 3.24 (1H, dd, J=3.6, 11.6 Hz), 1.45 (9H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 168.8, 163.2, 156.6, 145.0, 139.4, 134.0, 132.1, 131.5, 125.0, 124.6, 115.4, 96.8, 82.0, 74.6, 69.2, 68.2, 54.4, 51.1, 28.1 ppm; HR FAB MS (negative) $[M-H]^-$ Found m/z 551.1769, $C_{27}H_{27}O_9N_4$ requires 551.1778; IR (film) ν: 3397, 2102, 1710, 1606, 1511, 1390 cm^{-1}; $[\alpha]_D^{20}$ −10.7° (c 1.0, $CHCl_3$).

4.1.30. *N*-Biotinylglycylglycylglycine (40). Compound **46** (212.7 mg, 0.50 mmol) was dissolved in 1 M KOH aq (0.6 mL) and the solution was stirred for an hour at rt. After the solution was adjusted to pH 7.0 by Amberlite IR-120B, the resulting precipitate was dissolved in MeOH, and dried in vacuo to give **40** (180.8 mg, 88%).

Compound **40**. ^{1}H NMR (400 MHz, DMSO-d_6, 30 °C): 8.12 (1H, t, J=5.6 Hz), 8.07 (1H, t, J=5.6 Hz), 8.03 (1H, t, J=5.6 Hz), 6.38 (2H, s), 4.30 (1H, dd, J=4.9, 7.9 Hz), 4.12 (1H, dd, J=4.9, 7.9 Hz), 3.12–3.07 (1H, m), 2.81 (1H, dd, J=5.4, 12.2 Hz), 2.57 (1H, d, J=12.2 Hz), 2.13 (2H, t, J=7.3 Hz) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): 172.4, 170.8, 169.2, 168.9, 162.5, 60.9, 59.2, 55.2, 42.0, 41.7, 40.5, 39.8, 34.9, 28.1, 28.0, 25.0 ppm: HR FAB MS (negative) $[M-H]^-$ Found m/z 414.1461 $C_{16}H_{24}O_6N_5S$ requires 414.1447 IR (film) ν: 3285, 3085, 1735, 1718, 1685, 1560, 1508, 1420 cm^{-1}; $[\alpha]_D^{20}$ −3.5° (c 1.0, DMSO).

4.1.31. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-[*N*-biotinylglycyl-glycylglycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-hydroxyphenyl-2-acrylate (41). To the solution of **39** (40.5 mg, 73 μmol) in MeOH (1 mL) was added 5% Pd–$CaCO_3$, and the mixture was stirred for 2 h under hydrogen atmosphere at rt. The reaction mixture was filtered with Celite and dried in vacuo to give crude amine (39.8 mg).

Resulting crude amine (39.8 mg, ca.73 μmol) in DMF (1.0 mL) was mixed with HOBt (14.8 mg, 0.11 mmol) and **40** (31.5 mg, 75 μmol). After cooling to 0 °C, DCC (17.8 mg, 86 μmol) was added to this solution and stirred for 41 h. After filtration by hyflosupercell, the filtrate was dried up in vacuo to remove DMF and the residue was purified by ODS-TLC (H_2O/CH_3CN 5:4) to give **41** (12.9 mg, 27% in two steps).

Compound **41**. ^{1}H NMR (400 MHz CD_3OD, rt): 7.83–7.73 (4H, m), 7.54 (2H, d, J=8.8 Hz), 6.68 (1H, s), 6.67 (2H, d, J=8.8 Hz), 5.71 (1H, d, J=8.1 Hz), 4.59 (1H, dd, J=8.1, 11.0 Hz), 4.52 (1H, dd, J=6.6, 11.0 Hz), 4.44 (1H, dd, J=4.4, 7.9 Hz), 4.28 (1H, dd, J=4.4, 7.9 Hz), 3.93–3.70 (7H, m), 3.73 (2H, m), 3.47 (1H, dd, J=7.6, 14.0 Hz), 3.39 (1H, dd, J=6.6, 14.0 Hz), 3.20–3.15 (1H, m), 2.89 (1H,dd, J=4.7, 12.7 Hz), 2.67 (1H, d, J=12.7 Hz), 2.35 (2H, t, J=7.3 Hz), 1.75–1.49 (6H, m), 1.45 (9H, s) ppm; ^{13}C NMR (100 MHz, CD_3OD, rt): 176.9, 172.9, 172.1, 170.2, 169.7, 166.0, 164.6, 159.4, 141.0, 135.2, 135.0, 133.4, 132.9, 125.6, 125.2, 124.2, 123.8, 99.1, 82.9, 74.4, 69.1, 69.0, 63.2, 61.6, 56.9, 55.6, 44.0, 41.1, 40.4, 36.4, 29.6, 29.4, 28.5, 26.7, 26.6 ppm; HR FAB MS (negative) $[M-H]^-$ Found m/z 922.3298, $C_{43}H_{52}N_7O_{14}S$ requires 922.3293; IR (film) ν: 3328, 1704, 1515, 1375, 1259, 1153 cm^{-1}; $[\alpha]_D^{21}$ −55.1° (c 1.0, MeOH).

4.1.32. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-[4-[methyl-phenyl]-phenyl-methanone]-amino-6′-[*N*-biotinyl glycylglycyl-glycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-hydroxy-phenyl-2-acrylate (43). Hydrazine monohydrate (10 μL) was added to the solution of **41** (12.9 mg, 14 μmol) in EtOH (1.0 mL) and the solution was stirred for 23 h at rt. The reaction mixture was evaporated and dried in vacuo to remove hydrazine, and then purified by ODS-TLC (H_2O/CH_3CN=5:4 containing 1% AcOH) to give crude amine (9.9 mg).

TEA(3.5 μL, 25 μmol) and 4-Bromomethylbenzophenone (**42**, 3.75 mg, 13.7 μmol) were added to the solution of crude amine (9.9 mg, crude) in DMF(0.5 mL), and the mixture was stirred for 10 h at rt. After dried in vacuo to remove DMF, the residue was purified by ODS-TLC (H_2O/CH_3CN=2:3 containing 1% AcOH) to give **43** (6.3 mg, 52% in two steps).

Compound **43**. ^{1}H NMR (400 MHz, CD_3OD, rt): 7.80 (2H,d, J=7.6 Hz), 7.78 (2H, d, J=8.8 Hz), 7.72 (2H, d, J=8.4 Hz), 7.67–7.62 (4H, m), 7.56 (1H, t, J=7.6 Hz), 7.51 (2H, t, J=8.0 Hz), 7.14 (1H, s), 6.76 (2H, d, J=8.8 Hz), 5.20 (1H, d, J=8.4 Hz), 4.47 (1H, m), 4.27 (1H, dd, J=4.4, 8.6 Hz), 4.08 (1H, dd, J=4.4, 8.6 Hz), 3.88 (2H, s), 3.84 (4H, s), 3.73 (1H, d, J=2.8 Hz), 3.60–3.39 (4H, m), 3.21–3.14 (1H, m), 2.90 (1H, dd, J=4.4, 12.8 Hz), 2.67 (1H, d, J=12.8 Hz), 2.30 (2H, t, J=7.2 Hz), 1.68–1.34 (6H, m), 1.28 (9H, s) ppm; ^{13}C NMR (100 MHz CD_3OD, rt): 197.7, 177.1, 173.1, 172.3, 172.2, 167.1, 162.7, 160.9, 139.9, 139.5, 138.4, 134.5, 134.1, 131.5, 131.4, 130.9, 129.9, 129.6, 124.8, 116.3, 101.1, 85.0, 74.6, 70.6, 68.8, 63.2, 61.6, 56.9, 54.8, 52.4, 44.0, 43.7, 43.6, 41.0, 40.1, 36.4, 29.6, 29.5, 28.4, 28.0, 26.6, 8.1 ppm; HR FAB MS (negative) $[M-H]^-$ Found m/z 986.3984 $C_{49}H_{60}O_{13}N_7S$ requires 986.3970: IR (film) ν: 3290, 1668, 1612, 1454, 1279, 1203, 1147 cm^{-1}; $[\alpha]_D^{22}$ −9.5° (c 0.6, MeOH).

4.1.33. (*Z*)-2-(2′-Deoxy-2′-[4-[methyl-phenyl]-phenyl-methanone]-amino-6′-[*N*-biotinylglycylglycylglycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-hydroxyphenyl-2-acrylate (4). Compound **43** (3.6 mg, 3.65 μmol) in TFA (100 μL) was stirred for 25 min. After dried in vacuo to remove TFA immediately, the residue was purified by ODS-TLC (H_2O/CH_3CN 1:5 containing 1% AcOH) to give **4** (2.4 mg, 71%).

Compound **4**. ^{1}H NMR (400 MHz, CD_3OD, rt): 7.79 (2H, d, J=8.0 Hz), 7.77 (2H, d, J=8.0 Hz), 7.69 (4H, m), 7.63 (1H, t, J=7.6 Hz), 7.47 (2H, t, J=7.6 Hz), 7.22 (1H, s), 6.75 (2H, d, J=8.8 Hz), 5.18 (1H, d, J=8.8 Hz), 4.73 (1H, d, J=13.2 Hz), 4.53 (1H, d, J=13.2 Hz), 4.46 (1H, dd, J=4.4, 7.6 Hz), 4.28 (1H, dd, J=4.4, 7.6 Hz), 4.05–3.42 (12H, m), 3.17 (1H, m), 2.89 (1H, dd, J=5.2, 8.8 Hz), 2.68 (1H, d, J=8.8 Hz), 2.31 (2H, m), 1.79–1.58 (6H, m) ppm; ^{13}C NMR (100 MHz CD_3OD, rt): 181.6, 175.3, 172.8, 172.6, 172.3, 166.7, 162.0, 138.4, 137.3, 133.9, 133.6, 131.5, 131.4, 130.9, 129.6, 129.5, 120.5, 116.1, 98.2, 79.5, 76.3, 74.9, 73.0, 71.6, 64.2, 63.3, 61.7, 56.9, 53.6, 43.9, 43.7, 41.0, 36.4, 33.1, 30.7, 29.6, 26.6, 23.7, 8.0 ppm; HR FAB MS (negative): $[M-H]^-$ Found m/z 930.3368, $C_{45}H_{52}O_{13}N_7S$ requires m/z 930.3344; IR (film) ν: 1680, 1205, 1140 cm^{-1}; $[\alpha]_D^{25}$ +5.00° (c 0.5, MeOH).

4.1.34. *N-tert*-Butoxycarbonyl glycylglycylglycine methyl ester (45). To the solution of *N-tert*-Butoxycarbonyl glycylglycylglycine (**44**) (200 mg, 0.69 mmol) in DMF(7.0 mL) was added $CaCO_3$ (143 mg, 1.03 mmol) and CH_3I (0.13 mL, 0.83 mmol), and the mixture was stirred for an hour. Then, the reaction mixture was mixed with water, extracted with ethyl acetate. And the organic layer was washed with brine, dried over abs Na_2SO_4. After dried in vacuo, **45** (234 mg, quant.) was obtained.

Compound **45**. ^{1}HNMR (270 MHz CD_3OD, rt): 3.92 (2H, s), 3.89 (2H, s), 3.71 (2H, s), 3.67 (3H, s), 1.40 (9H, s) ppm; ^{13}C NMR (68 MHz, CD_3OD, rt): 173.2, 172.3, 171.8, 158.7, 81.0, 52.7, 44.9, 43.3, 41.9, 28.8 ppm; HR FAB MS (positive): $[M+H]^+$ Found m/z 304.1516 $C_{12}H_{22}O_6N_3$ requires 304.1509; IR (film) ν: 3315, 1747, 1668, 1533, 1438, 1369, 1216 cm^{-1}; $[\alpha]_D^{25}$ −22.2° (c 1.0, MeOH).

4.1.35. *N*-Biotinylglycylglycylglycine methyl ester (46). Compound **45** (161.1 mg 0.53 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and TFA(2.5 mL), and the mixture was stirred for 20 min at rt. After careful evaporation to remove TFA completely, the residue was dissolved in DMF (5.0 mL). After adjusted to pH 7.0 by the addition of TEA at 0 °C, the reaction mixture was mixed with *N*-Hydroxyl succinimidobiotin (341 mg, 0.532 mmol) and stirred for 35 h. After careful evaporation to remove DMF, the residue was washed with MeOH, and dried in vacuo to give **46** (222.5 mg, 88%).

Compound **46**. ^{1}H NMR (400 MHz, DMSO-d_6, 30 °C): 8.18 (1H, t, J=5.6 Hz), 8.05 (1H, t, J=5.6 Hz), 7.98 (1H, t, J=5.6 Hz), 6.71 (1H, s), 6.64 (1H, s), 4.62 (1H, dd, J=5.3, 7.4 Hz), 4.44 (1H, dd, J=5.3, 7.4 Hz), 4.16 (2H, d, J=5.6 Hz), 4.05 (2H, d, J=5.6 Hz), 4.02 (2H, d, J=5.6 Hz), 3.13 (1H, dd, J=4.8, 10.6 Hz), 2.89 (1H, d, J=10.6 Hz), 2.42 (1H, t, J=7.4 Hz) 1.98–1.56 (6H, m) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 30 °C): 172.4, 170.0, 169.2, 169.1, 162.5, 61.0, 59.1, 55.3, 51.6, 48.6, 45.8, 42.0, 40.5, 34.9, 28.1, 28.0, 25.0 ppm; HR FAB MS (negative) $[M-H]^-$ Found m/z 428.1597 $C_{17}H_{26}O_6N_5S$ requires 428.1604 IR (film) ν: 3267, 1751, 1709, 1637, 1560, 1421, 1205 cm^{-1}; $[\alpha]_D^{20}$ −2.7° (c 1.0, DMSO).

4.1.36. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-*O*-*p*-toluenesulfonyl-β-D-galactopyranosyloxy)-3-*p*-methoxy-phenyl-2-acrylate (47). Compound **38** (72 mg, 106 μmol) was dissolved in MeOH/CH_3CN 1:9. To this solution, diisopropylethylamine (DIPEA)(22 μL, 127 μmol), and then $TMSCHN_2$ (2 M, 69 μL, 137 μmol) were added. The reaction mixture was stirred for 5 h at rt under argon atmosphere, concentrated in vacuo, mixed with water and then extracted three times with ethyl acetate. The organic layer was washed with brine, dried over abs Na_2SO_4., and separated with silica gel column chromatography (*n*-hexane/ethyl acetate 1:3) to give **47** (59 mg, 81%).

Compound **47**. ^{1}H NMR (300 MHz, $CDCl_3$, rt): 7.80–7.54 (8H, m), 7.29 (2H, d, J=9.2 Hz), 6.78 (1H, s), 6.75 (2H, d, J=9.2 Hz), 5.80 (1H, d, J=8.1 Hz), 4.57 (2H, dd, J=3.3, 3.6 Hz), 4.24 (1H, dd, J=6.8, 10.3 Hz), 4.08–3.70 (3H, m), 3.82 (3H, s), 2.41 (3H, s), 1.46 (9H, s) ppm; HR ESI MS

(positive): $[M+Na]^+$ Found *m/z* 718.1935, $C_{35}H_{37}NNaO_{12}S$ requires *m/z* 718.1929.

4.1.37. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-azido-β-D-galactopyranosyloxy)-3-*p*-methoxyphenyl-2-acrylate (48). Compound **47** (61 mg, 88 μmol), sodium azide (57 mg, 880 μmol), and 15-crown-5 (175 μL, 880 μmol) were dissolved in DMF (1 mL), and this mixture was stirred overnight at 70 °C under argon atmosphere. After the reaction mixture was allowed to stand at rt, the reaction mixture was mixed with water, extracted with ethyl acetate, and separated by silica gel column chromatography (toluene/acetone 2:1) to give **48** (45 mg, 90%).

Compound **48**. ^{1}H NMR (300 MHz, $CDCl_3$, rt): 7.85–7.67 (4H, m), 7.60 (2H, d, $J=8.7$ Hz), 6.84 (1H, s), 6.77 (2H, d, $J=8.7$ Hz), 5.82 (1H, d, $J=6.4$ Hz), 4.64 (2H, m), 3.97 (1H, d, $J=4.0$ Hz), 3.79–3.65 (3H, m), 3.79 (3H, s), 1.48 (9H, s) ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 589.1905, $C_{28}H_{30}N_4NaO_9$ requires *m/z* 589.1905.

4.1.38. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-phthalimide-6′-[*N*-biotinylglycylglycylglycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-methoxy-phenyl-2-acrylate (49). Compound **48** (20 mg, 34.6 μmol) was dissolved in MeOH (1 mL). After addition of catalytic amount of Pd–$CaCO_3$, the solution was stirred overnight at rt under hydrogen atmosphere. The reaction mixture was filtered with Celite, concentrated in vacuo, and dissolved in DMF (1 mL). To this solution, biotin unit **40** (17 mg, 41.6 μmol), HOBt (7 mg, 51.9 μmol), and then DCC (9 mg, 41.6 μmol) were added. After overnight stirring, the reaction mixture was filtered with Hyflo-Super Cel (Wako Pure chemical Industry Co., Ltd), and the filtrate was concentrated to dryness using vacuum line, and the residue was separated with ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 10:1) to give **49** (6.8 mg, 18% in two steps).

Compound **49**. ^{1}H NMR (300 MHz CD_3OD, rt): 7.88–7.76 (4H, m), 7.65 (2H, d, $J=8.8$ Hz), 6.82 (2H, d, $J=8.8$ Hz), 6.72 (1H, s), 5.73 (1H, d, $J=8.1$ Hz), 4.61 (1H, dd, $J=8.0$, 11.0 Hz), 4.53 (1H, dd, $J=3.0$, 9.0 Hz), 4.46 (1H, dd, $J=4.8$, 7.7 Hz), 4.32 (1H, dd, $J=5.4$, 7.7 Hz), 3.70–3.15 (12H, m), 3.58 (3H, s), 2.65 (1H, dd, $J=4.8$, 12.9 Hz), 2.43 (1H, d, $J=12.9$ Hz), 2.09 (2H, t, $J=7.3$ Hz), 1.61–1.31 (6H, m), 1.24 (9H, s) ppm; ^{13}C NMR (400 MHz, CD_3OD, rt): 177.1, 173.1, 172.2, 172.1, 170.3, 169.9, 166.1, 164.7, 161.7, 141.8, 135.3, 135.2, 133.3, 126.9, 124.8, 124.3, 123.9, 114.9, 99.2, 83.1, 74.5, 69.1, 69.0, 63.2, 61.7, 56.9, 55.8, 55.6, 44.0, 43.6, 41.0, 40.4, 36.4, 34.8, 29.6, 29.4, 28.4 ppm; IR (film) *ν*: 33 23, 1772, 1718, 1680, 1640, 1604, 1510 cm^{-1}; $[\alpha]_D^{20}$ −55.1° (*c* 1.0, MeOH); ESI MS (positive): [M+Na] Found *m/z* 960.3422, $C_{44}H_{55}N_7NaO_{14}S$ requires *m/z* 960.3420.

4.1.39. *t*-Butyl (*Z*)-2-(2′-deoxy-2′-[4-benzoylphenyl] methylamino-6′-[*N*-biotinylglycylglycylglycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-methoxyphenyl-2-acrylate (50). Compound **49** (5.7 mg, 6.1 μmol) and hydrazine monohydrate (5 μL) were dissolved in MeOH (0.5 mL), and this solution was stirred for 18 h at rt. After stirring, excess hydrazine was removed completely by using vacuum line, and the residue was purified with ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 5:4, containing 1% acetic acid). Resulting crude product was dissolved in DMF (0.5 mL), and 4-(bromomethyl)benzophenone (2.0 mg, 7.3 μmol) and triethylamine (1.7 μL, 12.2 μmol) were added to this solution. The reaction mixture was stirred for 24 h under light-shielded condition. After removal of DMF using vacuum line, the residue was separated by ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 1:7, containing 1% TFA) to give **50** (1.2 mg, 20% in two steps).

Compound **50**. ^{1}H NMR (300 MHz CD_3OD, rt): 7.88–7.63 (10H, m), 7.50 (2H, d, $J=9.1$ Hz), 7.17 (1H, s), 6.89 (2H, d, $J=9.1$ Hz), 5.24 (1H, d, $J=8.4$ Hz), 4.46 (1H, dd, $J=4.7$, 7.7 Hz), 4.32 (1H, dd, $J=4.0$, 7.7 Hz), 3.90–3.45 (12H, m), 3.81 (3H, s), 2.90 (1H, dd, $J=5.1$, 12.8 Hz), 2.67 (1H, d, $J=12.8$ Hz), 2.29 (2H, t, $J=7.0$ Hz), 1.72–1.62 (6H, m), 1.58 (9H, s) ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 1024.4105, $C_{50}H_{63}N_7NaO_{13}S$, requires *m/z* 1024.4097.

4.1.40. (*Z*)-2-(2′-Deoxy-2′-[4-benzoylphenyl]methylamino-6′-[*N*-biotinylglycylglycylglycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-methoxyphenyl-2-acrylate (51). TFA (neat, 150 μL) was added to **50** (1.2 mg, 1.2 μmol). This mixture was stirred for 15 min under argon atmosphere in complete light-shielded condition. After stirring, residual TFA was removed with vaccum line, and the residue was separated by ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 1:5, containing 1% TFA) to give **51** (0.5 mg, 44%) with recovered **50** (0.6 mg, 50%).

Compound **51**. ^{1}H NMR (600 MHz CD_3OD, rt): 7.86–7.63 (10H, m), 7.49 (2H, d, $J=8.1$ Hz), 6.96 (1H, s), 6.88 (2H, d, $J=8.1$ Hz), 5.09 (1H, d, $J=9.0$ Hz), 4.58 (1H, d, $J=12.2$ Hz), 4.50 (1H, dd, $J=4.0$, 8.0 Hz), 4.43 (1H, d, $J=12.2$ Hz), 4.32 (1H, dd, $J=4.5$, 7.9 Hz), 3.95–3.65 (6H m), 3.82 (3H, s), 3.48 (3H, m), 3.42 (3H, m), 2.92 (1H, dd, $J=5.1$, 12.9 Hz), 2.72 (1H, d, $J=12.9$ Hz), 2.33 (2H, m),1.75–1.60 (6H, m) ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 968.3478, $C_{46}H_{55}N_7NaO_{13}S$, requires *m/z* 968.3471.

4.1.41. *t*-Butyl 2-(2′-deoxy-2′-phthalimide-β-D-galactopyranosyl-oxy)-3-*p*-benzyloxyphenylpropanate (52). Compound **24** (117 mg, 0.157 mmol) in methanol (1.5 mL) was mixed with sodium methoxide (17.1 mg, 0.316 mmol) at −20 °C, and the mixture was stirred under argon atmosphere. After 4 h stirring, Amberlite IR-120 B was added to this solution to adjust pH at 7.0. After filtration, the filtrate was concentrated in vacuo, and purified by silica gel column chromatography ($CHCl_3$/MeOH= 15:1) to give **52** (91.6 mg, 0.148 mmol, 94%).

Compound **52**. ^{1}H NMR (300 MHz, $CDCl_3$, rt): 7.79 (2H, br), 7.71–7.65 (2H, m), 7.42–7.30 (5H, m), 7.05 (2H, d, $J=8.8$ Hz), 6.76 (2H, d, $J=8.8$ Hz), 5.15 (1H, d, $J=8.1$ Hz), 4.98 (2H, s), 4.51–4.27 (3H, m), 4.10 (1H, br), 3.85 (2H, br), 3.60 (1H, t, $J=4.6$ Hz), 2.87 (2H, d, $J=6$, 4 Hz), 1.14 (9H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, rt): 170.3, 168.9, 157.7, 137.2, 134.0, 132.2, 131.1, 129.0, 128.7, 128.0, 127.6, 123.5, 114.4, 97.3, 81.6, 77.5, 73.5, 70.0, 69.9, 68.3, 63.1, 54.1, 38.0, 27.7 ppm; HR ESI MS (positive): $[M+Na]^+$

Found *m/z* 642.2309, $C_{34}H_{37}NNaO_{10}$ requires *m/z* 642.2310; IR (film) *ν*: 3470, 2978, 2934, 1774, 1717, 1512 cm^{-1}; $[\alpha]_D^{18}$ −23.6° (*c* 0.1, MeOH).

4.1.42. *t*-Butyl 2-(2′-deoxy-2′-phthalimide-6′-*O*-*p*-toluenesulfonyl-β-D-galactopyranosyloxy)-3-*p*-benzyloxyphenylpropanate (53). *p*-Toluene sulfonyl chloride (9.9 mg 52 μmol) was added to the solution of **52** (26.9 mg, 43 μmol) in dichloromethane (0.5 mL) at −20 °C, and the mixture was stirred for 5 h under argon atmosphere. The mixture was evaporated with toluene, and the residue was purified by preparative TLC using Merck 60 F_{254} ($CHCl_3$/MeOH=10:1) to give **53** (18.5 mg, 55%) with recovered **52** (4.9 mg, 18%).

Compound **53**. ^{1}H NMR (300 MHz, $CDCl_3$, rt): 7.82–7.79 (4H, m), 7.69–7.65 (2H, m), 7.40–7.30 (7H, m), 7.00 (2H, d, *J*=8.4 Hz), 6.73 (2H, d, *J*=8.4 Hz), 5.10 (1H, d, *J*=8.4 Hz), 4.96 (2H, s), 4.53 (1H, br), 4.33–4.28 (3H, m), 4.14 (1H, dd, *J*=6.4, 10.5 Hz), 4.01 (1H, br), 3.87 (1H, t, *J*=6.2 Hz), 2.85 (2H, d, *J*=6.1 Hz), 2.44 (3H, s), 1.11 (9H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$, rt): 169.6, 168.5, 157.3, 145.2, 137.0, 133.8, 132.3, 131.9, 130.7, 130.0, 128.5, 128.4, 128.0, 127.8, 127.4, 123.2, 114.2, 97.2, 81.5, 72.0, 69.8, 67.9, 67.7, 67.6, 45.9, 38.0, 29.7, 27.7, 21.7 ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 796.2396, $C_{41}H_{43}NNaO_{12}S$ requires *m/z* 796.2398; IR (film) *ν*: 3474, 2978, 2930, 1717, 1661, 1512, 1392, 1367 cm^{-1}; $[\alpha]_D^{18}$ −30.7° (*c* 0.1,MeOH).

4.1.43. *t*-Butyl 2-(2′-deoxy-2′-phthalimide-6′-*O*-*p*-toluenesulfonyl-β-D-galactopyranosyloxy)-3-hydroxyphenylpropanate (54). Compound **53** (220 mg, 0.284 mmol) was dissolved in THF (3 mL) and catalytic amount of 20% $Pd(OH)_2$ was added to this solution. This solution was stirred for 30 h under hydrogen atmosphere and filtered with Celite. After evaporation, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH=23:1) to give **54** (161 mg, 83%.).

Compound **54**. ^{1}H NMR (300 MHz, MeOH, rt): 7.85–7.78 (6H, m), 7.42 (2H, d, *J*=8.1 Hz), 6.92 (2H, d, *J*=8.4 Hz), 6.55 (2H, d, *J*=8.4 Hz), 5.04 (1H, d, *J*=8.4 Hz), 4.49 (1H, dd, *J*=3.1, 11.2 Hz), 4.37–4.12 (4H, m), 3.87–3.81 (2H, m), 2.79 (2H, ddd, *J*=6.2, 14.7, 21.1 Hz), 2.44 (3H, s), 1.13 (9H, s) ppm; ^{13}C NMR (75 MHz, MeOH, rt): 171.6, 170.2, 170.0, 157.1, 146.7, 135.2, 134.2, 133.6, 133.2, 131.9, 131.2, 131.1, 129.1, 128.1, 124.2, 123.9, 98.9, 82.7, 78.6, 74.2, 70.8, 69.7, 68.7, 55.1, 40.1, 28.0, 21.7 ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 706.1932, $C_{34}H_{37}NNaO_{12}S$ requires *m/z* 706.1929; IR (film) *ν*: 3464, 1776, 1713, 1516, 1392, 1366 cm^{-1}; $[\alpha]_D^{19}$ 30.9° (*c* 0.1,MeOH).

4.1.44. *t*-Butyl 2-(2′-deoxy-2′-phthalimide-6′-azido-β-D-galactopyranosyloxy)-3-hydroxyphenylpropanate (55). Compound **54** (35.3 mg, 51.5 μmol), sodium azide (5.1 mg, 77 μmol) were dissolved in DMF/H_2O 10:1 (0.6 mL), and this mixture was stirred for 11 h at 100 °C. After the reaction mixture was allowed to stand at rt, the reaction mixture was mixed with water, extracted with ethyl acetate, and then the organic layer was dried over abs Na_2SO_4. The organic layer was separated by preparative TLC using Merck 60F_{254} ($CHCl_3$/MeOH 7:1) to give **55** (26.4 mg, 93%).

Compound **55**. ^{1}H NMR (300 MHz, MeOH, rt): 7.91–7.87 (1H, m), 7.80 (3H, br), 6.92 (2H, d, *J*=8.4 Hz), 5.12 (1H, d, *J*=8.4 Hz), 4.55 (1H, dd, *J*=3.3, 11.0 Hz), 4.46–4.37 (2H, m), 3.86–3.69 (3H, m), 3.26 (1H, dd, *J*=3.3, 12.8 Hz), 2.82 (2H, ddd, *J*=5.9, 14.0, 25.5 Hz), 1.12 (9H, s) ppm; ^{13}C NMR (75 MHz, MeOH, rt): 171.8, 170.3, 170.0, 157.1, 135.3, 135.2, 133.7, 133.2, 131.9, 128.0, 124.2, 123.9, 115.7, 98.9, 82.6, 78.6, 76.4, 70.4, 68.8, 55.2, 52.5, 39.2, 28.0 ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 577.1903, $C_{27}H_{30}N_4NaO_{12}S$ requires *m/z* 577.1905; IR (film) *ν*: 3445, 2106, 1776, 1709, 1516, 1394 cm^{-1}; $[\alpha]_D^{19}$ −40.2° (*c* 0.1,MeOH).

4.1.45. *t*-Butyl 2-(2′-deoxy-2′-phthalimide-6′-[*N*-biotinylglycylglycylglycyl]amino-β-D-galactopyranosyloxy)-3-hydroxyphenylpropanate (56). Compound **55** (41.5 mg, 74.9 μmol) was dissolved in MeOH (1.5 mL). After addition of catalytic amount of Pd–$CaCO_3$, the solution was stirred for 3 h at rt under hydrogen atmosphere. The reaction mixture was filtered with Celite, concentrated in vacuo to give crude amine (38.6 mg). The resulting amine (38.6 mg, 73.1 μmol, crude) was dissolved in DMF (1 mL). To this solution, biotin unit **40** (46.3 mg, 112 μmol), HOBt (22.5 mg, 164 μmol) were added, and then 6.6 μL of 1 M DCC in DMF was added 10-times in every 20 min. After 24 h stirring, the reaction mixture was filtered with Hyflo-Super Cel (Wako Pure chemical Industry Co., Ltd), and the filtrate was concentrated to dryness using vacuum line, and the residue was separated with ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 5:4) to give **56** (40.1 mg, 58% in two steps).

Compound **56**. ^{1}H NMR (300 MHz, MeOH, rt): 7.91–7.88 (1H, m), 7.81–7.78 (3H, m), 6.95 (2H, d, *J*=8.5 Hz), 6.54 (2H, d, *J*=8.5 Hz), 5.06 (1H, d, *J*=8.4 Hz), 4.56–4.45 (3H, m), 4.42–4.30 (2H, m), 3.97–3.80 (7H, m), 3.72 (1H, t, *J*=6.7 Hz), 3.51 (2H, d, *J*=6.8 Hz), 3.25–3.12 (1H, m), 2.94–2.76 (3H, m), 2.69 (1H, d, *J*=12.8 Hz), 2.32 (2H, t, *J*=7.4 Hz), 1.77–1.31 (6H, m) ppm; ^{13}C NMR (75 MHz, MeOH, rt): 177.1, 173.1, 172.3, 172.2, 171.9, 170.3, 170.1, 166.2, 157.0, 135.2, 135.1, 133.7, 133.2, 131.9, 128.1, 124.2, 123.9, 115.7, 98.9, 82.6, 78.5, 74.1, 69.6, 68.8, 63.2, 61.7, 56.9, 55.3, 44.1, 43.7, 43.6, 41.1, 41.0, 39.2, 36.4, 29.6, 29.4, 28.0, 26.6 ppm; HR ESI MS (positive): $[M+Na]^+$ Found *m/z* 948.3427, $C_{43}H_{55}N_7NaO_{14}S$ requires *m/z* 948.3420; IR (film) *ν*: 3306, 2932, 1713, 1663, 1542, 1516, 1393 cm^{-1}; $[\alpha]_D^{19}$ 12.8° (*c* 0.1,MeOH).

4.1.46. *t*-Butyl 2-(2′-deoxy-2′-[4-benzoylphenyl]methylamino-6′-[*N*-biotinylglycylglycylglycyl]amino-β-D-galactopyranosyloxy)-3-hydroxyphenylpropanate (57). Compound **56** (26.7 mg, 28.7 μmol) was dissolved in 0.2 M hydrazine monohydrate in EtOH (2.0 mL), and this solution was stirred for 25 h at 30 °C, and then stirred for 4 h at 40 °C. After stirring, excess hydrazine was removed completely by using vacuum line, and the residue was purified with ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 3:2, containing 5% acetic acid). Resulting crude amine (25.4 mg) was dissolved in DMF (0.7 mL), and 4-(bromomethyl)benzophenone (9.7 mg, 35.1 μmol) and triethylamine (8.9 μL, 63.9 μmol) were added to this solution at 0 °C. The reaction mixture was stirred for 3.5 h at rt under argon atmosphere in

light-shielded condition. After removal of DMF using vacuum line, the residue was separated by ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 2:3, containing 5% TFA) to give **57** (18.4 mg, 64% in two steps).

Compound **57**. ^{1}H NMR (600 MHz, MeOH, rt): 7.81–7.70 (5H, m), 7.59 (2H, t, J=7.8 Hz), 7.31 (2H, d, J=8.2 Hz), 7.21 (2H, d, J=8.5 Hz), 6.75 (2H, d, J=8.5 Hz), 4.88 (1H, t, J=4.6 Hz), 4.76 (1H, d, J=8.5 Hz), 4.57–4.53 (2H, m), 4.40–4.35 (2H, m), 3.94–3.86 (7H, m), 3.71–3.69 (2H, m), 3.56 (1H, dd, J=5.6, 13.7 Hz), 3.48 (1H, dd, J=7.7, 13.7 Hz), 3.38 (1H, m), 3.28–3.23 (1H, m), 3.19 (1H, d, J=4.6 Hz), 2.98 (1H, dd, J=5.0, 12.8 Hz), 2.75 (1H, d, J=12.7 Hz), 2.35 (2H, m), 1.83–1.65 (4H, m), 1.58–1.48 (11H, m) 1.10 (9H, s) ppm; ^{13}C NMR (150 MHz, MeOH, rt): 197.6, 177.2, 174.7, 173.2, 172.4, 172.3, 166.1, 157.9, 139.8, 138.4, 136.7, 134.2, 132.5, 131.4, 131.3, 131.1, 129.7, 127.6, 116.1, 99.3, 85.6, 78.4, 74.8, 71.0, 69.5, 63.3, 62.4, 61.7, 57.0, 53.8, 44.3, 43.9, 43.7, 41.1, 41.0, 38.7, 36.5, 29.7, 29.5, 28.4, 26.6 ppm; HR ESI MS (positive): $[M+H]^+$ Found m/z 990.4283, $C_{49}H_{64}N_7O_{13}S$ requires m/z 990.4277; IR (film) ν: 3290, 2932, 1670, 1202, 1136 cm^{-1}; $[\alpha]_D^{18}$ 21.9° (c 0.1, MeOH).

4.1.47. (*Z*)-2-(2′-Deoxy-2′-[4-benzoylphenyl]methylamino-6′-[*N*-biotinylglycylglycylglycyl]-amino-β-D-galactopyranosyloxy)-3-*p*-methoxyphenyl-2-acrylate (58). TFA (neat, 700 μL) was added to **57** (4.8 mg, 48.4 μmol). This mixture was srirred for 1 h under argon atmosphere in complete light-shielded condition. After stirring, residual TFA was removed with vaccum line, and the residue was separated by ODS-preparative TLC (RP-18W, Merck Co., Ltd) (water/acetonitrile 1:1, containing 5% TFA) to give **58** (2.3 mg, 51%) with recovered **57** (2.2 mg, 46%).

Compound **58**. ^{1}H NMR (600 MHz, MeOH, rt): 7.81 (2H, d, J=8.1 Hz), 7.75–7.70 (3H, m), 7.59 (2H, t, J=7.8 Hz), 7.25 (2H, d, J=8.1 Hz), 7.19 (2H, d, J=8.4 Hz), 6.73 (2H, d, J=8.4 Hz), 4.90 (1H, m), 4.77 (1H, d, J=8.6 Hz), 4.55–4.53 (1H, m), 4.41 (1H, J=12.8 Hz), 4.37–4.35 (1H, m), 4.24 (1H, d, J=12.8 Hz), 3.97–3.84 (7H, m), 3.67–3.66 (2H, m), 3.57 (1H, dd, J=5.4, 13.8 Hz), 3.46 (1H, dd, J=7.8, 13.8 Hz), 3.30–3.24 (2H, m), 3.20–3.18 (2H, m), 2.97 (1H, dd, J=5.0, 12.7 Hz), 7.74 (1H, d, J=12.7 Hz), 2.39–2.30 (2H, m), 1.83–1.47 (6H, m) ppm; ^{13}C NMR (150 MHz, MeOH, rt): 197.7, 177.2, 173.1, 172.4, 172.3, 171.1, 165.1, 157.7, 139.6, 138.4, 136.8, 134.1, 132.3, 131.3, 131.3, 131.1, 129.6, 128.1, 116.1, 99.8, 74.8, 71.2, 69.5, 63.3, 62.7, 61.7, 57.0, 54.3, 44.2, 43.8, 43.7, 41.1, 41.0, 38.6, 36.5, 30.8, 29.7, 29.5, 26.6 ppm; HR ESI MS (positive): $[M+H]^+$ Found m/z 934.3658, $C_{45}H_{56}N_7O_{13}S$ requires m/z 934.3651; IR (film) ν: 3315, 1663, 1202 cm^{-1}; $[\alpha]_D^{18}$ 24.2° (c 0.1, MeOH).

4.2. Bioassay

The young leaves detached from the stem of the plant *Cassia mimosoides* L. with a sharp razor blade were used for the bioassay. One leaf was placed in H_2O (ca. 1.0 mL) using a 20 mL glass tube in the greenhouse kept at 25–35 °C and allowed to stand overnight. The leaves, which opened again the next morning (around 10:00 am.) were used for the bioassay. Each test solution was carefully poured into test tubes with a microsyringe around 10:00 am. The bioactive fraction was judged by the leaf-opening after the leaf-closing of the plant leaf in the blank solution containing no sample.

4.3. Photoaffinity labeling of membrane protein in plant motor cell

A large amount of plant motor cells was collected by cutting off a large number of sections of plant pulvini (size; ca. 0.5 mm×0.5 mm) containing the motor cell one by one from plant leaves under a stereoscopic microscope. Collected pulvini were immersed in the extraction buffer (0.25 M sucrose, 3 mM EDTA-2K, 2.5 mM DTT, 25 mM Tris–MES, 1 tablet of complete™/50 mL, pH 7.2) immediately. One cross-linking experiment needed about nine hundred plant sections.

Successive homogenization (15,000 rpm, 10 min) in extraction buffer (0.25 M sucrose, 3 mM EDTA, 2.5 mM DTT, 25 mM Tris–MES, pH 7.8) at 4 °C, filtration with nylon mesh (50 μm), and twice centrifugation (Beckman Coulter Optima TLX, 1st; 3000×g, 15 min, 4 °C, 2nd; 100,000×g, 60 min, 4 °C) gave a pellet of the crude membrane fraction. The content of total protein in that fraction was determined to be 68.7 μg by the Bradford method with BSA as a reference. The membrane ATPase activity was determined to be 0.67 μmol/mg protein·min by Sandstom's method.[15] The crude membrane fractions were suspended in the 50 μL of extraction buffer, and 14 μL of this solution containing 10 μg of crude membrane protein was incubated with probe **2** or **3** (3 μM concentration) for 20 min at rt, respectively. After cross-linking by irradiation of UV-light (365 nm) for 60 min at 4 °C. Electrophoresis buffer (0.3 M Tris–Cl, 10% SDS, 30% glycerol, 9.3% DTT, pH 6.8) was added to this reaction mixture and the solution was heated at 90 °C for 5 min. The reaction mixture was analyzed by SDS-PAGE (Ready Gel J 7.5%, BIO-RAD Co. Ltd) with molecular weight marker (Biotinylated SDS-PAGE standard, High-Range, BIO-RAD Co. Ltd). After western blotting using PVDF membrane (Hybond-P, Amersham Bioscience Co. Ltd), detection of the bands of the potential receptor for leaf-opening substance was carried out by chemiluminescence detection with ECL Advance Western Blotting Detection Kit (Amersham Bioscience Co. Ltd) with ECL mini-camera (Amersham Bioscience Co. Ltd).

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Synthesis of fused or not β-lactam-biaryl hybrids by free radical aryl–aryl coupling of 2-azetidinone-tethered haloarenes

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Dedicated to Professor Joaquín Plumet on the occasion of his 60th birthday

Abstract—*cis* and *trans*-Aryl-2-azetidinone-tethered haloarenes can be stereoselectively prepared using the ketene–imine cyclization. These β-lactam-tethered haloarenes were used for the regiocontrolled preparation of β-lactam-biaryl hybrids including fused tetracyclic biaryl-2-azetidinones as well as C4-dearylated not fused biphenyl-2-azetidinones via aryl–aryl radical cyclization and/or rearrangement. Alternatively, *trans*-dibenzocarbacephems could be stereoselectively prepared, both in racemic and enantiopure form, through the Staudinger reaction between phenanthridine and activated ketenes.

1. Introduction

The importance of the stereoselective synthesis of β-lactams is ever increasing in connection with the structure–activity relationship study and the development of new derivatives of the β-lactam antibiotics and inhibitors of β-lactamases.[1] Besides the utility of β-lactams as biologically active agents, they are used as intermediates in α- and β-amino acid synthesis, as well as building blocks for alkaloids, heterocycles, taxoids, and other types of compounds of biological and medicinal interest.[2] Consequently, the development of new approaches to the stereocontrolled synthesis of β-lactam systems is a subject of great interest. On the other hand, the importance of molecules possessing the biaryl unit as a central feature has been widely recognized.[3] Aryl radical addition onto an aromatic ring has become an important tool in organic synthesis as it provides mild and often very efficient routes to condensed carbo(hetero)aromatic ring systems.[4] In connection with our current research interest in the preparation and synthetic utility of β-lactams,[5] we report herein a full study[6] of the aryl–aryl radical coupling route to novel fused or not β-lactam-biaryl hybrids[7] by radical aryl–aryl coupling of 2-azetidinone-tethered arenes.

2. Results and discussion

Starting substrates, aryl-β-lactam-tethered haloarenes **1**–**4** (Scheme 1), were prepared in the racemic form using the ketene–imine cycloaddition as the key step.[8] 2-Azetidinones **1**, **2** and **4** were obtained from the corresponding imine[9] through Staudinger reaction with the appropriate acid chloride in the presence of Et_3N (Scheme 2, Table 1). For the preparation of β-lactams **3**, dichlorophenylphosphate was used as the condensating agent, starting from the corresponding imine and the α-substituted acetic acid.[10] Compounds **1** were obtained as single *trans*-diastereomers. In contrast, β-lactams **2**–**4** were obtained as their *cis*-diastereoisomers with total stereoselectivity. 2-Azetidinone **4f** was obtained as a *cis*–*trans* mixture with low *cis*-selectivity (70:30), the *cis*-isomer being easily separated by column chromatography.

The *trans* and *cis* isomers were easily distinguished by the value of $J_{3,4}$, the *cis* value (4.5–5.4 Hz) always being larger than the *trans* (1.5–2.1 Hz) in such compounds.[11] Regarding the stereochemistry of the above reactions, it is well known that *C*,*N*-diarylimines with acyloxy acid chlorides (Bose–Evans ketenes) give *cis*-β-lactams under Staudinger

Keywords: Lactams; Nitrogen heterocycles; Biaryls; Radical reactions; Polycycles; Cycloaddition.
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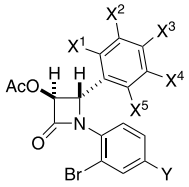
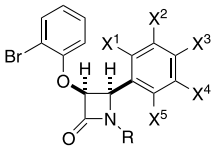
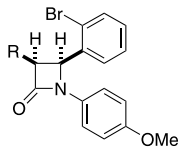


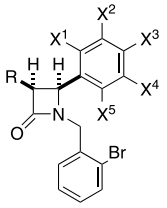
1a $X^1 = X^2 = X^4 = X^5 = Y = H$; $X^3 = OMe$
1b $X^1 = X^2 = X^4 = X^5 = H$; $X^3 = OMe$; Y = Me
1c $X^1 = X^2 = X^3 = X^4 = X^5 = Y = H$
1d $X^1 = X^3 = X^5 = Y = H$; $X^2 = X^4 = OMe$
1e $X^1 = X^3 = X^5 = OMe$; $X^2 = X^4 = Y = H$
1f $X^1 = X^3 = X^5 = Me$; $X^2 = X^4 = Y = H$



3a R = PMP; $X^1 = X^2 = X^4 = X^5 = H$; $X^3 = OMe$
3b R = Bn; $X^1 = X^2 = X^4 = X^5 = H$; $X^3 = OMe$
3c R = Bn; $X^1 = X^3 = X^5 = H$; $X^2 = X^4 = OMe$
3d R = Bn; $X^1 = X^3 = OMe$; $X^2 = X^4 = X^5 = H$
3e R = Bn; $X^1 = X^5 = OMe$; $X^2 = X^3 = X^4 = H$
3f R = Bn; $X^1 = X^3 = X^5 = OMe$; $X^2 = X^4 = H$
3g R = Bn; $X^1 = X^3 = X^5 = Me$; $X^2 = X^4 = H$

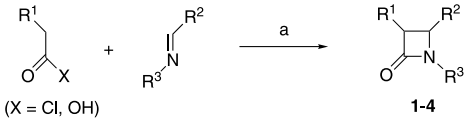


2a R = AcO
2b R = PhO



4a R = PhO; $X^1 = X^2 = X^4 = X^5 = H$; $X^3 = OMe$
4b R = AcO; $X^1 = X^3 = X^5 = H$; $X^2 = X^4 = OMe$
4c R = AcO; $X^1 = X^3 = OMe$; $X^2 = X^4 = X^5 = H$
4d R = AcO; $X^1 = X^5 = OMe$; $X^2 = X^3 = X^4 = H$
4e R = AcO, $X^1 = X^3 = X^5 = OMe$; $X^2 = X^4 = H$
4f R = $X^1 = X^3 = X^5 = Me$; $X^2 = X^4 = H$

Scheme 1. Starting β-lactam-tethered haloarenes **1**–**4**.



Scheme 2. Reagents and conditions: (a) For compounds **1**, **2** and **4**: R^1CH_2COCl, Et_3N, dichloromethane, rt, 12 h. For compound **3**: R^1CH_2-COOH, $PhOP(O)Cl_2$, Et_3N, dichloromethane, rt, 16 h.

reaction conditions.[8e] The exclusive formation of *trans*-β-lactams **1** with imines derived from *o*-bromoanilines and aromatic aldehydes in their reactions with acetoxyketene is noteworthy. The synthesis of some *trans*-2-azetidinones using imines derived from naphthylamines and related polyaromatic amines was also reported.[12] From these studies it seems that the presence of a bulky aryl substituent at the nitrogen of the imines has a crucial role in controlling the stereochemical result of the β-lactam formation.

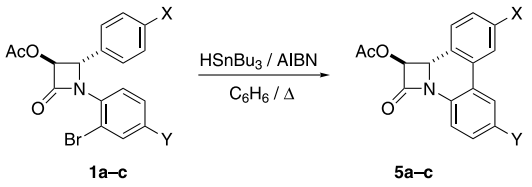
Having obtained the monocyclic precursors, the next stage was set to carry out the key radical aryl–aryl coupling. β-Lactam-tethered haloarenes **1**–**4** were reacted with

Table 1. Preparation of β-lactams **1**–**4**[a]

Compound	R^1	R^2	R^3	Yield (%)[b]	*cis*–*trans* ratio[c]
1a	AcO	PMP	*o*-BrC_6H_4	60	0:100
1b	AcO	PMP	Ar[6]	72	0:100
1c	AcO	Ph	*o*-BrC_6H_4	63	0:100
1d	AcO	Ar[1]	*o*-BrC_6H_4	80	0:100
1e	AcO	Ar[2]	*o*-BrC_6H_4	33	0:100
1f	AcO	Ar[3]	*o*-BrC_6H_4	62	0:100
2a	AcO	*o*-BrC_6H_4	PMP	59	100:0
2b	PhO	*o*-BrC_6H_4	PMP	75	100:0
3a	*o*-BrC_6H_4O	PMP	PMP	61	100:0
3b	*o*-BrC_6H_4O	PMP	Bn	80	100:0
3c	*o*-BrC_6H_4O	Ar[1]	Bn	56	100:0
3d	*o*-BrC_6H_4O	Ar[4]	Bn	85	100:0
3e	*o*-BrC_6H_4O	Ar[5]	Bn	53	100:0
3f	*o*-BrC_6H_4O	Ar[2]	Bn	71	100:0
3g	*o*-BrC_6H_4O	Ar[3]	Bn	68	100:0
4a	PhO	PMP	*o*-$BrC_6H_4CH_2$	57	100:0
4b	AcO	Ar[1]	*o*-$BrC_6H_4CH_2$	49	100:0
4c	AcO	Ar[4]	*o*-$BrC_6H_4CH_2$	65	100:0
4d	AcO	Ar[5]	*o*-$BrC_6H_4CH_2$	51	100:0
4e	AcO	Ar[2]	*o*-$BrC_6H_4CH_2$	80	100:0
4f	AcO	Ar[3]	*o*-$BrC_6H_4CH_2$	66	70:30

[a] PMP = 4-$OMeC_6H_4$. Ar^1 = 3,5-$(OMe)_2C_6H_3$. Ar^2 = 2,4,6-$(OMe)_3C_6H_2$. Ar^3 = 2,4,6-$Me_3C_6H_2$. Ar^4 = 2,4-$(OMe)_2C_6H_3$. Ar^5 = 2,6-$(OMe)_2C_6H_3$. Ar^6 = 2-Br-4-MeC_6H_3.
[b] Yield of pure, isolated product (or mixture of isomers, for **4f**) with correct analytical and spectral data.
[c] The ratio was determined by integration of well-resolved signals in the ^1H NMR spectra of the crude reaction mixtures before purification.

tributyltin hydride and AIBN in benzene at reflux under high dilution conditions (215 mL per mmol of starting 2-azetidinone). It was observed that the outcome of the reaction of the radicals generated from compounds **1**–**4** strongly depends on the relative position of both, the radical precursor and the radical acceptor in the 2-azetidinone ring. Thus, treatment of *trans*-β-lactams **1a**–**c** with Bu_3SnH/AIBN smoothly formed the corresponding condensed tetracyclic biaryl-2-azetidinones **5a**–**c** in good yields as single *trans*-diastereomers after chromatographic purification (Scheme 3, Table 2) together with small amounts of reduced starting material.



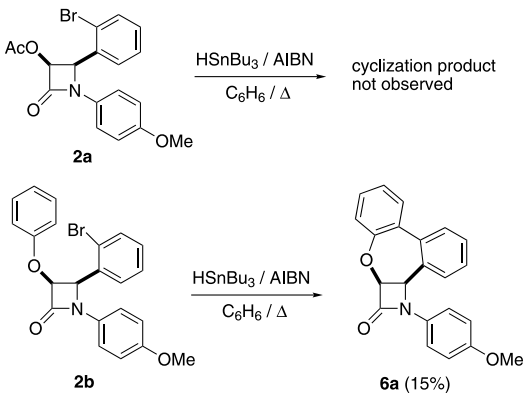
Scheme 3. Preparation of tetracyclic biaryl-2-azetidinones **5a**–**c**.

Table 2. Preparation of tetracyclic β-lactams **5a**–**c**

Substrate	X	Y	Product	Yield (%)[a]
1a	MeO	H	**5a**	70
1b	MeO	Me	**5b**	62
1c	H	H	**5c**	66

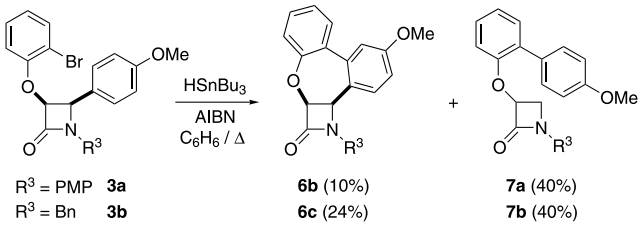
[a] Yield of pure, isolated product with correct analytical and spectral data.

On the other hand, reaction of β-lactam **2a** with the radical precursor on C4 and the aromatic acceptor on the lactam nitrogen does not form any cyclized products. In fact, the crude reaction mixtures contained debrominated 3-acetoxy-4-phenyl-1-(*p*-methoxyphenyl)-2-azetidinone[12b] (70%) together with some unreacted starting material. However, albeit in low yield, 2-azetidinone **2b**, having an extra radical acceptor on C3, formed biaryl-2-azetidinone **6a** (15%, isolated yield), with the debrominated 3-phenoxy-4-phenyl-1-(*p*-methoxy-phenyl)-2-azetidinone[12b] being the main reaction product (60%) (Scheme 4).



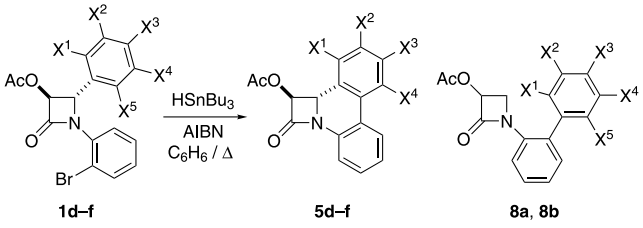
Scheme 4. Radical reaction of 2-azetidinones **2a** and **2b**.

Interestingly, 2-azetidinones **3a**, **3b**, and **4a**, with the same radical acceptor (PMP) on C4, but bearing an extra link (O or CH_2) on the radical precursor at C3 or N1 of the β-lactam ring behaved differently. The radical reaction of compounds **3a** and **3b**, afforded the expected cyclization products **6b** and **6c** (minor products) together with the new C4-dearylated C3-biphenyl 2-azetidinones **7a** and **7b** as the main reaction products, and dehalogenated starting 2-azetidinones (10% for **3a** and 15% for **3b**) (Scheme 5). However, compound **4a** gave a complex mixture under the same radical reaction conditions. In the ^1H NMR spectra of this crude material, cyclization product, rearranged product, and dehalogenated starting material were detected. However, we were unable to isolate any pure product by column chromatography.



Scheme 5. Radical reaction of 2-azetidinones **3a** and **3b**.

Next, we decided to explore the influence of more substituted radical acceptor aromatic rings in the regioselectivity of the above processes. 2-Azetidinones **1d**–**f**, **3c**–**g**, and **4b**–**f**, bearing methoxy or methyl groups at the different positions of the aromatic ring, were selected as starting substrates. The reaction pattern of *N*-(2-bromophenyl)-β-lactams **1d**–**f**, was different depending on both the number and position of the substituents on the acceptor ring at C4. Thus, while haloaryl 2-azetidinone **1d** afforded exclusively the cyclization product **5d**, β-lactams **1e** and **1f** formed, along with cyclization products **5e** and **5f**, C4-dearylated *N*-biphenyl 2-azetidinones **8a** and **8b**, respectively, and dehalogenated starting 2-azetidinones (Scheme 6, Table 3).



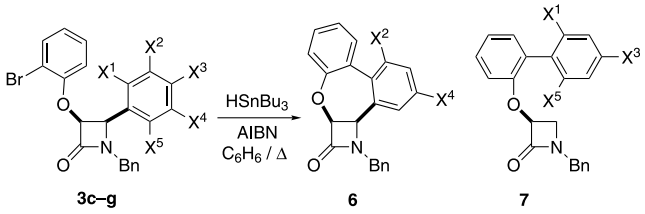
Scheme 6. Radical reaction of 2-azetidinones **1d**–**f**.

2-Azetidinones **3c**–**g**, bearing the radical precursor at C3 of the β-lactam nucleus, gave the best results, both on regioselectivity as well as chemical yield (Scheme 7, Table 4). No reduction products were detected in the ^1H NMR spectra of the crude reaction mixtures. While the radical reaction of 2-azetidinone-tethered arenes **3d**, **3f**, and **3g** afforded the corresponding rearranged products **7c**, **7d**, and **7e**, respectively, 2-azetidinone **3c** yielded the cyclization adduct **6d** as the sole product. However, β-lactam **3e** gave a complex reaction mixture. The above results point out the importance of the presence of a 4-methoxy group at the aromatic ring for the success of the rearrangement taking place.

Table 3. Radical reaction of 2-azetidinones **1d–f**

Substrate	X^1	X^2	X^3	X^4	X^5	Product	**5**:**8** Ratio[a]	Yield **5**:**8** (%)[b]
1d	H	OMe	H	OMe	H	**5d**	100:0	51
1e	OMe	H	OMe	H	OMe	**5e**:**8a**	40:30[c]	38:14
1f	Me	H	Me	H	Me	**5f**:**8b**	80:10[d]	58[e]

[a] The ratio was determined by integration of well-resolved signals in the ^{1}H NMR spectra of the crude reaction mixtures before purification.
[b] Yield of pure, isolated product with correct analytical and spectral data.
[c] The ^{1}H NMR spectra of the crude mixture showed **5e** and **8a**, together with 30% of the corresponding dehalogenated β-lactam.
[d] The ^{1}H NMR spectra of the crude mixture showed **5f** and **8b**, together with 10% of the corresponding dehalogenated β-lactam.
[e] Fractions containing pure compound **8b** could not be isolated.



Scheme 7. Radical reaction of 2-azetidinones **3c–g**.

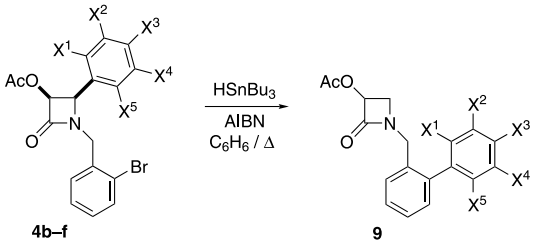
gave exclusively the C4-dearylated *N*-biphenyl-2-azetidinones **9a–c**, which were obtained in good yields after chromatographic purification (Scheme 8, Table 5). By contrast, the treatment of β-lactams **4b** and **4d** under the same conditions gave complex reaction mixtures.

The results above show that either cyclization or C4-dearylated rearranged products may be regioselectively obtained by choosing the appropriate structure of the precursor. Thus, placing three methoxy (methyl) groups at

Table 4. Radical reaction of 2-azetidinones **3c–g**

Substrate	X^1	X^2	X^3	X^4	X^5	Product[a]	Yield (%)[b]
3c	H	OMe	H	OMe	H	**6d**	60
3d	OMe	H	OMe	H	H	**7c**	45
3e	OMe	H	H	H	OMe	[c]	
3f	OMe	H	OMe	H	OMe	**7d**	80
3g	Me	H	Me	H	Me	**7e**	65

[a] Additional isomers were not detected in the ^{1}H NMR spectra of the crude reaction mixtures before purification.
[b] Yield of pure, isolated product with correct analytical and spectral data.
[c] The reaction gave a complex mixture. Fractions containing pure products could not be isolated.



Scheme 8. Radical reaction of *N*-halobenzyl 2-azetidinones **4b–f**.

the 2, 4, and 6 positions or two methoxy groups at the 2 and 4 positions led exclusively to *ipso*-substitution reactions (non-fused biaryl 2-azetidinones **7c–e** and **9a–c**), whereas the presence of two methoxy groups at the 3 and 5 positions resulted in the formation of *ortho*-substitution products (fused biaryl β-lactams **5e** and **6d**).

The behavior of the tested substrates may be rationalized through a competition between *ortho*-addition over an *ipso*-addition from the initially formed aryl radical **10** (Scheme 9).[13] For *N*-(2-bromoaryl)-β-lactams ($n=0$, substrates **1a–f**), formation of phenanthridine condensed

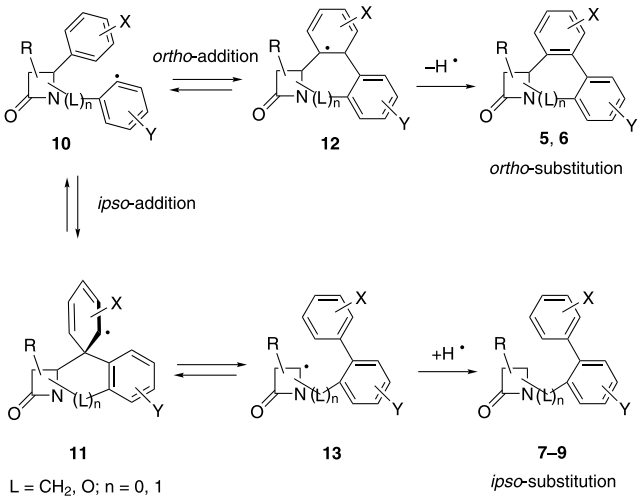
Table 5. Radical reaction of *N*-halobenzyl 2-azetidinones **4b–f**

Substrate	X^1	X^2	X^3	X^4	X^5	Prod.[a]	Yield (%)[b]
4b	H	OMe	H	OMe	H	[c]	
4c	OMe	H	OMe	H	H	**9a**	40
4d	OMe	H	H	H	OMe	[c]	
4e	OMe	H	OMe	H	OMe	**9b**	70
4f	Me	H	Me	H	Me	**9c**	50

[a] Additional isomers were not detected in the ^{1}H NMR spectra of the crude reaction mixtures before purification.
[b] Yield of pure, isolated product with correct analytical and spectral data.
[c] The reaction gave a complex mixture. Fractions containing pure products could not be isolated.

Next, we decided to explore the extension of the radical reaction of 2-azetidinones bearing the proradical center at N1 moving from *N*-haloaryl-β-lactams **1a–f** to *N*-halobenzyl-β-lactams **4b–f**. The treatment of *N*-(2-bromobenzyl)-β-lactams **4c**, **4e**, and **4f** under standard conditions

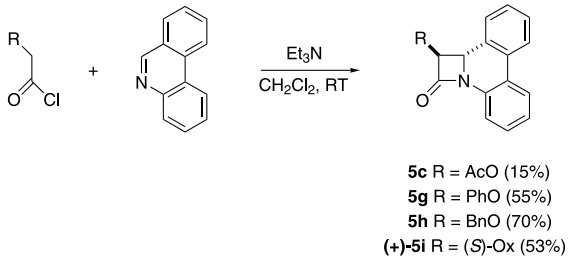
2-azetidinones **5a–c** would be accounted through cyclohexadienyl radical **12** resulting from 1,6-addition (*ortho* addition) in the former radical **10**. The 1,5-aryl migration produced minor rearrangement products **8a** and **8b**. For compounds **3a–g** and **4a–f**, with an extra link between the



Scheme 9. Proposed reaction pathways to β-lactam-biaryl hybrids **5–9**.

two aromatic rings ($n=1$; $L=O$, CH_2), the competition between the 1,6-addition (*ipso*), and the 1,7-addition (*ortho*) could explain the obtained results. Thus, addition of radical **10** to the neighbouring aromatic ring can operate through an *ipso*-addition to give the spiro-cyclohexadienyl radical **11** or alternatively *via* an *ortho*-addition to form the isomeric cyclohexadienyl radical **12**. Radical **11**, that benefits from the additional stabilization offered by the oxygen atoms of the methoxy groups placed on appropriate positions (*ortho* or *para*) in the aromatic acceptor ring, promotes the breakage of the C4–C_{ipso} bond. In this way, rearranged compounds of type **7** and **9** are obtained as the major or exclusive products of the reactions. The regeneration of aromaticity and formation of a more stable azetidin-2-on-4-yl radical **13**,[14] could provide the necessary driving force for these reactions. Alternatively, the cyclohexadienyl radical **12** derived from *ortho* attack would evolve to cyclisation products **6**, but not to rearranged products. The presence of *meta*-methoxy groups in the acceptor ring could lead to direct stabilization of the intermediate radical **12**, and cyclization product **6d** is the only observed product in this case. This result clearly indicates that the location of two *meta* methoxy groups exerts a dominant directing effect, which totally eliminates *ipso*-substitution products (type **7**).

Compounds **5a–f** are, formally, phenanthridine derived 2-azetidinones. The reaction of phenanthridine and different ketenes could give fused biaryl-2-azetidinones related to **5**. As a consequence, an alternative synthesis of some fused biaryl β-lactams related to **5** through Staudinger ketene–imine cycloaddition using phenanthridine as the imine component of the reaction is presented next.[6b] Phenanthridine reacts smoothly with acid chlorides having electron-withdrawing substituents at the α-position, in the presence of Et_3N, in dichloromethane at room temperature, to give the corresponding fused tetracyclic 2-azetidinones **5c** and **5g–i** in reasonable yields (Scheme 10). All products were obtained as single *trans* isomers. Enantiopure compound **5i** was obtained using the ketene derived from the Evans and Sjögren chiral 2-oxazolidinone.[15,16] However, reactions with alkanoyl chlorides (i.e., propanoyl- and isovaleryl chlorides), fail to give the corresponding β-lactam.[17] Therefore, more electrophilic ketenes seen to



Scheme 10. Preparation of tetracyclic biaryl-2-azetidinones **5c** and **5g–i**. (*S*)-Ox = (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl.

provide the driving force for the reaction to occur. Moreover, the exclusive *trans*-selectivity observed must be due to the cyclic imine structure and not a consequence of the nature of the ketene and the reaction conditions used.[18]

In conclusion, easily available 2-azetidinone-tethered haloarenes have proved to be appropriate substrates for the synthesis of fused or not β-lactam-biaryl hybrids by aryl–aryl radical cyclization and/or rearrangement. This novel rearrangement of the 2-azetidinone nucleus allows the synthesis of novel 4-unsubstituted-2-azetidinones, a structural feature present in different compounds of interest such as nocardicines and tabtoxines. In addition, an alternative synthesis of fused biaryl *trans*-2-azetidinones (*trans*-dibenzocarbacephems) using phenanthridine as the imine component in the Staudinger reaction and activated ketenes has been achieved. Efforts to develop these methodologies for the preparation of more elaborate biaryl policyclic products as suicide inhibitors of β-lactamases are currently underway in our research group.

3. Experimental

3.1. General methods

Melting points were taken using a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-300, Varian VRX-300S or Bruker AC-200. NMR spectra were recorded in $CDCl_3$ solutions, except otherwise stated. Chemical shifts are given in ppm relative to TMS (^1H, 0.0 ppm), or $CDCl_3$ (^{13}C, 76.9 ppm). Mass spectra were recorded on a Hewlett-Packard 5989A spectrometer. Microanalyses were performed in the UCM Microanalysis Service (Facultad de Farmacia, UCM, Madrid). Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Specific rotation $[\alpha]_D$ is given in 10^{-1} deg cm^2 g^{-1} at 25 °C, and the concentration (c) is expressed in g per 100 mL. All commercially available compounds were used without further purification. THF was distilled from Na–benzophenone. Benzene, dichloromethane and triethylamine were distilled from CaH_2. Flame-dried glassware and standard Schlenk techniques were used for moisture sensitive reactions. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). Identification of products was made by TLC (Kiesegel 60F-254). UV light ($\lambda=254$ nm), and a vanillin solution in sulfuric acid and 95% EtOH (1 g vanillin, 5 mL H_2SO_4, 150 mL EtOH) was used to develop the plates.

3.2. Materials

The following chemicals were prepared according to previously reported procedures: (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl-acetic acid,[19] (2-bromophenoxy)acetic acid.[20]

3.3. General procedure for the synthesis of β-lactam-tethered haloarenes 1–4

A mixture of aldehyde (10 mmol), 2-bromoaniline (10 mmol) and a catalytic amount of $ZnCl_2$/α-phenyl–ethylamine complex (0.1 mmol) in benzene (50 mL) was heated at reflux (2–4 h) on a Dean–Stark apparatus. Then, the mixture was filtered and the solvent was removed under reduced pressure. To a cooled (0 °C) solution of the imine in anhydrous dichloromethane (50 mL), Et_3N (4.16 mL, 30 mmol), the corresponding acid or acid chloride (15 mmol), [and $PhOP(O)Cl_2$, only when 2-(*o*-bromophenyl)acetic acid is used, 2.25 mL, 15 mmol] were successively added under argon. The resulting mixture was allowed to warm to room temperature, and was stirred for 16 h. The crude mixture was diluted with dichloromethane (100 mL) and washed with saturated $NaHCO_3$ (2× 20 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure.

3.3.1. *trans*-3-Acetoxy-1-(2-bromophenyl)-4-(4-methoxyphenyl)-azetidin-2-one (1a). From 1.36 g (10 mmol) of *p*-anisaldehyde, 1.72 g (10 mmol) of *o*-bromoaniline and 1.63 g (12 mmol) of acetoxyacetyl chloride, 2.3 g (60%) of compound **1a** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 4:1). Mp 130–132 °C (hexanes/ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3H), 3.68 (s, 3H), 5.36 (d, 1H, J= 1.8 Hz), 5.44 (d, 1H, J=1.8 Hz), 6.77 (d, 2H, J=8.7 Hz), 6.99 (m, 1H), 7.19 (d, 2H, J=8.7 Hz), 7.20 (m, 1H), 7.42 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 169.5, 162.7, 160.0, 134.0, 133.6, 128.3, 128.2, 128.1, 127.0, 126.5, 117.0, 114.3, 81.9, 66.1, 55.2, 20.5. IR (KBr, cm^{-1}): ν 1760, 1740, 1615, 1490, 1370. Anal. Calcd for $C_{18}H_{16}NO_4Br$: C, 55.40; H, 4.13; N, 3.59. Found: C, 55.23; H, 4.01; N, 3.80.

3.3.2. *trans*-3-Acetoxy-1-(2-bromotolyl)-4-(4-methoxyphenyl)-azetidin-2-one (1b). From 560 mg (3 mmol) of *p*-anisaldehyde, 0.56 g (3 mmol) of *o*-bromotoluidine and 612 mg (4.5 mmol) of acetoxyacetyl chloride, 860 mg (72%) of compound **1b** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 4:1). White solid. Mp 102–104 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 2.12 (s, 3H), 2.18 (s, 3H), 3.68 (s, 3H), 5.30 (d, 1H, J=2.1 Hz), 5.42 (d, 1H, J= 2.1 Hz), 6.76 (d, 2H, J=8.7 Hz), 7.0 (m, 1H), 7.17 (d, 2H, J=8.7 Hz), 7.27 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 169.6, 162.8, 160.0, 138.8, 134.2, 131.0, 128.8, 128.2, 127.1, 126.3, 117.0, 114.3, 81.8, 66.1, 55.2, 20.7, 20.5. IR (KBr, cm^{-1}): ν 1765, 1750, 1500, 1380. Anal. Calcd for $C_{19}H_{18}NO_4Br$: C, 56.45; H, 4.49; N, 3.46. Found: C, 56.77; H, 4.25; N, 3.70.

3.3.3. *trans*-3-Acetoxy-1-(2-bromophenyl)-4-phenyl-azetidin-2-one (1c). From 530 mg (5 mmol) of benzaldehyde, 860 mg (5 mmol) of *o*-bromoaniline and 910 mg (6.8 mmol) of acetoxyacetyl chloride, 1.13 g (63%) of compound **1c** was obtained as a pale yellow solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 118–120 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H), 5.42 (d, 1H, J=1.8 Hz), 5.46 (d, 1H, J=1.8 Hz), 6.95–7.50 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 169.7, 162.8, 137.3, 135.3, 134.2, 133.7, 129.0, 128.5, 128.2, 126.9, 126.5, 116.9, 81.9, 66.5, 20.6. IR ($CHCl_3$, cm^{-1}): ν 1775, 1760, 1490, 1370, 1150. Anal. Calcd for $C_{17}H_{14}NO_3Br$: C, 56.68; H, 3.92; N, 3.89. Found: C, 56.51; H, 3.61;N, 3.52.

3.3.4. *trans*-3-Acetoxy-1-(2-bromophenyl)-4-(3,5-dimethoxyphenyl)-azetidin-2-one (1d). From 340 mg (2 mmol) of 3,5-dimethoxybenzaldehyde, 340 mg (2 mmol) of *o*-bromoaniline and 410 mg (3 mmol) of acetoxyacetyl chloride, 720 mg (80%) of compound **1d** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 160–162 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3H), 3.65 (s, 6H), 5.30 (d, 1H, J=1.5 Hz), 5.46 (d, 1 H, J= 1.5 Hz), 6.29 (s, 1H), 6.40 (d, 2H, J=2.1 Hz) 7.00–7.40 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 169.5, 162.5, 161.2, 137.6, 134.1, 133.8, 128.5, 128.2, 126.1, 117.3, 104.7, 100.8, 81.6, 66.1, 55.3, 20.5. IR ($CHCl_3$, cm^{-1}): ν 1775 (br), 1700, 1600. Anal. Calcd for $C_{19}H_{18}NO_5Br$: C, 54.30; H, 4.32; N, 3.33. Found: C, 54.17; H, 4.60; N, 3.54.

3.3.5. *trans*-3-Acetoxy-1-(2-bromophenyl)-4-(2,4,6-trimethoxyphenyl)-azetidin-2-one (1e). From 390 g (2 mmol) of 2,4,6-trimethoxybenzaldehyde, 340 mg (2 mmol) of *o*-bromoaniline and 410 mg (3 mmol) of acetoxyacetyl chloride, 300 mg (33%) of compound **1e** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 2:1). Mp 166–167 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): 2.18 (s, 3H), 3.74 (s, 3H), 3.78 (s, 6H), 5.98 (d, 1H, J=2.1 Hz), 6.02 (s, 2H), 6.11 (d, 1H, J=2.1 Hz), 6.97 (m, 1H), 7.20 (m, 1H), 7.43 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 169.7, 164.4, 161.8, 160.1, 134.6, 133.5, 127.6, 127.5, 126.5, 117.0, 102.0, 90.6, 79.3, 59.1, 55.7, 55.2, 20.7. IR ($CHCl_3$, cm^{-1}): ν 1770, 1740, 1620, 1600, 1485, 1375. Anal. Calcd for $C_{20}H_{20}NO_6Br$: C, 53.35; H, 4.48; N, 3.11. Found: C, 53.54; H, 4.33; N, 3.05.

3.3.6. *trans*-3-Acetoxy-1-(2-bromophenyl)-4-(mesityl)-azetidin-2-one (1f). From 300 mg (2 mmol) of mesitaldehyde, 340 mg (2 mmol) of *o*-bromoaniline and 410 mg (3 mmol) of acetoxyacetyl chloride, 500 mg (62%) of compound **1f** was obtained as a pale yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H), 2.15 (s, 3H), 2.34 (s, 6H), 5.85 (s, 2H), 6.73 (s, 2H), 6.99 (t, 1H, J= 6.4 Hz), 7.20 (m, 1H), 7.42 (m, 2H).^{13}C NMR ($CDCl_3$): δ 169.5, 163.1, 138.3, 137.0, 134.6, 134.3, 130.8, 128.1, 127.9, 127.0, 125.5, 115.6, 79.5, 63.4, 20.8, 20.8, 20.5. IR ($CHCl_3$, cm^{-1}): ν 1760, 1490, 1380. Anal. Calcd for $C_{20}H_{20}NO_3Br$: C, 59.71; H, 5.01; N, 3.48. Found: C, 59.55; H, 5.21; N, 3.19.

3.3.7. *cis*-3-Acetoxy-4-(2-bromophenyl)-1-(4-methoxyphenyl)-azetidin-2-one (2a). From 370 mg (2 mmol) of *o*-bromobenzaldehyde, 250 mg (2 mmol) of *p*-anisidine and

340 mg (2.5 mmol) of acetoxyacetyl chloride, 460 mg (59%) of compound **2a** was obtained as a pale yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR ($CDCl_3$): δ 1.70 (s, 3H), 3.70 (s, 3H), 5.69 (d, 1H, J=5.1 Hz), 6.12 (d, 1H, J=5.1 Hz), 6.77 (d, 2H, J=9.3 Hz), 7.20 (m, 5H), 7.55 (d, 1H, J=7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 168.8, 161.6, 156.8, 133.2, 131.9, 130.2, 130.1, 129.0, 127.5, 123.8, 118.8, 114.6, 75.5, 60.8, 55.6, 20.1. IR ($CHCl_3$, cm^{-1}): ν 1760, 1740, 1370. Anal. Calcd for $C_{18}H_{16}NO_4Br$: C, 55.40; H, 4.13; N, 3.59. Found: C, 55.60; H, 4.24; N, 3.70.

3.3.8. *cis*-4-(2-Bromophenyl)-1-(4-methoxyphenyl)-3-phenoxy-azetidin-2-one (2b). From 125 mg (1 mmol) of *o*-bromobenzaldehyde, 120 mg (1 mmol) of *p*-anisidine and 260 mg (1.5 mmol) of phenoxyacetyl chloride, 320 mg (75%) of compound **2b** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 124–126 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 3.70 (s, 3H), 5.53 (d, 1H, J=5.1 Hz), 5.74 (d, 1H, J=5.1 Hz), 6.70–6.90 (m, 5H) 7.00–7.30 (m, 7H), 7.45 (d, 1H). ^{13}C NMR ($CDCl_3$): δ 162.4, 156.9, 156.4, 132.5, 131.9, 130.0, 129.8, 129.1, 129.0, 127.3, 123.6, 122.2, 118.6, 116.0, 114.3, 81.3, 60.8, 55.3. IR (KBr, cm^{-1}): ν 1760, 1600, 1515, 1300, 1130. Anal. Calcd for $C_{22}H_{18}NO_3Br$: C, 62.28; H, 4.28; N, 3.30. Found: C, 61.98; H, 4.45; N, 3.60.

3.3.9. *cis*-3-(2-Bromophenoxy)-1,4-[bis-(4-methoxyphenyl)]-azetidin-2-one (3a). From 270 g (2 mmol) of *p*-anisaldehyde, 250 mg (2 mmol) of *p*-anisidine, 580 mg of *o*-bromophenoxyacetyl acid (2.5 mmol) and 0.84 mL (2.5 mmol) of phenyldichlorophosphate, 550 mg (61%) of compound **3a** was obtained as a yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR ($CDCl_3$): δ 3.68 (s, 3H), 3.70 (s, 3H), 5.27 (d, 1H, J=4.8 Hz), 5.44 (d, 1H, J=4.8 Hz), 6.70–7.30 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 162.2, 160.0, 156.5, 153.6, 133.4, 130.4, 129.6, 128.3, 124.3, 123.3, 119.0, 115.5, 114.4, 113.9, 112.3, 81.6, 61.3, 55.4, 55.3. IR ($CHCl_3$, cm^{-1}): ν 1760, 1510, 1240. Anal. Calcd for $C_{23}H_{20}NO_4Br$: C, 60.81; H, 4.44; N, 3.08. Found: C, 60.99; H, 4.11; N, 2.87.

3.3.10. *cis*-1-Benzyl-3-(2-bromophenoxy)4-(4-methoxyphenyl)-azetidin-2-one (3b). From 140 mg (1 mmol) of *p*-anisaldehyde, 110 mg (1 mmol) of benzylamine, 280 mg (1.2 mmol) of *o*-bromophenoxyacetic acid, and 0.42 mL (1.2 mmol) of phenyldichlorophosphate, 300 mg (80%) of compound **1g** was obtained as a colorless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 3H), 3.80 (d, 1H, J=14.7 Hz), 4.66 (d, 1H, J=4.5 Hz), 4.81 (d, 1H, J=14.7 Hz), 5.31 (d, 1H, J=4.5 Hz), 6.70–7.30 (m, 13H). ^{13}C NMR ($CDCl_3$): δ 165.0, 159.9, 153.4, 134.6, 133.2, 130.0, 128.8, 128.6, 128.0, 127.9, 124.1, 123.0, 114.9, 113.7, 112.0, 82.3, 60.4, 55.2, 44.0. IR ($CHCl_3$, cm^{-1}): ν 1770, 1620, 1590, 1260, 1040. Anal. Calcd for $C_{23}H_{20}NO_3Br$: C, 63.02; H, 4.60; N, 3.20. Found: C, 62.77;H, 4.55; N, 3.33.

3.3.11. *cis*-1-Benzyl-3-(2-bromophenoxy)-4-(3,5-di-methoxyphenyl)-azetidin-2-one (3c). From 170 mg (1 mmol) of 3,5-dimethoxybenzaldehyde, 110 mg (1 mmol) of benzylamine, 280 mg (1.2 mmol) of *o*-bromophenoxyacetic acid and 0.19 mL (1.2 mmol) of phenyldichlorophosphate 260 mg (56%) of compound **3c** was obtained as a colorless oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 6H), 4.00 (d, 1H, J=14.8 Hz), 4.71 (d, 1H, J=4.7 Hz), 4.87 (d, 1H, J=14.8 Hz), 5.42 (d, 1H, J=4.7 Hz), 6.40 (t, 1H, J=2.4 Hz), 6.45 (d, 2H, J=2.4 Hz), 6.75–6.8 (m, 2H), 7.1–7.4 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 165.0, 160.6, 153.4, 134.8, 134.7, 133.3, 128.8, 128.7, 128.1, 128.0, 127.5, 123.1, 114.8, 106.5, 101.1, 82.2, 61.0, 55.4, 44.5. IR ($CHCl_3$, cm^{-1}): ν 1760, 1680, 1610, 1485. Anal. Calcd for $C_{24}H_{22}NO_4Br$: C, 61.55; H, 4.73; N, 2.99. Found: C, 61.77; H, 4.99; N, 3.11.

3.3.12. *cis*-1-(Benzyl)-3-(2-bromophenoxy)-4-(2,4-dimethoxyphenyl)-azetidin-2-one (3d). From 80 mg (0.5 mmol) of 2,4-dimethoxybenzaldehyde, 50 mg (0.5 mmol) of benzylamine, 140 mg (0.6 mmol) of *o*-bromophenoxyacetic acid and 0.16 mL (0.6 mmol), 200 mg (85%) of compound **3d** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 2:1). Mp 111–113 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 3.56 (s, 3H), 3.68 (s, 3H), 3.86 (d, 1H, J=15.0 Hz), 4.82 (d, 1H, J=15.0 Hz), 5.11 (d, 1H, J=4.5 Hz), 5.27 (d, 1H, J=4.5 Hz), 6.23 (d, 1H, J=1.8 Hz), 6.39 (dd, 1H, J=2.4, 8.4 Hz), 6.64 (t, 1H, J=7.8 Hz), 6.89 (dd, 1H, J=1.5, 8.4 Hz), 7.00 (m, 1H), 7.2 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 165.6, 160.9, 158.8, 153.6, 135.0, 135.0, 129.2, 128.75, 128.6, 127.9, 127.8, 122.7, 114.9, 113.2, 112.0, 104.1, 98.3, 82.3, 55.3, 55.2, 55.0, 44.3. IR (KBr, cm^{-1}): ν 1760, 1620, 1590, 1480. Anal. Calcd for $C_{24}H_{22}NO_4Br$: C, 61.55; H, 4.73; N, 3.00. Found: C, 61.22; H, 4.64; N, 2.85.

3.3.13. *cis*-1-Benzyl-3-(2-bromophenoxy)-4-(2,6-dimethoxyphenyl)-azetidin-2-one (3e). From 170 mg (1 mmol) of 2,6-dimethoxybenzaldehyde, 110 mg (1 mmol) of benzylamine, 280 mg (1.2 mmol) of *o*-bromophenoxyacetic acid and 0.19 mL (1.2 mmol) of phenyldichlorophosphate, 250 mg (53%) of compound **3e** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 101–102 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 3.6–3.8 (m, 6H), 3.75 (d, 1H, J=15.0 Hz), 4.72 (d, 1H, J=15.0 Hz), 5.34 (d, 1H, J=4.8 Hz), 5.45 (d, 1H, J=4.8 Hz), 6.40 (br s, 2H), 6.65 (t, 1H, J=7.3 Hz), 6.87 (d, 1H, J=8.5 Hz), 7.10–7.30 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 166.1, 153.9, 136.5, 135.6, 133.0, 130.0, 128.5, 128.5, 127.9, 127.4, 122.3, 114.1, 111.4, 107.6, 81.2, 76.2, 55.9, 53.3, 44.6. IR (KBr, cm^{-1}): ν 1760, 1600, 1485,1260. Anal. Calcd for $C_{24}H_{22}NO_4Br$: C, 61.55; H, 4.73; N, 2.99. Found: C, 61.66; H, 4.53; N, 3.21.

3.3.14. *cis*-1-Benzyl-3-(2-bromophenoxy)4-(2,4,6-trimethoxyphenyl)-azetidin-2-one (3f). From 140 mg (0.7 mmol) of 2,4,6-trimethoxybenzaldehyde, 80 mg (0.7 mmol) of benzylamine, 190 mg (0.8 mmol) of 2-bromophenoxyacetic acid and 0.27 mL (0.8 mmol) of phenyldichlorophosphate, 240 mg (71%) of compound **3f** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 132–133 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$) δ 3.70 (br s, 6H), 3.79 (s, 3H), 3.81 (d, 1H, J=15.0 Hz), 4.77 (d, 1H, J=15.0 Hz), 5.38 (d, 1H, J=5.4 Hz), 5.45 (d, 1H, J=5.4 Hz), 5.99 (br s, 1H), 6.09 (br s, 1H), 6.74 (t, 1H, J=7.8 Hz), 6.91

(dd, 1H, $J=1.5$, 8.4 Hz) 7.08–7.2 (m, 3H), 7.15–7.4 (m, 4H), 7.41 (d, 1H, $J=6.9$ Hz). ^{13}C NMR ($CDCl_3$): δ 166.2, 161.6, 154.0, 135.7, 133.1, 131.8, 128.5, 128.5, 128.5, 127.9, 127.4, 122.3, 114.2, 111.2, 110.6, 81.3, 80.4, 55.2, 53.2, 44.3. IR (KBr, cm^{-1}): ν 1760, 1615, 1490. Anal. Calcd for $C_{25}H_{24}NO_5Br$: C, 60.25; H, 4.85; N, 2.81. Found: C, 59.99; H, 5.11; N, 2.71.

3.3.15. *cis*-1-Benzyl-3-(2-bromophenoxy)-4-(mesityl)-azetidin-2-one (3g). From 150 mg (1 mmol) of mesitaldehyde, 110 mg (1 mmol) of benzylamine, 280 mg (1.2 mmol) of *o*-bromophenoxyacetic acid, and 0.19 mL (1.2 mmol) of phenyldichlorophosphate, 290 mg (68%) of compound **3g** was obtained as a yellowish oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3H), 2.17 (s, 3H), 2.49 (s, 3H), 3.64 (d, 1H, $J=14.4$ Hz), 4.88 (d, 1H, $J=14.4$ Hz), 5.04 (d, 1H, $J=5.0$ Hz), 5.35 (d, 1H, $J=5.0$ Hz), 6.70–7.30 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 165.1, 153.9, 139.0, 137.9, 137.1, 134.5, 133.3, 131.9, 129.5, 128.8, 128.1, 124.8, 123.2, 115.2, 112.4, 82.5, 57.0, 44.9, 22.1, 21.0, 20.9. IR ($CHCl_3$, cm^{-1}): ν 1760, 1610, 1575. Anal. Calcd for $C_{25}H_{24}NO_2Br$: C, 66.67; H, 5.37; N, 3.11. Found: C, 66.81; H, 5.65; N, 2.97.

3.3.16. *cis*-1-(2-Bromobenzyl)-3-phenoxy-4-(4-methoxyphenyl)-azetidin-2-one (4a). From 126 mg (0.9 mmol) of *p*-anisaldehyde, 169 mg (0.9 mmol) of 2-bromobenzylamine and 230 mg (1.3 mmol) of phenoxyacetic chloride, 230 mg (57%) of compound **4a** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 2:1). Mp 144–146 °C (hexanes–AcOEt). ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3H), 4.16 (d, 1H, $J=14.8$ Hz), 4.80 (d, 1H, $J=4.4$ Hz), 4.95 (d, 1H, $J=14.8$ Hz), 5.45 (d, 1H, $J=4.4$ Hz), 6.75–7.40 (m, 12H), 7.60 (d, 1H, $J=8.0$ Hz). ^{13}C NMR ($CDCl_3$): δ 165.5, 159.6, 156.7, 136.4, 133.9, 132.8, 131.0, 129.7, 128.9, 127.5, 124.3, 123.7, 121.7, 115.3, 113.4, 81.8, 61.4, 55.0, 44.2. IR ($CHCl_3$, cm^{-1}): ν 1750, 1720. Anal. Calcd for $C_{23}H_{20}NO_3Br$: C, 63.02; H, 4.60; N, 3.20. Found: C, 63.20; H, 4.88; N, 3.05.

3.3.17. *cis*-3-Acetoxy-1-(2-bromobenzyl)-4-(3,5-dimethoxyphenyl)-azetidin-2-one (4b). From 270 mg (1.6 mmol) of 3,5-dimethoxybenzaldehyde, 300 mg (2.3 mmol) of 2-bromobenzylamine and 330 mg (2.4 mmol) of acetoxyacetyl chloride, 340 mg (49%) of compound **4b** was obtained as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ 1.69 (s, 3H), 3.68 (s, 6H), 4.15 (d, 1H, $J=14.8$ Hz), 4.63 (d, 1H, $J=4.7$ Hz), 4.81 (d, 1H, $J=14.8$ Hz), 5.73 (d, 1H, $J=4.7$ Hz), 6.25 (d, 2H, $J=2.3$ Hz), 6.32 (t, 1H, $J=2.3$ Hz), 7.05–7.20 (m, 3H), 7.46 (d, 1H, $J=7.0$ Hz). ^{13}C NMR ($CDCl_3$): δ 169.1, 164.7, 135.1, 133.8, 133.1, 131.2, 129.8, 127.8, 127.2, 123.9, 106.1, 100.7, 77.1, 61.6, 55.3, 45.1, 20.0. IR ($CHCl_3$, cm^{-1}): ν 1760, 1720, 1370. Anal. Calcd for $C_{20}H_{20}NO_5Br$: C, 55.31; H, 4.64; N, 3.22. Found: C, 55.61; H, 4.29; N, 3.12.

3.3.18. *cis*-3-Acetoxy-1-(2-bromobenzyl)-4-(2,4-dimethoxyphenyl)-azetidin-2-one (4c). From 240 mg (1.4 mmol) of 2,4-dimethoxybenzaldehyde, 260 mg (1.4 mmol) of 2-bromobenzylamine and 280 mg (2.2 mmol) of acetoxyacetyl chloride, 410 mg (65%) of compound **4c** was obtained as a yellow solid after purification by flash chromatography (hexanes/ethyl acetate, 2:1). Mp 145–147 °C (hexanes/AcOEt). ^{1}H NMR ($CDCl_3$): δ 1.73 (s, 3H), 3.69 (s, 3H), 3.80 (s, 3H), 4.12 (d, 1H, $J=15.0$ Hz), 4.85 (d, 1H, $J=15.0$ Hz), 5.15 (d, 1H, 4.5 Hz), 5.81 (d, 1H, $J=4.5$ Hz), 6.38 (d, 1H, $J=2.4$ Hz), 6.45 (d, 1H, $J=8.7$ Hz), 7.08–7.25 (m, 4H), 7.50 (d, 1H, $J=8.1$ Hz). ^{13}C NMR ($CDCl_3$): δ 168.9, 165.4, 161.0, 159.1, 134.1, 133.0, 130.8, 129.6, 129.3, 127.7, 123.9, 113.0, 104.1, 98.4, 77.0, 55.6, 55.5, 55.3, 44.8, 20.0. IR (KBr, cm^{-1}): ν 1770, 1750, 1620,1600. Anal. Calcd for $C_{20}H_{20}NO_5Br$: C, 55.31; H, 4.64; N, 3.22. Found: C, 55.11; H, 4.75; N, 3.03.

3.3.19. *cis*-3-Acetoxy-1-(2-bromobenzyl)-4-(2,6-dimethoxyphenyl)-azetidin-2-one (4d). From 380 mg (2.3 mmol) of 2,6-dimethoxybenzaldehyde, 430 mg (2.3 mmol) of 2-bromobenzylamine and 460 mg (3.4 mmol) of acetoxyacetyl chloride, 500 mg (51%) of compound **4d** was obtained as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ 1.77 (s, 3H), 3.77 (s, 3H), 3.99 (d, 1H, $J=15.2$ Hz), 4.77 (d, 1H, $J=15.2$ Hz), 5.52 (d, 1H, $J=4.9$ Hz), 5.87 (d, 1H, $J=4.9$ Hz), 6.52 (d, 2H, $J=8.4$ Hz), 7.00–7.30 (m, 4H), 7.50 (d, 1H, $J=7.9$ Hz). ^{13}C NMR ($CDCl_3$): δ 169.2, 166.1, 135.1, 134.8, 132.7, 130.6, 130.2, 129.2, 127.4, 123.8, 107.7, 104.0, 76.2, 55.9, 53.7, 45.0, 20.1. IR ($CHCl_3$, cm^{-1}): ν 1760, 1600, 1480, 1120. Anal. Calcd for $C_{20}H_{20}NO_5Br$: C, 55.31; H, 4.64; N, 3.22. Found:C, 55.47; H, 4.55; N, 3.15.

3.3.20. *cis*-3-Acetoxy-1-(2-bromobenzyl)-4-(2,4,6-trimethoxyphenyl)-azetidin-2-one (4e). From 320 mg (1.6 mmol) of 2,4,6-trimethoxybenzaldehyde, 300 mg (1.6 mmol) of 2-bromobenzylamine and 320 mg (2.4 mmol) of acetoxyacetyl chloride, 600 mg (80%) of compound **4e** was obtained as a yellowish oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 1.73 (s, 3H), 3.66 (s, 3H), 3.73 (s, 6H), 3.86 (d, 1H, $J=15.0$ Hz), 4.65 (d, 1H, $J=15.0$ Hz), 5.33 (d, 1H, $J=5.1$ Hz), 5.73 (d, 1H, $J=5.1$ Hz), 6.00 (s, 2H), 7.00–7.20 (m, 3H), 7.41 (d, 1H, $J=6.9$ Hz). ^{13}C NMR ($CDCl_3$): δ 169.3, 166.2, 161.6, 134.8, 132.7, 130.5, 129.1, 127.3, 123.7, 99.9, 90.6, 90.1, 76.2, 55.8, 55.2, 53.5, 44.7, 20.2. IR ($CHCl_3$, cm^{-1}): ν 1760, 1615. Anal. Calcd for $C_{21}H_{22}NO_6Br$: C, 54.32; H, 4.78; N, 3.02. Found: C, 54.40; H, 4.58; N, 3.15.

3.3.21. 3-Acetoxy-1-(2-bromobenzyl)-4-mesityl-azetidin-2-one (4f). From 180 mg (1.2 mmol) of mesitaldehyde, 220 mg (1.2 mmol) of 2-bromobenzylamine and 250 mg (1.9 mmol) of acetoxyacetyl chloride, 150 mg (66%) of compound **4f** was obtained as a *cis–trans* mixture (70:30). The *cis* isomer was isolated after purification by flash chromatography (hexanes/ethyl acetate, 2:1).

Data for cis-isomer. Colourless oil. ^{1}H NMR ($CDCl_3$): δ 2.12 (br s, 9H), 2.26 (s, 3H), 3.87 (d, 1H, $J=14.8$ Hz), 4.69 (s, 1H), 4.96 (d, 1H, $J=14.8$ Hz), 5.76 (s, 3H), 6.81 (s, 2H), 7.10–7.30 (m, 3H), 7.57 (d, 1H, $J=7.5$ Hz). ^{13}C NMR ($CDCl_3$): δ 169.6, 164.1, 138.2, 137.0, 133.7, 133.7, 133.0, 131.3, 129.7, 127.7, 126.2, 124.0, 79.9, 59.6, 45.3, 20.8, 20.6, 20.5. IR ($CHCl_3$, cm^{-1}): ν 1765, 1450, 1400, 1380. Anal. Calcd for $C_{21}H_{22}NO_3Br$: C, 60.59; H, 5.33; N, 3.36. Found: C, 60.28; H, 5,11; N, 3.22.

3.4. General procedure for the radical cyclization reaction. Synthesis of dibenzocarbacephems 5 from haloaryl-β-lactams 1

A solution of the corresponding 2-azetidinone **1** (1 mmol), Bu_3SnH (1.2 mmol), and AIBN (25% w/w) in dry benzene (215 mL) was refluxed under argon atmosphere until complete disappearance of the starting substrate (TLC, 2–4 h). After cooling, the resulting crude reaction mixture was treated with 10% aqueous solution of KF (200 mL) and was stirred for 2 h at room temperature. The organic layer was separated, dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography of the residue eluting with hexanes/ethyl acetate mixtures gave analytically pure compound **5**.

3.4.1. Dibenzocarbacephem 5a. From 300 mg (0.8 mmol) of compound **1a**, 220 mg (70%) of compound **5a** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 4:1). Mp 138–140 °C (hexanes/ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3H), 3.81 (s, 3H), 4.83 (s, 1H), 5.68 (d, 1H, J=1.5 Hz), 6.88 (dd, 1H, J=1.8, 8.4 Hz), 7.19–7.30 (m, 4H), 7.61 (d, 1H, J=8.4 Hz), 7.75 (dd, 1H, J=0.9, 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 170.2, 163.0, 160.0, 132.0, 131.6, 129.3, 127.4, 126.1, 125.6, 123.8, 123.2, 121.0, 114.5, 109.5, 82.9, 58.4, 55.6, 20.7. IR (KBr, cm^{-1}): ν 1785, 1750, 1630, 1575, 1500. Anal. Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.55; H, 4.99; N, 4.19.

3.4.2. Dibenzocarbacephem 5b. From 300 mg (0.8 mmol) of compound **1b**, 150 mg (62%) of compound **11b** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 160–162 °C (hexanes/ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3H), 2.34 (s, 3H), 3.82 (s, 3H), 4.80 (s, 1H), 5.66 (d, 1H, J=1.5 Hz), 6.87 (dd, 1H, J=2.1, 8.4 Hz), 7.10 (d, 1H, J=8.4 Hz), 7.26 (d, 1H, J=1.2 Hz), 7.33 (d, 1H, J=7.8 Hz), 7.55 (s, 1H), 7.61 (d, 1H, J=8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 170.1, 162.7, 159.7, 135.6, 131.5, 129.7, 129.4, 127.1, 125.2, 124.1, 123.1, 120.6, 114.2, 109.2, 82.7, 58.2, 55.4, 23.3, 20.5. IR (KBr, cm^{-1}): ν 1780, 1645, 1620, 1500, 1430. Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.75; H, 5.22; N, 4.09.

3.4.3. Dibenzocarbacephem 5c. From 300 mg (0.8 mmol) of compound **1b**, 140 mg (66%) of compound **11b** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H), 4.94 (s, 1H), 5.80 (d, 1H, J=1.5 Hz), 7.20–7.40 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 170.0, 162.8, 130.6, 129.1, 129.0, 128.8, 126.3, 126.0, 126.0, 125.5, 123.7, 123.7, 120.8, 117.6, 82.9, 58.4, 20.7. IR ($CHCl_3$, cm^{-1}): ν 1780, 1755, 1500. Anal. Calcd for $C_{17}H_{13}NO_3$: C, 73.11; H, 4.69; N, 5.01. Found: C, 73.44; H, 4.55; N, 4.83.

3.4.4. Dibenzocarbacephem 5d. From 100 mg (0.2 mmol) of compound **1d**, 40 mg (51%) of compound **5d** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR($CDCl_3$): δ 2.19 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 4.71 (s, 1H), 5.67 (s, 1H), 6.45 (s, 1H), 6.95 (s, 1H), 7.20 (m, 2H), 7.43 (d, 1H, J=6.4 Hz), 8.42 (d, 1H, J=7.4 Hz). ^{13}C NMR ($CDCl_3$): δ 170.3, 161.5, 161.4, 160.8, 159.1, 134.9, 131.2, 128.8, 127.1, 125.5, 124.9, 120.3, 83.0, 58.5, 55.7, 55.4, 20.6. IR ($CHCl_3$, cm^{-1}): ν 1780, 1615, 1180. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.36; H, 4.92; N, 4.37.

3.5. Preparation of dibenzocarbapenem 5e and *N*-biaryl-β-lactam 8a by radical reaction of haloaryl β-lactam 1e

According to the general procedure described in Section 3.4. From 180 mg (0.4 mmol) of β-lactam **1e**, and after flash chromatography eluting with hexanes/ethyl acetate (2:1), 50 mg (38%) of the less polar compound **5e** and 20 mg (14%) of the more polar compound **8a** were obtained.

3.5.1. Dibenzocarbapenem 5e. White solid. Mp 111–113 °C. ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 4.79 (d, 1H, J=0.7 Hz), 5.76 (d, 1H, J=1.3 Hz), 6.54 (d, 1H, J=2.4 Hz), 7.03 (d, 1H, J=0.7 Hz), 7.25 (m, 2H), 7.50 (dd, 1H, J=2.0, 7.0 Hz), 8.51 (dd, 1H, J=1.3, 2.4 Hz). ^{13}C NMR ($CDCl_3$): δ 170.3, 161.5, 160.8, 159.2, 134.9, 131.2, 128.8, 127.1, 125.4, 124.9, 120.3, 120.3, 101.6, 99.7, 83.0, 58.5, 55.7, 55.5, 20.6. IR (KBr, cm^{-1}): ν 1770, 1615, 1170. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.48; H, 5.00; N, 4.27.

3.5.2. *N*-Biaryl-β-lactam 8a. Colourless oil. ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3H), 2.87 (dd, 1H, J=2.1, 7.1 Hz), 3.30 (dd, 1H, J=5.1, 7.1), 3.64 (s, 6H), 3.81 (s, 3H), 5.47 (dd, 1H, J=2.1, 5.1 Hz), 6.11 (s, 2H), 7.05–7.30 (m, 3H), 7.97 (d, 1H, J=8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 169.9, 161.6, 158.9, 158.8, 136.3, 133.0, 127.8, 124.9, 124.4, 121.8, 90.4, 90.2, 74.3, 55.8, 55.3, 50.5, 20.5. IR ($CHCl_3$, cm^{-1}): ν 1760, 1615, 1600. Anal. Calcd for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.58; H, 5.53; N, 3.53.

3.6. Preparation of dibenzocarbapenem 5f by radical reaction of haloaryl β-lactam 1f

According to the general procedure described in Section 3.4. From 100 mg (0.2 mmol) of β-lactam **1f** a mixture (8:1) of compounds **5f** and **8b** was obtained, together with some minor dehalogenated starting material. After flash chromatography eluting with hexanes/ethyl acetate (2:1), 50 mg (58%) of compound **5f** was obtained as a white solid. Mp 160–162 °C (hexanes/ethyl acetate). ^{1}H NMR ($CDCl_3$): 2.11 (s, 3H), 2.18 (s, 3H), 2.26 (br s, 3H), 5.27 (s, 1H), 5.72 (s, 1H), 6.70 (s, 2H), 7.00 (m, 1H), 7.15–7.20 (m, 3H). IR (KBr, cm^{-1}): ν 1770, 1615. Anal. Calcd for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.58; N, 4.56. Found: C, 74.33; H, 5.41; N, 4.28. Compound **8b** could not be obtained in pure form.

3.7. Preparation of fused biaryl-β-lactam 6a by radical reaction of haloaryl β-lactam 2b

According to the general procedure described in Section 3.4. From 100 mg (0.2 mmol) of β-lactam **2b**, 20 mg (15%) of compound **6a** was obtained as a pale yellow oil after flash chromatography eluting with hexanes/ethyl acetate (2:1). ^{1}H NMR ($CDCl_3$): δ 3.61 (s, 3H), 5.04 (d, 1H, J=5.0 Hz), 5.75 (d, 1H, J=5.0 Hz), 6.61 (d, 2H, J=9.0 Hz), 7.00 (d, 2H, J=9.0 Hz), 7.10–7.50 (m, 7H), 7.51 (d, 1H, J=7.4 Hz).

^{13}C NMR ($CDCl_3$): δ 161.9, 156.4, 152.7, 138.1, 133.2, 132.4, 131.9, 131.8, 130.7, 130.0, 129.5, 129.4, 128.0, 126.3, 123.4, 118.8, 114.1, 93.4, 64.6, 55.3. IR ($CHCl_3$, cm^{-1}): ν 1755, 1420. Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.74; H, 4.82; N, 3.90.

3.8. Preparation of compounds 6b and 7a by radical reaction of haloaryl β-lactam 3a

According to the general procedure described in Section 3.4. From 150 mg (0.3 mmol) of β-lactam **3a**, and after flash chromatography eluting with hexanes/ethyl acetate (2:1), 10 mg (10%) of the less polar compound **6b** and 50 mg (40%) of the more polar compound **7a** were obtained.

3.8.1. Fused biaryl-β-lactam 6b. Colourless oil. ^{1}H NMR ($CDCl_3$): δ 3.61 (s, 3H), 3.79 (s, 3H), 5.01 (d, 1H, J= 5.4 Hz), 5.70 (d, 1H, J=5.4 Hz), 6.61 (d, 2H, J=9.3 Hz), 6.89 (m, 2H), 7.00 (d, 2H, J=9.3 Hz), 7.10–7.30 (m, 4H), 7.41 (d, 1H, J=9.0 Hz). ^{13}C NMR ($CDCl_3$): δ 162.0, 160.7, 156.5, 152.8, 139.5, 133.9, 133.2, 130.8, 129.7, 129.4, 126.4, 124.1, 123.6, 119.0, 117.3, 114.2, 113.3, 93.2, 64.2, 55.5, 55.4. IR ($CHCl_3$, cm^{-1}): ν 1760, 1610, 1520. Anal. Calcd for $C_{23}H_{19}NO_4$: C, 73.98; H, 5.13; N, 3.75. Found: C, 73.64; H, 4.95; N, 3.95.

3.8.2. 3-(4′-Methoxybiphenyl-2-yloxy)-1-(4-methoxyphenyl)-azetidin-2-one 7a. Colourless oil. ^{1}H NMR ($CDCl_3$): δ 3.57 (dd, 1H, J=2.1, 6.0 Hz), 3.72 (s, 3H), 3.77 (s, 3H), 3.88 (dd, 1H, J=4.8, 6.0 Hz), 5.29 (dd, 1H, J=2.1, 4.8 Hz), 6.74 (m, 1H), 6.81 (d, 2H, J=9.0 Hz), 6.89 (d, 2H, J=9.0 Hz), 7.05 (m, 1H), 7.23 (m, 2H), 7.23 (d, 2H, J=9.0 Hz), 7.43 (d, 2H, J=9.0 Hz). ^{13}C NMR ($CDCl_3$): δ?162.1, 158.8, 156.6, 154.3, 130.9, 130.6, 129.4, 129.2, 128.3, 122.8, 118.9, 118.0, 114.5, 113.9, 113.6, 79.4, 55.5, 55.3, 47.5. IR ($CHCl_3$, cm^{-1}): ν 1760, 1520. Anal. Calcd for $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73. Found: C, 73.34; H, 5.36; N, 3.55.

3.9. Preparation of compounds 6c and 7b by radical reaction of haloaryl β-lactam 3b

According to the general procedure described in Section 3.4. From 100 mg (0.2 mmol) of β-lactam **3b**, and after flash chromatography eluting with hexanes/ethyl acetate (2:1), 20 mg (24%) of the less polar compound **6c** and 30 mg (40%) of the more polar compound **7b** were obtained.

3.9.1. Fused biaryl-β-lactam 6c. Colourless oil. ^{1}H NMR ($CDCl_3$): δ 3.74 (d, 1H, J=15.3 Hz), 3.78 (s, 3H), 4.22 (d, 1H, J=15.3 Hz), 4.42 (d, 1H, J=4.8 Hz), 5.56 (d, 1H, J= 4.8 Hz), 6.75 (m, 1H), 6.86 (m, 2H), 6.96 (d, 1H, J= 9.3 Hz), 7.10–7.40 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 164.5, 152.7, 134.7, 133.5, 133.2, 129.6, 129.4, 129.1, 128.7, 128.6, 128.5, 127.6, 126.1, 123.8, 123.6, 117.0, 112.9, 93.8, 63.0, 55.4, 44.7. IR ($CHCl_3$, cm^{-1}): ν 1760, 1620. Anal. Calcd for $C_{23}H_{19}NO_3$: C, 77.29; H, 5.36; N, 3.92. Found: C, 77.14; H, 5.12; N, 3.68.

3.9.2. 1-Benzyl-3-(4′-methoxybiphenyl-2-yloxy)-azetidin-2-one 7b. Pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 3.12 (dd, 1H, J=2.1, 5.7 Hz), 3.37 (t, 1H, J=5.7 Hz), 3.77 (s, 3H), 4.35 (s, 2H), 5.17 (dd, 1H, J=2.1, 4.5 Hz), 6.87 (d, 2H, J= 8.4 Hz), 7.00 (m, 1H), 7.10–7.30 (m, 8H), 7.40 (d, 2H, J= 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 165.8, 158.7, 154.3, 137.4, 134.8, 130.9, 130.8, 130.6, 130.2, 128.9, 128.3, 128.3, 128.0, 122.7, 113.5, 80.4, 55.3, 47.6, 45.9. IR ($CHCl_3$, cm^{-1}): ν 1760. Anal. Calcd for $C_{23}H_{21}NO_3$: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.70; H, 5.65; N, 3.64.

3.10. Preparation of fused biaryl-β-lactam 6d by radical reaction of haloaryl β-lactam 3c

According to the general procedure described in Section 3.4. From 100 mg (0.2 mmol) of β-lactam **3c**, 60 mg (60%) of compound **6d** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 3.66 (d, 1H, J= 14.8 Hz), 3.66 (s, 3H), 3.73 (s, 3H), 4.23 (d, 1H, J= 14.8 Hz), 4.30 (d, 1H, J=4.7 Hz), 5.49 (d, 1H, J=4.7 Hz), 6.16 (d, 1H, J=2.3 Hz), 6.51 (d, 1H, J=2.3 Hz), 6.90 (m, 2H), 7.10–7.30 (m, 6H), 7.35 (dd, 1H, J=1.7, 7.8 Hz). ^{13}C NMR ($CDCl_3$): δ 164.3, 159.7, 158.2, 152.3, 133.7, 132.4, 128.7, 128.5, 128.4, 128.1, 127.7, 124.5, 123.3, 118.6, 108.9, 99.9, 93.9, 63.5, 55.9, 55.3, 44.9. IR ($CHCl_3$, cm^{-1}): ν 1750. Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.22; H, 5.37; N, 3.29.

3.11. Preparation of 1-benzyl-3-(2′,4′-dimethoxybiphenyl-2-yloxy)-azetidin-2-one 7c by radical reaction of haloaryl β-lactam 3d

According to the general procedure described in Section 3.4. From 150 mg (0.3 mmol) of β-lactam **3d**, 50 mg (45%) of compound **7c** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 3.06 (dd, 1H, J=2.0, 6.0 Hz), 3.34 (dd, 1H, J=5.0, 6.0 Hz), 3.61 (s, 3H), 3.76 (s, 3H), 4.32 (AB system, 2H, J=15.1 Hz), 5.13 (dd, 1H, J= 2.0, 5.0 Hz), 6.40–6.50 (m, 2H), 6.99 (t, 1H, J=7.0 Hz), 7.10–7.30 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 166.0, 160.4, 157.8, 155.2, 134.8, 131.7, 131.6, 128.8, 128.4, 128.4, 128.2, 127.9, 122.3, 119.9, 114.9, 104.2, 98.6, 80.7, 55.4, 55.3, 47.7, 45.8. IR ($CHCl_3$, cm^{-1}): ν 1760, 1610, 1500. Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 75.19; H, 6.20; N, 3.38.

3.12. Preparation of 1-benzyl-3-(2′,4′,6′-trimethoxybiphenyl-2-yloxy)-azetidin-2-one 7d by radical reaction of haloaryl β-lactam 3f

According to the general procedure described in Section 3.4. From 100 mg (0.2 mmol) of β-lactam **3f**, 70 mg (80%) of compound **7d** was obtained as a yellowish oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 3.12 (dd, 1H, J=2.0, 5.7 Hz), 3.39 (dd, 1H, J=4.7, 5.7 Hz), 3.61 (s, 3H), 3.71 (s, 3H), 3.85 (s, 3H), 4.38 (AB system, 2H, J=15.1 Hz), 5.17 (dd, 1H, J=2.0, 4.7 Hz), 6.16 (d, 1H, J=2.4 Hz), 6.21 (d, 1H, J=2.4 Hz), 7.00–7.40 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 166.2, 160.9, 158.7, 158.4, 155.9, 135.1, 133.1, 129.0, 128.5, 128.0, 124.5, 122.2, 115.3, 90.9, 90.8, 81.0, 56.0, 55.7, 55.4, 48.0, 45.9. IR ($CHCl_3$, cm^{-1}): ν 1760, 1620, 1600. Anal. Calcd for $C_{25}H_{25}NO_5$: C, 71.58; H, 6.01; N, 3.34. Found: C, 71.43; H, 6.23; N, 3.11.

3.13. Preparation of 1-benzyl-3-(2′,4′,6′-trimethyl-biphenyl-2-yloxy)-azetidin-2-one 7e by radical reaction of haloaryl β-lactam 3g

According to the general procedure described in Section 3.4. From 140 mg (0.2 mmol) of β-lactam **3g**, 80 mg (65%) of compound **7e** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 1.85 (s, 3H), 1.92 (s, 3H), 2.23 (s, 3H), 2.95 (dd, 1H, J=2.0, 6.0 Hz), 3.25 (dd, 1H, J=5.0, 6.0 Hz), 4.30 (s, 2H), 5.09 (dd, 1H, J=2.0, 5.0 Hz), 6.82 (d, 2H, J=7.8 Hz), 6.95–7.30 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 165.9, 154.7, 136.7, 136.5, 135.9, 134.8, 134.6, 131.4, 130.1, 128.9, 128.2, 127.9, 127.8, 122.5, 114.8, 80.3, 47.6, 45.8, 21.1, 20.4. IR ($CHCl_3$, cm^{-1}): ν 760. Anal. Calcd for $C_{25}H_{25}NO_2$: C, 80.83; H, 6.78; N, 3.77. Found: C, 81.02; H, 6.59; N, 3.70.

3.14. Preparation of 3-acetoxy-1-(2′,4′-dimethoxy-biphenyl-2-ylmethyl)-azetidin-2-one 9a by radical reaction of haloaryl β-lactam 4c

According to the general procedure described in Section 3.4. From 200 mg (0.5 mmol) of β-lactam **4c**, 70 mg (40%) of compound **9a** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3H), 2.86 (d, 1H, J=6.0 Hz), 3.27 (s, 1H), 3.68 (s, 3H), 3.78 (s, 3H), 4.24 (s, 2H), 5.35 (s, 1H), 6.50 (m, 2H), 7.05 (m, 2H), 7.30 (m, 3H). IR ($CHCl_3$, cm^{-1}): ν 1760, 1615, 1590. Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.66; H, 6.10; N, 4.03.

3.15. Preparation of 3-acetoxy-1-(2′,4′,6′-trimethoxy-biphenyl-2-ylmethyl)-azetidin-2-one 9b by radical reaction of haloaryl β-lactam 4e

According to the general procedure described in Section 3.4. From 230 mg (0.5 mmol) of β-lactam **4e**, 170 mg (65%) of compound **9b** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 3H), 2.95 (dd, 1H, J=1.5, 6.3 Hz), 3.32 (dd, 1H, J=4.8, 6.3 Hz), 3.69 (s, 6H), 3.86 (s, 3H), 4.24 (AB system, 2H, J=15.0 Hz), 5.44 (dd, 1H, J=1.5, 4.8 Hz), 6.22 (s, 2H), 7.15 (m, 1H), 7.33 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 169.7, 164,3, 161.0, 158.0, 158.0, 134.1, 134.0, 132.0, 128.5, 127.4, 90.7, 90.5, 74.3, 55.5, 55.3, 47.6, 43.8, 20.4. IR ($CHCl_3$, cm^{-1}): ν 1760, 1615, 1590. Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.21; H, 6.33; N, 3.44.

3.16. Preparation of 3-acetoxy-1-(2′,4′,6′-trimethyl-biphenyl-2-ylmethyl)-azetidin-2-one 9c by radical reaction of haloaryl β-lactam 4f

According to the general procedure described in Section 3.4. From 70 mg (0.2 mmol) of β-lactam **4f**, 30 mg (50%) of compound **9c** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR ($CDCl_3$): δ 1.82 (s, 6H), 2.04 (s, 3H), 2.26 (s, 3H), 2.97 (dd, 1H, J=2.1, 6.0 Hz), 3.33 (dd, 1H, J=4.5, 6.0 Hz), 4.02 (AB system, 2H, J=15.6 Hz), 5.49 (dd, 1H, J=2.1, 4.5 Hz), 6.87 (s, 2H), 7.05 (m, 1H), 7.30 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 169.8, 164.7, 140.2, 137.1, 135.7, 135.5, 132.6, 130.0, 128.3, 128.3, 128.2, 127.6, 74.7, 48.2, 43.1, 26.8, 21.0, 20.5, 20.3. IR ($CHCl_3$, cm^{-1}): ν 1760. Anal. Calcd for $C_{21}H_{23}NO_3$: C, 74.75; H, 6.87; N, 4.15. Found: C, 74.94; H, 6.75; N, 3.91.

3.17. General procedure for the synthesis of dibenzo-carbacephems 5c and 5g–i by Staudinger ketene–phenantridine cycloaddition

To a solution of phenantridine (1 mmol) in anhydrous dichloromethane (15 mL), Et_3N (0.42 mL, 3 mmol) and the corresponding acid or acid chloride (1.5 mmol), [and $PhOP(O)Cl_2$, only when (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl-acetic acid is used, 0.22 mL, 1.5 mmol] were successively added under argon, and the resulting mixture was stirred at room temperature for 16 h. The crude mixture was diluted with dichloro-methane (10 mL) and washed with saturated $NaHCO_3$ (2×5 mL) and brine (10 mL) and water (10 mL). After drying with $MgSO_4$ the product was purified by column chromatography to afford the tetracyclic 2-azetidinone **5**.

3.17.1. Dibenzocarbacephem 5c. From 180 mg (1 mmol), of phenantridine and 205 mg (1.5 mmol) of acetoxyacetyl chloride, 30 mg (15%) of compound **5c** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). See data in Section 3.4.3.

3.17.2. Dibenzocarbacephem 5g. From 180 mg (1 mmol), of phenantridine and 260 mg (1.5 mmol) of phenoxyacetyl chloride, 200 mg (65%) of compound **5g** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 2:1). Mp 118–119 °C. ^{1}H NMR ($CDCl_3$): δ 5.16 (s, 1H), 5.42 (d, 1H, J=1.8 Hz), 7.03 (t, 1H), 7.18–7.34 (m, 9H), 7.47 (d, 1H, J=6.6 Hz), 7.80 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 165.1, 157.5, 132.0, 131.4, 130.4, 129.9, 129.3, 128.9, 128.7, 125.9, 125.4, 125.3, 124.0, 123.7, 123.0, 121.0, 116.7, 89.2, 57.7. IR (KBr, cm^{-1}): ν 1760, 1600, 1490. Anal. Calcd for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.82; N, 4.47. Found: C, 80.38; H, 4.96; N, 4.39.

3.17.3. Dibenzocarbacephem 5h. From 360 mg (2 mmol), of phenantridine and 540 mg (3 mmol) of phenoxyacetyl chloride, 460 mg (70%) of compound **5h** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 2:1). Mp 149–151 °C. ^{1}H NMR ($CDCl_3$): δ 4.88 (d, 1H, J=11.7 Hz), 4.97 (d, 1H, J=1.8 Hz), 5.02 (d, 1H, J=11.7 Hz), 5.03 (s, 1H), 7.06 (d, 1H, J=7.3 Hz), 7.20–7.55 (m, 10H), 7.84 (t, 2H, J=7.8 Hz). ^{13}C NMR ($CDCl_3$): δ 166.0, 136.8, 132.0, 131.6, 130.2, 129.0, 128.7, 128.6, 128.3, 128.3, 128.1, 125.6, 125.2, 125.1, 123.8, 123.5, 120.7, 90.5, 72.8, 57.6. IR (KBr, cm^{-1}): ν 1760, 1500, 1460. Anal. Calcd for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N, 4.28. Found: C, 80.45; H, 5.10; N, 4.37.

3.17.4. (+)-Dibenzocarbacephem 5i. From 180 mg (1 mmol), of phenantridine, 330 mg (1.5 mmol) of (*S*)-(2-oxo-4-phenyl-oxazolidin-3-yl)-acetic acid, and 220 μL (1.5 mmol) of phenyl dichlorophosphate, 200 mg (70%) of compound **5h** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). $[\alpha]_D$ +343 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$):

δ 4.21 (d, 1H, $J=1.8$ Hz), 4.37 (dd, 1H, $J=6.0$, 9.0 Hz), 4.79 (t, 1H, $J=9.0$ Hz), 5.20 (dd, 1H, $J=6.0$, 9.0 Hz), 5.27 (d, 1H, $J=1.8$ Hz), 7.10–7.40 (m, 6H) 7.45 (m, 3H), 7.70 (t, 2H, $J=7.5$ Hz). ^{13}C NMR ($CDCl_3$): δ 165.1, 157.5, 132.0, 131.4, 130.4, 129.9, 129.3, 128.9, 128.7, 125.9, 125.4, 125.3, 124.0, 123.7, 123.0, 121.0, 116.7, 89.2, 57.7. IR (KBr, cm^{-1}): ν 1760, 1610, 1500, 1450. Anal. Calcd for $C_{24}H_{18}N_2O_3$: C, 75.38; H, 4.74; N, 7.33. Found: C, 75.31; H, 4.92; N, 7.47.

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Tuning the reactivity and chemoselectivity of electron-poor pyrroles as dienophiles in cycloadditions with electron-rich dienes

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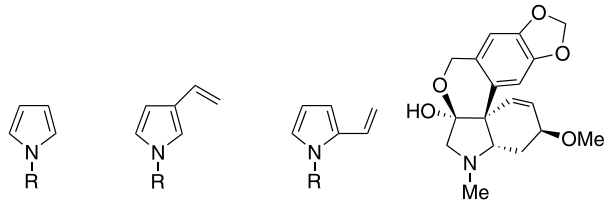
Abstract—Activation by Lewis acid catalysis and high pressure allows pyrrole derivatives to react with electron-rich dienes in normal electron demand [4+2] cycloadditions, provided that the aromatic ring is substituted by at least two electron-withdrawing groups. The dienophilic behavior of the heterocycle is expressed through the involvement of either the aromatic carbon–carbon double bond in an all-carbon process or the carbonyl moiety of the substituent in a heterocycloaddition reaction. In this regard, the nature of the heterocyclic substituents is shown to have a dramatic influence and to direct both the reactivity and the chemoselectivity of the cycloaddition.

1. Introduction

The Diels–Alder (DA) cycloaddition represents an efficient tool for the convergent and stereocontrolled synthesis of functionalized polycyclic systems.[1] The scope of the reaction is large and allows the synthesis of cyclohexene derivatives but also of a variety of heterocycles by swapping carbon atoms on the diene and/or dienophile with heteroatoms (O, N, S for instance; the so-called hetero-Diels–Alder reaction (HDA)).[2]

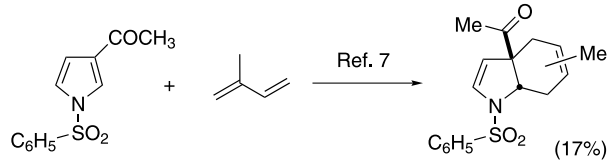
The use of five-membered aromatic heterocycles in these cycloadditions is almost as old as the reaction itself, and different possibilities have been described, depending on the substrate.[3] In this context, pyrrole **1** was soon considered as diene by involving either all four π-electrons of the heterocycle or only two of them, as in **2** and **3** (Fig. 1).[4]



1 **2** **3** **6**

Figure 1. Structures of pyrroles **1–3** and tazettine **6**.

In contrast, the literature mentions only a few reports on pyrrole derivatives acting as dienophiles in inverse electron-demand cycloaddition reactions.[5] Even scarcer are the cases relating to the involvement of this heteroaromatic cycle as dienophile in normal electron-demand [4+2]. To our knowledge, before our work,[6] the only example was published 15 years ago.[7] This behavior required the presence of two electron-withdrawing substituents on the N–C=C moiety of the aromatic ring. Thus, pyrrole **4**, bearing both an acetyl group on carbon 3 of the heterocycle and a benzenesulfonyl unit on position 1, reacted with isoprene to deliver compounds **5** as a 1:2 regioisomeric mixture (Scheme 1). Interestingly, these cycloadducts feature a quaternary carbon at the ring junction and are irreversibly dearomatized during the process. However, harsh conditions (195 °C, 72 h) had to be used and the isolated yield was low, thus forbidding the practical development of this approach and its use in the synthesis of products encompassing a five-membered nitrogen heterocycle (e.g., tazettine **6**). This result nevertheless pointed out the feasibility of such a reaction.



4 **5**

Scheme 1.

Keywords: Pyrrole; Diels–Alder reaction; Heterocycloaddition; Chemoselectivity; High pressure.
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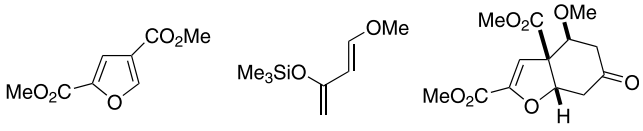
We recently reported that the analogous process involving the more reactive indole derivatives can be carried out in an efficient manner when activated by the combined action of a Lewis acid and high pressures.[8]

The more pronounced aromatic character of pyrrole led us to consider the activating effect of a third electron withdrawing substituent to generate a usable cycloaddition process. Thus, the known and easily accessible trisubstituted pyrrole **7**[9] was selected as the first substrate to study this dienophilic behavior. The choice of the 2,4-regioisomer was dictated by the nearly complete lack of reactivity of electron-poor five-membered heterocycles bearing an electron withdrawing group on carbon 2, thereby warranting a probable site selectivity on the heterocycle.[6,10] In addition the documented interaction between 2,4-biscarbomethoxy-furan **8** and Danishefsky's diene **9** was reported to involve the C4,C5 carbon–carbon double bond in a complete regioselective fashion, exclusively furnishing cycloadduct **10** after hydrolysis (Fig. 2).[11]



8 9 10

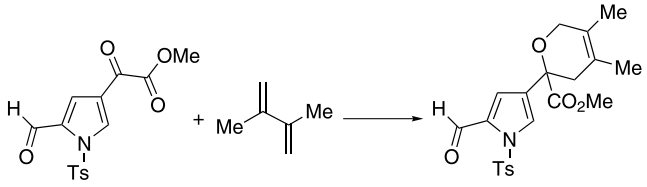
Figure 2. Structures of furan **8**, diene **9** and cycloadduct **10**.

2. Results and discussion

Reacting pyrrole **7** with excess 2,3-dimethylbuta-1,3-diene **11** at 100 °C for 72 h in the presence of zinc chloride (10% mol) led to an 85% consumption of the starting substrate. Purification of the crude residue furnished a material whose mass spectrometry indicated the presence of a 1:1 cycloadduct (21% isolated yield). Further analyses led to the identification of structure **12**, the result of an hetero Diels–Alder process between the 4-keto unit and the diene (Table 1, entry 1). Activation by either high pressure alone (entry 2), or a combination of high pressure and Lewis acid (entry 3) did not change the course of the reaction. Low conversion rates are believed to be the result of the competitive polymerization process which (i) slows the diffusion rate down and (ii) decreases the diene concentration.[12] As the resultant polymer also renders isolation difficult, attempts to solve these problems included diminishing the number of equivalents of diene. This led to a slightly better but still modest 28% isolated yield (entry 4).

The use of Danishefsky's diene **9** led to an analogous chemoselectivity, the 4-keto moiety proving once again to

Table 1. Reaction between pyrrole **7** and diene **11**

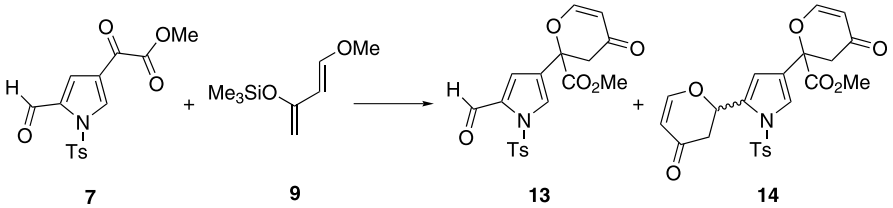


7 11 12

Entry	**11** (equiv)	*P* (GPa)	*T* (°C)	Cat.[a]	*t* (h)	Conv.[b] (%)	Yield (%)
1	12	10^{-4}	100	$ZnCl_2$	72	85	21
2	12	1.6	25	—	24	14	—[c]
3	12	1.6	25	$ZnCl_2$	24	36	—[c]
4	4	1.2	25	$ZnCl_2$	72	52	28

[a] 0.1 Catalysed (0.1 equiv) was used was used.
[b] Conversion.
[c] Not isolated.

Table 2. Cycloaddition between pyrrole **7** and diene **9**

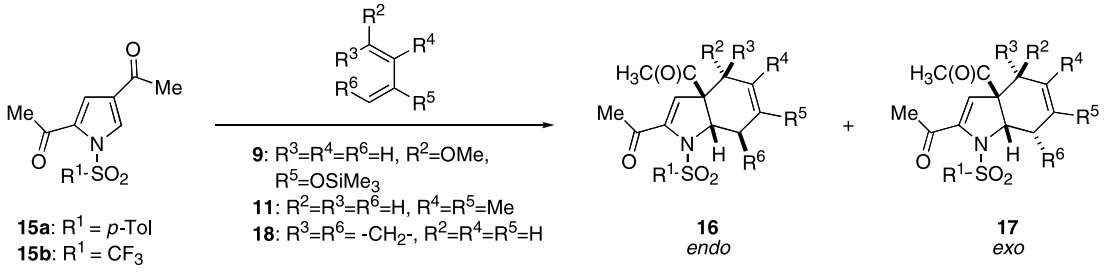


7 9 13 14

Entry	**7** (equiv)	*P* (GPa)	Cat.[a]	*T* (h)	Conv.[b] (%)	Ratio **13**:**14**	Yield (%)	
							13	**14**
1	1.5	10^{-4}	—	20	100	92:8	90	5
2	2	1.2	—	72	100	76:24	66	18
3	12	1.6	EuFOD	24	53	45:55	18	24

[a] 0.1 Catalysed (0.1 equiv) was used was used.
[b] Conversion.

Table 3. Cycloaddition between diacetylpyrroles **15** and dienes **9**, **11** and **18**



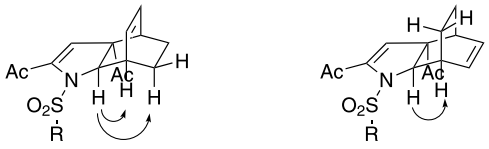
Entry	Dienophile	Diene (equiv)	*P* (GPa)	*T* (°C)	Cat.[a]	*t* (h)	Conv.(%)[b]	Product	*endo*/*exo*	Yield (%)
1	**15a**	**11** (12)	10^{-4}	130	$ZnCl_2$	168	38	**16a**	—	19
2	**15a**	**11** (12)	1.2	50	—	72	0	**16a**	—	—
3	**15a**	**11** (6)	1.2	50	$ZnCl_2$	72	47	**16a**	—	40
4	**15a**	**11** (6)	1.2	50	$ZnCl_2$	72	59	**16a**	—	48[c]
5	**15a**	**18** (6)	1.2	50	$ZnCl_2$	36	44	**16b/17b**	7/93	31
6	**15a**	**9** (6)	1.6	50	EuFOD	72	23	**16c/17c**	—	0[d]
7	**15b**	**11** (12)	10^{-4}	130	$ZnCl_2$	24	100	**16d**	—	0[e]
8	**15b**	**11** (6)	1.2	50	$ZnCl_2$	72	100	**16d**	—	80
9	**15b**	**11** (6)	1.2	50	$ZnCl_2$	72	96	**16d**	—	64[c]
10	**15b**	**18** (6)	1.2	50	$ZnCl_2$	36	76	**16e/17e**	—	—[f]
11	**15b**	**18** (6)	1.6	50	$ZnCl_2$	36	100	**16e/17e**	35/65	70
12	**15b**	**9** (6)	1.6	50	EuFOD	36	69	**16f/17f**	58/42	48[g,h]
13	**15b**	**9** (6)	1.6	50	EuFOD	72	80	**16f/17f**	60/40	61[g,h]

[a] Unless otherwise indicated, 0.1 equiv of catalyst was used.
[b] Conversion.
[c] One equivalent of catalyst was used.
[d] Compound **19a** was isolated in 16% yield (see text).
[e] Complete degradation occured.
[f] Not isolated.
[g] Diasteromeric cycloadducts **20a** and **20b** were isolated after hydrolysis of the silyl enol ether.
[h] Compound **19b** was isolated in 15 and 18% yield, respectively (see text).

be the most reactive site. Thus treatment of pyrrole **7** with **9** (1.5 equiv) at room temperature led to a complete conversion after 20 h (Table 2, entry 1). Purification of the crude oil led to the isolation of cycloadduct **13** in a much better, 90% yield. The minor formation of bisadducts **14**, the result of two sequential hetero Diels–Alder processes, was also observed (5% isolated yield). Carrying out the reaction under a pressure of 1.2 GPa decreased the ratio **13**:**14** (entry 2). Attempts to maximize the formation of **14** involved the use of higher pressures, increasing the number of equivalents of diene, and activation by a Lewis acid. This led to the formation of a 45:55 mixture of **13**:**14**, which were separated by chromatography on silica (entry 3).

The exclusive hetero Diels–Alder processes arising from reactions between **7** and dienes **9** or **11** point to the low reactivity of the pyrrole carbon–carbon double bonds. Both aldehyde and ketoester carbonyl units have been reported to react as heterodienophiles and to constitute a useful access to dihydropyrans.[2] Although this reaction is in general much slower than the classical all-carbon Diels–Alder cycloaddition, it may become a competitive or exclusive pathway when the all-carbon dienophile becomes less reactive. Recently, work from this laboratory has shown that tenuous changes in electron-poor indoles may induce a complete reversal of chemoselectivity in their cycloaddition reactions with dienes.[8b]

The acetyl unit was next selected as electron withdrawing group. Indeed, literature data indicated that involvement of a simple ketone as heterodienophile is much less common.[2,13] Hence 2,4-diacetyl-1-*p*-tosylpyrrole (**15a**)[14,15] as singled out as the best available candidate to pursue the present study. Heating a toluene solution of **15a** and dimethylbutadiene **11** at 130 °C (pressure tube) for 7 days in the presence of zinc chloride (0.1 equiv) led to a 38% conversion (Table 3, entry 1). Purification of the crude resulting sample gave the all-carbon cycloadduct **16a** in low yield. However, this result was very encouraging as chemoselectivity and site selectivity in favor of the C_4,C_5 carbon–carbon double bond proved to be complete.[16] This result was in complete accordance with previous data gathered on indoles and furans.[6,8,11] The need for Lewis acid activation was verified by carrying out the same reaction in either the presence or the absence of zinc chloride under high pressure (entries 2 and 3).[17] Thus, conducting the reaction under a pressure of 1.2 GPa, at 50 °C and in the presence of the same Lewis acid led to a slightly higher conversion which, this time translated into an isolated yield of 40% (entry 3). Increasing the amount of $ZnCl_2$ to one full equivalent led to an optimized yield of 48% (entry 4). The use of cyclohexa-1,3-diene **18**, a four π-electron partner frozen in a *cisoid* conformation, led to essentially the same result, the expected diastereomeric cycloadducts **16b** and **17b** being isolated in moderate yield (entry 5). The relative stereochemistry was assigned according to NOESY experiments (Fig. 3). Interestingly, the major diastereomer produced in this case is the one defined as *exo*, that is corresponding to the approach where the dienic part and the β-acetyl group are superimposed.[18]



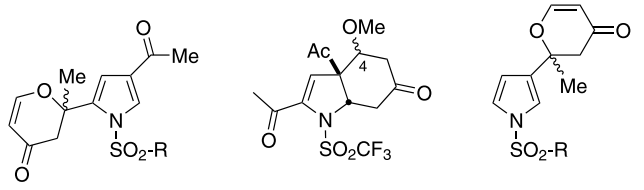
16b: R = *p*-Tol
16e: R = CF_3

17b: R = *p*-Tol
17e: R = CF_3

Figure 3. NOESY correlations observed on **16b** and **16e**, and **17b** and **17e**.

The reaction between **15a** and Danishefsky's diene **9** in the presence of EuFOD indicated a low conversion (entry 6). Purification of the crude mixture led to the isolation of a 1:1 adduct, whose analytical data pointed to structure **19a**, the result of an heterocycloaddition involving the carbonyl unit on position 2. Presumably, the electron-poor heterocycle acts as a global electron withdrawing substituent, thereby inducing the ketone behavior to parallel the known ketoester and ketoamide reactivities.[2,8b]

Attempts to further increase the reactivity and reverse the chemoselectivity in favor of the pyrrolyl C_2,C_3 carbon–carbon double bond included replacing the *p*-tosyl group with a trifluoromethanesulfonyl (triflyl) unit, a more powerful activating group.[19] Hence, pyrrole **15b** was prepared by interacting 2,3-diacetylpyrrole[15] with *N*,*N*-bistriflylphenylimide under basic conditions (78% isolated yield).[20] The



19a: R = *p*-Tolyl
19b: R = CF_3

20a : (4*R**) *endo*
20b : (4*S**) *exo*

24a: R = *p*-Tolyl
24b: R = CF_3

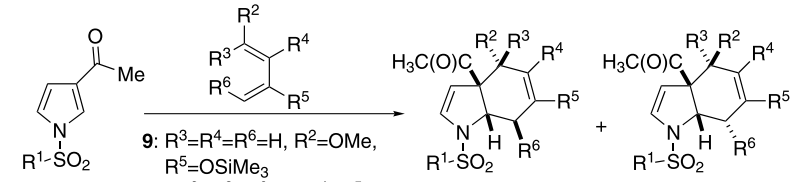
Figure 4. Structures of cycloadducts **19a**, **19b**, **20a**, **20b**, **24a** and **24b**.

thermal sensitivity of the substrate was highlighted by its complete degradation when heated at 130 °C in the presence of $ZnCl_2$ (entry 7). Activation by high pressures proved once again to be crucial. Thus subjecting substrate **15b** and 6 equiv of **11** to a pressure of 1.2 GPa at 50 °C and in the presence of $ZnCl_2$ led to a complete conversion and the regioselective formation of adduct **16d** (80% isolated yield) (entry 8). In the case of **15b**, increasing the amount of $ZnCl_2$ from 0.1 to 1 equiv resulted in a drop of the yield (compare entries 8 and 9). The reaction with cyclohexa-1,3-diene **18** illustrated once again the dramatic improvement induced by the triflyl group (entries 10 and 11). Optimized conditions led to a complete conversion and furnished a 70% isolated yield of a 35:65 mixture of *endo* and *exo* stereoisomers (**16e** and **17e**, respectively, entry 11), the latter being the major one. Reaction with the electronically enriched diene **9** yielded a 69% conversion after 36 h under 1.6 GPa, and 80% conversion after 72 h (entries 12 and 13). Hydrolysis generated a mixture of three adducts which were separated by chromatography. The two first adducts proved to be the expected *endo* and *exo* adducts resulting from reaction between the diene and the C_4=C_5 aromatic double bond (a 6:4 diastereomeric mixture), isolated as keto derivatives **20a** and **20b** in 48 and 61% yield (entries 12 and 13, respectively). Further elution afforded heterocycloadduct **19b** (15 and 18% isolated yield, respectively). In this case, the site selectivity was unambiguously determined by carrying out HMBC NMR experiments.

Thus the triflyl group did succeed in playing a dual role: not only does it increase the reactivity of the C_4,C_5 carbon–carbon double bond of the aromatic five-membered cycle, but, in addition, it is able to reverse the chemoselectivity, the carbon–carbon double bond being now the favor site of reactivity.

These results induced us to examine the effect of the triflyl group on the less reactive, monoacetylated pyrrole, and to compare it to the analogous tosylated substrate **21a** (Fig. 4).

Table 4. Cycloaddition between acetylpyrroles **21** and dienes **9**, **11** and **18**



9: R^3=R^4=R^6=H, R^2=OMe, R^5=$OSiMe_3$
11: R^2=R^3=R^6=H, R^4=R^5=Me
18: R^3=R^6= -CH_2-, R^2=R^4=R^5=H

21a: R^1 = *p*-Tolyl
21b: R^1 = CF_3

22 *endo*

23 *exo*

Entry	R^1	Diene (equiv)	*P* (GPa)	Cat.[a]	*t* (h)	Conv. (%)[b]	*endo*/*exo*	Yield (%)
1	*p*Tol	**11** (6)	1.2	$ZnCl_2$	36	Traces	—	—[c]
2	*p*Tol	**18** (6)	1.6	$ZnCl_2$	36	50	5/95	25
3	*p*Tol	**9** (6)	1.6	EuFOD	72	7	—	—[d]
4	CF_3	**11** (6)	1.2	$ZnCl_2$	36	31	—	24
5	CF_3	**11** (6)	1.6	$ZnCl_2$	36	91	—	80
6	CF_3	**18** (6)	1.6	$ZnCl_2$	36	97	13/87	49
7	CF_3	**9** (6)	1.6	EuFOD	72	23	—	—[e]

[a] 0.1 equiv of catalyst was used.
[b] Conversion.
[c] Not isolated.
[d] Compound **24a** was isolated in 7% yield.
[e] Compound **24b** was isolated in 15% yield.

Not surprisingly, reaction between the latter and any of the above diene (**9**, **11** or **18**) under hyperbaric conditions proved sluggish, delivering the desired adducts in 25% isolated yield, at best (Table 4, entries 1–3). Reaction of the corresponding triflyl derivative **21b**[21,22] with dimethylbutadiene, however, confirmed the above observations: under a pressure of 1.2 GPa and in the presence of 10% mol $ZnCl_2$, a 31% conversion was observed after 36 h, which became nearly quantitative when the same reaction was carried out under 1.6 GPa (entries 4 and 5). The desired cycloadduct **22a** was then isolated in 80% yield. A complete conversion was also obtained with cyclohexa-1,3-diene **18** and a 13:87 mixture of *endo*/*exo* cycloadducts were isolated in 49% yield (entry 6). The use of Danishefsky's diene, however, did not lead in this case to a reversal of chemoselectivity and heterocycloadduct **24b**, resulting from a pericyclic process involving the 3-carbonyl unit as the 2π component, was obtained in low yield (entry 7).[23]

The results described clearly indicate that similar levels of energy exist for the carbon–carbon double bond of aromatic pyrrole, when substituted by electron withdrawing substituents, on the one hand, and the acyl group of the substituent(s) in position 2 or 4, on the other hand. The structural and electronic nature of the diene definitely plays a role on the chemoselectivity of the reaction. The triflyl group, however, may counteract the tendency of electron-rich dienes to react in a hetero Diels–Alder fashion, and will induce in most cases the pyrrole C_2–C_3 double bond (1,3-disubstituted cases) or C_4–C_5 double bond (1,2,4-trisubstituted cases) to behave instead as the reacting dienophile.

3. Conclusion

The nature of the substituents on the *N*-tosylpyrrole nucleus plays a crucial role in the course of the cycloaddition reaction. Thus, formyl- and ketoester- substituted pyrroles undergo a hetero-Diels–Alder process resulting from their involvement as heterodienophiles, while the carbonyl group of unactivated ketones is almost inert, thereby allowing the aromatic carbon–carbon double bond of the pyrrole to behave as a dienophile—albeit in low yields. Biactivation by high pressures and Lewis acid leads to an increase in isolated yields. In this regard, replacement of the *p*-tosyl group with a triflyl one results in a dramatic change by further activating the latter reaction enough to afford good isolated yields of dearomatized cycloadducts encompassing a quaternary center at the ring junction. This methodology may now be used in the synthesis of natural and non-natural products, and in the production of scaffolds for the preparation of libraries.

4. Experimental

Unless otherwise stated, ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in deuterated chloroform relative to $(CH_3)_4Si$ and $CDCl_3$, respectively. Chemical shifts are expressed in parts per million (ppm). Low resolution and high-resolution mass spectra were recorded on Unicam ATI Automass and Jeol 500 spectrometers, respectively. IR spectra were recorded on Perkin–Elmer 16PC FT-IR spectrometers. The crude organic extracts were dried over magnesium sulfate. Unless otherwise stated, the products are colorless oils.

4.1. General procedure for the tosylation of pyrrole

A mixture of the pyrrole derivative (1 equiv), DMAP (cat.), $(^i Pr)_2EtN$ (1.3 equiv), and TsCl (1–1.3 equiv) was stirred at room temperature for 1 h under argon. After quenching with a 1 N HCl aqueous solution, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were dried and concentrated under reduced pressure.

4.1.1. [5-Formyl-1-(toluene-4-sulfonyl)-1*H*-pyrrol-3-yl]-oxo-acetic acid methyl ester (7). Prepared according to the general procedure using (5-formyl-1*H*-pyrrol-3-yl)oxo-acetic acid, methyl ester (1.81 g, 10 mmol), DMAP (20 mg), $(^i Pr)_2EtN$ (2.26 mL, 13 mmol), and TsCl (1.91 g, 10 mmol) in CH_2Cl_2 (20 mL). The residue was purified by crystallization from CH_2Cl_2/heptane to give **7** as a white solid (2.80 g, 84%, mp 149 °C). ^{1}H NMR δ 2.44 (s, 3H), 3.98 (s, 3H), 7.38 (d, J=7.9 Hz, 2H), 7.65 (d, J=1.9 Hz, 1H), 7.91 (d, J=7.9 Hz, 2H), 8.64 (d, J=1.9 Hz, 1H), 9.90 (s, 1H). ^{13}C NMR δ 21.8, 53.3, 122.2, 124.2, 128.3 (2C), 130.3 (2C), 133.7, 135.3, 147.0 (2C), 161.3, 176.9, 178.4. IR (NaCl) ν 3360, 3154, 1728, 1682, 1548, 1380, 1180, 1146, 1052 cm^{-1}. MS (EI) *m*/*z* (relative intensity) 335 [$M^{+\cdot}$] (5), 276 (100), 155 (78), 91 (94), 65 (29). HRMS calcd for $C_{21}H_{26}NO_4S$: ($M^{+\cdot}$) 335.0464. Found: 335.0451.

4.1.2. 1-[5-Acetyl-1-(toluene-4-sulfonyl)-1*H*-pyrrol-3-yl] ethanone (15a). Prepared according to the general procedure using 1-(5-acetyl-1*H*-pyrrol-3-yl)-ethanone (0.76 g, 5 mmol), DMAP (10 mg), $(^i Pr)_2EtN$ (1.13 mL, 6.5 mmol), and TsCl (1.24 g, 6.5 mmol) in CH_2Cl_2 (10 mL). The residue was purified by chromatography on silica and elution with a mixture heptane/EtOAc (3:2) to give **15a** as a white solid (1.42 g, 93%, mp 170 °C). ^{1}H NMR δ 2.38 (s, 3H), 2.44 (s, 3H), 2.51 (s, 3H), 7.35 (d, J=7.9 Hz, 2H), 7.41 (d, J=1.9 Hz, 1H), 7.94 (d, J=7.9 Hz, 2H), 8.33 (d, J=1.9 Hz, 1H). ^{13}C NMR δ 21.5, 26.8, 27.0, 121.6, 125.3, 128.5 (2C), 129.3 (2C), 132.6, 133.8, 134.5, 145.4, 186.1, 191.9. IR (NaCl) ν 3334, 3144, 1682, 1552, 1470, 1362, 1126 cm^{-1}. MS (EI) *m*/*z* (relative intensity) 305 [$M^{+\cdot}$] (6), 241 (28), 226 (20), 155 (48), 91 (100), 65 (21). Anal. Calcd for $C_{15}H_{15}NO_4S$: C, 59.00; H, 4.95; N, 4.59; S, 10.50. Found: C, 59.46; H, 5.28; N, 4.67; S, 10.12.

4.1.3. 1-(5-Acetyl-1-trifluoromethanesulfonyl-1*H*-pyrrol-3-yl)ethanone (15b). A mixture of the pyrrole 1-(5-acetyl-1*H*-pyrrol-3-yl)-ethanone (0.61 g, 4 mmol), DMAP (20 mg), $(^i Pr)_2EtN$ (1.39 mL, 8 mmol), and $PhNTf_2$ (1.79 g, 5 mmol) in CH_2Cl_2 (10 mL) was stirred in CH_2Cl_2 (10 mL) at room temperature for 4 days under argon. After quenching with saturated aqueous $NaHCO_3$, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were washed with 1 N HCl, dried and concentrated under reduced pressure. The residue was purified by flash chromatography on silica and elution with a mixture cyclohexane/EtOAc (9:1) to give **15b** as a white solid (0.88 g, 78%, mp 78 °C). ^{19}F NMR δ −67.42. ^{1}H NMR δ 2.51 (s, 3H), 2.54 (s, 3H), 7.52 (d, J=1.7 Hz, 1H), 8.01 (d,

$J=1.7$ Hz, 1H). ^{13}C NMR δ 26.8, 27.2, 117.3, 122.5, 127.7, 133.2, 135.8, 185.9, 191.2. IR (NaCl) ν 3132, 1686, 1416, 1212, 1132 cm^{-1}. MS (EI) *m/z* (relative intensity) 283 [$M^{+\cdot}$] (85), 268 (100), 118 (67), 69 (92). Anal. Calcd for $C_9H_8F_3NO_4S$: C, 38.17; H, 2.85; N, 4.95; S, 11.32. Found: C, 38.19; H, 2.74; N, 4.88; S, 11.41.

4.1.4. 1-(1-Trifluoromethanesulfonyl-1*H*-pyrrol-3-yl) ethanone (21b). A solution of 1-(1*H*-Pyrrol-3-yl)-ethanone (436 mg, 4 mmol) and (^{i}Pr)$_2$EtN (1.4 mL, 8 mmol) in CH_2Cl_2 (20 mL) was cooled to −78 °C under argon. Triflic anhydride (845 μL, 5 mmol) was added dropwise, and after 30 min of stirring at the same temperature, the resultant solution was poured into 2 N NaOH aqueous solution and extracted with CH_2Cl_2. The combined organic layers were then poured into a 6 N HCl aqueous solution, stirred vigorously at room temperature for 15 min and finally extracted with CH_2Cl_2. Drying of the organic layers, filtration and evaporation under reduced pressure delivered the crude product which was immediately purified by Kügelrohr distillation (0.05 mmHg, 80–90 °C) to give an oil (761 mg, 79%, mp 34 °C) which crystallized upon standing. ^{19}F NMR δ −76.28. ^{1}H NMR δ 2.48 (s, 3H), 6.88 (m, 1H), 7.15 (m, 1H), 7.68 (m, 1H). ^{13}C NMR δ 27.4, 114.3, 118.9, 123.3, 125.8, 131.2, 192.0. IR (NaCl) ν 3129, 1687, 1235, 1210, 1153, 1060 cm^{-1}. MS (EI) *m/z* (relative intensity) 241 [$M^{+\cdot}$] (50), 226 (100), 142 (6), 93 (81), 69 (75). Anal. Calcd for $C_7H_6F_3NO_3S$: C, 34.86; H, 2.51; N, 5.81; S, 13.29. Found: C, 34.82; H, 2.55; N, 5.69; S, 13.26.

4.2. General procedure for the high-pressure reactions

Non-catalyzed reactions. To a solution of the requisite pyrrole, in dry dichloromethane (0.2 M) at room temperature under argon, was added the freshly distilled diene (6 equiv). The resultant mixture was transferred into a high-pressure vessel and compressed at the requisite pressure and temperature. After decompression, the solvent and excess diene were evaporated under reduced pressure. Chromatography of the residue on silica and elution led to the isolation of the cycloadduct(s).

The ketones resulting from hydrolysis of the silyl enol ether in the case of cycloadducts derived from Danishefsky diene were obtained in the following manner: after the reaction, removal of excess diene was achieved by bulb-to-bulb distillation under reduced pressure (50 °C/0.1 bar). The residue (0.3 mmol scale) was then stirred overnight in methanol (2 mL) in the presence of silica. Filtration and purification as above delivered the desired products.

4.3. Catalyzed reactions under high pressure

The experimental procedure is identical, except that the Lewis acid at room temperature was first added to the pyrrole solution. The mixture was stirred for 30 min and the diene (6 equiv) were added.

4.4. General procedure for thermal cycloadditions

A vessel containing the requisite pyrrole, the diene (12 equiv), hydroquinone (10 mg per mmol of substrate) in dry degassed toluene was sealed and heated in a sand bath behind a safety shield at the desired temperature. After cooling, the solvents and excess diene were removed under reduced pressure and the residue was chromatographed.

4.4.1. 2-[5-Formyl-1-(toluene-4-sulfonyl)-1*H*-pyrrol-3-yl]-4,5-dimethyl-3,6-dihydro-2*H*-pyran-2-carboxylic acid methyl ester (12). Elution with a mixture of heptane/EtOAc (4:1) gave the product. ^{1}H NMR δ 1.42 (s, 3H), 1.62 (s, 3H), 2.30 (d, $J=17.0$ Hz, 1H), 2.33 (s, 3H), 2.70 (d, $J=17.0$ Hz, 1H), 3.64 (s, 3H), 3.91 (d, $J=15.1$ Hz, 1H), 4.19 (d, $J=15.1$ Hz, 1H), 7.09 (d, $J=2.1$ Hz, 1H), 7.24 (d, $J=8.5$ Hz, 2H), 7.58 (d, $J=2.1$ Hz, 1H), 7.72 (d, $J=8.5$ Hz, 2H), 9.83 (s, 1H). ^{13}C NMR δ 13.6, 18.3, 21.6, 37.6, 52.6, 66.7, 75.6, 121.4, 121.9, 123.6, 126.2, 127.5 (2C), 128.1, 130.0 (2C), 133.2, 134.8, 146.0, 171.8, 178.7. IR (NaCl) ν 2922, 1738, 1674, 1378, 1178, 1092 cm^{-1}. MS (CI) *m/z* (relative intensity) 418 [MH^+] (100), 264 (14), 157 (11). HRMS calcd for $C_{21}H_{24}NO_6S$: (MH^+) 418.1324. Found: 418.1327.

4.4.2. 2-[5-Formyl-1-(toluene-4-sulfonyl)-1*H*-pyrrol-3-yl]-4-oxo-3,4-dihydro-2*H*-pyran-2-carboxylic acid methyl ester (13). Elution with a 3:2 mixture of heptane/EtOAc yielded cycloadduct **13**. ^{1}H NMR δ 2.37 (s, 3H), 2.93 (d, $J=16.8$ Hz, 1H), 3.25 (d, $J=16.8$ Hz, 1H), 3.71 (s, 3H), 5.43 (d, $J=6.0$ Hz, 1H), 7.10 (d, $J=1.9$ Hz, 1H), 7.29 (d, $J=7.9$ Hz, 2H), 7.35 (d, $J=6.0$ Hz, 1H), 7.64 (d, $J=1.9$ Hz, 1H), 7.76 (d, $J=7.9$ Hz, 2H), 9.87 (s, 1H). ^{13}C NMR δ 21.7, 43.9, 53.8, 81.9, 108.2, 120.9, 124.3, 126.1, 127.7 (2C), 130.3 (2C), 133.6, 134.5, 146.5, 160.9, 169.0, 178.6, 188.6. IR (NaCl) ν 2956, 1740, 1682, 1596, 1460, 1378, 1224, 1178, 1090 cm^{-1}. MS (CI) *m/z* (relative intensity) 404 [MH^+] (100). HRMS calcd for $C_{19}H_{18}NO_7S$: (MH^+) 404.0804. Found: 404.0806.

4.4.3. 4-Oxo-2-[5-(4-oxo-3,4-dihydro-2*H*-pyran-2-yl)-1-(toluene-4-sulfonyl)-1*H*-pyrrol-3-yl]-3,4-dihydro-2*H*-pyran-2-carboxylic acid methyl ester (14). Biscycloadduct was obtained by elution with a mixture of heptane/EtOAc (1:1) (two diastereomers). ^{1}H NMR δ 2.37 and 2.37 (s, 3H), 2.62 and 2.65 (dd, $J=4.1$, 17.0 Hz, 1H), 2.83 (dd, $J=12.8$, 17.0 Hz, 1H), 2.93 (d, $J=16.6$ Hz, 1/2H), 2.94 (d, $J=16.9$ Hz, ½H), 3.21 (d, $J=16.6$ Hz, ½H), 3.22 (d, $J=16.9$ Hz, ½H), 3.71 (s, 3H), 5.38–5.43 (m, 2H), 5.86 and 5.87 (dd, $J=4.1$, 12.8 Hz, 1H), 6.40 (m, 1H), 6.99 and 7.03 (d, $J=6.0$ Hz, 1H), 7.27 (d, $J=8.3$ Hz, 2H), 7.32 and 7.33 (d, $J=6.0$ Hz, 1H), 7.43 and 7.43 (d, $J=1.5$ Hz, 1H), 7.65 (d, $J=8.3$ Hz, 2H). ^{13}C NMR δ 21.6, 40.4 and 40.4, 43.6 and 43.7, 53.6, 71.7, 82.0, 107.5 and 108.0, 112.4 (2C), 122.1 and 122.2, 123.2 and 123.2, 127.1 and 127.2 (2C), 129.9 and 130.0 (2C), 131.4, 135.3, 145.8 and 145.8, 161.0 and 161.0, 161.5 and 161.7, 169.2 and 169.3, 188.9 and 188.9, 190.9. IR (NaCl) ν 3060, 1682, 1596, 1400, 1374, 1224, 1174, 1092, 1038 cm^{-1}. HRMS calcd for $C_{23}H_{22}NO_8S$: (MH^+) 472.1066. Found: 472.1046.

4.4.4. 1-[2-Acetyl-5,6-dimethyl-1-(toluene-4-sulfonyl)-1,4,7,7a-tetrahydroindol-3a-yl]ethanone (16a). Elution with a 7:3 mixture of heptane/EtOAc furnished derivative **16a**. ^{1}H NMR δ 1.53 (s, 3H), 1.58 (s, 3H), 1.77 (s, 3H), 1.96 (m, 2H), 2.14–2.24 (m, 1H), 2.32–2.46 (m, 1H), 2.35 (s, 3H), 2.39 (s, 3H), 4.33 (dd∼t, $J=4.1$ Hz, 1H), 5.40 (s, 1H), 7.23 (d, $J=7.9$ Hz, 2H), 7.46 (d, $J=7.9$ Hz, 2H). ^{13}C NMR

δ 19.4, 19.5, 21.6, 25.8, 28.7, 37.0, 37.6, 64.0, 64.7, 123.2, 125.1, 127.6, 128.5 (2C), 129.6 (2C), 130.8, 144.6, 147.4, 194.9, 206.0. IR (NaCl) ν 2926, 1702, 1354, 1166, 1090, 814, 660 cm^{-1}. HRMS calcd for $C_{21}H_{26}NO_4S$: (MH^+) 388.1583. Found: 388.1584.

4.4.5. 1-[4-Acetyl-3-(toluene-4-sulfonyl)-3-aza-tricyclo[5.2.2.0.^{2,6}]undeca-4,8-dien-6-yl]ethanone (16b/17b) Elution with a mixture of cyclohexane/EtOH (95:5) led to the isolation of the major, *exo* diastereomer **17b**. ^1H NMR δ 1.17–1.25 (m, 2H), 1.61–1.70 (m, 1H), 1.70 (s, 3H), 1.83–1.93 (m, 1H), 2.40 (s, 3H), 2.58 (s, 3H), 2.73–2.75 (m, 1H), 3.03–3.04 (m, 1H), 4.17 (d, J=3.0 Hz, 1H), 5.65 (s, 1H), 6.05 (dd∼t, J=7.3 Hz, 1H), 6.24 (dd∼t, J=7.3 Hz, 1H), 7.28 (d, J=8.3 Hz, 2H), 7.55 (d, J=8.3 Hz, 2H). ^{13}C NMR δ 16.8, 21.6, 21.8, 25.1, 28.4, 34.5, 34.7, 64.3, 66.5, 121.8, 128.3 (2C), 129.6 (2C), 131.2, 132.8, 132.9, 144.5, 146.0, 195.1, 202.9. IR (NaCl) ν 2946, 1701, 1615, 1597, 1354, 1164 cm^{-1}. MS (EI) *m/z* (relative intensity) 385 [$M^{+\bullet}$] (4), 264 (17), 187 (18), 155 (31), 91 (100). HRMS calcd for $C_{21}H_{24}NO_4S$: (MH^+) 386.1426. Found: 386.1410.

4.4.6. 1-(2-Acetyl-5,6-dimethyl-1-trifluoromethanesulfonyl-1,4,7,7a-tetrahydro-indol-3a-yl)ethanone (16d). Product **16d** was obtained by eluting with a 85:15 mixture of cyclohexane/EtOAc. ^{19}F NMR δ −71.68. ^1H NMR δ 1.65 (s, 3H), 1.76 (s, 3H), 2.17–2.41 (m, 4H), 2.26 (s, 3H), 2.33 (s, 3H), 5.11 (dd∼t, J=4.9 Hz, 1H), 5.81 (s, 1H). ^{13}C NMR δ 19.1, 19.4, 26.4, 28.1, 36.2, 36.9, 64.8, 65.4, 119.6, 125.4, 125.8, 126.6, 144.0, 190.3, 204.7. IR (NaCl) ν 2921, 1707, 1625, 1389, 1200, 1153 cm^{-1}. MS (EI) *m/z* (relative intensity) 366 [$M^{+\bullet}$] (15), 278 (63), 189 (50), 174 (45), 146 (100), 131 (36), 91 (40). Anal. Calcd for $C_{15}H_{18}F_3NO_4S$: C, 49.31; H, 4.97; N, 3.83; S, 8.78. Found: C, 49.24; H, 5.04; N, 3.79; S, 8.52.

4.4.7. 1-(4-Acetyl-3-trifluoromethanesulfonyl-3-azatricyclo[5.2.2.0^{2,6}]undeca-4,8-dien-6-yl)ethanone (16e/17e). Elution with a 3:1 mixture of heptane/EtOAc delivered the major diastereomer **17e** (*exo*). ^{19}F NMR δ −71.09. ^1H NMR δ 1.30–1.80 (m, 4H), 2.21 (s, 3H), 2.43 (s, 3H), 2.98–3.02 (m, 2H), 4.90 (d, J=26 Hz, 1H), 5.90 (s, 1H), 6.17–6.30 (m, 2H). ^{13}C NMR δ 15.8, 21.9, 25.9, 28.0, 34.4 (2C), 66.1, 66.9, 120.0, 123.9, 132.2, 133.7, 143.3, 190.6, 202.6. IR (NaCl) ν 2953, 1707, 1617, 1390, 1199 cm^{-1}. MS (CI) *m/z* (relative intensity) 364 [MH^+] (100), 284 (8), 232 (13), 152 (10). HRMS calcd for $C_{15}H_{16}F_3NO_4S$: (MH^+) 364.0831. Found: 364.0826.

4.4.8. 2-[4-Acetyl-1-(toluene-4-sulfonyl)-1*H*-pyrrol-2-yl]-2-methyl-2,3-dihydro-pyran-4-one (19a). Eluent: cyclohexane/EtOAc (7:3). ^1H NMR δ 1.74 (s, 3H), 2.35 (s, 3H), 2.42 (s, 3H), 2.82 (d, J=16.6 Hz, 1H), 2.94 (d, J=16.6 Hz, 1H), 5.43 (d, J=6.2 Hz, 1H), 6.97 (d, J=1.9 Hz, 1H), 7.25 (d, J=6.2 Hz, 1H), 7.32 (d, J=8.3 Hz, 2H), 7.74 (d, J=1.9 Hz, 1H), 7.88 (d, J=8.3 Hz, 2H). ^{13}C NMR δ 21.9, 27.2, 27.9, 47.3, 80.5, 106.8, 121.2, 126.7, 127.8, 128.7 (2C), 129.6 (2C), 133.8, 135.5, 145.3, 161.2, 186.2, 191.4. IR (NaCl) ν 2920, 1733, 1676, 1595, 1173 cm^{-1}. MS (EI) *m/z* (relative intensity) 373 [$M^{+\bullet}$] (21), 239 (16), 155 (30), 148 (32), 91 (100), 65 (31). HRMS calcd for $C_{19}H_{20}NO_5S$: (MH^+) 374.1062. Found: 374.1059.

4.4.9. 2-(4-Acetyl-1-trifluoromethanesulfonyl-1*H*-pyrrol-2-yl)-2-methyl-2,3-dihydro-pyran-4-one (19b). Eluted with mixtures of cyclohexane/EtOAc (7:3–3:7). ^{19}F NMR δ −68.17. ^1H NMR δ 1.73 (s, 3H), 2.50 (s, 3H), 2.83 (d, J=16.6 Hz, 1H), 2.92 (d, J=16.6 Hz, 1H), 5.46 (d, J=6.0 Hz, 1H), 7.11 (d, J=1.9 Hz, 1H), 7.26 (d, J=6.0 Hz, 1H), 7.42 (d, J=1.9 Hz, 1H). ^{13}C NMR δ 26.9, 27.3, 46.9, 79.9, 106.8, 119.5, 122.2, 126.8, 130.5, 135.6, 160.6, 185.5, 190.5. IR (NaCl) ν 2925, 1688, 1681, 1598, 1415, 1223 cm^{-1}. MS (CI) *m/z* (relative intensity) 352 [MH^+] (100), 251 (18), 220 (42). HRMS calcd for $C_{13}H_{12}F_3NO_5S$: (MH^+) 352.0466. Found: 352.0469.

4.4.10. 2,3a-Diacetyl-4-methoxy-1-trifluoromethanesulfonyl-1,3a,4,5,7,7a-hexahydro-indol-6-one (20a/20b). Elution with mixtures of cyclohexane/EtOAc (7:3–3:7) gave the major, *endo* diastereomer **20a**. ^{19}F NMR δ −72.21. ^1H NMR δ 2.32 (dd, J=9.8, 181 Hz, 1H), 2.35 (s, 3H), 2.39 (s, 3H), 2.70 (dd, J=5.7, 160 Hz, 1H), 2.74 (dd, J=4.5, 181 Hz, 1H), 2.78 (dd, J=4.9, 160 Hz, 1H), 3.32 (s, 3H), 4.08 (dd, J=4.5, 98 Hz, 1H), 5.17 (dd, J=4.9, 5.7 Hz, 1H), 6.26 (s, 1H). ^{13}C NMR δ 28.2, 29.4, 40.0, 43.4, 57.3, 63.7, 66.2, 77.4, 119.4, 121.8, 144.6, 189.9, 203.6, 206.8; IR (NaCl) ν 2925, 1712, 1624, 1602, 1391, 1211 cm^{-1}. MS (CI) *m/z* (relative intensity) 384 [MH^+] (100), 352 (32), 252 (82), 220 (11). HRMS calcd for $C_{14}H_{17}F_3NO_6S$: (MH^+) 384.0729. Found: 384.0732. Further elution delivered the minor, *exo* diastereomer **20b**. ^{19}F NMR δ −72.05. ^1H NMR δ 2.28 (dd, J=2.8, 185 Hz, 1H), 2.33 (s, 3H), 2.36 (s, 3H), 2.82 (dd, J=3.8, 185 Hz, 1H), 2.88 (d, J=41 Hz, 2H), 3.31 (s, 3H), 4.15 (dd, J=2.8, 38 Hz, 1H), 5.75 (t, J=4.1 Hz, 1H), 5.85 (s, 1H). ^{13}C NMR δ 25.9, 28.4, 37.5, 41.6, 57.2, 61.2, 66.4, 78.8, 118.8, 119.3, 144.9, 190.1, 199.7, 204.2. IR (NaCl) ν 2925, 1712, 1624, 1602, 1391, 1211 cm^{-1}. MS (CI) *m/z* (relative intensity) 384 [MH^+] (31), 352 (100), 252 (88), 220 (22). HRMS calcd for $C_{14}H_{17}F_3NO_6S$: (MH^+) 384.0729. Found: 384.0724.

4.4.11. 1-[3-(Toluene-4-sulfonyl)-3-aza-tricyclo[5.2.2.0^{2,6}]-undeca-4,8-dien-6-yl]-ethanone (22b/23b). Elution with a 3:2 mixture of cyclohexane/CH_2Cl_2 yielded the major, *exo* diastereomer **23b**. ^1H NMR δ 1.03–1.30 (m, 2H), 1.66–1.82 (m, 1H), 1.70 (s, 3H), 1.94–2.04 (m, 1H), 2.39 (s, 3H), 2.78 (dt, J=2.6, 5.2 Hz, 1H), 3.05–3.10 (m, 1H), 4.13 (dd, J=1.1, 3.8 Hz, 1H), 4.89 (d, J=4.1 Hz, 1H), 6.10–6.20 (m, 2H), 6.45 (d, J=4.1 Hz, 1H), 7.28 (d, J=8.3 Hz, 2H), 7.65 (d, J=8.3 Hz, 2H). ^{13}C NMR δ 17.5, 21.6, 21.7, 25.1, 34.2, 34.6, 63.0, 69.6, 111.6, 127.6 (2C), 129.8 (2C), 132.7, 132.9, 133.7, 133.8, 144.1, 205.2. IR (NaCl) ν 2941, 1706, 1594, 1351, 1163 cm^{-1}. MS (CI) *m/z* (relative intensity) 344 [MH^+] (68), 264 (100), 154 (95), 136 (70), 91 (46). HRMS calcd for $C_{19}H_{22}NO_3S$: (MH^+) 344.1320. Found: 344.1328.

4.4.12. 1-(5,6-Dimethyl-1-trifluoromethanesulfonyl-1,4,7,7a-tetrahydroindol-3a-yl)-ethanone (22d). Obtained from elution with a 65:35 mixture of cyclohexane/CH_2Cl_2. ^{19}F NMR δ −73.76. ^1H NMR δ 1.68 (s, 3H), 1.74 (s, 3H), 2.15–2.33 (m, 3H), 2.23 (s, 3H), 2.50 (dd, J=4.7, 14.9 Hz, 1H), 4.97–5.03 (m, 1H), 5.15 (d, J=4.3 Hz, 1H), 6.28 (d, J=4.3 Hz, 1H). ^{13}C NMR δ 19.4, 19.7, 26.0, 36.0, 37.2, 62.9, 66.8, 114.4, 120.1, 126.1, 126.6, 130.6, 205.9. IR

(NaCl) ν 2916, 1712, 1398, 1227, 1192 cm^{-1}. HRMS calcd for $C_{13}H_{16}F_3NO_3S$: (MH^+) 324.0894. Found: 324.0881.

4.4.13. 1-(3-Trifluoromethanesulfonyl-3-azatricyclo-[5.2.2.0^{2,6}]undeca-4,8-dien-6-yl)-ethanone (22e/23e). The major diastereomer **23e** (*exo*) by elution with a 92:8 mixture of cyclohexane/EtOAc. ^{19}F NMR δ −74.24. ^1H NMR δ 1.09–1.36 (m, 2H), 1.76–1.82 (m, 2H), 2.17 (s, 3H), 2.95 (ddd∼dt, J=2.6, 5.1 Hz, 1H), 3.06–3.13 (m, 1H), 4.77 (dd, J=1.1, 2.6 Hz, 1H), 5.20 (d, J=4.1 Hz, 1H), 6.15–6.26 (m, 2H), 6.34 (d, J=4.1 Hz, 1H). ^{13}C NMR δ 16.6, 22.0, 25.5, 34.3 (2C), 64.4, 69.6, 113.3, 120.0, 130.4, 132.4, 133.8, 204.0. IR (NaCl) ν 2950, 1713, 1398, 1225, 1195 cm^{-1}. HRMS calcd for $C_{13}H_{14}F_3NO_3S$: ($M^{+\cdot}$) 321.0643. Found: 321.0646.

4.4.14. 2-Methyl-2-(1-trifluoromethanesulfonyl-1*H*-pyrrol-3-yl)-2,3-dihydro-pyran-4-one (24b). Elution with a 4:1 mixture of cyclohexane/EtOAc afforded the desired product. ^{19}F NMR δ −76.52. ^1H NMR δ 1.74 (s, 3H), 2.90 (d, J=16.6 Hz, 1H), 3.00 (d, J=16.6 Hz, 1H), 5.53 (d, J=6.0 Hz, 1H), 6.50 (dd, J=3.4, 1.5 Hz, 1H), 7.07–7.10 (m, 1H), 7.10–7.15 (m, 1H), 7.27 (d, J=6.0 Hz, 1H). ^{13}C NMR δ 27.3, 47.1, 80.3, 106.6, 113.7, 118.7, 119.0, 123.2, 133.5, 160.9, 191.3. IR (NaCl) ν 3142, 2982, 1677, 1597, 1419, 1233, 1209, 1147 cm^{-1}. MS (EI) m/z (relative intensity) 309 [$M^{+\cdot}$] (1), 239 (86), 148 (12), 104 (100). HRMS calcd for $C_{11}H_{11}NO_4S$: (MH^+) 310.0361. Found: 310.0355.

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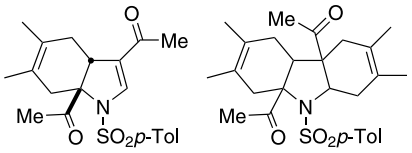
15. 2,4-Diacetylpyrrole was tosylated according to literature procedure. See: Masquelin, T.; Broger, E.; Müller, K.; Schmid, R.; Obrecht, D. *Helv. Chim. Acta* **1994**, *77*, 1395–1411.

16. Examples of possible side-products include cycloadduct **i** and biscycloadduct **ii**; however, none of these were isolated, or even detected in the crude mixture.



i **ii**

17. Various Lewis acids (among which $AlMe_3$, $MeAlCl_2$, Me_2AlCl, $ZnCl_2$, EuFOD, for example) were checked for their abilities to catalyze the reaction. Zinc chloride proved to be the most efficient one.
18. *endo* Addition can be defined as 'that particular arrangement of reactants in which the more bulky side of the diene is under the more bulky side of the dienophile', meaning the pyrrole part in this case[1c] In this precise case, the volume of the transition state leading to the *exo* diasteromer is probably more compact than the one delivering the *endo* cycloadduct.
19. Analogous reactions conducted with indole derivatives have shown the beneficial effect of the triflyl group, allowing the desired transformation to reach completion under milder conditions and in shorter times. Chataigner, I.; Piettre, S. R. Unpublished results.
20. Pyrrolyl substrate **15b** was easily prepared by reacting the pyrrole derivative with *N*,*N*-bistriflylimide. See: Hendrickson, J. B.; Bergeron, R. *Tetrahedron Lett.* **1973**, 4607–4610.
21. 3-Acetylpyrrole was prepared according to the following procedure: Kakushima, M.; Hamel, P.; Frenette, R.; Rokak, J. *J. Org. Chem.* **1983**, *48*, 3214–3219.
22. 3-Acetylpyrrole was triflated using the procedure of Abell. See: Abell, A. D.; Nabbs, B. K.; Battersby, A. R. *J. Am. Chem. Soc*. **1998**, *120*, 1741–1746.
23. The exclusive site selectivity observed in the hetero Diels–Alder reaction of **15a** and **15b** to generate dihydropyrans **19a** and **19b**, respectively, in connection with the formation of **24a** and **24b**, seems to indicate the higher reactivity of an acyl group in α versus β position to the sulfonamide. Such a behavior might result from the possible chelation of the Lewis acid by both the reacting carbonyl group and the sulfonyl unit in the former case only.

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Towards new camptothecins. Part 2: Synthesis of the ABCD ring scaffold substituted by a carboxyl group in the 5-position ☆

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Abstract—In the context of formation of camptothecins substituted by a carbonyl function on position 5 of cycle C, synthesis of a new keto tetrahydroindolizine was realized. This compound was obtained from the reaction of Bredereck's reagent with an indolizine derived from pyroglutamic acid. That yielded a dimethylaminovinyl group whose $NaIO_4$ oxidation gave a ketone. The indolizinone obtained was reacted in Friedlander condition to give the ABCD ring scaffold of camptothecins substituted by a methoxycarbonyl group on the 5-position. It was also shown that, if it is desired, a 5-carboxamide group does not need to be introduced at the beginning of the synthesis sequence.

1. Introduction

The isolation and structure of camptothecin **1** were reported in 1966 by Wall[1a] and many reviews devoted to this compound revealed its paramount importance.[1b,c] Interest in this cytotoxic drug and hemisynthetic analogs was stimulated when its mode of action was discovered. When the cleavable complex between topoisomerase I and DNA is stabilized by camptothecin, collision of the replication fork with this reversible complex[2] leads to cell death by preventing DNA religation.[3] The crystal structure of the DNA–topoisomerase I–camptothecin complex was resolved and two models of camptothecin–DNA–topoisomerase I interaction were formulated.[4] Irinotecan and topotecan have emerged from these studies and are used in cancer treatment.[5] In the camptothecin series, structure–activity relationships are well established as regards modifications in rings A, B, D and E. They also indicated the need for a hydroxy lactone ring[6] and the capacity of substituents in positions 7, 9, 10 and 11 to maintain or improve biological activity. However, contradictory results have been observed concerning position 5. Indeed introduction of hydroxy, methoxy, oxycarbonyl or amino groups in this position generally results in inactive products,[7a] although 5-(2-hydroxyethoxy)camptothecin (DRF-1042) is in phase II clinical trial.[7b] In the same way, 5-ethylidenecamptothecin exhibits the same order of potency as campthothecin[7c] and some 5-methylenecarbonyl substituents are also tolerated whilst the inhibitory function is maintained[8] (Scheme 1).

Moreover, positions 5, 6 and 7 are face to face with the major groove of the DNA.[9] Improving the water solubility of camptothecins analogs in order to decrease the toxicity of the compound is also a major concern.[10]

As part of a program focusing on potential anti-cancer agents,[11] we wished to elucidate the influence of a carbonyl (acid, ester, amide) substituent on position 5 of cycle C. We wanted also to check if a 5-carboxamide group interacted with the major groove and improved the water solubility of camptothecin derivatives. Many total syntheses of camptothecins have already been published,[1b–d,12] and we search to apply our knowledge of the chemistry of pyroglutamic acid[13] and indolizines[14a,15] to the general approach of Danishefsky.[16] In this retrosynthetic scheme, a strategic point is a Friedlander condensation between aminobenzaldehyde and a keto indolizine (analog to compound **2**). Starting from racemic **3** we have recently obtained indolizines **4** and **5**, but we did not manage to introduce a heteroatom such as oxygen (**6**), bromine (**7**) or nitrogen (**8**) on the 1-position of compound **4** (Scheme 1).[17]

In this paper, we have focused on a new method to obtain a keto indolizine. The poor results previously observed for the reaction of selenium oxide or isoamyl nitrite with triester **4** were partly due to the Het-CH_2–CO group that reacted primarily with the reagent.[17] We have now chosen to add in

☆ Part 1 in this series: see Ref. 17.

Keywords: Keto tetrahydroindolizine; Camptothecin; Bredereck's reagent.
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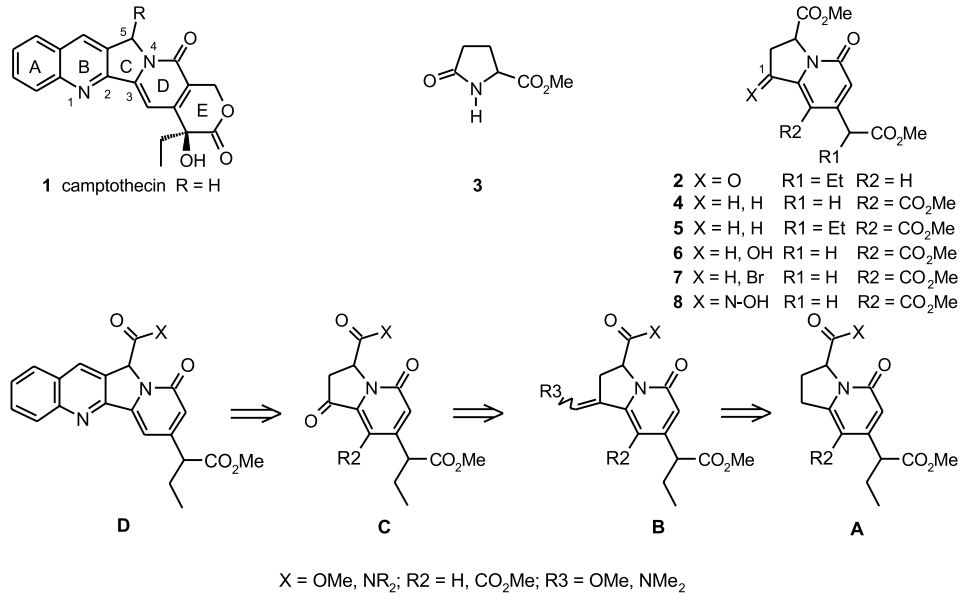


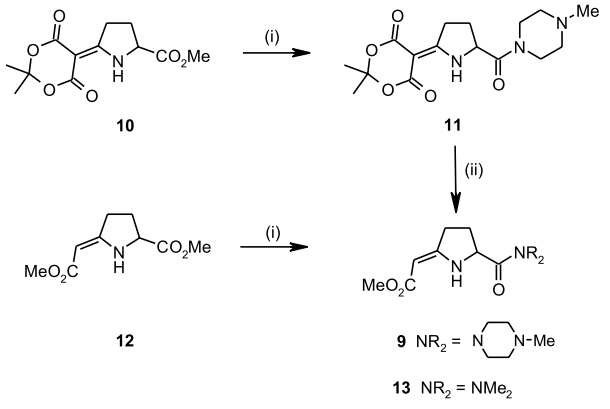
Scheme 1.

the ethyl chain at the beginning of the sequence in order to lower reactivity on this carbon (compounds A). We planned the formation of the needed ketone group (compounds C) by oxidation of an enol or enamide (compounds B), and the formation of indolizino[1,2-*b*]quinolin-9(11*H*)-one D as models for future camptothecins syntheses (Scheme 1). A carboxamide chain being a substituent of some target compounds, we checked also the possibility to introduce this group early in the synthesis.

2. Results and discussion

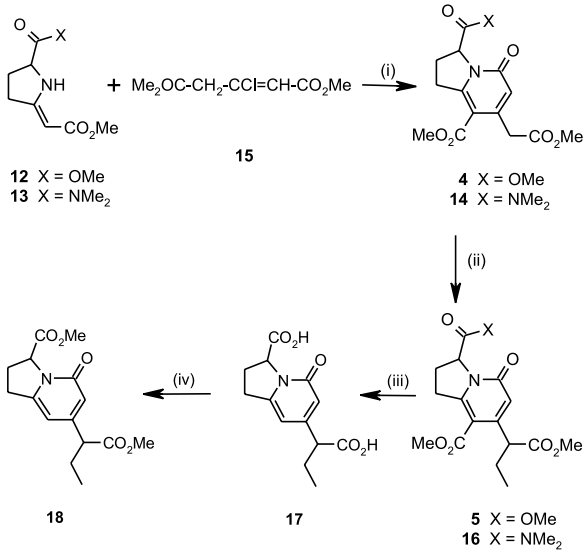
2.1. Synthesis of the starting pyridones

The Danishefsky approach to pyridones begins with enaminoesters.[16] We synthesized the enaminoester **9** substituted by a 5-carboxamide group from reaction of *N*-methylpiperazine with ester **10**.[18] This yielded amide **11** (44%), without opening of the Meldrum's ring.[19] Reflux of this product with a sodium methylate solution[14,20] then led to enamide **9** (38%). Alternatively, treatment of enaminoester **12**[14] with *N*-methylpiperazine gave 32% of compound **9** (Scheme 2). Although not optimized, the yields of amides **9** and **11** were low, probably because the free amino group of these *N*-acyl methylpiperazines posed problems during the isolation of products. Given the poor results obtained in the preceding reactions, a dimethylamide group was then chosen, and condensation of diester **12** with dimethylamine in methanol easily gave 77% of pure amide **13**.



Scheme 2. Reaction conditions: (i) *N*-methylpiperazine (**9**, reflux, 72 h, 32%; **11**, reflux, 40 h, 44%) or Me_2NH (**13**, MeOH, reflux, 4 h, 77%); (ii) MeONa, MeOH, reflux, 51 h (**9**, 38%).

We have already described[17] that pyridones **4** and **5** can be obtained according to the general method of Danishefsky.[16] In the same way, pyridone **14** was obtained in 95% yield by reacting dimethyl 3-chloroglutaconate (**15**)[16] with amide **13** in refluxing methanol. The use of ethanol as the solvent for this reaction led to the *trans*-esterification of one ester

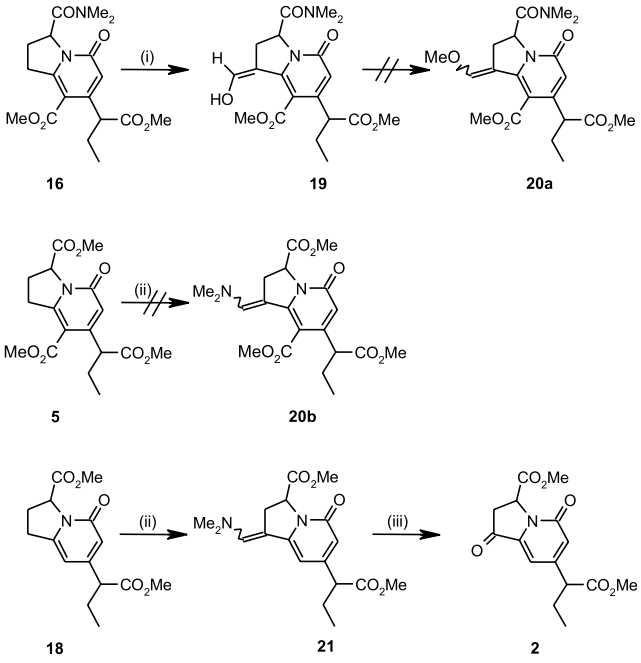


Scheme 3. Reaction conditions: (i) Et_3N, MeOH, MeOH, reflux, 29 h (**4**, 93%; **14**, 95%); (ii) EtI, NaH, THF, rt, 40 h (**5**, 92%; **16**, 78%); (iii) HBr, MeOH, reflux, 5h (**17**, 97%); (iv) MeOH, $MeSO_3H$, $CHCl_3$, molecular sieves 3 Å, reflux, 48 h (**18**, 97%).

group. An ethyl group was then easily introduced into compound **14**, resulting in diester **16** (78%) obtained as a mixture of diastereoisomers (Scheme 3). As for triester **5**, it was treated with 48% boiling HBr to give the pyridone **17** (97%). This diacid was then esterified with MeOH, while drying the ternary azeotrope H_2O/MeOH/$CHCl_3$.[21] That resulted in a very good yield of diester **18** (97%). (Scheme 3).

2.2. Synthesis of ketone 2

In previous works,[16] an oxygen atom was introduced into position 1 of indolizines by selenium oxidation. Because this approach was inefficient in our case, we thought that an indirect method might lead to the desired ketone **2**. Oxidation of double bonds can give ketones, and we planned to oxidize an enol ether group. As a preliminary test, we began the sequence described in Scheme 4, starting from indolizine **16**. A Vilsmeier and Haack related reaction[22] easily yielded 96% of an aldehyde in the desired position (in the enol form **19** according to ^{1}H NMR), but this compound proved to be rather unstable[23] and enol ether **20a** could not be obtained (Scheme 4).

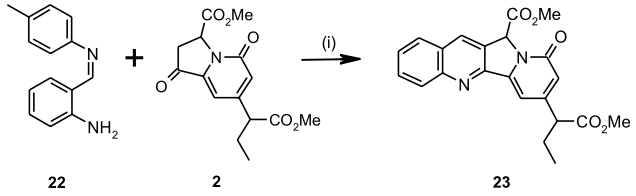


Scheme 4. Reaction conditions: (i) DMF, $POCl_3$, 80 °C, 24 h (**19**, 96%); (ii) *tert*-BuOCH(NMe_2)$_2$, 110 °C, 2 h (**21**, 98%); (iii) $NaIO_4$, H_2O, THF, rt, 30 min (**2**, 96%).

Another way to obtain the desired keto group starts from the Bredereck's reagent (*tert*-BuOCH(NMe_2)$_2$). This is an interesting amide acetal which easily reacts with a variety of heterocyclic compounds containing an active methylene,[24] resulting in a dimethylaminovinyl group. It is known that this type of double bond can be oxidized to a ketone by photochemistry[25] or by using $NaIO_4$.[26] Although triester **5** does not react with Bredereck's reagent, a 98% yield of crude enamine **21** was obtained when diester **18** was refluxed for 2 h in a slight excess of the reagent. This unstable black oil was used directly in the next step. Oxidation was then performed with $NaIO_4$ in a THF/H_2O mixture, giving ketone **2**. This compound was stable enough to be purified with 96% yield, as orange oil, by silica gel flash chromatography (Scheme 4). Because the carboxamide function of indolizine **16** cannot survive to the reflux in concentrated HBr needed to remove the aryl ester group, introduction of an amide functionality must not be realized at the beginning of the synthesis sequence.

2.3. Obtention of ABCD camptothecin rings

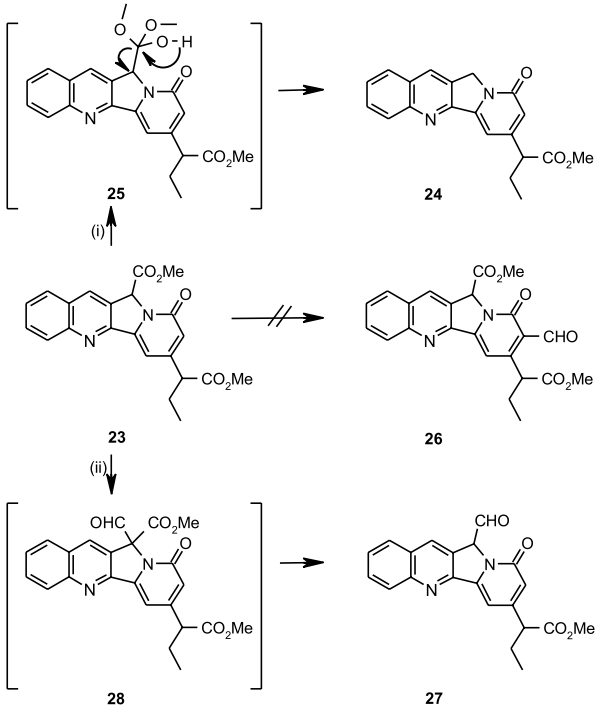
Friedlander reaction[27] has often been used for the synthesis of the quinoline ring of camptothecins.[1b,c,16,28] Because of the low stability of aminobenzaldehyde, it is often replaced by benzylidene toluidine **22**, obtained by reducing the corresponding nitroaromatic[29] with sodium sulphide.[30] Thus, ketone **2** was refluxed with **22** in acetic acid for 1 h, giving 73% of the target heterocycle **23** possessing the ABCD rings of camptothecin, substituted in position 5 by a methyl ester group (Scheme 5).



Scheme 5. Reaction conditions: (i) AcOH, reflux, 1 h (**23**, 73%).

2.4. Reactivity of the 5-methoxycarbonyl group

The 5-methoxycarbonyl group of **23** proved to be a rather labile group which is removed during refluxing in methanol. That led to 57% of the known camptothecin precursor **24**.[12a,31–33] A mechanism can be suggested for this reaction,



Scheme 6. Reaction conditions: (i) MeOH, reflux, 48 h (**24**, 57%); (ii) $POCl_3$, DMF, 80 °C, 24 h (**27**, 84%).

in which an anion is eliminated from intermediate **25** (Scheme 6). It has recently been demonstrated that an anion can easily be formed in the 5-position of camptothecins,[34] and loss of dimethyl carbonate when esters α-substituted by a withdrawing group are treated with alcohol, is a known process.[35]

Another example of the lability of a 5-methoxycarbonyl group in that scaffold is observed in the following reaction: although it was not the main goal of the present study, we have reacted diester **23** in the Vilsmeier–Haack condition in the hope to obtain aldehyde **26** which could be converted into a lactone heterocycle.[22,36] The Vilsmeier reaction has already been used for such a purpose (although in that case the pyridone ring was substituted by the electron-giving group –OMe).[22] Aldehyde **27** (84%) was the only compound isolated from that reaction. This product is of low stability and decomposes on SiO_2 or in the air. Diester **28** was an obvious intermediate in the formation of **27** (Scheme 6).

3. Conclusion

In this paper, we have resolved the crucial question of the formation of a ketone on the 1-position of tetrahydroindolizines and we have utilized this compound to built the ABCD ring scaffold of camptothecin. We have also shown that, if it is desired, a 5-carboxamide group does not need to be introduced at the beginning of the synthesis of modified camptothecins. Further application of this strategy towards the synthesis of 5-methoxycarbonyl camptothecin, is currently underway in this laboratory.

4. Experimental

4.1. Materials

Melting points were determined using an electrothermal apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained on a Varian Gemini 2000 at 200, 50 MHz, respectively. IR spectra were obtained in ATR mode on an FTIR Bruker Tensor 27. Thin layer chromatography (TLC) was performed on pre-coated Kieselgel $60F_{254}$ plates. Microanalyses were performed by the 'Service de Microanalyses' of LSEO, Université de Bourgogne, Dijon, France or by the 'Service Central de Microanalyses' of CNRS in Vernaison, France. The methyl pyroglutamate used was racemic.

4.1.1. Methyl 7-[1-(methoxycarbonyl)propyl]-1,5-dioxo-1,2,3,5-tetrahydro-3-indolizinecarboxylate (2). A mixture of enamide **21** (5 g, 14.4 mmol) and sodium periodate (9.22 g, 43.1 mmol) in tetrahydrofuran (50 mL) and water (50 mL) was stirred for 30 min. The solid obtained upon filtration was washed with dichloromethane (30 mL). The liquid phase was partitioned with dichloromethane and the combined organic phases were dried (Na_2SO_4) and then evaporated. The residue was purified by flash chromatography on SiO_2 (EtOAc) giving a mixture of two diastereoisomers of ketone **2** as an orange oil (96%). Due to his low stability, this product was only analyzed by NMR, and the crude compound was used for the next step; TLC R_f (EtOAc): 0.72; ^1H NMR ($CDCl_3$): δ ppm 0.92 and 0.93 (2t, $J=7.4$ Hz, 3H), 1.66–1.88 (m, 1H), 1.95–2.18 (m, 1H), 2.85 (dd, $J=19.1$, 3.7 Hz, 1H), 3.21 (dd, $J=19.1$, 9.1 Hz, 1H), 3.43 (t, $J=7.4$ Hz, 1H), 3.70 and 3.71 (2s, 3H), 3.83 (s, 3H), 5.20 (dd, $J=9.4$, 3.7 Hz, 1H), 6.75 (d, $J=1.4$ Hz, 1H), 6.92 (t, $J=1.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ ppm 11.8 (CH_3), 25.5 and 25.6 (CH_2), 38.3 (CH_2), 52.3 (CH), 52.7 (CH_3), 53.2 (CH), 54.7 (CH_3), 103.9 and 104.0 (CH), 125.1 and 125.2 (CH), 139.1 (C), 152.4 (C), 160.3 (C), 169.3 (C), 172.0 (C), 193.8 (C).

4.1.2. Methyl 5-[(4-methyl-1-piperazinyl)carbonyl]-2-pyrrolidinylideneacetate (9). *From* **12**. A stirred solution of enaminoester **12** (1 g, 5 mmol) in *N*-methylpiperazine (1.6 mL, 1.4 g, 14.4 mmol) was refluxed for 72 h (N_2). After cooling at rt, the mixture was purified by chromatography (SiO_2, MeOH), giving amide **9** as a grey powder (32%), mp (MeOH): 165–167 °C; TLC R_f (MeOH): 0.30; IR (KBr): ν cm^{-1} 1650, 1600, 1220; ^1H NMR ($CDCl_3$): δ ppm 1.99–2.17 (m, 1H), 2.17–2.31 (m, 1H), 2.37 (s, 3H), 2.41–2.55 (m, 4H), 2.55–2.68 (m, 1H), 2.68–2.85 (m, 1H), 3.51–3.63 (m, 2H), 3.63–3.77 (m, 2H), 3.64 (s, 3H), 4.60 (dd, $J=8.0$, 6.0 Hz, 1H), 4.61 (s, 1H), 8.03 (s, 1H, deuterium oxide exchangeable); ^{13}C NMR ($CDCl_3$): δ ppm 26.4 (CH_2), 31.3 (CH_2), 41.8 (CH_2), 44.8 (CH_2), 45.8 (CH_3), 50.2 (CH_3), 54.4 (CH_2), 58.8 (CH_2), 72.2 (CH), 77.1 (CH), 165.1 (C), 169.2 (C), 170.4 (C).

Anal. Calcd for $C_{13}H_{21}N_3O_3$: C, 58.41; H, 7.92; N, 15.72; O, 17.95. Found: C, 58.01; H, 7.89; N, 15.49; O, 18.21.

From **11**. A stirred solution of amide **11** (0.3 g, 5 mmol) and sodium methoxide (0.097 g, 1.8 mmol) in methanol (5 mL) was refluxed for 51 h (N_2). The solution was acidified to about pH 7 with diluted HCl, and then evaporated. The residue was dissolved in dichloromethane (10 mL), and the solution was washed with water (7 mL), leading to amide **9** as a grey powder (38%). Physical properties are identical to those of the compound obtained from ester **12**.

4.1.3. 2,2-Dimethyl-5-{5-[(4-methylpiperazin-1-yl)carbonyl]pyrrolidin-2-ylidene}-1,3-dioxane-4,6-dione (11). A stirred mixture of ester **10** (10 g, 37.2 mmol) and *N*-methylpiperazine (10 mL, 9.0 g, 90 mmol) was refluxed for 40 h (N_2). After cooling at rt, diethyl ether (150 mL) was added and the mixture was refluxed for 1 h. The solid obtained on filtration was refluxed again in diethyl ether (150 mL) for 1 h then purified by chromatography (SiO_2, CH_2Cl_2/MeOH 96:4) to give amide **11** as a white powder (44%), mp (Et_2O): 154–156 °C; TLC R_f (MeOH): 0.20; IR (KBr): ν cm^{-1} 1770, 1600, 1220; ^1H NMR ($CDCl_3$): δ ppm 1.70 (s, 3H); 1.72 (s, 3H), 2.07–2.26 (m, 1H), 2.37 (br s, 3H), 2.42–2.61 (m, 5H), 3.47 (m, 2H), 3.42–3.62 (m, 2H), 3.62–3.82 (m, 2H), 4.84 (dd, $J=9.0$, 6.3 Hz, 1H), 10.21 (s, 1H, deuterium oxide exchangeable); ^{13}C NMR ($CDCl_3$): δ ppm 25.7 and 25.9 (CH_3), 26.4 (CH_2), 26.6 and 26.8 (CH_3), 34.4 and 34.8 (CH_2), 41.9 and 44.9 (CH_2), 43.0 (CH_2), 45.5 and 45.7 (CH_3), 51.8 (CH_2), 54.2 and 54.5 (CH_2), 60.2 (CH), 81.3 and 82.3 (C), 103.0 and 103.3 (C), 163.0 and 163.1 (C), 166.0 and 166.2 (C), 167.7 (C), 175.8 (C), 176.4 (C).

Anal. Calcd for $C_{16}H_{23}N_3O_5$, 0.25 H_2O: C, 56.21; H, 6.93; N, 12.29; O, 24.57. Found: C, 55.98; H, 6.88; N, 12.45; O, 24.57.

4.1.4. 5-Ethylidene-pyrrolidine-2-carboxylic acid dimethylamide (13). A stirred solution of diester **12** (20 g, 100 mmol) in methanol (100 mL) was heated at 50 °C for 4 h while dimethylamine was bubbled until saturation. After cooling at 0 °C for 12 h the solid was filtered then washed with ether, giving a mixture of two geometrical isomers of amide **13** as a white powder (77%), mp (MeOH): 147–149 °C; TLC R_f (CH_2Cl_2/MeOH, 95:5): 0.44; IR: ν cm^{-1} 3320, 1650, 1635, 1590, 1190; ^1H NMR ($CDCl_3$): δ ppm 1.99–2.19 (m, 1H), 2.19–2.38 (m, 1H), 2.52–2.71 (m, 1H), 2.71–2.87 (m, 1H), 2.97 (s, 3H), 3.08 (s, 3H), 3.64 (s, 3H), 4.63 (dd, J=7.9, 4.9 Hz, 1H), 4.80 (s, 1H, deuterium oxide exchangeable); the NH group was not observed; ^{13}C NMR ($CDCl_3$): δ ppm 25.8 (CH_2), 30.7 and 31.0 (CH_3), 35.5 and 36.2 (CH_2), 49.8 and 52.1 (CH_3), 58.4 and 59.9 (CH), 78.0 (CH), 164.6 and 165.3 (C), 170.3 and 170.7 (C), 172.1 (C).

Anal. Calcd for $C_{10}H_{16}N_2O_3$: C, 56.59; H, 7.60; N, 13.20. Found: C, 56.94; H, 7.28; N, 12.85.

4.1.5. Methyl 3-[(dimethylamino)carbonyl]-7-(2-methoxy-2-oxoethyl)-5-oxo-1,2,3,5-tetrahydro-8-indolizinecarboxylate (14). A stirred mixture of amide **13** (15 g, 70 mmol) and diester **15** (19.1 g, 100 mmol) in methanol (100 mL) and triethylamine (19.6 mL, 140 mmol) was refluxed for 29 h. The solution was evaporated, dichloromethane (200 mL) was added and the solution was partitioned with water (3×50 mL). The organic phase was dried (Na_2SO_4) then evaporated. The residue crystallized from methanol giving the pyridone **14** as slightly yellow crystals (95%), mp (MeOH): 150–152 °C; TLC R_f (CH_2Cl_2/MeOH, 95:5): 0.34; IR: ν cm^{-1} 1740, 1710, 1650, 1595, 1525, 1445, 1100; ^1H NMR ($CDCl_3$): δ ppm 2.10–2.28 (m, 1H), 2.28–2.52 (m, 1H), 3.0 (s, 3H), 3.23 (s, 3H), 3.60 (dd, J=9.8, 5.8 Hz, 2H), 3.61 (d, J=16.8 Hz, 1H), 3.71 (s, 3H), 3.78 (s, 3H), 3.96 (d, J=16.8 Hz, 1H), 5.50 (dd, J=9.4, 2.3 Hz, 1H), 6.26 (s, 1H); ^{13}C NMR ($CDCl_3$): 25.4 (CH_2), 34.0 (CH_2), 35.9 (CH_3), 37.1 (CH_3), 41.2 (CH_2), 51.5 (CH_3), 52.0 (CH_3), 59.6 (CH), 106.3 (C), 120.0 (CH), 147.8 (C), 158.3, 160.4 (C), 165.7 (C), 169.0 (C), 170.8 (C).

Anal. Calcd for $C_{16}H_{20}N_2O_6$: C, 57.14; H, 5.99; N, 8.33. Found: C, 56.83; H, 6.06; N, 8.18.

4.1.6. Methyl 3-[(dimethylamino)carbonyl]-7-[1-(methoxycarbonyl)propyl]-5-oxo-1,2,3,5-tetrahydro-indolizine-8-carboxylate (16). Sodium hydride (157.4 mg, 6.5 mmol) then diester **14** (2 g, 5.9 mmol) was added to tetrahydrofuran (38 mL) (glove bag). The suspension was stirred at rt for 20 min. Ethyl iodide (1.54 mL, 17.7 mmol) was added (syringe), and then the mixture was stirred at rt for 40 h (N_2). Methanol (12 mL) was added and the mixture was evaporated. Dichloromethane (100 mL) was added and the solution was partitioned with 0.1 N HCl (2×25 mL). The organic phase was dried (Na_2SO_4) then evaporated, giving a mixture of diastereoisomers **16** which crystallized from ethyl acetate (78%), mp (EtOAc): 82–84 °C; TLC R_f (CH_2Cl_2/MeOH, 95:5): 0.4; IR: ν cm^{-1} 1740, 1710, 1645, 1590, 1520, 1445, 1195; ^1H NMR ($CDCl_3$): δ ppm 0.94 and 0.95 (2t, J=7.4 Hz, 3H), 1.62–1.89 (m, 1H), 1.95–2.27 (m, 2H), 2.27–2.51 (m, 1H), 3.01 (s, 3H), 3.23 (s, 3H), 3.34–3.63 (m, 2H), 3.66 and 3.67 (2s, 3H), 3.81 and 3.82 (2s, 3H), 3.95–4.18 (m, 1H), 5.49 and 5.50 (2dd, J=9.5, 2.3 Hz, 1H), 6.36 and 6.37 (2s, 1H); ^{13}C NMR ($CDCl_3$): δ ppm 12.4 (CH_3), 25.4 and 25.5 (CH_2), 25.7 (CH_2), 33.8 (CH_2), 36.0 (CH_3), 37.2 (CH_3), 49.8 and 49.9 (CH), 51.6 (CH_3), 52.0 (CH_3), 59.4 (CH), 106.6 (C), 117.3 (CH), 152.4 (C), 157.1 (C), 160.5 (C), 165.9 (C), 168.9 (C), 172.8 and 173.1 (C).

Anal. Calcd for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69. Found: C, 58.95; H, 6.71; N, 7.73.

4.1.7. 7-(1-Carboxypropyl)-5-oxo-1,2,3,5-tetrahydro-3-indolizinecarboxylic acid (17). A stirred solution of triester **5** (10 g, 28.5 mmol) in 48% hydrobromic acid (60 mL) was heated at 135 °C for 5 h. The residue obtained upon evaporation crystallized from acetone, giving the mixture of two diastereoisomers **17** as a white powder (97%), mp (acetone): 183–185 °C; TLC R_f (MeOH): 0.60; IR: ν cm^{-1} 3500, 3360, 1710, 1645, 1550, 1525, 1460, 1200; ^1H NMR (D_2O/NaOD): δ ppm 0.87 (t, J=7.3 Hz, 3H), 1.67–1.87 (m, 1H), 1.90–2.09 (m, 1H), 2.27–2.46 (m, 1H), 2.50–2.75 (m, 1H), 3.19 (dd, J=9.1, 6.2 Hz, 2H), 3.53 (t, J=7.6 Hz, 1H), 5.13 (dd, J=9.8, 3.3 Hz, 1H), 6.42 (d, J=1.3 Hz, 1H), 6.55 (d, J=1.3 Hz, 1H); ^{13}C NMR (D_2O/NaOD): δ ppm 14.4 (CH_3), 28.0 (CH_2), 29.4 (CH_2), 32.7 (CH_2), 59.5 and 59.6 (CH), 67.4 (CH), 107.4 and 107.7 (CH), 116.2 and 116.3 (CH), 154.7 and 154.9 (C), 160.9 (C), 165.7 (C), 179.9 (C), 183.6 (C).

Anal. Calcd for $C_{13}H_{15}NO_5$, 0.5 H_2O: C, 56.93; H, 5.88; N, 5.11. Found: C, 56.71; H, 6.04; N, 5.67.

4.1.8. Methyl 7-[1-(methoxycarbonyl)propyl]-5-oxo-1,2,3,5-tetrahydro-3-indolizinecarboxylate (18). A solution of diacid **17** (10 g, 37.7 mmol) and methanesulfonic acid (0.3 mL, 4.6 mmol) in chloroform (200 mL) and methanol (300 mL) was refluxed for 48 h while drying the solvent by condensing it in a soxhlet-type apparatus containing 3 Å molecular sieves (100 g). Dichloromethane (100 mL) was added to the residue obtained upon evaporation and the solution was washed with an $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4) and then evaporated. Compound **18** (97%) crystallized from ethyl acetate as a mixture of two diastereoisomers, mp (EtOAc): 73–75 °C; TLC R_f (EtOAc): 0.43; IR: ν cm^{-1} 1725, 1660, 1590, 1530, 1430, 1205; ^1H NMR ($CDCl_3$): δ ppm 0.91 and 0.92 (2t, J=7.4 Hz, 3H), 1.61–1.86 (m, 1H), 1.89–2.14 (m, 1H), 2.20–2.41 (m, 1H), 2.41–2.62 (m, 1H), 2.93–3.23 (m, 2H), 3.23–3.34 (t, J=7.6 Hz, 1H), 3.69 and 3.70 (2s, 3H), 3.79 (s, 3H), 5.08 and 5.09 (2 dd, J=9.4, 3.3 Hz, 1H), 6.15 and 6.16 (2s, 1H), 6.30 (s, 1H); ^{13}C NMR ($CDCl_3$): δ ppm 11.8 (CH_3), 25.4 (CH_2), 26.0 (CH_2), 30.2 (CH_2), 52.0 (CH), 52.6 (CH_3), 52.8 (CH_3), 60.8 (CH), 100.9 (CH), 116.3 (CH), 149.9 (C), 152.5 (C), 161.2 (C), 170.3 (C), 172.7 (C).

Anal. Calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.49; H, 6.78; N, 5.17.

4.1.9. Methyl 3-[(dimethylamino)carbonyl]-1-formyl-7-[1-(methoxycarbonyl)propyl]-5-oxo-1,2,3,5-tetrahydro-8-indolizinecarboxylate (19). Phosphorus oxychloride

(0.47 mL, 3 mmol) was added to dimethylformamide (1.5 mL, 19.4 mmol) at 0 °C. The solution was stirred for 30 min (N_2), and then a solution of pyridone **16** (1 g, 2.74 mmol in dimethylformamide (1.5 mL) was added. The mixture was heated at 80 °C for 24 h (N_2). After cooling at rt, water (50 mL) and dichloromethane (50 mL) were added. The organic phase was partitioned with water (3×50 mL) and then dried (Na_2SO_4). The residue obtained upon evaporation was purified by flash chromatography on $C_{18}SiO_2$ (MeOH/H_2O 70:30). Aldehyde **19** was obtained as a mixture of two diastereoisomers of as a coloured power (96%); TLC ($C_{18}SiO_2$) R_f (MeOH/H_2O 60:40): 0.5l, mp (EtOAc): 135–137 °C; IR: ν cm^{-1} 1740, 1715, 1670, 1655, 1605, 1590, 1555, 1230; ^1H NMR ($CDCl_3$): δ ppm 1.08 and 1.09 (2t, J=7.4 Hz, 3H); 1.91–2.27 (m, 2H), 3.04 and 3.06 (2s, 3H), 3.15 (ddd, J=15.9, 4.2, 1.7 Hz, 1H), 3.28 and 3.29 (2s, 3H), 3.42 (s, 3H), 3.45 (ddd, J=15.9, 9.8, 1.7 Hz, 1H), 3.68 and 3.75 (2s, 3H), 5.58 and 5.59 (2dd, J=9.8, 4.2 Hz, 1H), 6.52 and 6.53 (2s, 1H), 7.13 (t, J=1.5 Hz, 1H), 10.01 (s, 1H). This compound was no further analyzed.

4.1.10. Methyl 1-[(dimethylamino)methylene]-7-[1-(methoxycarbonyl)propyl]-5-oxo-1,2,3,5-tetrahydro-3-indolizinecarboxylate (21). A stirred mixture of diester **18** (10 g, 34.1 mmol) and Bredereck's reagent (7 g, 40.9 mmol) was heated at 110 °C for 2 h (N_2). After cooling at rt, dichloromethane (150 mL) was added, and then the solution was washed with water (3×100 mL). The organic phase was dried (Na_2SO_4) then evaporated, giving a mixture of two diastereoisomers of enamide **21** as a black oil (98%). This crude compound was used for the next step; TLC ($C_{18}SiO_2$) R_f (EtOAc): 0.2; IR: ν cm^{-1} 1730, 1645, 1515, 1430, 1200; ^1H NMR ($CDCl_3$): δ ppm 0.90 and 0.91 (2t, J=7.3 Hz, 3H), 1.61–1.88 (m, 1H), 1.88–2.16. (m, 1H), 3.00 (s, 6H), 3.11 (ddd, J=15.0, 3.1, 1.3 Hz, 1H), 3.22. (t, J=7.8 Hz, 1H), 3.44 (dd, J=15.0, 10.8 Hz, 1H), 3.68 and 3.69 (2s, 3H), 3.77 (s, 3H), 5.01 (dd, J=10.8, 4.3 Hz, 1H), 5.97 (q, J=1.6 Hz, 1H), 5.97 (s, 1H), 6.68 (t, J=1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ ppm 11.8 (CH_3), 25.5 (CH_2), 30.0 (CH_2), 42.0 (CH_3), 51.9 (CH), 52.4 (CH_3), 53.0 (CH_3), 59.3 (CH), 91.8 (CH), 97.0 (C), 110.3 (CH), 138.6 (C), 152.6 (C), 153.0 (C), 161.5 (C), 170.8 (C), 173.1 (C).

4.1.11. Methyl 7-[1-(methoxycarbonyl)propyl]-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinoline-11-carboxylate (23). A solution of ketone **2** (1 g, 3.2 mmol) and aniline **21** (0.82 g, 3.9 mmol) in acetic acid (10 mL) was refluxed for 1 h (N_2). After cooling, water (50 mL) was added and the solution was partitioned with dichloromethane (50 mL). The organic phase was washed with brine, with a saturated $NaHCO_3$ solution, and then dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on SiO_2 (EtOAc), resulting in **23** as a white powder (73%), mp (EtOAc): 171–173 °C; IR: ν cm^{-1} 1740, 1690, 1655, 1615, 1585, 1545, 1500, 1170; ^1H NMR ($CDCl_3$): δ ppm 0.98 and 0.99 (2t, J=7.3 Hz, 3H), 1.80–2.02 (m, 1H), 2.06–2.30 (m, 1H), 3.50 (t, J=7.8 Hz, 1H), 3.73 and 3.75 (2s, 3H), 3.84 (s, 3H), 6.06 and 6.07 (2d, J=0.8 Hz, 1H), 6.66 (t, J=1.7 Hz, 1H), 7.31 and 7.32 (2d, J=1.7 Hz, 1H), 7.66 (td, J=6.8, 1.0 Hz, 1H), 7.84 (td, J=6.8, 1.5 Hz, 1H), 7.94 (dd, J=8.0, 1.2 Hz, 1H), 8.22 (bd, J=8.0 Hz, 1H), 8.44 (br s, 1H); $^{13}C(CDCl_3)$: δ ppm 11.9 (CH_3), 25.5 and 25.6 (CH_2), 52.2 (CH_3), 53.1 (CH), 53.4 (CH_3), 62.1 (CH), 101.1 (CH), 120.1 (C), 127.3 (CH), 127.8 (CH), 127.9 (CH), 128.2 (CH), 129.6 (CH), 130.8 (C), 131.0 (CH), 145.1 (C), 149.2 (C), 152.0 (C), 153.0 (C), 160.5 (C), 166.4 (C), 172.4 (C).

Anal. Calcd for $C_{22}H_{20}N_2O_5$, H_2O: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.49; H, 5.13; N, 7.21.

4.1.12. Methyl 2-(9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-7-yl)butanoate (24). A solution of diester **23** (1 g, 3.2 mmol) in methanol (20 mL) was refluxed for 2 days. On evaporation the residue was purified by flash chromatography on SiO_2 (EtOAc), giving ester **24** as a white power (57%), mp (EtOAc): 225–227 °C (229–230 °C);[33] TLC R_f (EtOAc): 0.38; IR: ν cm^{-1} 1735, 1660, 1600, 1455, 1435, 1150; ^1H NMR ($CDCl_3$): δ ppm 0.97 (t, J=7.6 Hz, 3H), 1.80–2.04 (m, 1H), 2.04–2.31 (m, 1H), 3.50 (t, J=7.9 Hz, 1H), 3.73 (s, 3H), 5.25 (d, J=1.2 Hz, 2H), 6.66 (dd, J=2.1, 1.7 Hz, 1H), 7.33 (d, J=1.7 Hz, 1H), 7.64 (dt, J=7.9, 1.3 Hz, 1H), 7.82 (dt, J=7.9, 1.6 Hz, 1H), 7.92 (dd, J=8.1, 1.5 Hz, 1H), 8.22 (dd, J=8.3, 1.2 Hz, 1H), 8.36 (dd, J=1.2, 0.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ ppm 12.0 (CH_3), 25.5 (CH_2), 49.6 (CH_2), 52.2 (CH_3), 53.1 (CH), 100.9 (CH), 119.3 (CH), 127.6 (C), 127.9 (CH), 128.0 (CH), 128.6 (CH), 129.5 (CH), 130.3 (C), 130.8 (CH), 145.8 (C), 148.6 (C), 152.6 (C), 152.7 (C), 161.1 (C), 172.6 (C).

Anal. Calcd for $C_{20}H_{18}N_2O_3$, 0.5 H_2O: C, 69.96; H, 5.58; N, 8.16. Found: C, 69.58; H, 5.42; N, 7.96.

4.1.13. Methyl 2-(11-formyl-9-oxo-9,11-dihydroindolizino[1,2-*b*]quinolin-7-yl)butanoate (27). Phosphorus oxychloride (0.37 mL, 2.4 mmol) was added to dimethylformamide (1 mL, 12.8 mmol) at 0 °C. The solution was stirred for 30 min (N_2), and then a solution of diester **23** (0.5 g, 1.6 mmol) in dimethylformamide (1 mL) was added. The mixture was heated at 80 °C for 24 h (N_2). After cooling at rt, water (25 mL) and dichloromethane (25 mL) were added. The organic phase was partitioned with water (3×25 mL) then washed with a saturated solution of $NaHCO_3$. After drying (Na_2SO_4), the solution was evaporated giving aldehyde **27** which crystallized from ethyl acetate as a brown powder (84%) that rapidly decomposed. This unstable compound also decomposed considerably during SiO_2 flash chromatography (EtOAc) to give a very poor yield of impure **27**; IR: ν cm^{-1} 1730, 1655, 1595, 1515, 1165; ^1H NMR ($CDCl_3$): δ ppm 0.95 (s, 3H), 1.75–1.90 (m, 1H), 1.95–2.25 (m, 1H), 3.49 (t, J=7.7 Hz, 1H), 3.75 (s, 3H), 6.10 (s, 1H), 6.72 (s, 1H), 7.41 (s, 1H), 7.64 (t, J=6.9 Hz, 1H), 7.85 (t, J=6.9 Hz, 1H), 7.94 (d, J=7.9 Hz, 1H), 8.23 (d, J=7.9 Hz, 1H), 8.49 (s, 1H), 9.82 (s, 1H).

Anal. Calcd for $C_{21}H_{18}N_2O$: C, 69.90; H, 5.01; N, 7.73. Found: C, 69.12; H, 5.42; N, 7.53.

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New C_2-symmetric 2,2′-bipyridine crown macrocycles for enantioselective recognition of amino acid derivatives

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Abstract—A series of new C_2-symmetric 2,2′-bipyridine-contaning crown macrocycles **1**–**4** has been developed for enantiomeric recognition of amino acid derivatives. These new macrocycles have been showed to be strong complexing agents for primary organic ammonium salts (with K up to 4.83×10^5 M^{-1} and $-\Delta G_0$ up to 32.4 kJ mol^{-1}) and also useful chromophores for UV–vis titration studies. These macrocyclic hosts exhibited enantioselective binding towards the (*S*)-enantiomer of phenylglycine methyl ester hydrochloride (**Am1**) with $K_{(S)}/K_{(R)}$ up to 2.10 ($\Delta\Delta G_0 = -1.84$ kJ mol^{-1}) in CH_2Cl_2 with 0.25% CH_3OH. The structure–binding relationship studies showed that the aromatic subunit and the ester group of the ammonium guests are both important for good enantioselectivity. In addition, the host–guest complexes have been studied using various NMR experiments.

1. Introduction

One of the most remarkable features of many biomolecules, such as enzymes, is their abilities to distinguish enantiomers in biological reactions. Most of the amino acids and their derivatives, which are basic building blocks of many biologically important molecules, are chiral. The development of artificial receptors for these interesting compounds becomes an important research area because it can provide valuable information for a better understanding of the interactions between molecules in nature. Moreover, the studies of their recognition properties may also lead to the development of useful molecular devices and materials in biochemical[1,2] and pharmaceutical studies,[3] separation processes,[4] catalysis[5] and sensing.[6]

In the passed decade, chiral pyridine-containing macrocycles have been an attractive research area due to their ability of chiral discrimination towards organic ammonium salts and the amino acid derivatives.[7–11] The pyridine subunit of these macrocycles were reported to be important for the tripod hydrogen bonding formation with the primary ammonium salts and the π–π interaction with the aromatic moiety of the ammonium guests.[7] Since, we have been developing various types of pyridine-containing ligands for asymmetric catalysis,[12] we are interested in developing new artificial receptors for enantiomeric recognition of amino acid derivatives based on 2,2′-bipyridine (bpy).

Bpys have been used widely as metal chelating ligands due to their strong chelating ability towards various metals and ease of functionalization.[13] For instance, the bpy–ruthenium complexes have been studied extensively due to their interesting photochemical and other properties.[14] The results of these studies had led to the development of various types of bpy–ruthenium complexes-based sensors.[15] In contrast, the study of the interaction between bpy-containing macrocycles[16] and organic cationic substrates remains an unexplored area. Herein, we report the synthesis of a series of new bpy crown macrocycles (Fig. 1), and the study of their enantiomeric recognition properties towards amino acid derivatives and chiral organic ammonium salts.

2. Results and discussion

2.1. Preparation of new chiral bpy crown macrocycles

Bpy crown macrocycles **1**–**4** could be readily prepared via the two-step one-pot protocol shown in Scheme 1, which involved deprotonation of (*R*,*R*)-6,6′-bipyridinediol **5**[17] followed by cyclization with the appropriate ethyl glycol

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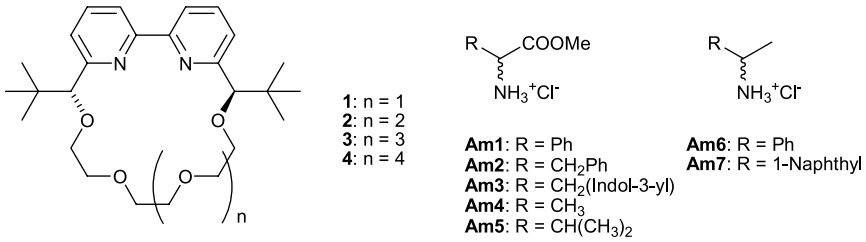
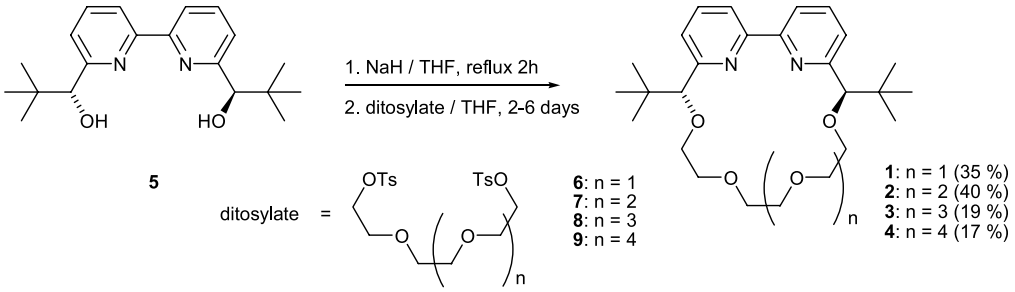


Figure 1. Chiral bpy crown macrocycles **1**–**4** (hosts) and chiral organic ammonium salts **Am1**–**Am7** (guests).



Scheme 1. Synthesis of chiral bpy crown macrocycles **1**–**4**.

ditosylate (**6**–**9**). To determine the optimal conditions for the cyclization process, reaction of diol **5** with triethylene glycol ditosylate (**6**) was first investigated using potassium hydride in THF under high dilution conditions. Since bpy crown macrocycle **1** contains a pseudo 18-crown-6 frame work, the presence of potassium ions was expected to give the optimal template effect on the cyclization process.[18] After 2 days stirring at room temperature, diol **5** was consumed and the MS-ESI analysis of the crude product mixture shows the formation of the bpy crown macrocycle. However, only 15% of the cyclization product (**1**) was isolated. Bpy crown macrocycle **1** was characterized unambiguously by elemental analysis, MS-ESI and NMR experiments. The ^{1}H and ^{13}C NMR spectra of **1** show only one set of signals for the two pyridine rings of the bpy subunit and a singlet for the two *t*-butyl groups, which indicate that the C_2-symmetry of bpy diol **5** is unaffected after the incorporation of the crown moiety. Moreover, the signals corresponding to the ethylene glycol units are also well resolved, which suggested that the bpy crown macrocycle is well accommodated in the pseudo 18-crown-16 frame work.

The cyclization protocol was then investigated using sodium hydride under the same reaction conditions. Surprisingly, these conditions afforded the cyclization product in a much higher isolated yield (35%). These results suggested that the template effect of alkaline metal ion may not be effective in this system, due to the rigid bpy subunit of the macrocycle. In addition, the solvent effect had also been studied. Cyclization using NaH in DMF under the high dilution condition gave a similar yield of **1** (33%) in 2 days. However, switching to DMSO resulted in only trace amounts of the cyclization product with 56% of diol **5** recovered under the same conditions.

After optimizing the cyclization conditions, diol **5** was deprotonated using sodium hydride in THF followed by treatment of the corresponding ditosylate (**7**–**9**) under the high dilution conditions to afford the desired chiral bpy crown macrocycles **2**–**4** in 17–40% isolated yields (Scheme 1). The structures of this new series of bpy crown macrocycles were characterized using the same methods as **1**. However, reaction between **5** and di(ethylene glycol)ditosylate gave only trace amounts of the expected cyclization product. This poor result may be due to the high ring strain of the bipyridino-15-crown-5 structure of the cyclization product.

2.2. Enantiomeric recognition studies using UV–vis method

With the new macrocyclic hosts prepared, we first examined the binding properties of bpy crown macrocycle **1** towards (*R*)-(−)-2-phenylglycine methyl ester hydrochloride ((*R*)-**Am1** in Fig. 1) using the UV–vis titration method. The concentration of the macrocyclic host was fixed at 2.5×10^{-5} M in CH_2Cl_2 with 0.25% of CH_3OH because of the solubility of the guest. The UV−vis signals with the guest concentration varied from 0 to 6×10^{-5} M were then observed. As shown in Figure 2, the absorption peak at

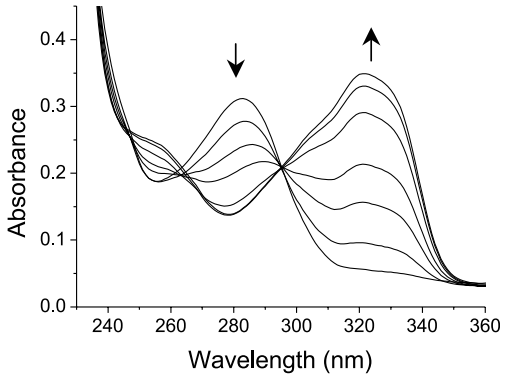


Figure 2. UV–vis titration of **1** with (*R*)-**Am1** at 298 K in CH_2Cl_2 containing 0.25% CH_3OH: [**1**]$=2.5\times10^{-5}$ M and [(*R*)-**Am1**]$=0$, 0.5, 1, 1.5, 3, 4.5, 6×10^{-5} M.

298 nm for **1** decreased gradually upon addition of the guest and a new absorption peak for the inclusion complex started to appear at 320 nm forming the isosbestic point at 305 nm. In contrast, binding studies using dimethylether of **5** led to only small and irregular change in the UV spectrum. This result indicated that the crown portion of the macrocycle is essential for binding. The Job's plot based on the absorbance at 320 nm supported the 1:1 stoichiometry of the host–guest complex (Fig. 3).

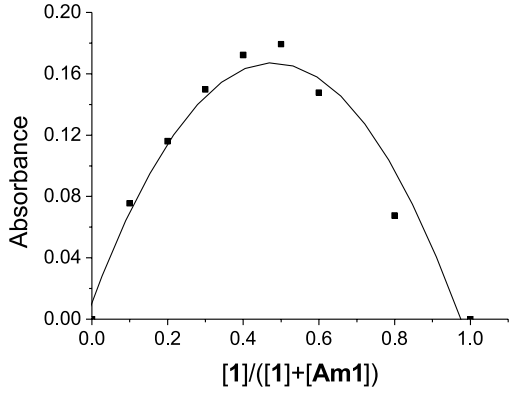


Figure 3. Job's plot of bpy crown macrocycle **1** and (*R*)-**Am1** in CH_2Cl_2 containing 0.25% CH_3OH at 298 K with [**1**]+[(*R*)-**Am1**]=5.0×10^{-5} M.

$$\frac{A_0}{A_0 - A} = \frac{\varepsilon_0}{\varepsilon_0 - \varepsilon}\left(1 + \frac{1}{K[\mathbf{Am}]_i}\right) \quad (1)$$

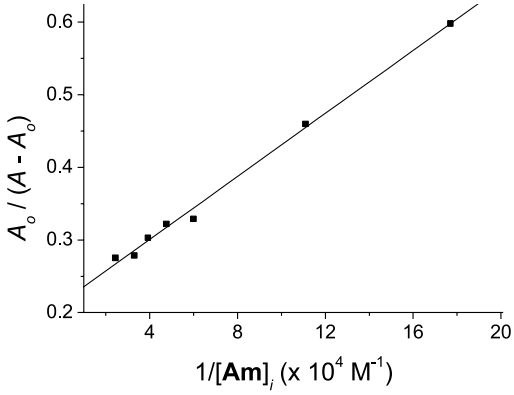


Figure 4. The plot of $A_0/(A-A_0)$ versus $1/[\mathbf{Am}]_i$ at 320 nm based on the 1:1 binding model of bpy crown macrocycle **1** with (*R*)-**Am1**.

The equilibrium binding constant (K) was estimated using Eq. 1, where A and A_0 are the absorbance of the inclusion complex at $\lambda_{max}=320$ nm with concentration of free **Am**=$[\mathbf{Am}]_i$ and 0, respectively, ε_0 and ε are the molar absorption coefficients for the free and **Am**-bound bpy macrocycle at $\lambda_{max}=320$ nm, respectively. Since the assumption of $[\mathbf{Am}]_i$ being equal to the total concentration of **Am** is not adequate in our system, the values of $[\mathbf{Am}]_i$ were calculated using the Taylor's series approximation.[19] The plot of $A_0/(A_0-A)$ against $1/[\mathbf{Am}]_i$ showed good linear relationship with $R=0.998$ for (*R*)-**Am1** (Fig. 4) and 0.992 for (*S*)-**Am1**. The binding constants were determined by the ratio of *y*-intercept to the slope,[20] which gave $K_{(R)}=9.86\pm0.77\times10^4\ M^{-1}$ and $K_{(S)}=20.7\pm1.29\times10^4\ M^{-1}$. These equilibrium binding constants are considered to be large among the analogous pyridine-containing crown macrocycles.[7,8]

To study the effect on the cavity size of the macrocyclic hosts, the binding properties of bpy crown macrocycles **2**–**4** towards **Am1** were studied using the same UV–vis titration method. As shown in Table 1, all the bpy crown macrocycles showed enantioselectivity towards (*S*)-**Am1** regardless of the cavity size of the macrocycles. Bpy crown macrocycle **1** showed the best enantiomeric recognition ability towards **Am1** with $K_{(S)}/K_{(R)}$ equals 2.1 ($\Delta\Delta G_0=-1.84$ kJ mol^{-1}). The high enantioselectivity of **1** might due to its pseudo 18-crown-6 frame work, which seems to provide a good environment for hydrogen bonding and π–π interaction with the guest molecule.[7]

After optimizing the cavity size of the macrocyclic host, the effect on the structure of the ammonium guest was then investigated. A number of amino acid derivatives and chiral organic ammonium salts were submitted for the enantiomeric recognition studies with bpy crown macrocycle **1** and the results were summarized in Table 2. Generally, the ammonium guests containing an aromatic side-chain exhibited higher enantioselectivity than those containing an alkyl side-chain. The ammonium guest bearing a phenyl side-chain (**Am1**) showed the strongest binding between the host and the guest, and it also gave the highest $K_{(S)}$ to $K_{(R)}$ ratio (Table 2, entries 1–4). However, the benzyl derivative, phenylalanine methyl ester hydrochloride (**Am2**), showed a significantly lower enantioselectivity. These results suggested that the extra methylene group of the benzyl moiety in **Am2** may orientate the phenyl ring of **Am2** poorly for π–π interaction with the bpy subunit of the host. In addition, tryptophan methyl ester hydrochloride (**Am3**) was

Table 1. Enantiomeric recognition studies of bpy crown macrocycles **1**–**4** towards **Am1** in CH_2Cl_2 with 0.25% CH_3OH at 298 K using UV–vis titration method[a]

Entry	Host	Guest[b]	K ($\times10^4$ M^{-1})	$K_{(S)}/K_{(R)}$	$-\Delta G_0$ (kJ mol^{-1})	$\Delta\Delta G_0$[c] (kJ mol^{-1})	R
1	**1**	(*R*)-**Am1**	9.9±0.8	2.1	29	−1.9	0.998
2	**1**	(*S*)-**Am1**	21±1.3		30		0.992
3	**2**	(*R*)-**Am1**	7.5±0.5	1.6	28	−1.2	0.996
4	**2**	(*S*)-**Am1**	12±0.6		29		0.998
5	**3**	(*R*)-**Am1**	7.6±0.6	1.3	28	−0.6	0.997
6	**3**	(*S*)-**Am1**	9.7±1.6		28		0.986
7	**4**	(*R*)-**Am1**	7.8±0.8	1.8	28	−1.4	0.991
8	**4**	(*S*)-**Am1**	14±1.1		29		0.992

[a] The concentration of the hosts: 2.5×10^{-5} mol dm^{-3}.
[b] **Am1**: 2-Phenylglycine methyl ester hydrochloride.
[c] $\Delta\Delta G_0=-nRT\ln(K_{(S)}/K_{(R)})$.

Table 2. Enantiomeric recognition studies of bpy crown macrocycle **1** towards amino acid derivatives and chiral organic ammonium salts at 298 K using UV–vis and NMR titration methods

Entry	Method	Guest[a]	K ($\times 10^2$ M^{-1})	$K_{(S)}/K_{(R)}$	$-\Delta G_0$ (kJ mol^{-1})	$\Delta\Delta G_0$[b] (kJ mol^{-1})	R
1	UV–vis[c]	(*R*)-**Am1**	990±77	2.1	29	−1.9	0.998
2	UV–vis[c]	(*S*)-**Am1**	2100±130		30		0.992
3	UV–vis[c]	(*R*)-**Am2**	300±42	1.6	26	−1.2	0.996
4	UV–vis[c]	(*S*)-**Am2**	480±41		27		0.998
5	UV–vis[c]	(*R*)-**Am4**	470±37	1.0	27	−0.1	0.998
6	UV–vis[c]	(*S*)-**Am4**	490±66		27		0.995
7	UV–vis[c]	(*R*)-**Am5**	81±9	1.3	22	−0.8	0.993
8	UV–vis[c]	(*S*)-**Am5**	110±9		23		0.988
9	NMR[d]	(*R*)-**Am1**	2.7	3.7	14	−3.2	—
10	NMR[d]	(*S*)-**Am1**	10		17		—
11	NMR[d]	(*R*)-**Am6**	6.0	1.1	16	0.1	—
12	NMR[d]	(*S*)-**Am6**	5.7		16		—

[a] **Am1**: 2-Phenylglycine methyl ester hydrochloride; **Am2**: Phenylalanine methyl ester hydrochloride; **Am4**: Alanine methyl ester hydrochloride; **Am5**: Valine methyl ester hydrochloride; **Am6**: (α-Phenylethyl)ammonium chloride.
[b] $\Delta\Delta G_0 = -nRT \ln (K_{(S)}/K_{(R)})$.
[c] The concentration of **1** is 2.5×10^{-5} mol dm^{-3} in CH_2Cl_2 with 0.25% CH_3OH.
[d] The initial concentration of **1** is 5.1×10^{-3} M in CD_2Cl_2 with 10% CD_3OD.

also submitted for the recognition study. However, the equilibrium binding constant could not be determined because of overlap between the UV signals corresponding to the host and the guest.

Among the ammonium guests bearing alkyl side-chains, alanine methyl ester hydrochloride (**Am4**) showed almost no enantioselectivity and the more bully valine methyl ester hydrochloride (**Am5**) showed slightly higher enantioselectivity than **Am4**. These results suggested that the steric effect of the ammonium guest is important for good enantioselectivity when no aromatic group is available for π–π interaction. In addition, (α-phenylethyl)ammonium chloride (**Am6**) and (α-(1-naphthyl)ethyl)ammonium chloride (**Am7**) were used to study effect of the binding properties without the ester subunit. Unfortunately, no new UV signal corresponding to the host–guest complex was observed upon addition of **Am6**, and the UV signals between **Am7** and the macrocyclic host **1** was found to be overlapped.

2.3. Enantiomeric recognition studies using NMR titration method

Since the binding studies with **Am6** and **Am7** could not be carried out using UV–vis spectroscopic method, their binding studies with bpy crown macrocycle **1** were then carried out using the NMR titration method.[21–23] The binding study with **Am1** using the NMR titration method was also carried out for comparison. A 10% of CD_3OD in CD_2Cl_2 solution was used due to the solubility of the ammonium guests. The initial concentration of **1** was set to be 5.1×10^{-3} M in order to obtain reasonable ^1H NMR signals and this solution was titrated with 40.5×10^{-3} M of the ammonium guest in the same solvent system with tetramethylsilane (TMS) as the internal standard. By using the non-linear least squares treatment, the equilibrium binding constants and the chemical shift of the host–guest complex signal of interested (δ_c) can be calculated through the minimization of the error function F (Eq. 2),[24]

$$F = \Sigma(\delta_{obsd} - \delta_{ave})^2$$

$$= \Sigma[\delta_{obsd,i} - X_{f,i}\delta_f - (1 - X_{f,i})\delta_c]^2 \quad (2)$$

where δ_{obsd} is the chemical shift of the bpy crown macrocycle signal of interest and δ_{ave} is the weighted average of the same signal or the free and complexed bpy crown macrocycle. δ_f is the chemical shift of the same signal of the free crown macrocycle and X_f is the mole fraction of the free bpy crown macrocycle.

As shown in Table 2 entries 9–12, the equilibrium binding constants for **Am1** and **Am6** observed under the NMR conditions (10% CD_3OD in CD_2Cl_2) are much smaller (about 200-fold for (*S*)-**Am1** and more than 300-fold for (*R*)-**Am1**) than those observed under the UV–vis conditions (0.25% CH_3OH in CH_2Cl_2). To investigate this dramatic effect on different methanol content, the recognition study between **1** and **Am1** was carried out in CH_2Cl_2 with 10% CH_3OH using the UV–vis method. These conditions also gave small K values ($K_{(S)}=3500$ M^{-1} and $K_{(R)}=1800$ M^{-1}), which is roughly in the same order of magnitude as the results obtained by the NMR titration method. Though the NMR study showed almost no enantioselectivity for **Am6**, it showed the good binding ability of **Am6** towards **1**, which was not observed by the UV–vis method. These results suggested that the ester subunit of the ammonium guest is important for the formation of the new UV signal for the host–guest complex, and it is also important for the high enantiomeric recognition ability. The equilibrium binding constant between **Am7** and **1** could not be determined due to overlap between signals corresponding to the naphthalane ring and the bpy moiety.

2.4. Characterization of the host–guest complex

The host–guest complexes were characterized using various NMR experiments. 2D NOESY and ROESY spectra of a roughly 1:1 mixture of **1** and (*S*)-**Am1** in CD_2Cl_2 with 5%

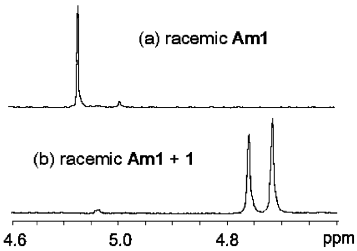


Figure 5. The signals correspond to the α-protons of (±)-**Am1** (5.0×10^{-3} M) in CD_2Cl_2 with 5% CD_3OD with (a) no bpy crown macrocycle, and (b) bpy crown macrocycle **1** (5.0×10^{-3} M).

CD_3OD did not show any correlation between the phenyl protons of the ammonium guest and the bpy protons of the macrocyclic host. These results could be due to the fast exchange rate between the host and the guest molecules.[7,23] The ^{1}H NMR spectrum of (±)-**Am1** (with the (*S*)-enantiomer in slightly excess) showed a singlet signal for the α-protons (Fig. 5a), which was spilt into two peaks in the presence of **1** (Fig. 5b). Moreover, the ^{1}H NMR spectrum of **1**·(*S*)-**Am1** complex showed a distinct 2:3 pattern for the phenyl proton signals (Fig. 6), indicating the π-systems of **1** and (*S*)-**Am1** are in close vicinity.

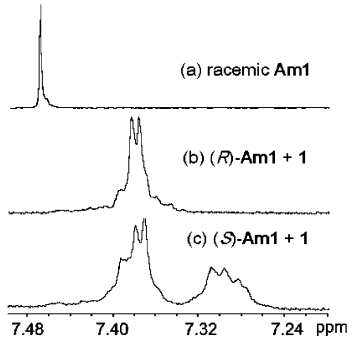


Figure 6. The signals correspond to the benzene rings of (a) (±)-**Am1** alone (5.0×10^{-3} M); (b) (*R*)-**Am1** (5.0×10^{-3} M) and (c) (*S*)-**Am1** (5.0×10^{-3} M) with bpy crown macrocycle **1** (5.0×10^{-3} M) in CD_2Cl_2 with 5% CD_3OD.

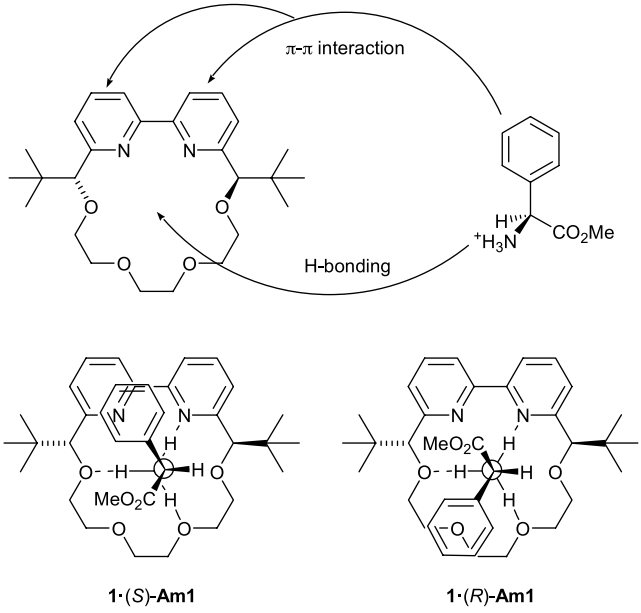


Figure 7. Interactions between bpy crown macrocycle **1** with **Am1**.

Although, the 2D NOESY and ROESY experiments showed no correlation between **1** and (*S*)-**Am1**, the structure–binding relationship studies and the ^{1}H NMR spectra strongly suggested that the presence of π–π interaction between bpy crown macrocycle **1** and **Am1**. Figure 7 showed the conformations of the **1**·(*S*)-**Am1** and **1**·(*R*)-**Am1** complexation models with the ester group and the side-chain at the α-position pointing away from the *t*-butyl group of the macrocyclic host. The **1**·(*S*)-**Am1** complexation model is considered to be more stable due to the extra stabilization energy from the π–π interaction between the bpy subunit of the macrocyclic host and the phenyl ring of the ammonium guest. These transition state models seem to be able to account for the high enantioselectivity of (*S*)-**Am1**.

3. Conclusion

In summary, we have developed a series of new C_2-symmetric bpy crown macrocycles (**1**–**4**) and studied their enantiomeric recognition properties towards a number of amino acid derivatives and chiral organic ammonium salts using UV–vis and NMR methods. The macrocycles were found to be strong chelating agents for organic ammonium salts (with K up to 4.8×10^5 M^{-1}) and useful chromophore for UV–vis titration studies. Bpy crown macrocycle **1**, bearing the pseudo 18-crown-6 type structure, exhibited the highest enantioselectivity towards (*S*)-**Am1** with $K_{(S)}/K_{(R)}$ equals 2.1. The structure–binding relationship studies showed that the π–π interaction between the phenyl group of the ammonium guest and the bpy subunit of the macrocycle host is important for high enantioselectivity. This observation was also supported by the NMR studies of the **1**·(*S*)-**Am1** complex. Moreover, the recognition study of **1** with **Am6** suggested that the ester group of the ammonium guests is important for both high enantioselectivity and the new UV signal formation for the host–guest complex.

4. Experimental

4.1. Materials and apparatus

2,6-Bipyridinediol (**5**) was prepared according to the literature procedures.[15] AR-grade CH_2Cl_2 and CH_3OH were used for the UV–vis titration experiments. The ^{1}H and ^{13}C NMR spectra were obtained by a 300 MHz instrument. The 2D NOESY and ROESY spectra were obtained by a 500 MHz instrument.

4.2. General procedures for the synthesis of ethylene glycol ditosylate 6–9

The corresponding ethylene glycol (3.0 mmol) in dry THF (20 mL) was added dropwise to a vigorously stirred suspension of NaH (0.23 g, 9.6 mmol) in dry THF (5 mL) at 0 °C. The reaction mixture was slowly warmed and refluxed for 4 h and then cooled to 0 °C. After addition of tosyl chloride (1.43 g, 7.5 mmol) in dry THF (10 mL) at 0 °C, the mixture was warmed to room temperature and

stirred for 48 h. The reaction mixture was concentrated under reduced pressure and the residue was treated cautiously with water and extracted with diethyl ether (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by silca gel column chromatography (petroleum ether/ethyl acetate) to provide the corresponding ditosylate compound.

4.2.1. Tri(ethyl glycol)ditosylate (6) and penta(ethylene glycol)ditosylate (7). Both compounds are commercially available.

4.2.2. Tetra(ethylene glycol)ditosylate (8). The above procedures were followed with tetra(ethylene glycol) as the starting material. The isolated yield for **8** is 1.39 g (92%): ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 6H), 3.55–3.58 (m, 8H), 3.64–3.70 (m, 4H), 4.11–4.17 (m, 4H), 7.34 (d, J=8.1 Hz, 4H), 7.79 (d, J=8.7 Hz, 4H).

4.2.3. Hexa(ethylene glycol)ditosylate (9). The above procedures were followed with hexa(ethylene glycol) as the starting material. The isolated yield for **9** is 1.68 g (95%): ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 6H), 3.58–3.70 (m, 20H), 4.14–4.17 (m, 4H), 7.35 (d, J=7.8 Hz, 4H), 7.80 (d, J=8.4 Hz, 4H).

4.3. General procedures for the synthesis of bpy crown macrocycle 1–4

To a vigorously stirred suspension of NaH (0.080 g, 3.2 mmol) in dry THF (3 mL) at 0 °C was added dropwise a solution of di-*tert*-butyl-2,6-bipyridinedimethanol **6** (0.33 g, 1.0 mmol) in dry THF (10 mL). The resulting mixture was stirred at 0 °C for 30 min and heated under reflux for 2 h. The mixture was then cooled to room temperature and treated with a solution of the corresponding ethylene glycol di-*p*-tosylate (1.2 mmol) in dry THF (25 mL) dropwise. The resulting mixture was stirred at room temperature for 2–6 days. After removal of the solvent under aspirator vacuum, the residue was treated with water and extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by silca gel column chromatography (petroleum ether/ethyl acetate) to yield bpy crown macrocycle **1**–**4**.

4.3.1. (*R*,*R*)-7,18-Di-*tert*-butyl-8,11,14,17-tetraoxa-23,24-diaza-tricyclo[17.3.1.1^{2,6}]tetracosa-1(23),2(24),3,5,19,21-hexaene (1). The above procedures were followed with tri(ethylene glycol)di-*p*-tosylate **6**. Bpy crown macrocycle **1** was isolated as an off-white solid (0.15 g, 35%): mp 142–146 °C $[\alpha]_D^{25}$ −107.6 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2968, 1577, 1109; ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 18H), 2.59–2.68 (m, 2H), 2.76–2.84 (m, 2H), 3.15–3.21 (m, 2H), 3.22–3.29 (m, 2H), 3.40–3.47 (m, 2H), 3.76–3.83 (m, 2H), 4.05 (s, 2H), 7.23 (d, J=7.8 Hz, 2H), 7.72 (t, J=7.8 Hz, 2H), 7.94 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.4, 35.7, 68.5, 70.2, 71.0, 91.2, 120.4, 123.0, 135.8, 157.3, 160.4. Anal. Calcd for $C_{26}H_{38}N_2O_4$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.46; H, 8.55; N, 6.49; Positive ion MS-ESI *m*/*z*: 443 (MH^+), 465 ($M+Na^+$), 481 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{26}H_{38}N_2O_4$: 442.2832; found 442.2834.

4.3.2. (*R*,*R*)-7,21-Di-*tert*-butyl-8,11,14,17,20-pentaoxa-26,27-diaza-tricyclo[20.3.1.1^{2,6}]heptacosa-1(26),2(27),3,5,22,24-hexaene (2). The above procedures were followed with tetra(ethylene glycol)di-*p*-tosylate **7**. Bpy crown macrocycle **2** was isolated as an amorphous solid (0.19 g, 40%): $[\alpha]_D^{25}$ +15.6 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2953, 2868, 1571, 1438, 1107; ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 18H), 2.66–2.81 (m, 2H), 2.84–3.02 (m, 4H), 3.04–3.14 (m, 2H), 3.22–3.34 (m, 2H), 3.40–3.50 (m, 2H), 3.62–3.73 (m, 2H), 3.87–3.99 (m, 2H), 4.32 (s, 2H), 7.41 (d, J=7.5 Hz, 2H), 7.77 (t, J=7.8 Hz, 2H), 8.26 (d, J=7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.2, 35.9, 69.0, 69.8, 71.5, 72.0, 92.5, 119.1, 122.3, 135.9, 154.7, 161.3; Positive ion MS-ESI *m*/*z*: 487 (MH^+), 509 ($M+Na^+$), 525 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{28}H_{42}N_2O_5$: 486.3094; found 486.3090.

4.3.3. (*R*,*R*)-7,24-Di-*tert*-butyl-8,11,14,17,20,23-hexaoxa-29,30-diaza-tricyclo[23.3.1.1^{2,6}]triaconta-1(29),2(30),3,5,25,27-hexaene (3). The above procedures were followed with penta(ethylene glycol)di-*p*-tosylate **8**. Bpy crown macrocycle **3** was isolated as a pale yellow oil (0.10 g, 19%): $[\alpha]_D^{25}$ +19.3 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2953, 2868, 1571, 1438, 1107; ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 18H), 3.13–3.82 (m, 20H), 4.34 (s, 2H), 7.44 (d, J=7.8 Hz, 2H), 7.78 (t, J=7.8 Hz, 2H), 8.23 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.4, 36.0, 70.3, 70.5, 70.7, 70.9, 71.8, 92.1, 119.4, 122.4, 136.3, 154.9, 160.2; Positive ion MS-ESI *m*/*z*: 531 (MH^+), 553 ($M+Na^+$), 569 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{30}H_{46}N_2O_6$: 530.3356; found 530.3354.

4.3.4. (*R*,*R*)-7,27-Di-*tert*-butyl-8,11,14,17,20,23,26-hepta-oxa-32,33-diaza-tricyclo[26.3.1.1^{2,6}]tritriaconta-1(32),2(33),3,5,28,30-hexaene (4). The above procedures were followed with hexa(ethylene glycol)di-*p*-tosylate **9**. Bpy crown macrocycle **5** was isolated as a pale yellow oil (0.10 g, 17%): $[\alpha]_D^{25}$ −46.4 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2953, 1572, 1441, 1107; ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 18H), 3.37–3.65 (m, 24H), 4.29 (s, 2H), 7.44 (d, J=7.8 Hz, 2H), 7.80 (d, J=7.8 Hz, 2H), 8.26 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.5, 35.8, 69.7, 70.6, 70.8, 70.9, 71.2, 91.8, 119.5, 122.4, 136.6, 155.0, 160.8; positive ion MS-ESI *m*/*z*: 575 (M^+), 597 ($M+Na^+$), 613 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{32}H_{50}N_2O_7$: 574.3618; found 574.3616.

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24. The association constant (K) for 1:1 complexation can be expressed by Eq. 3

$$K = \frac{[C]}{[H][G]} = \frac{[C]}{([H]_0 - [C])([G]_0 - [C])} \tag{3}$$

where $[H]_0$ and $[G]_0$ are the initial concentrations of the host and guest, respectively, and [H], [G] and [C] are the concentrations of the host, guest and complex at equilibrium, respectively. By substitution of $[C]=[H]_0(1-X_f)$ into Eq. 3, X_f became the unknown of the quadratic equation (Eq. 4):

$$[H]_0 X_f^2 + \left([G]_0 - [H]_0 + \frac{1}{K}\right)X_f - \frac{1}{K} = 0 \tag{4}$$

Therefore,

$$X_f = \frac{-([G]_0 - [H]_0 + 1/K) \pm \sqrt{([G]_0 - [H]_0 + 1/K)^2 - 4[H]_0(-1/K)}}{2[H]_0} \tag{5}$$

Since

$$F = \Sigma(\delta_{obsd} - \delta_{ave})^2$$

$$= \Sigma[\delta_{obsd,i} - X_{f,i}\delta_f - (1 - X_{f,i})\delta_c]^2 \tag{2}$$

K and δ_c were calculated by minimization of the error function F using a computer program developed by us.

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Non-biaryl atropisomers derived from carbohydrates. Part 3: Rotational isomerism of sterically hindered heteroaryl imidazolidine-2-ones and 2-thiones☆

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Abstract—The present work describes in detail the preparation and structural characterization of a series of heteroaryls in which an *o*,*o*′-disubstituted phenyl ring is connected through a single C–N bond to a heterocyclic fragment of a chiral imidazolidine-2-one or 2-thione. As a consequence of hindered rotation, some of these substances exist as stable rotamers at room temperature and can easily be separated and characterized. Molecular mechanic calculations have also been carried out to evaluate the barriers to rotation.

1. Introduction

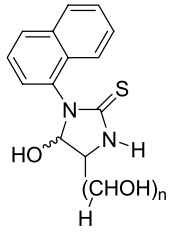
It is now unnecessary to emphasize the importance of biaryl atropisomers with a well-defined sense of helicity, as these substances have found numerous applications in asymmetric catalysis and materials chemistry.[3]

In stark contrast, non-biaryl atropisomers still represent an underestimated family of chiral conveyors. This concept, however, is hardly new. Atropisomerism in heterocycles bearing naphtyl and *o*-substituted phenyl groups have been previously reported.[4] Anyhow, atroposelective reactions with this kind of substances are a rather unexplored domain and offer promising perspectives.[5]

More recent challenges involving atropisomerism and conformational control include the design of chiral motors or switches, which can eventually be incorporated into parts of data storage units or electronic circuits,[6] as well as the mimicing of allosteric functions characteristic of biological systems.[7]

Controlling molecular motion in axially chiral systems, however, requires the easy preparation, isolation, and characterization of single molecules, together with an in-depth understanding of their physical properties.

A few years ago we introduced a new family of heteroaryl atropisomers that combine both central and axial chirality.[2,8,9] Thus, 1-naphtylimidazolidine-2-thiones appended to an acyclic sugar fragment with different configurations (**1**), exhibit atropisomerism due to hindered rotation around the C–N bond between the aryl group and the heterocyclic ring. Compounds **1** can easily be converted into more rigid structures such as **2** by acid-catalyzed cyclization. However, the barriers to rotation for compounds **1** and **2** were too low to allow us their separation at room temperature.



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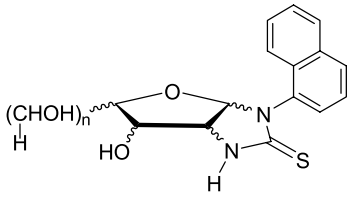

2

We have also synthesized a large variety of compounds featuring structures **3**–**5** (X=S or O) which bear and *ortho*-substituted benzene ring. In all cases and, like **1** and **2**, their barriers to rotation are low and cannot be separated.[2] The

☆ See Refs. 1,2.

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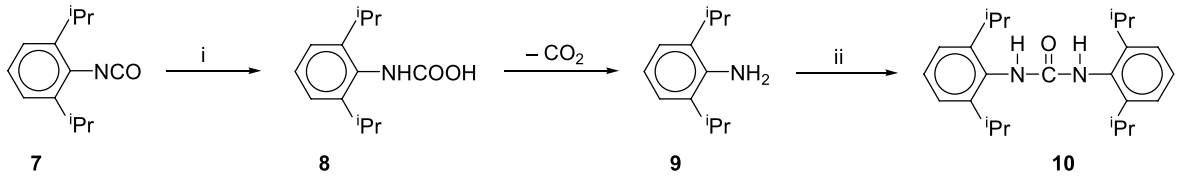
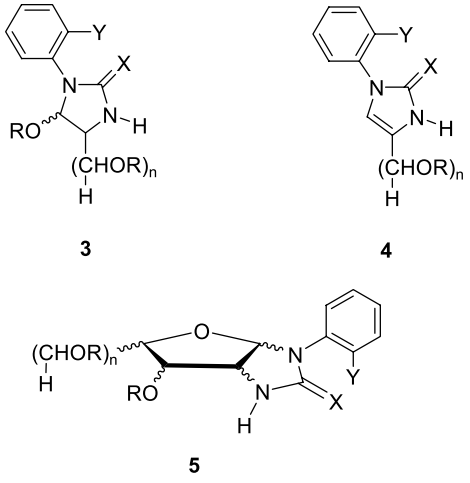


Scheme 1. (i) H_2O; (ii) **7**.

presence of a C=S bond is specially noticeable as this structural feature largely increases the barrier to rotation, whereas lower barriers were observed for their oxoanalogs. The MM2 calculations support these experimental results.

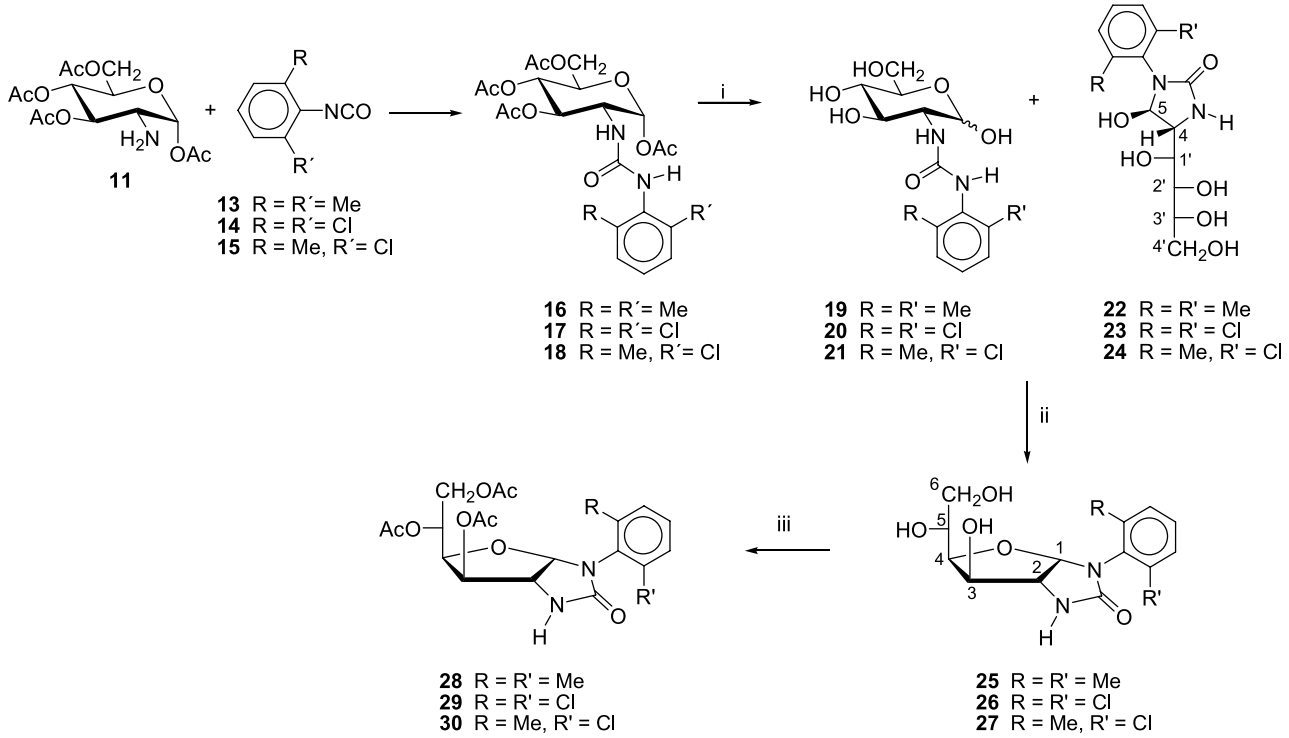


In two consecutive papers we now extend these previous results to imidazolidine-2-one and 2-thione derivatives linked to *o*,*o*′-disubstituted phenyl rings. Such rotational isomers[10] are stable entities at room temperature, and therefore the diastereomeric rotamers could be isolated by chromatography and/or crystallization, and unequivocally identified by NMR and X-ray diffraction analyses.

2. Results

2.1. Synthesis of *o*,*o*′-disubstituted 1-aryl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-ones

Our first attempts to prepare *o*,*o*′-disubstituted 1-aryl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-ones involved the well-established reaction between 2-amino-2-deoxy-D-glucopyranose (**6**) and aryl isocyanates in aqueous media, followed by acid-catalyzed cyclization of the ureido derivative.[2b,11] Nevertheless, aryl isocyanates bearing large groups at their *ortho* positions failed to react with aminosugars in an aqueous medium owing to steric hindrance. Under such circumstances, hydrolysis of the isocyanate occurs leading to the unwanted *N*,*N*′-diarylurea. Thus, for example, the reaction of **6** with 2,6-diisopropylphenyl isocyanate (**7**) afforded exclusively 1,3-bis(2,6-diisopropylphenyl)urea (**10**), through the intermediate carbamic acid **8**, and not the expected ureidosugar derivative (Scheme 1).



Scheme 2. (i) NH_3/MeOH; (ii) AcOH 30%, △; (iii) Ac_2O/C_5H_5N, −20 °C.

Conversely, it is possible to accomplish successfully the synthesis of *o*,*o*′-disubstituted derivatives starting from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose[12] (**11**), thereby facilitating the condensation with aryl isocyanates in an aprotic solvent and avoiding hydrolysis side reactions. Following this procedure and using 2,6-dimethylphenyl, 2,6-dichlorophenyl and 2-chloro-6-methylphenyl isocyanates (**13**–**15**), the corresponding arylureido derivatives **16**–**18** were obtained (Scheme 2). Although symmetrically substituted isocyanates **13** and **14** cannot induce atropisomerism, such derivatives are useful in determining experimental rotational barriers and have been employed to optimize the synthetic procedure.

Compounds **16**–**18** were prepared in good to excellent yields; however variable amounts of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (**12**) were often obtained as by-product. This substance was unequivocally identified by comparison of its physical properties and spectroscopic data with an authentic sample[13] (see Section 3).

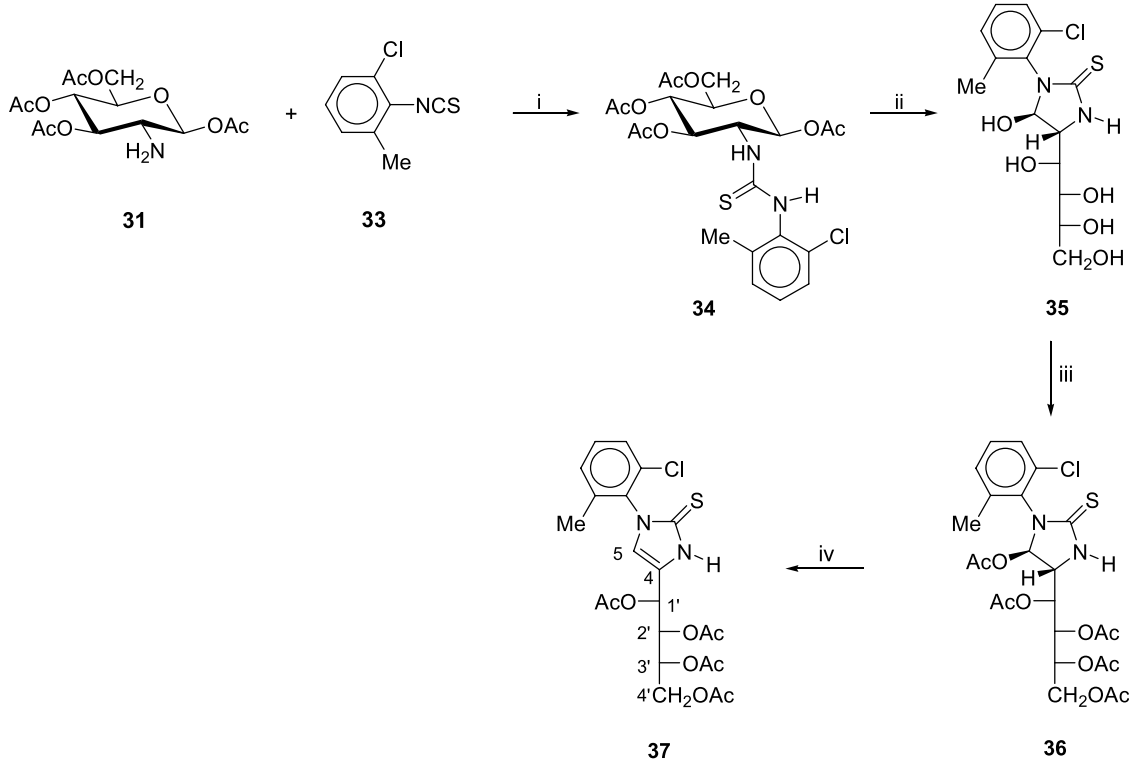
The structures attributed to **16**–**18** are supported by their physical, spectroscopic, and polarimetric data, analogous to those of other per-*O*-acetylated sugar ureas previously described.[2b,11] IR spectra show both the stretching (3400–3300 cm^{-1}) and deformation (~1500 cm^{-1}) bands of the NH group as well as the carbonyl stretching band (~1690 cm^{-1}), different from the ester carbonyl absorption (~1750 cm^{-1}). The α-anomeric configuration agrees with the small value of $J_{1,2}$ (<4 Hz) and the high values of optical rotation.

Further treatment of compounds **16**–**18** with ammonia in methanol at room temperature provided, after complete deacetylation, mixtures of the unprotected ureido derivatives **19**–**21** and the corresponding 5-hydroxyimidazolidine-2-ones **22**–**24**. The later substances are generated in different ratios by easy cyclization of ureas **19**–**21**.[11] Such mixtures could not be separated, although were identified by their spectroscopic data and used in subsequent steps without purification.

The subsequent treatment of these mixtures with hot aqueous acetic acid leads to a high-yielding preparation of the corresponding 1-aryl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-ones **25**–**27**. The structures of these compounds are consistent with their elemental analyses, polarimetric and spectroscopic data, analogous to similar bicycles.[2b,11] The small value of $J_{2,3}$ (~0 Hz) rules out a pyranose structure and confirms that **25**–**27** are glycofuranoses in which H-2 and H-3 display a *trans* arrangement. On the contrary, $J_{1,2}$ values (>6.2 Hz) show the existence of *cis*-fused rings. The ^{13}C NMR spectra show that C-4 (and not C-5) is the most deshielded signal, a fact also accounting for the furanoid character of the sugar moiety.

As expected, ^{1}H and ^{13}C NMR spectra for **27** show duplicated and close signals corresponding to a mixture of two atropisomers in ~2:1 (*P*:*M*) ratio,[14] which could not be separated by crystallization. Isolation of these rotamers was attempted by preparing their per-*O*-acetyl derivatives. In this way, treatment of **25**–**27** with acetic anhydride and pyridine at −20 °C provided the corresponding **28**–**30** in high yields.

^{1}H NMR spectra show the absence of *N*-acetylation and IR data for **28** show absorption bands at 3500–3000 cm^{-1} due



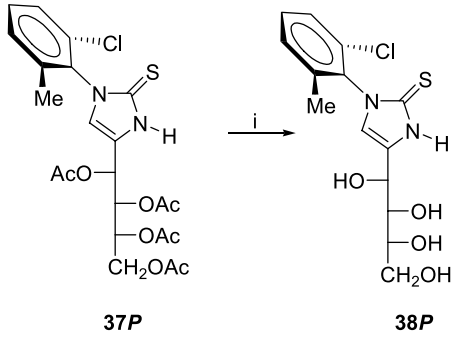
Scheme 3. (i) CH_2Cl_2, △; (ii) NH_3/MeOH; (iii) Ac_2O/C_5H_5N, −20 °C; (iv) $KHCO_3/C_6H_6$, △.

to the presence of water in the crystal lattice.[15] Again, NMR spectra show **30** as a mixture of *M* and *P* atropisomers, each separated by preparative chromatography (benzene–acetone 3:1) and crystallized from ethanol. The absolute configuration of **30***P* was determined by X-ray diffraction analysis.[1,16]

2.2. Synthesis of *o*,*o*′-disubstituted 1-aryl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thiones

Initially we followed a synthetic pathway analogous to that described for aryl ureido derivatives, but using the protected amino sugar **31** as starting material,[17] in order to avoid the formation of compound **12**. Condensation at room temperature with 2-chloro-6-methylphenyl isothiocyanate (**33**) allowed the preparation of thioureido derivative **34** (Scheme 3). When the reaction was carried out under reflux, significant amounts of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (**32**) were formed. This substance was identified by comparison of its physical and spectroscopic properties with an authentic sample.[18]

Pure imidazolidine-2-thione **35** was obtained in high yield by treatment of compound **34** with a saturated solution of ammonia in methanol at room temperature. The ^{1}H NMR spectrum of a crude sample of **35** showed a mixture of two *M*:*P* rotamers in a 70:30 ratio.[16] Both of them presented *R* absolute configuration at C-5 as it could be inferred from the small values of $J_{4,5}$ (~0 Hz) upon the addition of D_2O.[2b,8,11] Compound **35** could be characterized by treatment with acetic anhydride and pyridine at low temperature to afford the per-*O*-acetyl derivative **36** in almost quantitative yield and, as mixture of two atropisomers again. The major rotamer **36***P* could be isolated by fractional crystallization.[16] The small value of $J_{4,5}$ (1.4 Hz) reveals that configuration at C-5 should be *R*.

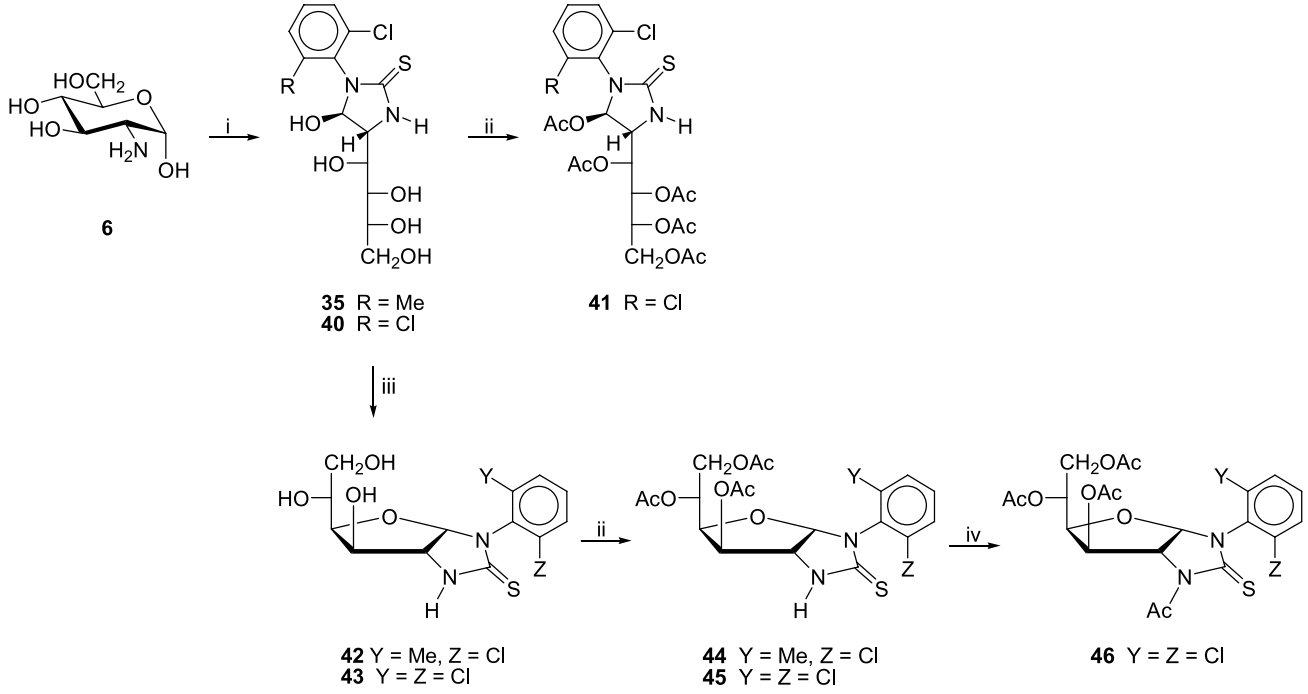


37*P* **38***P*

Scheme 4. (i) NH_3/MeOH.

Following the procedure previously described in one of our former works[2a] and starting from the atropisomeric mixture of **36***M* and **36***P*, compound **37** was obtained by acetic acid elimination as a mixture of two rotamers (85:15). The major rotamer **37***P*[16] was isolated by fractional crystallization in 42% yield. Treatment of this atropisomer with a saturated solution of ammonia in methanol gave the corresponding deprotected *P* rotamer **38**[16] (Scheme 4). Again, the structures assigned to **36–38** are supported by their spectral data and elemental analyses.

Since compound **35** represents a salient precursor en route to the bicyclic imidazolidine-2-thiones we are aiming to obtain, a more direct synthetic route was attempted. *o*,*o*′-Disubstituted aryl isocyanates with large groups failed to react with aminosugars in an aqueous medium due to the hydrolysis of isocyanate. However, reactions with aryl isothiocyanates can advantageously be conducted in the presence of water because the competing hydrolysis proceeds slowly. The direct condensation between *o*,*o*′-disubstituted aryl isothiocyanates and **6** in ethanol–water proved to be completely satisfactory. Thus,



6

35 R = Me
40 R = Cl

41 R = Cl

42 Y = Me, Z = Cl
43 Y = Z = Cl

44 Y = Me, Z = Cl
45 Y = Z = Cl

46 Y = Z = Cl

Scheme 5. (i) 2-Cl-6-RC_6H_3NCS; (ii) Ac_2O, C_5H_5N, −20 °C; (iii) AcOH 30%, △, (iv) Ac_2O, C_5H_5N, 40 °C.

compounds **35** and **40** were obtained in high yields when reaction between **6** and 2-chloro-6-methylphenyl isothiocyanate (**33**) or 2,6-dichlorophenyl isothiocyanate (**39**) took place (Scheme 5). Although the symmetrically disubstituted derivative **40** does not allow atropisomerism, this condensation was carried out to test the feasibility of the process. The structure attributed to **40** was further characterized as its per-*O*-acetyl counterpart **41**.

Treatment of **35** or **40** with hot aqueous acetic acid resulted in high yields of the corresponding 1-aryl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thiones **42** or **43**, respectively. The structure assigned to these compounds is consistent with their spectroscopic data and elemental analyse. Compound **42** was found to be an unequal mixture of two atropisomers as revealed by NMR analyses. The major atropisomer **42***P* was isolated after several crystallizations from 96% aqueous ethanol.[16] Compounds **42** and **43** could be characterized as their acetylated derivatives **44** and **45**, which were prepared by reaction with acetic anhydride and pyridine at −20 °C.

Isolation of *M* and *P* rotamers of **44** by flash chromatography (ethyl acetate- *n*-hexane 1:2), followed by treatment with saturated solution of ammonia in methanol at room temperature, afforded the corresponding pure atropisomers **42***M* and **42***P*. ^{1}H and ^{13}C NMR spectra for these two rotamers showed a single signal set, thereby evidencing that the axial chirality in atropisomers of compound **44** remained unaffected by deacetylation. The absolute configurations of **42***M* and **42***P* could be unambiguously determined by single-crystal X-ray diffractometry as depicted in their ORTEP diagrams with the crystallographic numbering (Figs. 1 and 2).[19]

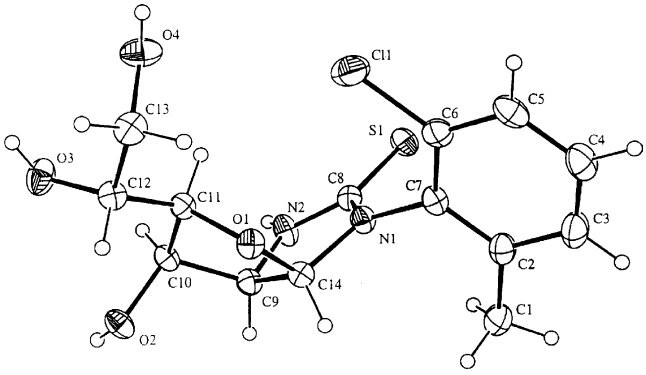


Figure 1. X-ray diffraction analysis of compound **42***P*.

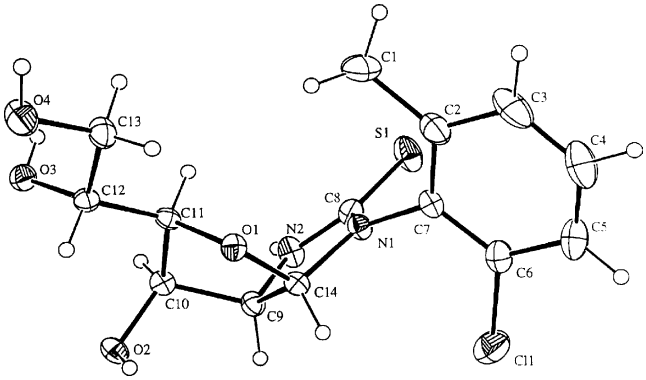


Figure 2. X-ray diffraction analysis of compound **42***M*.

Since all attempts to crystallize **45** were unsuccessful, this product was transformed into the *N*-acyl derivative **46** running the acetylation at 40 °C.[20] The IR spectra of **46** exhibits the amide band at 1675 cm^{-1} and its ^{1}H and ^{13}C NMR spectra show the characteristic chemical shifts at 2.92 and 27.1 ppm, respectively, due to the *N*-Ac methyl group. A comparison of the optical rotations of compounds **45** and **46** indicates that *N*-acetylation causes a significant decrease of ∼28°.[20]

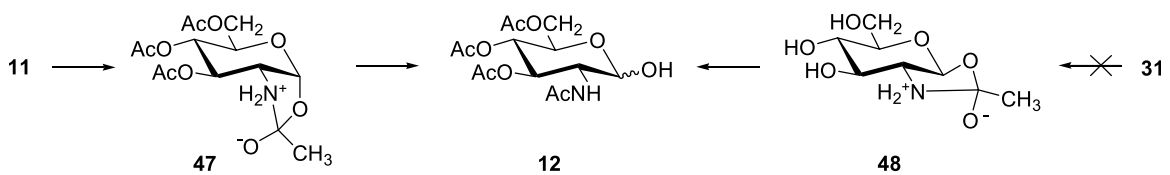
3. Discussion

As mentioned, condensation of 2-amino-2-deoxy-D-glucopyranose (**6**) with *o*,*o*′-disubstituted aryl isocyanates is more difficult than when monosubstituted aryl isocyanates are employed. On the other hand, with less reactive isothiocyanates, the direct condensation between **6** and *o*,*o*′-disubstituted aryl isothiocyanates was successfully carried out in aqueous media.

When 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose[12] (**11**) is used in an aprotic solvent, hydrolysis of isocyanate is avoided and the expected ureidosugars are formed. However, 2-acetamido-3,4,6-tri-*O*-acetyl-D-glucopyranose (**12**) is often obtained from **11**, probably due to an intramolecular migration of the acetyl group located at the anomeric position to the free amine group at C-2, through the intermediate **47** (Scheme 6). The rationale appears to be plausible as reactions employing the β-anomer **31** were not contaminated with **12**. The *trans* relationship between the anomeric acetate and the amino group at C-2 avoids the rearrangement because intermediate **48** is more strained and therefore less stable than **47**.[21]

These results along with the extensive formation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (**32**) in reactions at reflux, show that steric effects play a key role and undesirable reactions such as the rearrangement of **11** to **12** or the formation of **32** from **31** are favored.

It is interesting to point out that the thioureido derivative **34**



Scheme 6.

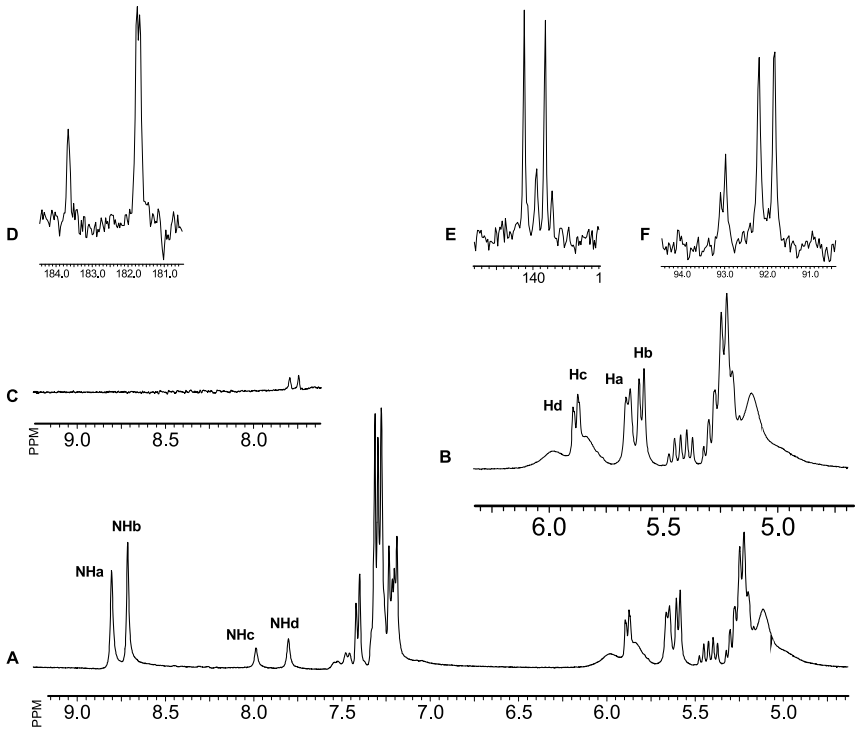


Figure 3. (A) ^{1}H NMR spectrum of **34** at −30 °C. (B) Amplified area of anomeric protons in the ^{1}H NMR spectrum. (C) D_2O exchange experiment. (D), (E) and (F) Amplified area of urea carbonyl groups, some aromatic carbons and anomeric carbons peaks, respectively, in the ^{13}C NMR spectrum of **34** at −23 °C.

Table 1. Selected spectroscopic data and population for rotamers of **34**[a]

Rotamer	NH	H-1	$J_{1,2}$	C-1	C=O	Population (%)[b]
a	8.65	5.65	7.6	91.88	139.93	38.9
b	8.56	5.59	8.4	91.53	139.36	42.2
c	7.94	5.87	8.8	92.79	139.16	7.6
d	7.78	5.88	8.8	92.66	139.60	11.4

[a] In $CDCl_3$.
[b] From digital integration of ^{1}H NMR signals at 250 K.

has an important barrier to rotation. The ^{1}H NMR spectrum at room temperature showed broad signal sets. When this spectrum was recorded at 60 °C all signals coalesced, while at −30 °C they were split into four, corresponding to a complex conformational equilibrium (Fig. 3). Some spectroscopic data as well as the relative populations observed at −30 °C for the four rotamers are showed in Table 1.

Considering that H-2 and the NH bonded to C-2 always show an antiperiplanar disposition, as it has been previously described for other sugar-based ureas and thioureas,[22] the possible conversion among the different conformers are reduced to eight (Fig. 4).

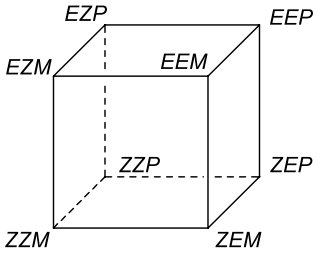
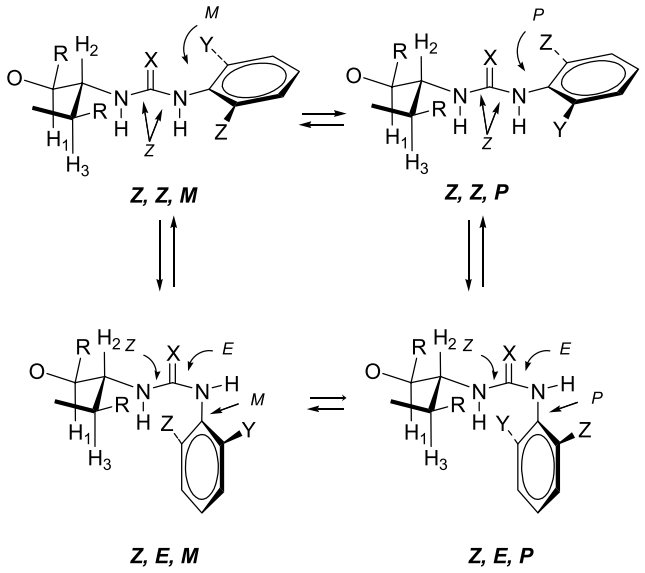


Figure 4. Diagram showing the interconversion of ureido and thioureido conformers.

Of such conformers, the more stable ones are depicted in Scheme 7 and show a less steric hindrance. Rotamers **a** and **b** should correspond to *ZZM* and *ZZP* geometries, as these would show similar chemical shifts for NH, H-1 and C-1. On the same way, geometries *ZEM* and *ZEP* would correspond to rotamers **c** and **d**. In addition, NH_c and NH_d peaks collapse at 9 °C (T_c=282 K) and NH_a and NH_b at 19 °C (T_c=292 K). The barriers to rotation calculated from these data and the difference in frequency units (Hz) between the NH signals for **c** and **d** ($\Delta\nu$=61.20 Hz) and for **a** and **b** ($\Delta\nu$=35.20 Hz) in the spectrum recorded at low temperature are 13.7 and 14.6 kcal mol^{-1}, respectively.[23] Both barriers are of the same order and should correspond to conversion between *M* and *P* atropisomers. A more accurate study on these equilibria lie beyond the scope of the present study and will be subject of future research.

Both ureido and thioureido compounds (**49**, X=O or S) were deacetyled by treatment with a saturated solution of ammonia in methanol to give the unprotected derivatives **50** (X=O or S) and hence the corresponding imidazolidine-2-ones and 2-thiones **51** (X=O or S). These substances were



Scheme 7. Stable rotamers of (thio)ureido derivatives (priority order according to Cahn–Ingold–Prelog rules: Y>Z).

later transformed into bicyclic structures **52**, under mild acid catalysis (Scheme 8). These results support the mechanism previously proposed for these transformations.[9,11]

Structures **51** and **52** with different substituents at both *ortho* positions could be resolved as stable atropisomers and fully characterized. The same fact happens with the imidazoline-2-thione derivatives **37** and **38**, suggesting that the substituent at C-5 does not affect markedly the magnitude of the barrier to rotation. This value is due exclusively to the interactions between an *ortho* substituent and either O or S atoms at C-2.[2] When both *ortho* substituents have different sizes, one should expect a large atropisomeric ratio, as it happens with Cl and methyl group, a fact in agreement with the magnitude of their steric parameters: $E_s=-0.97$ for chlorine and $E_s=-1.24$ for the methyl group.[24]

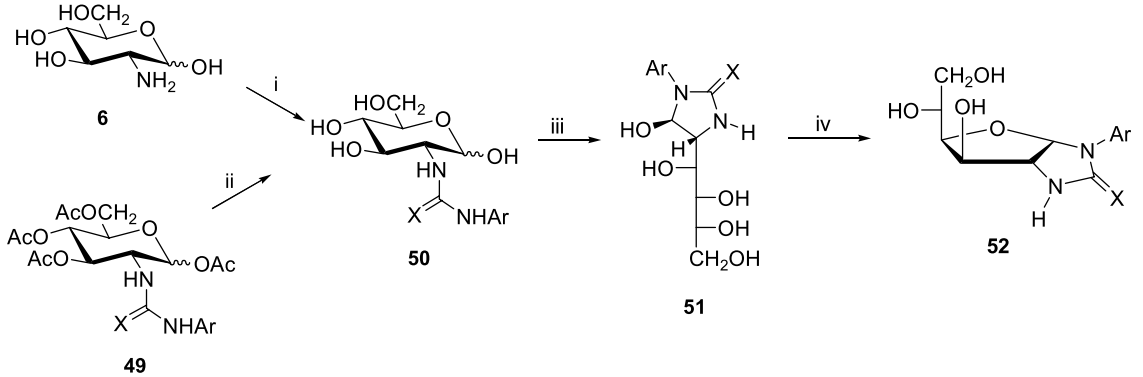
In previous papers we have described an experimental and theoretical study on several heteroaryl imidazolidine-2-one(thione) and imidazoline-2-thione derivatives with hindered rotation around a single bond.[2] Some structural variations were evaluated such as length of bond types, C=O versus C=S; nature of substituents at C-5; *cis* or *trans* stereochemistry between the heterocyclic ring and the carbohydrate moiety, and nature of substituents at the *ortho* position of the aromatic ring.

Free rotation around a single bond converts the *M* (a*R*) isomer into its *P* (a*S*) counterpart and vice versa.[14] Such an interconversion takes place by rotation around the N–C(aryl) single bond and the corresponding transition states are the peaks of highest potential energy through a 360° rotation. The potential energy was determined by means of molecular mechanics (MM2) calculations.[25,26] Starting from the *P* isomer, which is a conformation of low potential energy, rotation of the dihedral angle θ [$C_{(sp3)}$–$N_{(sp2)}$–$C_{(sp2)}$–$C_{(sp2)}$] at 30° intervals gives rise to a conformational energy diagram for the interconversion of atropisomers. A further refinement was also accomplished to determine the conformations when the energy is at a maximum, simply by rotation of the angle of torsion every 5° within the interval between −30 and +30° around the maxima previously reached. It was found that the conformations of lower potential energy corresponded to dihedral angles of ∼90° (E_P) and ∼270° (E_M). The points of higher potential energy are observed at ∼0° ($E^{\neq}_{min}$) and ∼180° ($E^{\neq}_{max}$) due to coplanarity of both the aromatic ring and the heterocyclic moiety. All of the studied cases showed barriers to rotation too low to make it possible to isolate rotational isomers at room temperature.[2]

However, molecular mechanics calculations predicted that *o*,*o*′-disubstitution will cause high enough barriers to rotation to allow isolation of atropisomers. According to our theoretical results for compounds **25**–**27** and their thioanalogous **42**, **43** and **53**, it will be possible to have barriers to rotation higher than 23 kcal mol^{-1} and hence rotamer separation at room temperature,[27] when the aromatic ring bears two substituents at the *ortho* positions. In this situation there will always be an important steric interaction between either substituent and the C=X group in both transition structures, **54** or **55** (Table 2).

However, the presence of two substituents at the *ortho* positions moved the peaks of higher potential energy from $\theta=0$ and 180° to $\theta=30$ and 210°, respectively. As an example, the potential energy diagram (MM2) for **53** is shown in Figure 5.

In order to confirm the above-mentioned predictions we have studied the thermal stability of the isolated



Scheme 8. (i) ArNCX; (ii) NH_3/MeOH; (iii) pH ≥7; (iv) AcOH 30%, △.

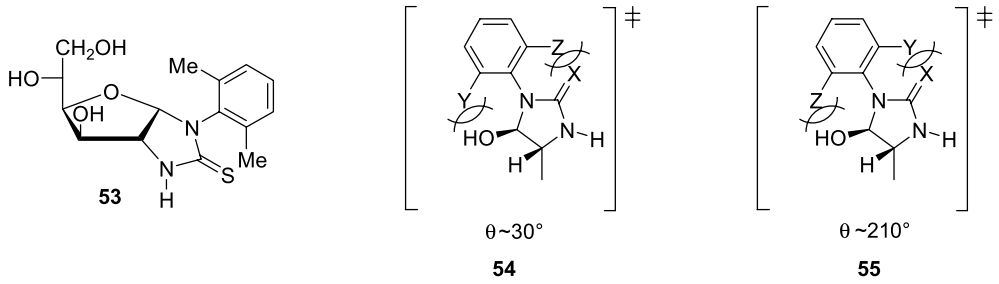


Table 2. Barriers to rotation (kcal mol^{-1})[a,b] for **25**–**27**, **42**, **43** and **53**

Compound	E_M	E_P	$\Delta H^{\ddagger}_{min}$	$\Delta H^{\ddagger}_{max}$	$\Delta\Delta H^{\ddagger}$	ΔH°
25	23.60		36.48		0.00	0.00
26	25.36		34.85		0.00	0.00
27	24.39	24.52	30.31	34.69	4.38	0.13
42	24.87	24.74	37.91	43.09	5.18	0.10
43	25.73		42.36		0.00	0.00
53	23.94		50.35		0.00	0.00

[a] Determined by MM2.
[b] E_M or E_P denotes the potential energy of *M* or *P* conformers; $E^{\ddagger}_{min}$ ($E^{\ddagger}_{max}$) is the potential energy for the transition structure of the lowest (highest) energy; $\Delta H^{\ddagger}_{min}$ ($\Delta H^{\ddagger}_{max}$)$=E^{\ddagger}_{min}$ ($E^{\ddagger}_{max}$)$-E_P$; $\Delta\Delta H^{\ddagger}=\Delta H^{\ddagger}_{max}$-$\Delta H^{\ddagger}_{min}$; $\Delta H^{\circ}=|E_M-E_P|$; 1 kcal$=$4.18 kJ.

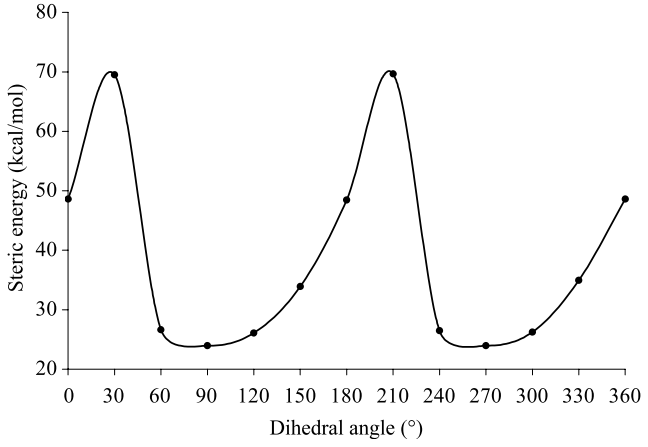


Figure 5. Plot of potential energy versus the angle of torsion for **53** (MM2 calculations).

atropisomers, using NMR techniques. All atropisomers are stable at room temperature as they did not show any change for a period of three weeks in DMSO-d_6. Furthermore, imidazolidine-2-thione derivatives **36***P*, **42***P*, **42***M*, **44***P* and **44***M* were heated for 4 h in DMSO-d_6 at 160 °C, and in some cases decomposition was observed but not the interconversion between atropisomers.

Experiments of dynamic NMR were conducted for some atropisomeric mixtures of **27**, **30**, **35**, **42** and **44**. Neither of such mixtures coalesced under heating up to 150 °C. This temperature ($T_c>423$ K) and the chemical shifts for analogous signals in each pair of atropisomers at room temperature, allowed us to establish a threshold value of 20–22 kcal mol^{-1} for their barriers to rotation,[23,28] in total agreement with our theoretical data obtained by MM2.

In conclusion, we have reported that MM2 calculations predict the possibility of obtaining room-temperature stable non-biaryl rotamers derived from carbohydrates. These predictions were found to be correct by a successful synthesis and isolation of these sort of compounds.

4. Experimental

4.1. General methods

All solvents were purchased from commercial sources and used as received unless otherwise stated. Melting points were determined on Gallenkamp and Electrothermal apparatus and are uncorrected. Analytical and preparative TLC were performed on precoated Merck 60 GF_{254} silica gel plates with a fluorescent indicator, and detection by means of UV light at 254 and 360 nm and iodine vapors. Flash chromatography[29] was performed on Merck 60 silica gel (230–400 mesh). Optical rotations were measured at the sodium line (589 nm) at 20$\pm$2 °C on a Perkin–Elmer 241 polarimeter. IR spectra were recorded in the range 4000–600 cm^{-1} on Perkin–Elmer 399 or a FT-IR MIDAC spectrophotometers. Solid samples were recorded on KBr (Merck) pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 AC/PC instrument at 400 and 100 MHz, respectively, or with a Bruker AC 200-E instrument at 200 and 50.3 MHz, respectively, in different solvent systems. Assignments were confirmed by homo- and hetero-nuclear double-resonance, DEPT (distortionless enhancement by polarization transfer), and variable temperature experiments. TMS was used as the internal standard ($\delta=$ 0.00 ppm) and all *J* values are given in Hz. Microanalyses were determined on a Leco 932 analyser at the Universidad de Extremadura (Spain), and by the Servei de Microanàlisi del CSIC at Barcelona (Spain), and the Instituto de Investigaciones Químicas del CSIC at Sevilla (Spain). High resolution mass spectra (chemical ionization) were recorded on a VG Autospec spectrometer by the Servicio de Espectrometría de Masas de la Universidad de Córdoba (Spain).

4.1.1. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[3-(2,6-dimethylphenyl)ureido]-α-D-glucopyranose (16). To a solution of

1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose[12] (**11**) (0.35 g, 1.0 mmol) in dichloromethane (5 mL) was added 2,6-dimethylphenyl isocyanate, **13** (1.0 mmol). The reaction was controlled by TLC (benzene–methanol 3:1). After three days the mixture was evaporated to dryness and the residue was crystallized from ethanol 96% giving **16** (83%), mp 196–198 °C, $[\alpha]_D$ +108.5 (*c* 0.5, $CHCl_3$); ν_{max} 3600–3200 (H_2O, NH)[15], 1540 (NH), 1740 (C=O), 1220 (C–O–C), 1630 (NC=O), 1030, 1000 (C–O), 755 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.10 (m, 3H, Ar), 6.98 (bs, 1H, Ar–NH), 6.15 (d, $J_{1,2}=2.8$ Hz, 1H, H-1), 5.15–5.04 (m, 3H, NH, H-3, H-4), 4.34 (m, 1H, H-2), 4.20 (dd, $J_{5,6}=4.1$ Hz, $J_{6,6'}=12.5$ Hz, 1H, H-6), 4.01 (dd, $J_{5,6'}=2.0$ Hz, $J_{6,6'}=12.4$ Hz, 1H, H-6′), 3.90 (m, 1H, H-5), 2.19 (s, 6H, CH_3), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (CH_3–*C*O), 170.5 (CH_3–*C*O), 169.0 (CH_3–*C*O), 168.4 (CH_3–*C*O), 156.0 (NH–*C*O–NH), 137.0 (2C), 133.2 (2C), 128.5 (2C) (aromatics), 90.7 (C-1), 70.4 (C-3), 69.5 (C-5), 67.5 (C-4), 61.4 (C-6), 51.3 (C-2), 20.5 (2C, *C*H_3–CO), 20.4 (2C, *C*H_3–CO), 17.8 (2C, CH_3). Anal. Calcd for $C_{23}H_{30}N_2O_{10}\cdot 1/2\ H_2O$: C, 54.87; H, 6.20; N, 5,56. Found: C, 54.98; H, 6.15; N, 5.62.

4.1.2. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[3-(2,6-dichlorophenyl)ureido]-α-D-glucopyranose (17). From 2,6-dichlorophenyl isocyanate (**14**) and following the above procedure, **17** was obtained (87%), mp 172–175 °C, $[\alpha]_D$ +77 (*c* 0.5, $CHCl_3$); ν_{max} 3320, 1540 (NH), 1730 (C=O), 1210 (C–O–C), 1635 (NC=O), 1030, 1000 (C–O), 760 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.06 (m, 4H, Ar, Ar–NH), 6.24 (d, $J_{1,2}=3.7$ Hz, 1H, H-1), 5.40 (bs, 1H, NH), 5.22 (m, 2H, H-3, H-4), 4.41 (ddd, $J_{1,2}=3.7$, $J_{2,NH}=10.2$ Hz, $J_{2,3}=9.1$ Hz, 1H, H-2), 4.24 (dd, $J_{5,6}=4.4$ Hz, $J_{6,6'}=12.4$ Hz, 1H, H-6), 4.05 (m, 1H, H-6′), 3.99 (m, 1H, H-5), 2.18 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4 (CH_3–*C*O), 170.7 (CH_3–*C*O), 169.1 (CH_3–*C*O), 168.7 (CH_3–*C*O), 154.7 (NH–*C*O–NH), 134.3, 131.9, 128.4 (4C) (aromatics), 90.9 (C-1), 70.5 (C-3), 69.6 (C-5), 67.6 (C-4), 61.8 (C-6), 51.7 (C-2), 20.7 (*C*H_3–CO), 20.6 (2C, *C*H_3–CO), 20.4 (*C*H_3–CO). Anal. Calcd for $C_{21}H_{24}Cl_2N_2O_{10}$: C, 47.12; H, 4.52; N, 5.23. Found: C, 47.00; H, 4.55; N, 5.15.

4.1.3. 1,3,4,6-Tetra-*O*-acetyl-2-[3-(2-chloro-6-methylphenyl)ureido]-2-deoxy-α-D-glucopyranose (18). From 2-chloro-6-methylphenyl isocyanate (**15**) and following the described procedure, **18** was obtained (99%), mp 162–165 °C, $[\alpha]_D$ +100 (*c* 0.5, $CHCl_3$); ν_{max} 3340, 3270, 1550 (NH), 1740 (C=O), 1230 (C–O–C), 1645 (NC=O), 1050, 1015 (C–O), 770 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.10 (m, 3H, Ar), 7.01 (s, 1H, ArNH), 6.21 (d, $J_{1,2}=3.6$ Hz, 1H, H-1), 5.22–5.14 (m, 3H, NH, H-3, H-4), 4.39 (m, 1H, H-2), 4.22 (dd, $J_{5,6}=4.3$ Hz, $J_{6,6'}=12.5$ Hz, 1H, H-6), 4.04 (dd, $J_{5,6'}=2.1$ Hz, $J_{6,6'}=12.4$ Hz, 1H, H-6′), 3.96 (m, 1H, H-5), 2.23 (s, 3H, CH_3), 2.11 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (CH_3–*C*O), 170.6 (CH_3–*C*O), 169.0 (CH_3–*C*O), 168.5 (CH_3–*C*O), 155.4 (NH–*C*O–NH), 139.0, 133.0, 132.3, 129.3, 128.3, 127.3 (aromatics), 90.8 (C-1), 70.4 (C-3), 69.5 (C-5), 67.5 (C-4), 61.6 (C-6), 51.7 (C-2), 20.5 (2C, *C*H_3–CO), 20.4 (2C, *C*H_3–CO), 18.3 (CH_3). Anal. Calcd for $C_{22}H_{27}ClN_2O_{10}$: C, 51.32; H, 5.29; N, 5.44. Found: C, 51.35; H, 5.36; N, 5.59.

4.1.4. 1-(2,6-Dimethylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (25). To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2,6-dimethylphenyl)-ureido]-α-D-glucopyranose, **16**, (0.5 g, 1.0 mmol) in methanol (16 mL), was added a saturated solution of ammonia in methanol (16 mL). The reaction was controlled by TLC (chloroform–methanol 3:1). After ten hours at room temperature the mixture was evaporated to dryness and the residue treated with acetic acid (15 mL), and heated at ~100 °C (external bath) for 30 min. The solution was evaporated to dryness and the resulting solid was crystallized from 96% aqueous ethanol affording **25** (0.17 g, 55%), mp 245–247 °C, $[\alpha]_D$ +96.5 (*c* 0.5, DMF); ν_{max} 3480, 3380, 3250 (OH, NH), 1470 (NH), 1660 (C=O), 1080, 1030 (C–O), 1580, 790, 780 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 7.21 (d, $J_{2,NH}=1.2$ Hz, 1H, NH), 7.15–7.07 (m, 3H, Ar), 5.65 (d, $J_{1,2}=6.2$ Hz, 1H, H-1), 5.21 (d, $J_{3,OH}=4.8$ Hz, 1H, C3–OH), 4.72 (d, $J_{5,OH}=6.0$ Hz, 1H, C5–OH), 4.45 (t, $J_{6,OH}=J_{6',OH}=5.6$ Hz, 1H, C6–OH), 4.10–4.07 (m, 2H, H-2, H-3), 3.86 (dd, $J_{3,4}=2.2$ Hz, $J_{4,5}=8.6$ Hz, 1H, H-4), 3.72 (m, 1H, H-5), 3.55 (m, 1H, H-6), 3.34 (m, 1H, H-6′), 2.18 (s, 3H, CH_3), 2.12 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3 (C=O), 139.0, 136.7, 135.5, 128.2, 128.1, 127.8 (aromatics), 91.1 (C-1), 79.7 (C-4), 74.5 (C-3), 68.8 (C-5), 64.1 (C-6), 61.8 (C-2), 18.4 (CH_3), 17.7 (CH_3). Anal. Calcd for $C_{15}H_{20}N_2O_5$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.09; H, 6.64; N, 9.19.

4.1.5. 1-(2,6-Dichlorophenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]-imidazolidine-2-one (26). From **17** and following the procedure described for **25**, compound **26** was obtained (95%), mp 255–257 °C, $[\alpha]_D$ +89.0 (*c* 0.5, DMF); ν_{max} 3300 (OH, NH), 1470 (NH), 1695 (C=O), 1080, 1020, 1010 (C–O), 775 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 7.59–7.40 (m, 4H, Ar, NH), 5.79 (d, $J_{1,2}=6.4$ Hz, 1H, H-1), 5.23 (d, $J_{3,OH}=5.1$ Hz, 1H, C3–OH), 4.74 (d, $J_{5,OH}=5.9$ Hz, 1H, C5–OH), 4.41 (t, $J_{6,OH}=J_{6',OH}=5.6$ Hz, 1H, C6–OH), 4.11 (d, $J_{1,2}=6.5$ Hz, 1H, H-2), 4.07 (m, 1H, H-3), 3.93 (dd, $J_{3,4}=2.0$ Hz, $J_{4,5}=8.7$ Hz, 1H, H-4), 3.78 (m, 1H, H-5), 3.53 (m, 1H, H-6), 3.33 (m, 1H, H-6′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.0 (C=O), 137.1, 134.9, 132.7, 130.6, 129.2, 128.9 (aromatics), 89.9 (C-1), 80.2 (C-4), 74.6 (C-3), 68.9 (C-5), 64.5 (C-6), 62.2 (C-2). Anal. Calcd for $C_{13}H_{14}Cl_2N_2O_5$: C, 44.72; H, 4.04; N, 8.02. Found: C, 44.59; H, 4.10; N, 7.87.

4.1.6. 1-(2-Chloro-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]-imidazolidine-2-one (27). From **18** and following the procedure described for **25**, compound **27** was obtained (83%) as mixture of rotamers with a 35:65 (*M*:*P*) ratio, mp 252–254 °C, $[\alpha]_D$ +87.5 (*c* 0.5, DMF); ν_{max} 3480, 3380, 3250 (OH, NH), 1475 (NH), 1660 (C=O), 1080, 1030, (C–O), 1585, 790, 780 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 7.40–7.16 (m, 8H, Ar, NH, *M* and *P*), 5.73 (d, $J_{1,2}=6.4$ Hz, 1H, H-1, *M*), 5.71 (d, $J_{1,2}=6.3$ Hz, 1H, H-1, *P*), 5.24 (d, $J_{3,OH}=5.2$ Hz, 1H, C3–OH, *M*), 5.19 (d, $J_{3,OH}=4.8$ Hz, 1H, C3–OH, *P*), 4.73 (d, $J_{5,OH}=6.2$ Hz, 1H, C5–OH, *M*), 4.70 (d, $J_{5,OH}=5.8$ Hz, 1H, C5–OH, *P*), 4.46 (t, $J_{6,OH}=J_{6',OH}=5.6$ Hz, 1H, C6–OH,

M), 4.38 (t, $J_{6,OH}=J_{6',OH}=5.7$ Hz, 1H, C6–OH, *P*), 4.08 (m, 4H, H-2, H-3, *M* and *P*), 3.94 (dd, $J_{3,4}=2.2$ Hz, $J_{4,5}=8.7$ Hz, 1H, H-4, *P*), 3.87 (dd, $J_{3,4}=2.2$ Hz, $J_{4,5}=8.7$ Hz, 1H, H-4, *M*), 3.72 (m, 2H, H-5, *M* and *P*), 3.55 (m, 2H, H-6, *M* and *P*), 3.33 (m, 2H, H-6′, *M* and *P*), 2.24 (s, 3H, CH_3, *M*), 2.20 (s, 3H, CH_3, *P*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.9 C=O, (*M*), 157.6 C=O, (*P*), 141.9, 139.8, 135.6, 133.7, 133.2, 129.6, 129.5, 129.3, 127.6, 127.5 (aromatics), 90.7 C-1 (*M*), 90.2 C-1 (*P*), 80.2 C-4 (*P*), 79.8 C-4 (*M*), 74.7 C-3 (*P*), 74.4 C-3 (*M*), 69.0 C-5 (*P*), 68.6 C-5 (*M*), 64.7 C-6 (*P*), 64.1 C-6 (*M*), 62.1 C-2 (*M*), 62.0 C-2 (*P*), 18.5 CH_3 (*M*), 18.0 CH_3 (*P*). Anal. Calcd for $C_{14}H_{17}ClN_2O_5$: C, 51.15; H, 5.21; N, 8.52. Found: C, 51.00; H, 5.24; N, 8.45.

4.1.7. 1-(2,6-Dimethylphenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (28). To a solution of 1-(2,6-dimethylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one, **25** (0.8 g, 2.6 mmol) in pyridine (10.0 mL), cooled at −20 °C, was added acetic anhydride (8.0 mL) and the reaction mixture was kept at that temperature for 12 h. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water and identified as **28** (0.8 g, 71%). Recrystallized from 96% aqueous ethanol it had mp 230–232 °C, $[\alpha]_D$ +116.0 (*c* 0.5, $CHCl_3$); ν_{max} 3600–3100 (H_2O, NH),[15] 1435 (NH), 1750 (C=O), 1710 (NC=O), 1230 (C–O–C), 1030, (C–O), 1580, 1470, 770 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.20–7.10 (m, 3H, Ar), 6.13 (d, $J_{2,NH}=1.8$ Hz, 1H, NH), 5.80 (d, $J_{1,2}=6.4$ Hz, 1H, H-1), 5.36 (d, $J_{3,4}=2.8$ Hz, 1H, H-3), 5.20 (m, 1H, H-5), 4.59 (m, 2H, H-4, H-6), 4.29 (dd, $J_{1,2}=6.4$, $J_{2,NH}=2.3$ Hz, 1H, H-2), 4.04 (dd, $J_{5,6'}=4.3$ Hz, $J_{6,6'}=12.4$ Hz, 1H, H-6′), 2.32 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.7 (CH_3–*C*O), 158.9 (C=O), 138.8, 136.2, 133.8, 128.9, 128.7 (2C) (aromatics), 92.1 (C-1), 75.8 (C-3), 75.7 (C-4), 67.6 (C-5), 62.9 (C-6), 60.5 (C-2), 20.8 (2C, *C*H_3–CO), 20.7 (*C*H_3–CO), 18.4 (CH_3), 18.0 (CH_3). Anal. Calcd for $C_{21}H_{26}N_2O_8\cdot\frac{1}{2}H_2O$: C, 56.88; H, 6.14; N, 6.32. Found: C, 56.56; H, 5.98; N, 5.92.

4.1.8. 1-(2,6-Dichlorophenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (29). From **26** and following the procedure described for **28**, compound **29** was obtained (96%), mp 214–216 °C, $[\alpha]_D$ +102.0 (*c* 0.5, $CHCl_3$), ν_{max} 3380 (NH), 1740 (C=O), 1690 (NC=O), 1220 (C–O–C), 1055, 1030, (C–O), 1560, 1470, 785, 770 cm^{-1}(aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.26 (m, 3H, Ar), 6.17 (d, $J_{2,NH}=1.7$ Hz, 1H, NH), 6.06 (d, $J_{1,2}=6.5$ Hz, 1H, H-1), 5.34 (d, $J_{3,4}=2.8$ Hz, 1H, H-3), 5.24 (m, 1H, H-5), 4.70 (dd, $J_{3,4}=2.8$ Hz, $J_{4,5}=9.4$ Hz, 1H, H-4), 4.55 (dd, $J_{5,6}=2.4$ Hz, $J_{6,6'}=12.3$ Hz, 1H, H-6), 4.31 (dd, $J_{2,NH}=2.2$ Hz, $J_{1,2}=6.5$ Hz, 1H, H-2), 4.03 (dd, $J_{5,6'}=5.4$ Hz, $J_{6,6'}=12.3$ Hz, 1H, H-6′), 2.07 (s, 6H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.7 (CH_3–*C*O), 157.4 (C=O), 137.2, 135.1, 131.4, 130.2, 129.1, 128.7 (aromatics), 90.5 (C-1), 76.2 (C-3), 75.7 (C-4), 67.5 (C-5), 63.1 (C-6), 60.7 (C-2), 20.8 (3C, *C*H_3–CO). Anal. Calcd for $C_{19}H_{20}Cl_2N_2O_8$: C, 48.02; H, 4.24; N, 5.89. Found: C, 47.82; H, 4.21; N, 5.90.

4.1.9. 1-(2-Chloro-6-methylphenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (30). From **27** and following the procedure described for **28**, the titled compound **30** was obtained as a mixture of rotamers in a ∼1:3 (*M*: *P*) ratio (88%). Both rotamers were isolated by preparative TLC (benzene–acetone 3:1). After extracting silica-gel with ethyl acetate and evaporating to dryness, they were crystallized from 96% aqueous ethanol.

Compound **30***P*. Cubic transparent crystals, mp 193–196 °C, $[\alpha]_D$ +93.5 (*c* 0.5, $CHCl_3$), $R_f=0.3$ (benzene–acetone 3:1); ν_{max} 3390 (NH), 1740 (C=O), 1690 (NC=O), 1220 (C–O–C), 1070, 1045, 1030 (C–O), 1590, 1560, 1470, 780, 750, 710 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.18 (m, 3H, Ar), 5.85 (d, $J_{1,2}=6.4$ Hz, 1H, H-1), 5.80 (d, $J_{2,NH}=1.6$ Hz, 1H, NH), 5.33 (d, $J_{3,4}=2.8$ Hz, 1H, H-3), 5.24 (m, 1H, H-5), 4.74 (dd, $J_{3,4}=2.8$ Hz, $J_{4,5}=9.4$ Hz, 1H, H-4), 4.56 (dd, $J_{5,6}=2.4$ Hz, $J_{6,6'}=12.3$ Hz, 1H, H-6), 4.29 (dd, $J_{2,NH}=2.2$ Hz, $J_{1,2}=6.4$ Hz, 1H, H-2), 4.04 (dd, $J_{5,6'}=5.6$ Hz, $J_{6,6'}=12.3$ Hz, 1H, H-6′), 2.27 (s, 3H, CH_3), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (CH_3–*C*O), 169.9 (CH_3–*C*O), 169.7 (CH_3–*C*O), 157.7 (C=O), 138.8, 135.8, 132.1, 129.6, 129.5, 128.2 (aromatics), 90.9 (C-1), 76.3 (C-3), 76.0 (C-4), 67.7 (C-5), 63.2 (C-6), 60.5 (C-2), 20.8 (2C, *C*H_3–CO), 20.7 (*C*H_3–CO), 18.2 (CH_3). Anal. Calcd for $C_{20}H_{23}ClN_2O_8$: C, 52.81; H, 5.10; N, 6.16. Found: C, 52.80; H, 5.06; N, 6.19.

Compound **30***M*. Needle shaped crystals, mp 170 °C, $[\alpha]_D$ +109 (*c* 0.5, $CHCl_3$), $R_f=0.4$ (benzene- acetone 3:1), ν_{max} 3300 (NH), 1730 (C=O), 1690 (NC=O), 1230 (C–O–C), 1040 (C–O), 1590, 1570, 1480, 750 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.19 (m, 3H, Ar), 6.35 (s, 1H, NH), 5.99 (d, $J_{1,2}=6.4$ Hz, 1H, H-1), 5.35 (d, $J_{3,4}=2.7$ Hz, 1H, H-3), 5.21 (m, 1H, H-5), 4.58 (m, 2H, H-4, H-6), 4.33 (dd, $J_{2,NH}=2.1$ Hz, $J_{1,2}=6.4$ Hz, 1H, H-2), 4.04 (dd, $J_{5,6'}=4.4$ Hz, $J_{6,6'}=12.4$ Hz, 1H, H-6′), 2.36 (s, 3H, CH_3), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.7 (CH_3–*C*O), 158.7 (C=O), 141.3, 133.6, 132.8, 129.6 (2C), 127.8 (aromatics), 91.9 (C-1), 75.9 (C-3), 75.6 (C-4), 67.6 (C-5), 63.0 (C-6), 60.7 (C-2), 20.7 (3C, *C*H_3–CO), 18.6 (CH_3). HRMS: *m/z* found 455.1225. $M+H^+$ required for $C_{20}H_{23}ClN_2O_8$: 455.1221.

4.1.10. 1,3,4,6-Tetra-*O*-acetyl-2-[3-(2-chloro-6-methylphenyl)thioureido]-2-deoxy-β-D-glucopyranose (34). To a solution of $NaHCO_3$ (2.74 g, 32.6 mmol) in water (130 mL) was added under stirring 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride **31**,[17] (10.4 g, 27.1 mmol) and dichloromethane (130 mL), keeping the stirring for 30 min. The organic layer was separated and the aqueous extracted with dichloromethane (50 mL). The combined organic fractions were washed with water and dried over anhydrous magnesium sulfate and concentrated to approx. 50 mL. 2-Chloro-6-methylphenyl isothiocyanate, **33**, (5.0 g, 27.2 mmol) was then added and the reaction mixture was left at room temperature for 48 h. Compound **34** separated spontaneously as a white solid, which was filtered and washed with cold diethyl ether (75%), mp 174–176 °C, $[\alpha]_D$ −15.0 (*c* 1.0, $CHCl_3$); ν_{max} 3300, 2940, 1530 (NH), 1730 (C=O), 1230 (C–O–C), 1070, 1030 (C–O), 770 cm^{-1} (aromatics); ^{1}H NMR

(400 MHz, $CDCl_3$, $T=333$ K) δ 7.50 (d, $J_{2,NH}=6.4$ Hz, 1H, NH), 7.34–7.19 (m, 3H, Ar), 5.64 (m, 2H, H-1, NH), 5.10 (m, 3H, H-2, H-3, H-4), 4.21 (dd, $J_{5,6}=4.7$, $J_{6,6'}=12.4$ Hz, 1H, H-6), 4.12 (dd, $J_{5,6'}=2.5$, $J_{6,6'}=12.4$ Hz, 1H, H-6′), 3.77 (m, 1H, H-5), 2.24 (s, 3H, CH_3), 2.11 (s, 3H, OAc), 2.05 (s, 6H, OAc), 1.98 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$, $T=295$ K) δ 182.3 (C=S), 171.1 (CH_3–*C*O), 170.7 (2C, CH_3–*C*O), 169.4 (CH_3–*C*O), 139.5, 133.9, 129.7 (2C), 128.1 (2C) (aromatics), 92.5 (C-1), 72.6 (C-3, C-5), 68.1 (C-4), 61.6 (C-6), 57.8 (C-2), 20.9 (*C*H_3–CO), 20.8 (*C*H_3–CO), 20.7 (*C*H_3–CO), 20.5 (*C*H_3–CO) 18.0 (CH_3). Anal. Calcd for $C_{22}H_{27}ClN_2O_9S$: C, 49.77; H, 5.13; N, 5.28; S, 6.04. Found: C, 49.66; H, 5.16; N, 5.28; S, 5.80.

4.1.11. (4*R*,5*R*)-1-(2-Chloro-6-methylphenyl)-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)imidazolidine-2-thione (35). *Procedure A*. To a solution of 2-amino-2-deoxy-α-D-glucopyranose hydrochloride, **6**, (10.8 g, 50.0 mmol) in water (60.0 mL) was added $NaHCO_3$ (4.6 g, 55.0 mmol) and 2-chloro-6-methylphenyl isothiocyanate (50.0 mmol) under stirring. The mixture was diluted with ethanol (90.0 mL) to obtain a homogeneous solution that was then heated at 45 °C (external bath) for 30 min. When the mixture was left to room temperature, **35** spontaneously crystallized as a white solid, mixture of two rotamers (72%) in a ~3:1(*P*:*M*) ratio, mp 173–175 °C (ethanol 96%), $[\alpha]_D$ −23.5 (*c* 1.0, DMF), ν_{max} 3500–3000 (OH, NH), 1450 (NH), 1470 and 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H, NH, *P*), 8.17 (s, 1H, NH, *M*), 7.37–7.25 (m, 6H, Ar, *P* and *M*), 6.80 (d, $J_{5,OH}=7.4$ Hz, 1H, C5–OH, *P*), 6.62 (d, $J_{5,OH}=7.9$ Hz, 1H, C5–OH, *M*), 5.26 (d, $J_{5,OH}=7.2$ Hz, 2H, H-5, *P* and *M*), 4.85 (d, $J_{1',OH}=6.9$ Hz, 1H, C1′–OH, *M*), 4.74 (d, $J_{1',OH}=6.5$ Hz, 1H, C1′–OH, *P*), 4.59 (d, $J_{2',OH}=5.6$ Hz, 1H, C2′–OH, *P*), 4.47 (d, $J_{3',OH}=8.3$ Hz, 1H, C3′–OH, *P*), 4.40 (t, $J_{4',OH}=J_{4'',OH}=5.7$ Hz, 1H, C4′–OH, *P*), 3.80–3.31 (m, 12H, H-4, H-1′, H-2′, H-3′, H-4′, H-4″, *P* and *M*), 2.36 (s, 3H, CH_3, *P*), 2.18 (s, 3H, CH_3, *M*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.6 (C=S, *M*), 180.2 (C=S, *P*), 142.7, 139.8, 136.1, 134.8, 134.6, 133.2, 129.4 (3C), 129.3, 127.6, 127.3 (aromatics, *P* and *M*), 87.7 (C-5, *M*), 87.0 (C-5, *P*), 71.4 (C-1′, *M*), 71.2 (C-1′, *P*), 70.2 (C-2′, *P* and *M*), 70.0 (C-3′, *P*), 69.7 (C-3′, *M*), 66.1 (C-4, *M*), 65.7 (C-4, *P*), 63.6 (C-4′, *P*), 63.4 (C-4′, *M*), 19.3 (CH_3, *P*), 18.1 (CH_3, *M*). Anal. Calcd for $C_{14}H_{19}ClN_2O_5S$: C, 46.35; H, 5.28; N, 7.78; S, 8.84. Found: C, 46.08; H, 5.34; N, 7.55; S, 8.63.

Procedure B. To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-chloro-6-methylphenyl)thioureido]-β-D-glucopyranose (**34**) (5.4 g, 10.2 mmol) in methanol (160 mL), was added under vigorous stirring a saturated solution of ammonia in methanol (160 mL). The process was followed by TLC (chloroform–methanol, 3:1) and after 6 h at room temperature the reaction mixture was evaporated to dryness. The crude was crystallized from 96% aqueous ethanol. The title compound was filtered and washed with cold 96% aqueous ethanol and diethyl ether (3.5 g, 94%).

4.1.12. (4*R*,5*R*)-4-(1,2,3,4-Tetra-*O*-acetyl-D-*arabino*-tetritol-1-yl)-5-acetoxy-1-(2-chloro-6-methylphenyl)-imidazolidine-2-thione (36*P*). To a solution of **35** (2.4 mmol) in pyridine (10.0 mL), cooled at −20 °C for 15 min, was added acetic anhydride (6.0 mL) and the reaction mixture was kept at this temperature for 24 h. The mixture was then poured into ice-water and the resulting solid was filtered and washed with cold water affording **36** (94%) as a rotamer mixture in a ~6:1 (*P*:*M*) ratio. Fractional crystallization from 96% aqueous ethanol allowed the major rotamer **36*P*** to be isolated, mp 181–183 °C, $[\alpha]_D$ +28.0 (*c* 0.5, $CHCl_3$), ν_{max} 3320 (NH), 1760, 1730 (C=O), 1240, 1220, 1200 (C–O–C), 1500, 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H, NH), 7.35–7.19 (m, 3H, Ar), 6.58 (d, $J_{4,5}=1.4$ Hz, 1H, H-5), 5.65 (dd, $J_{4,1'}=8.8$, $J_{1',2'}=1.6$ Hz, 1H, H-1′), 5.32 (dd, $J_{1',2'}=1.5$ Hz, $J_{2',3'}=9.0$ Hz, 1H, H-2′), 5.00 (m, 1H, H-3′), 4.21 (m, 2H, H-4′, H-4″), 3.93 (dd, $J_{4,5}=1.4$ Hz, $J_{4,1'}=8.7$ Hz, 1H, H-4), 2.37 (s, 3H, CH_3), 2.16 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.03 (s, 6H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.7 (C=S), 170.6 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.7 (2C, CH_3–*C*O), 169.3 (CH_3–*C*O), 141.3, 133.5, 132.8, 130.1, 129.5, 127.7, (aromatics), 85.6 (C-5), 68.7 (C-2′), 68.3 (C-1′), 67.6 (C-3′), 61.2 (C-4′), 61.1 (C-4), 20.8 (*C*H_3–CO), 20.7 (*C*H_3–CO), 20.6 (3C, *C*H_3–CO), 18.7 (CH_3). Anal. Calcd for $C_{24}H_{29}ClN_2O_{10}S$: C, 50.31; H, 5.10; N, 4.89; S, 5.59. Found: C, 50.58; H, 5.05; N, 4.96; S, 5.14.

4.1.13. Transformation of (4*R*,5*R*)-4-(1,2,3,4-tetra-*O*-acetyl-D-*arabino*-tetritol-1-yl)-5-acetoxy-1-(2-chloro-6-methylphenyl)imidazolidine-2-thione (36) into 4-(1,2,3,4-tetra-*O*-acetyl-D-*arabino*-tetritol-1-yl)-1-(2-chloro-6-methylphenyl)imidazoline-2-thione (37). A solution of **36** (0.08 g) in DMSO-d_6 (0.5 mL) was heated at 80 °C and the transformation was monitored by ^{1}H NMR. Compound **37** was characterized by NMR spectroscopy. ^{1}H NMR (400 MHz, DMSO-d_6, 295 K) δ 9.92 (s, 1H, NH), 7.46–7.32 (m, 6H, Ar, *P* and *M*), 7.09 (s, 1H, H-5, *P*), 7.07 (s, 1H, H-5, *M*), 5.90 (d, $J_{1',2'}=2.7$ Hz, 1H, H-1′, *M*), 5.89 (d, $J_{1',2'}=3.5$ Hz, 1H, H-1′, *P*), 5.47 (dd, $J_{1',2'}=3.4$ Hz, $J_{2',3'}=8.1$ Hz, 1H, H-2′, *P*), 5.46 (dd, $J_{1',2'}=3.3$ Hz, $J_{2',3'}=8.0$ Hz, 1H, H-2′, *M*), 5.16 (m, 2H, H-3′, *P* and *M*), 4.21 (dd, $J_{3',4'}=2.7$ Hz, $J_{4',4''}=12.5$ Hz, 2H, H-4′, *P* and *M*), 4.15 (dd, $J_{3',4'}=5.0$ Hz, $J_{4',4''}=12.4$ Hz, 1H, H-4″, *M*), 4.14 (dd, $J_{3',4''}=5.1$ Hz, $J_{4',4''}=12.4$ Hz, 1H, H-4″, *P*), 2.07 (s, 3H, CH_3), 2.02 (s, 6H, OAc), 2.01 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.95 (s, 6H, OAc).

4.1.14. 4-(1,2,3,4-Tetra-*O*-acetyl-D-*arabino*-tetritol-1-yl)-1-(2-chloro-6-methylphenyl)imidazoline-2-thione (37*P*). To a solution of **36** (0.6 g, 1.05 mmol) in benzene (23 mL) was added $KHCO_3$ (0.2 g) and heated at reflux under stirring for 20 h. The reaction was followed by TLC (benzene–acetone, 3:1). The salt was filtered and the organic phase washed twice with water, dried over magnesium sulphate and evaporated to dryness. The crude was crystallized from 96% aqueous ethanol, affording a mixture of both rotamers (0.32 g, 60%). Recrystallization from 96% aqueous ethanol afforded the major rotamer *P* (0.08 g, 42%), mp 176–178 °C, $[\alpha]_D$ −89.4 (*c* 0.5, $CHCl_3$), ν_{max} 3200 (NH), 1750 (C=O), 1270, 1210 (C–O–C), 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.84 (s, 1H, NH), 7.38–7.25 (m, 3H, Ar), 6.64 (s, 1H, H-5), 6.07 (d, $J_{1',2'}=3.1$ Hz, 1H, H-1′), 5.48 (dd, $J_{1',2'}=3.1$ Hz, $J_{2',3'}=8.6$ Hz, 1H, H-2′), 5.20 (m, 1H, H-3′), 4.23 (dd, $J_{3',4'}=2.7$ Hz, $J_{4',4''}=12.6$ Hz, 1H, H-4′), 4.13 (dd, $J_{3',4''}=4.3$ Hz, $J_{4',4''}=12.5$ Hz, 1H,

H-4″), 2.18 (s, 3H, CH_3), 2.17 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 169.9 (CH_3–*C*O), 169.7 (CH_3–*C*O), 169.5 (CH_3–*C*O), 163.3 (C=S), 139.2, 133.6, 132.7, 130.5, 129.4, 127.7 (aromatics), 124.5 (C-4), 116.1 (C-5), 70.6 (C-2′), 68.2 (C-3′), 64.4 (C-1′), 61.5 (C-4′), 20.8 (*C*H_3–CO), 20.7 (*C*H_3–CO), 20.6 (2C, *C*H_3–CO), 18.3 (CH_3). Anal. Calcd for $C_{22}H_{25}ClN_2O_8S$: C; 51.51, H; 4.91, N; 5.46, S; 6.25. Found: C; 51.14, H; 5.00, N; 5.60, S; 6.18.

4.1.15. 1-(2-Chloro-6-methylphenyl)-4-(D-*arabino*-tetritol-1-yl)imidazoline-2-thione (38*P*). Compound **37***P* (0.11 g, 0.21 mmol) was dissolved in methanol (3 mL), a saturated solution of ammonia in methanol (5.5 mL) was added and the mixture kept at room temperature overnight. The reaction was controlled by TLC (chloroform–methanol, 3:1). The mixture was then evaporated to dryness and crystallized from 96% aqueous ethanol, affording pure compound **38***P* (0.05 g, 62%), mp 207–209 °C, $[\alpha]_D$ −73.4 (*c* 0.5, DMF), ν_{max} 3530, 3500 (NH, OH), 1610 (C=C), 1120–1000 (C–O), 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (bs, 1H, NH), 7.47–7.34 (m, 3H, Ar), 6.82 (s, 1H, H-5), 5.09 (d, $J_{1',OH}$=6.9 Hz, 1H, C1′–OH), 4.71(d, $J_{1',OH}$=5.9 Hz, 1H, H-1′), 4.66 (s, 1H, C2′–OH), 4.60 (s, 1H, C3′–OH), 4.39 (t, $J_{4',OH}$=$J_{4'',OH}$= 5.3 Hz, 1H, C4′–OH), 3.62–3.35 (m, 4H, H-2′, H-3′, H-4′, H-4″), 2.08 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7 (C=S), 139.2, 134.8, 132.2, 132.1, 130.3, 129.5 (aromatics), 127.6 (C-4), 115.2 (C-5), 73.5 (C-2′), 71.4 (C-3′), 64.4 (C-1′), 63.4 (C-4′), 18.3 (CH_3). Anal. Calcd for $C_{14}H_{17}ClN_2O_4S$: C; 48.77, H; 4.97, N; 8.12, S; 9.30. Found: C; 48.31, H; 5.00, N; 8.12, S; 9.18.

4.1.16. (4*R*,5*R*)-1-(2,6-Dichlorophenyl)-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)imidazolidine-2-thione (40). From 2,6-dichlorophenyl isothiocyanate (**39**) and following the procedure described for **35**, compound **40** was obtained (71%) by spontaneous crystallization when the mixture was cooled to room temperature: mp 201–202 °C (96% aqueous ethanol), $[\alpha]_D$ −20.5 (*c* 1.0, DMF), ν_{max} 3460–3000 (OH, NH), 1470 (NH), 1550 and 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H, NH), 7.56–7.39 (m, 3H, Ar), 6.75 (d, $J_{5,OH}$=7.3 Hz, 1H, C5–OH), 5.30 (dd, $J_{4,5}$=3.5 Hz, $J_{5,OH}$=7.3 Hz, 1H, H-5), 4.76 (d, $J_{1',OH}$= 6.7 Hz, 1H, C1′–OH), 4.59 (d, $J_{2',OH}$=5.1 Hz, 1H, C2′–OH), 4.50 (d, $J_{3',OH}$=8.0 Hz, 1H, C3′–OH), 4.40 (t, $J_{4',OH}$=$J_{4'',OH}$=4.9 Hz, 1H, C4′–OH), 3.83–3.39 (m, 6H, H-4, H-1′, H-2′, H-3′, H-4′, H-4″); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.3 (C=S), 137.9, 135.0, 133.8, 130.6, 129.1, 128.7 (aromatics), 87.0 (C-5), 71.2 (C-1′), 70.4 (C-2′), 69.9 (C-3′), 65.9 (C-4), 63.6 (C-4′). Anal. Calcd for $C_{13}H_{16}Cl_2N_2O_5S$: C, 40.74; H, 4.21; N, 7.31; S, 8.37. Found: C, 40.49; H, 4.02; N, 7.35; S, 8.61.

4.1.17. (4*R*,5*R*)-4-(1,2,3,4-Tetra-*O*-acetyl-D-*arabino*-tetritol-1-yl)-5-acetoxy-1-(2,6-dichlorophenyl)imidazolidine-2-thione (41). From **40** and following the procedure described for **36**, compound **41** was obtained (97%), mp 191–193 °C, $[\alpha]_D$ +63.5 (*c* 0.5, $CHCl_3$), ν_{max} 3300 (NH), 1750, 1720 (C=O), 1260, 1200 (C–O–C), 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H, NH), 7.46–7.27 (m, 3H, Ar), 6.50 (s, 1H, H-5), 5.60 (dd, $J_{4,1'}$=9.5 Hz, $J_{1',2'}$=1.6 Hz, 1H, H-1′), 5.34 (dd, $J_{1',2'}$= 1.6 Hz, $J_{2',3'}$=9.2 Hz, 1H, H-2′), 5.00 (m, 1H, H-3′), 4.20 (m, 2H, H-4′, H-4″), 3.93 (d, $J_{4,1'}$=9.4 Hz, 1H, H-4), 2.17 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.4 (C=S), 170.6 (CH_3–*C*O), 169.8 (2C, CH_3–*C*O), 169.5 (2C, CH_3–*C*O), 137.1, 135.4, 132.8, 130.7, 129.0, 128.7 (aromatics), 84.4 (C-5), 68.5 (C-1′), 68.1 (C-2′), 67.1 (C-3′), 61.1 (C-4′), 61.0 (C-4), 20.8 (*C*H_3–CO), 20.7 (*C*H_3–CO), 20.6 (*C*H_3–CO), 20.5 (*C*H_3–CO), 20.4 (*C*H_3–CO). Anal. Calcd for $C_{23}H_{26}Cl_2N_2O_{10}S$: C, 46.55; H, 4.42; N, 4.72; S, 5.40. Found: C, 46.40; H, 4.60; N, 4.79; S, 5.53.

4.1.18. 1-(2-Chloro-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thione (42). A solution of **35** (1.5 g, 4.13 mmol) in aqueous acetic acid (53.0 mL) was heated at ~100 °C (external bath) for 30 min. The mixture was then evaporated to dryness and the residue crystallized from 96% aqueous ethanol, affording a crystalline product that was filtered and washed with cold ethanol, being a mixture of both rotamers of **42** (1.1 g, 75%).

Compound **42***P*. From a mixture of atropisomers of **42** (1.8 g, 5.12 mmol) the major rotamer was separated by fractional crystallization from 96% aqueous ethanol (0.342 g, 19%). An analytic sample showed R_f=0.6, mp 238–240 °C, $[\alpha]_D$ +92 (*c* 0.5, DMF), ν_{max} 3480, 3320, 3200 (OH, NH), 1475 (NH), 1040 (C–O), 790 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 1H, NH), 7.36–7.27 (m, 3H, Ar), 5.88 (d, $J_{1,2}$=6.6 Hz, 1H, H-1), 5.34 (d, $J_{3,OH}$=4.7 Hz, 1H, C3–OH), 4.74 (d, $J_{5,OH}$=6.1 Hz, 1H, C5–OH), 4.42 (t, $J_{6,OH}$=$J_{6'\text{-}OH}$=5.6 Hz, 1H, C6–OH), 4.27 (d, $J_{1,2}$=6.7 Hz, 1H, H-2), 4.13 (m, 1H, H-3), 3.85 (dd, $J_{3,4}$=2.3 Hz, $J_{4,5}$=8.7 Hz, 1H, H-4), 3.75 (m, 1H, H-5), 3,62 (m, 1H, H-6), 3.31 (m, 1H, H-6′), 2.17 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.5 (C=S), 139.6, 135.4, 134.8, 129.6, 129.5, 127.6 (aromatics), 94.1 (C-1), 80.5 (C-4), 74.1 (C-3), 68.8 (C-5), 66.3 (C-2), 64.5 (C-6), 17.9 (CH_3). Anal. Calcd for $C_{14}H_{17}ClN_2O_4S$: C, 48.77; H, 4.97; N, 8.12; S, 9.30. Found: C, 49.07; H, 5.14; N, 8.23; S, 8.99.

Rotamer **42***P* was also obtained by the following procedure: to a solution of **44***P* (0.47 g, 0.99 mmol) in methanol (16 mL), was added a saturated solution of ammonia in methanol (16 mL) and the mixture kept at room temperature overnight. The reaction was followed by TLC (chloroform–methanol, 3:1). After that time it was evaporated to dryness and the residue crystallized from 96% aqueous ethanol, affording **42***P* (0.28 g, 81%).

Compound **42***M*. To a solution of **44***M* (0.15 g, 0.31 mmol) in methanol (5 mL) was added a saturated solution of ammonia in methanol (5 mL) and the mixture was kept at room temperature overnight. The reaction was controlled by TLC (chloroform–methanol, 3:1). After that time it was evaporated to dryness and the residue crystallized from 96% aqueous ethanol, affording **42***M* (0.07g, 64%), R_f=0.7, mp 209–211 °C, $[\alpha]_D$ +193.0 (*c* 0.7, DMF), ν_{max} 3500–3000 (OH, NH), 1500 (NH), 1030 (C–O), 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H, NH), 7.42–7.27 (m, 3H, Ar), 5.85 (d, $J_{1,2}$=6.6 Hz, 1H, H-1), 5.40 (d,

$J_{3,OH}$=5.3 Hz, 1H, C3–OH), 4.78 (d, $J_{5,OH}$=6.0 Hz, 1H, C5–OH), 4.50 (t, $J_{6,OH}$=$J_{6',OH}$=5.5 Hz, 1H, C6–OH), 4.28 (d, $J_{1,2}$=6.6 Hz, 1H, H-2), 4.15 (dd, $J_{3,4}$=2.1 Hz, $J_{3,OH}$= 5.2 Hz, 1H, H-3), 3.78 (dd, $J_{3,4}$=2.1 Hz, $J_{4,5}$=8.7 Hz, 1H, H-4), 3.75–3.53 (m, 3H, H-5, H-6, H-6′), 2.29 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.6 (C=S), 141.9, 134.8, 132.8, 129.8, 129.6, 127.4 (aromatics), 94.4 (C-1), 80.3 (C-4), 73.9 (C-3), 68.3 (C-5), 66.3 (C-2), 63.9 (C-6), 18.8 (CH_3). Anal. Calcd for $C_{14}H_{17}ClN_2O_4S$: C, 48.77; H, 4.97; N, 8.12; S, 9.30. Found: C, 48.98; H, 5.11; N, 7.99; S, 8.93.

4.1.19. 1-(2,6-Dichlorophenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thione (43). A solution of **40** (1.0 g, 2.61 mmol) in aqueous acetic acid (30%, 33 mL), was heated at 100 °C (external bath) for 30 min. The solution was then evaporated to dryness and the white residue obtained was crystallized from 96% aqueous ethanol (0.67 g, 70%). An analytic sample obtained by recrystallization from 96% aqueous ethanol showed mp 245–246 °C, $[\alpha]_D$ +151.2 (*c* 0.5, DMF), ν_{max} 3400–3000 (OH, NH), 1560, 1295 (thioamide), 1440 (NH), 1500, 770 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H, NH), 7.61–7.42 (m, 3H, Ar), 5.91 (d, $J_{1,2}$= 6.6 Hz, 1H, H-1), 5.38 (d, $J_{3,OH}$=5.0 Hz, 1H, C3–OH), 4.77 (d, $J_{5,OH}$=5.9 Hz, 1H, C5–OH), 4.45 (t, $J_{6,OH}$=$J_{6'-OH}$= 5.5 Hz, 1H, C6–OH), 4.29 (d, $J_{1,2}$=6.7 Hz, 1H, H-2), 4.14 (d, $J_{3,4}$=2.4 Hz, 1H, H-3), 3.86–3.30 (m, 4H, H-4, H-5, H-6, H-6′); ^{13}C NMR (50.33 MHz, DMSO-d_6) δ 181.4 (C=S), 137.3, 134.8, 133.6, 131.0, 129.2, 128.9 (aromatics), 93.8 (C-1), 80.6 (C-4), 74.1 (C-3), 68.7 (C-5), 66.5 (C-2), 64.4 (C-6). Anal. Calcd for $C_{13}H_{14}Cl_2N_2O_4S$: C, 42.75; H, 3.86; N, 7.67; S, 8.78. Found: C, 42.68; H, 3.75; N, 7.74; S, 8.72.

4.1.20. 3,5,6-Tri-*O*-acetyl-1-(2-chloro-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thione (44). From **42** and following the procedure described for **28**, compound **44** was obtained (85%) as a mixture of rotamers in a ~1:4 (*M*:*P*) ratio. From a fraction of this mixture (1.3 g) both rotamers were separated by flash chromatography (ethyl acetate–hexane 1:2).

Compound **44***P*. R_f=0.6, (1.0 g, 78%). Recrystallized from 96% aqueous ethanol, mp 173–175 °C, $[\alpha]_D$ +125.4 (*c* 0.5, $CHCl_3$), ν_{max} 3310 (NH), 1740 (C=O), 1230 (C–O–C), 1050, 1030 (C–O), 1480, 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.21 (m, 4H, Ar and NH), 5.97 (d, $J_{1,2}$=6.7 Hz, 1H, H-1), 5.36 (d, $J_{3,4}$=2.9 Hz, 1H, H-3), 5.27 (m, 1H, H-5), 4.72 (dd, $J_{3,4}$=2.9, $J_{4,5}$=9.3 Hz, 1H, H-4), 4.60 (dd, $J_{5,6}$=2.4 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6), 4.44 (dd, $J_{1,2}$=6.6 Hz, $J_{2,3}$=1.0 Hz, 1H, H-2), 4.03 (dd, $J_{5,6'}$= 5.5 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6′), 2.25 (s, 3H, CH_3), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9 (C=S), 170.6 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.6 (CH_3–*C*O), 138.5, 135.6, 133.4, 129.9, 129.5, 128.2 (aromatics), 94.7 (C-1), 76.7 (C-3), 75.4 (C-4), 67.5 (C-5), 64.3 (C-2), 62.9 (C-6), 20.7 (2C, *C*H_3–CO), 20.6 (*C*H_3–CO), 18.1 (CH_3). Anal. Calcd for $C_{20}H_{23}ClN_2O_7S$: C, 51.01; H, 4.92; N, 5.95; S, 6.81. Found: C, 51.33; H, 4.81; N, 5.96; S, 6.86. HRMS: *m*/*z* found 471.1001. M+H$^+$ for $C_{20}H_{23}ClN_2O_7S$ required 471.0993.

Compound **44***M*. R_f=0.7, (0.20 g, 16%), mp 88 °C, $[\alpha]_D$ +182 (*c* 0.5, $CHCl_3$), ν_{max} 3320 (NH), 1740 (C=O), 1230 (C–O–C), 1030, (C–O), 1470, 770, 730 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.28 (m, 4H, Ar and NH), 6.18 (d, $J_{1,2}$=6.7 Hz, 1H, H-1), 5.44 (d, $J_{3,4}$=2.9 Hz, 1H, H-3), 5.31 (m, 1H, H-5), 4.68 (dd, $J_{5,6}$=2.3 Hz, $J_{6,6'}$= 12.4 Hz, 1H, H-6), 4.61 (dd, $J_{3,4}$=2.9 Hz, $J_{4,5}$=9.2 Hz, 1H, H-4), 4.55 (d, $J_{1,2}$=6.7 Hz, 1H, H-2), 4.09 (dd, $J_{5,6'}$= 4.3 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6′), 2.45 (s, 3H, CH_3), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.09 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.6 (C=S), 170.5 (CH_3–*C*O), 169.7 (CH_3–*C*O), 169.5 (CH_3–*C*O), 141.2, 133.9, 133.0, 129.9, 129.5, 127.7 (aromatics), 95.4 (C-1), 76.4 (C-3), 75.1 (C-4), 67.4 (C-5), 64.5 (C-2), 62.7 (C-6), 20.6 (2C, *C*H_3–CO), 20.5 (*C*H_3–CO), 18.6 (CH_3). HRMS: *m*/*z* found 471.0987. M+H$^+$ required for $C_{20}H_{23}ClN_2O_7S$ 471.0993.

4.1.21. 3,5,6-Tri-*O*-acetyl-1-(2,6-dichlorophenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thione (45). From **43** and following the procedure described for **28**, compound **45** was obtained (100%), mp 102–105 °C, $[\alpha]_D$ +152.6 (*c* 0.5, $CHCl_3$), ν_{max} 3300 (NH), 1740 (C=O), 1230 (C–O–C), 1040 (C–O), 1470, 1440, 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.27 (m, 4H, Ar, NH), 6.17 (d, $J_{1,2}$=6.8 Hz, 1H, H-1), 5.35 (d, $J_{3,4}$= 3.0 Hz, 1H, H-3), 5.30 (m, 1H, H-5), 4.68 (dd, $J_{3,4}$=3.0 Hz, $J_{4,5}$=9.3 Hz, 1H, H-4), 4.60 (dd, $J_{5,6}$=2.4 Hz, $J_{6,6'}$= 12.4 Hz, 1H, H-6), 4.47 (dd, $J_{2,NH}$=1.5 Hz, $J_{1,2}$=6.9 Hz, 1H, H-2), 4.02 (dd, $J_{5,6'}$=5.5 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6′), 2.10 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.03 (s, 3H, OAc); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 182.6 (C=S), 170.6 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.6 (CH_3–*C*O), 137.3, 134.8, 132.6, 130.6, 129.0, 128.7 (aromatics), 94.4 (C-1), 76.6 (C-3), 76.3 (C-4), 67.4 (C-5), 64.6 (C-2), 62.9 (C-6), 20.8 (*C*H_3–CO), 20.7 (*C*H_3–CO), 20.6 (*C*H_3–CO). Anal. Calcd for $C_{19}H_{20}Cl_2N_2O_7S$: C, 46.45; H, 4.10; N, 5.70; S, 6.52. Found: C, 46.25; H, 3.96; N, 5.63; S, 6.38.

4.1.22. 1-Acetyl-3-(2,6-dichlorophenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[1,2-*d*]imidazolidine-2-thione (46). A solution of **45** (0.13 g, 0.27 mmol) in pyridine (0.8 mL) and acetic anhydride (0.8 mL) was heated at 40 °C for 2 h. The reaction mixture was then poured over ice-water and a white solid was formed. This product was filtered and washed with cold water, (0.12 g, 86%), and then recrystallized from 96% aqueous ethanol, mp 202–204 °C, $[\alpha]_D$ +124.4 (*c* 0.5, $CHCl_3$), ν_{max} 3600–3100 (crystallization H_2O)[15], 1735, 1715 (C=O), 1675 (C=O), 1240, 1220 (C–O–C), 775 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.27 (m, 3H, Ar), 6.06 (d, $J_{1,2}$=7.0 Hz, 1H, H-1), 5.79 (d, $J_{3,4}$=3.1 Hz, 1H, H-3), 5.21 (m, 1H, H-5), 4.96 (d, $J_{1,2}$=7.0 Hz, 1H, H-2), 4.55 (m, 2H, H-4, H-6), 3.99 (dd, $J_{5,6}$=5.5 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6′), 2.92 (s, 3H, N–Ac), 2.10 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.65 (bs, crystallization H_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.6 (C=S), 171.5 (CH_3–*C*O), 170.6 (CH_3–*C*O), 169.8 (CH_3–*C*O), 168.5 (CH_3–*C*O), 136.9, 134.2, 132.6, 130.9, 129.2, 129.0 (aromatics), 90.0 (C-1), 76.9 (C-4), 73.9 (C-3), 67.0 (2C, C-2, C-5), 63.0 (C-6), 27.1 (N–Ac), 20.8 (2C, *C*H_3–CO), 20.7 (*C*H_3–CO). Anal. Calcd for $C_{21}H_{22}Cl_2N_2O_8S\cdot 2H_2O$: C, 44.30; H, 4.60; N, 4.92; S, 5.63. Found: C, 44.29; H, 4.41; N, 4.67; S, 5.92.

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19. The authors have deposited the atomic coordinates for these structures with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. Crystal data for **42***M*, (CCDC-239943), $C_{14}H_{17}ClN_2O_4S$, $M_r=344.81$, monoclinic, $P2_1$, $a=7.7947(3)$ Å, $b=7.5324(2)$ Å, $c=13.4087(5)$ Å, $V=783.93(5)$ Å^3, $Z=2$, $D_{calcd}=1.461$ g cm^{-3}, λ(Mo Kα)=0.71073 Å, $\mu=3.96$ cm^{-1}, $F(000)=360$, $T=293(2)$ K, GooF$^2=1.073$, independent reflections=2748 [$R_{int}=0.0660$] of a total of 9490 collected reflections, $R(F)$ obeying $F^2>2\sigma(F^2)=0.0347$, $wR(F^2)=0.0890$, R(all data)=0.0385, $wR(F^2)=0.0914$. Crystal data for **42***P*, (CCDC-239942), $C_{14}H_{17}ClN_2O_4S$, $M_r=344.81$, orthorhombic, $P2_12_12_1$, $a=9.7765(3)$ Å, $b=12.2006(6)$ Å, $c=13.1541(6)$ Å, $V=1569.01(12)$ Å^3, $Z=4$, $D_{calcd}=1.460$ g cm^{-3}, λ(MoKα)=0.71073 Å, $\mu=3.95$ cm^{-1}, $F(000)=720$, $T=293(2)$ K, GooF$^2=1.029$, independent reflections=2672 [$R_{int}=0.0381$] of a total of 6126 collected reflections, $R(F)$ obeying $F^2>2\sigma(F^2)=0.0362$, $wR(F^2)=0.0792$, R(all data)=0.0482, $wR(F^2)=0.0843$.
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Non-biaryl atropisomers derived from carbohydrates. Part 4: Absolute stereochemistry of carbohydrate-based imidazolidine-2-ones and 2-thiones with axial and central chirality☆

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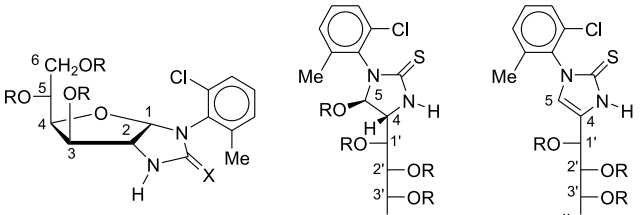
Abstract—NMR spectroscopy has proven to be an enabling methodology in elucidating the axial chirality of a series of non-biaryl atropisomers attached to a carbohydrate moiety, based on deshielding effects caused by the aromatic ring.

1. Introduction

In the preceding paper,[1] we reported the synthesis and isolation of some carbohydrate-appended imidazolidine-2-ones and -2-thiones (**1**–**6**) as well as imidazoline-2-thiones (**7** and **8**) as room temperature stable atropisomers. Free rotation around a single bond converts the *M* (a*R*) isomer into its *P* (a*S*) counterpart and vice versa.[2]



Such an interconversion takes place by rotation around the N–C (aryl) single bond and the transition structures correspond to the peaks of highest potential energy through a 360° rotation. There are two possible and unequally populated isomeric transition states, in which one of the substituents at the *ortho* position on the aromatic ring interacts with the C=X function and this steric crowding is largely responsible for the high barrier to rotation. Molecular mechanics calculations predict that *o*,*o*′-disubstitution will cause high enough barriers to rotation (>23 kcal mol^{-1}) for isolation of atropisomers to occur.[3] The peaks of higher potential energy were found at $\theta = 30$ and 210°, approximately[1] (Fig. 1).

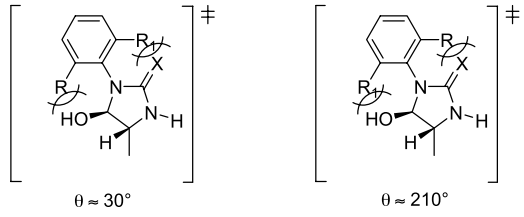


Figure 1. Transition states for interconversion of *P* and *M* rotamers.

In order to verify our theoretical predictions, we have synthesized a series of derivatives featuring the above-mentioned structural requirements, and establishing the absolute stereochemistry of the isolated atropisomers by means of NMR chemical shift correlations, some further confirmed by single-crystal X-ray diffractometry.

☆ See Ref. 1.

Keywords: Atropisomerism; Carbohydrates; Imidazolidines; Molecular mechanics; NMR spectroscopy.
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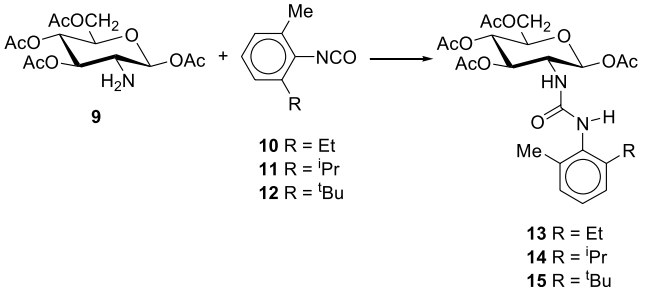
2. Results and discussion

The protocol utilized in these syntheses has been previously described.[1] Thus, condensation of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose[4] (**9**) with 2-ethyl-6-methylphenyl isocyanate (**10**), 2-isopropyl-6-methylphenyl isocyanate (**11**) and 2-*tert*-butyl-6-methylphenyl isocyanate (**12**), afforded the corresponding ureido derivatives **13**–**15** with good yields[5] (Scheme 1).



Scheme 1.

The ^{1}H NMR spectra for the ureido derivatives **14** and **15**, when registered at −30 °C, show four signal sets corresponding to four atropisomers (Table 1). This fact was also found in similar thioureido derivatives.[1] These experiments at variable temperature allowed us to calculate the experimental barriers to rotation. Thus, the barrier for compound **14** resulted to be 15.0 kcal mol^{-1} (T_c=298 K, $\Delta\nu$=28.96 Hz) while a value of 16.2 kcal mol^{-1} (T_c=328 K, $\Delta\nu$=50.77 Hz) was found for the ureido derivative **15**.[6]

Deacetylation at room temperature of compounds **13**–**15** gave anomeric mixtures of α and β ureido derivatives (**16**–**21**) together with 5-hydroxyimidazolidine-2-ones (**22**–**24**), although in the case of compounds **13** and **15**, pure α-anomers **19** and **21** were isolated, the latter being a mixture of two atropisomers. Formation of **19** and **21** not only involves *O*-deprotection of **13** and **15** to afford **16** and **18**, respectively, but also an anomeric change. Their glucopyranoid structures are supported by ^{13}C NMR data, in agreement with those of other ureidosugars.[7] The α-anomeric configuration is consistent with the small value of $J_{1,2}$ (3.6 Hz) (Scheme 2).

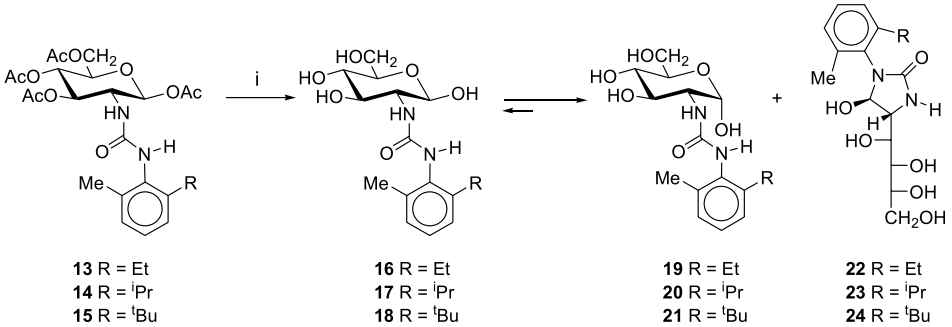
The 5-hydroxyimidazolidine-2-one **22**, arising from cyclization of ureas **16** and **19**, was also isolated as mixture of rotamers, as evidenced by the two signal sets observed in its ^{1}H and ^{13}C NMR spectra. The chemical shift for C-5 (~84 ppm) rules out both an ureido derivative and a bicyclic structure such as **1**, ($\delta_{C\text{-}1}$~90–92 ppm).[7,8] The *R* configuration at C-5, that is, the heterocyclic carbon bearing the hydroxyl group, could be assigned on the basis of the coupling constant $J_{4,5}$~0–3 Hz.[7,9] The two atropisomers were formed in a ~1:1 ratio and crystallized with a molecule of ethanol, as evidenced by their spectral data and elemental analyses. The structure of compound **22** was confirmed by preparing its per-*O*-acetyl derivative **25**, as mixture of rotamers, although leaving intact the initial stereochemistry. The methylenic hydrogens on the ethyl groups are diastereotopic and show complex ^{1}H NMR patterns.

Treatment of the crude mixtures, generated by deacetylation of **13**–**15**, with hot aqueous acetic acid led to the corresponding 1-aryl-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-ones (**26**–**28**). While **26** and **27** appeared as mixtures of two rotational isomers, only the (*M*)-atropisomer of **28** could be isolated. The structures of these compounds are consistent with their elemental analysis, as well as polarimetric and spectroscopic data analogous to similar bicycles.[1,7] The magnitudes of $J_{1,2}$

Table 1. Selected spectroscopic data and population for rotamers of **14** and **15**[a]

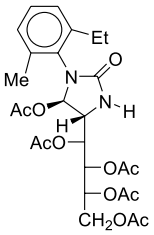
	14, Rotamers				**15**, Rotamers			
	a	**b**	**c**	**d**	**a**	**b**	**c**	**d**
NH	6.97	6.90	6.38	6.37	6.79	6.73	6.33	6.29
H-1	5.88	5.88	5.73	5.66	5.89	5.77	5.69	5.56
Populations[b]	26.0[c]		36.9	37.1	16.1	17.0	28.5	38.4

[a] In $CDCl_3$ at 258 K.
[b] In %.
[c] Combined population of rotamers **a** and **b**.



Scheme 2. (i) NH_3–MeOH.

(>6.2 Hz) and $J_{2,3}$ (~0 Hz) reveal the furanosic character of the sugar with a *cis* fusion of both rings. As expected, ^{1}H and ^{13}C NMR spectra for **26** and **27** showed duplicated signals corresponding in each case to a mixture of two atropisomers in ~1:1 (*P*:*M*) ratio,[2] which could not be resolved.



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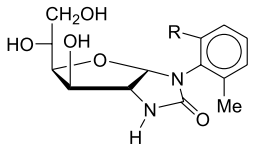
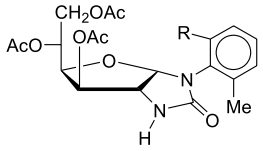


26 R = Et
27 R = ^{i}Pr
28 R = ^{t}Bu

The absolute configuration of **28*M*** could be unambiguously determined by single-crystal diffractometry as depicted in the ORTEP diagram with the crystallographic numbering[10] (Fig. 2).

We next attempted isolation of the remaining rotamers via their per-*O*-acetyl derivatives. Thus, treatment of atropisomeric mixtures of **26** or **27** with acetic anhydride and pyridine at −20 °C provided high yields of **29** or **30**, respectively. In a similar way, acetylation of **28*M*** led to **31*M*** in pure form as this *O*-protection does not affect the axial stereochemistry.

M and *P* atropisomers of **29** and **30** could be separated by preparative thin-layer chromatography (using ethyl ether as eluent). When silica gel was extracted with ethyl acetate pure *M* and *P* rotamers of **29** and **30** were obtained. However, when extraction of compound **29**, was carried out with methanol, its rotamer **29*P*** (R_f=0.5) underwent deacetylation giving rise to pure **26*P***, while the other rotamer (**29*M***, R_f=0.6) remained unaffected. This curious and unexpected result is not so surprising as the deacetylating properties of silica gel in methanol have been documented.[11] The structures attributed to the individual rotamers of **29** and **30** and **31*M*** are equally supported by their spectroscopic data. ^{1}H and ^{13}C NMR spectra reveal that the heterocyclic nitrogen remains unprotected. IR spectra for *M* and *P* rotamers of **29** show absorptions at 3500–3000 cm^{-1} due to the presence of water in the crystal lattice.[12]



29 R = Et
30 R = ^{i}Pr
31 R = ^{t}Bu

On the other hand, condensation of the *O*-protected aminosugar **9** with 2-ethyl-6-methylphenyl isothiocyanate (**32**) at room temperature afforded the thioureido derivative **33** (Scheme 3), although yields were invariably low. In an attempt to improve this yield, the reaction was also carried out under reflux but large amounts of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**34**) were formed. This substance was identified by comparison of its physical and spectroscopic properties with an authentic sample.[13]

The structure assigned to **33** is supported by its elemental analysis, spectral and polarimetric data. The room

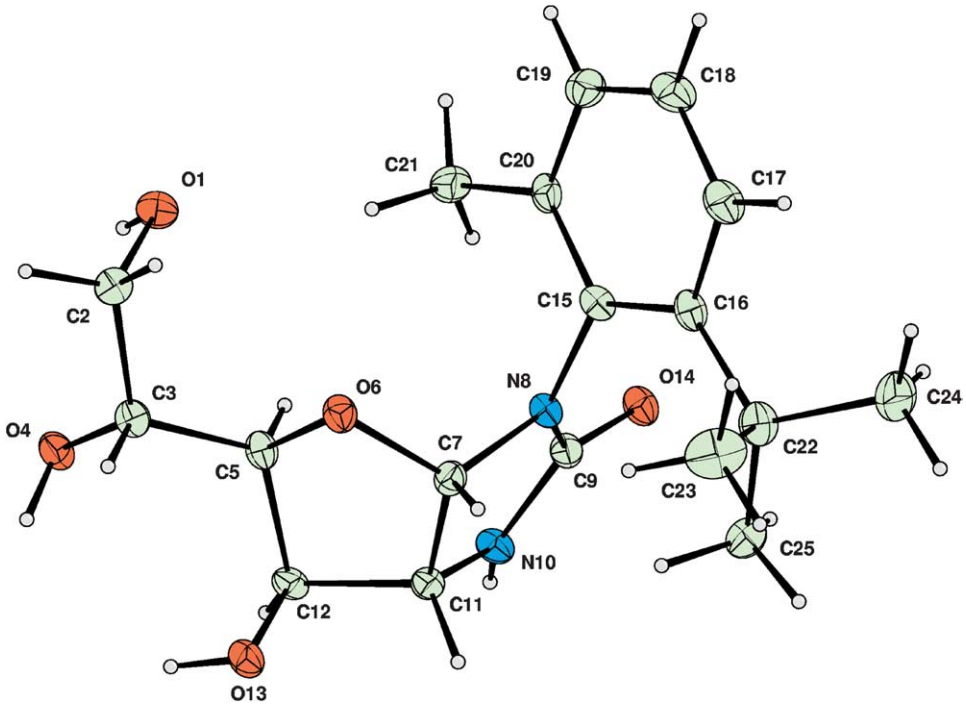
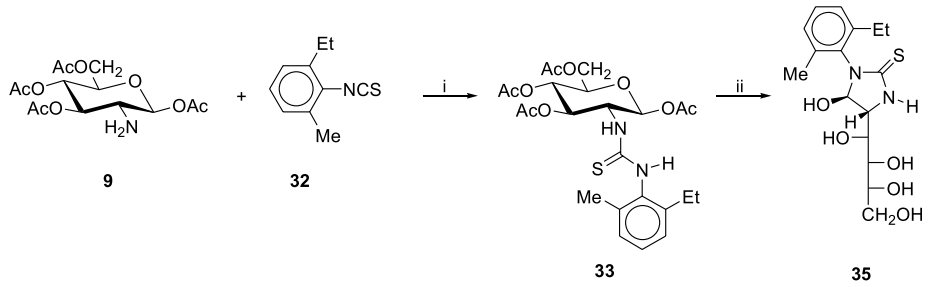


Figure 2. X-ray diffraction structure for **28*M***.



Scheme 3. (i) CH_2Cl_2, △ (ii) NH_3–MeOH.

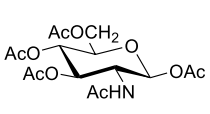
temperature ^{1}H NMR spectrum showed two well-separated signal sets of the corresponding atropisomers in ~1:1 ratio.

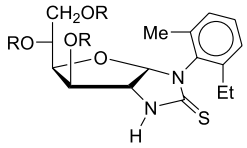
Further treatment of **33** with ammonia in methanol at room temperature provided the deacetylated derivative 5-hydroxyimidazolidine-2-thione (**35**) in high yield. Its *R*-configuration at C-5 is consistent with a zero value for $J_{4,5}$.[9]

The subsequent treatment of **35** with hot aqueous acetic acid led to the corresponding bicyclic imidazolidine-2-thione **36** with yields no higher than 25%.

The structure of **36** is consistent with its elemental analysis and spectroscopic data. NMR spectra showed a mixture of two rotamers in ~1:1 ratio. Rotamer **36*P*** was isolated by crystallization from aqueous ethanol.

Acetylation of the *M* and *P* mixture of **36** with acetic anhydride and pyridine at −20 °C provided the corresponding per-*O*-acetyl derivative **37**, also as mixture of atropisomers.





36 R = H
37 R = Ac

Compound **37*P*** could be separated by crystallization from aqueous ethanol. Again, its structure is supported by elemental analysis and spectral data found in similar bicycles.[1,9] Likewise, the atropisomers of **36** and **37** showed complex signals for the diastereotopic methylene protons on the ethyl groups.

The absolute stereochemistry of **37*P*** could also be unambiguously determined by single-crystal X-ray diffractometry as depicted in its ORTEP diagram with the crystallographic numbering (Fig. 3).[14]

All the new synthesized rotamers were stable at room temperature and no coalescence could be observed for either

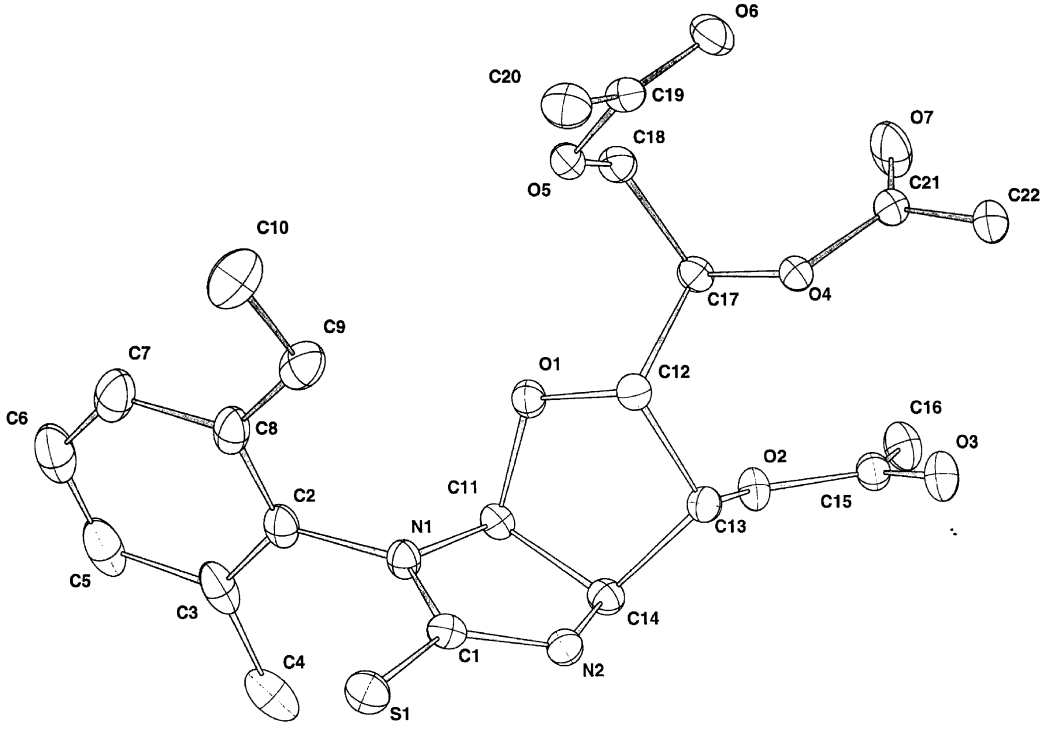


Figure 3. X-ray diffraction analysis of compound **37*P***.

atropisomeric mixture on heating up to 150 °C. This finding clearly suggest that coalescence should occur at a higher temperature ($T_c > 423$ K) and, even at that threshold value, the resulting rotational barriers (20–22 kcal mol^{-1})[6,15] are in full agreement with our theoretical data.[1]

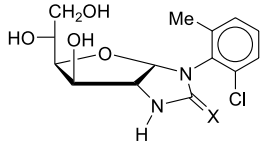
2.1. Assignment of the absolute stereochemistry of atropisomers derived from imidazolidine-2-one, imidazoline-2-one and their thioanalogues

The main aspect on elucidating the structure of the described atropisomers is to establish their axial absolute stereochemistry. Obviously, the most important technique is X-ray diffractometry, although it has sometimes a restricted use, hampered by availability of suitable crystals. This limitation could be overcome by stereochemical correlations with other products with well-known structures.

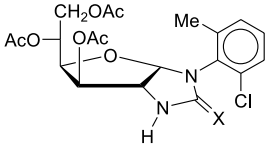
Since the structures of some characteristic rotamers could be unequivocally established,[1,16] we were able to assign the axial stereochemistry for all the synthesized atropisomers.

2.2. Axial stereochemistry of 1-arylimidazolidine-2-one and 2-thiones derived from 2-chloro-6-methylphenyl isocyanate and isothiocyanate

The synthesis of 1-(2-chloro-6-methylphenyl)imidazolidine-2-one (**1** and **2**) and imidazolidine-2-thione (**3** and **4**) has been already described.[1] Atropisomers of **2**, **3** and **4** could be isolated and the absolute stereochemistry for the two rotamers of **3** and the *P* rotamer of **2** were determined by means of X-ray diffractometry.[1,16] The unambiguous knowledge of these structures has been harnessed to establish a useful stereochemical correlation with the rest of atropisomers.



1 X = O
3 X = S



2 X = O
4 X = S

The axial stereochemistry for *M* and *P* atropisomers of **4** was unequivocally determined when each was transformed into the corresponding deacetylated derivatives (**3*M*** and **3*P***), as this reaction has no effect on the axial chirality.

Obviously, the major atropisomer of **3** has the same axial stereochemistry as the major atropisomer of **4**, both *P* (the presence of acetyl groups does not affect to the priority rules of the Cahn–Ingold–Prelog system).

On the other hand, X-ray diffractrometry showed that the major atropisomer of compound **2** had *P* configuration.[16] This fact immediately attributes the opposite *M*-configuration to the minor counterpart. Again, as the axial stereochemistry is not influenced by acetylation, one can reasonably conclude that major and minor atropisomers of compound **1** exhibit *P* and *M* axial conformations, respectively.

Having established the absolute stereochemistry for rotamers of compounds **1**–**4**, we accomplished a study of their NMR spectroscopic data (Table 2) and finding some regularities that can be used in a predictive way. Thus, all major rotamers show *P* configurations and their methyl groups on the aromatic ring resonate at higher field than the corresponding (*M*)-rotamers both in ^{1}H and ^{13}C NMR spectra.

Although $\Delta\delta_{CH_3}$ are not too large, the order of such chemical shifts remains constant. Unfortunately, this trend could not be observed for H-1.

It is interesting to point out how coherent these data are. A couple of atropisomers can only be distinguished from a stereochemical point of view, and therefore the different chemical shift of the methyl groups should reflect two distinctive chemical environments. The methyl group appears deshielded when placed in the concavity formed by the two heterocyclic rings (*M* atropisomers), whereas the same group located in the opposite spatial orientation (*P* atropisomers) shows more shielded resonances (Fig. 4). This variation can be observed for all compounds listed in Table 2 when going from *M* to *P* atropisomers.

These two spatial dispositions are present at the same time on compounds **38** and **39**,[1] where methyl groups bear both *ortho* positions of the aromatic ring. Accordingly, such methyl groups exhibit the chemical shift differences described in Figure 4.

Table 2. Selected NMR data for compounds **1**–**4**

Comp.	^{1}H NMR			^{13}C NMR		Abund.	Axial stereochemistry
	δ_{CH_3}	$\Delta\delta_{CH_3}$	$\delta_{H\text{-}1}$	δ_{CH_3}	$\Delta\delta_{CH_3}$	[a]	
1[b]	2.24	0.04	5.73	18.5	0.5	−	*M*
	2.20		5.71	18.0		+	*P*
2[c]	2.36	0.09	5.99	18.6	0.4	−	*M*
	2.27		5.85	18.2		+	*P*
3[b]	2.29	0.12	5.85	18.8	0.9	−	*M*
	2.17		5.88	17.9		+	*P*
4[c]	2.39	0.14	6.12	18.6	0.5	−	*M*
	2.25		5.97	18.1		+	*P*

[a] Symbols +/− indicate major/minor atropisomers.
[b] In DMSO-d_6.
[c] In $CDCl_3$.

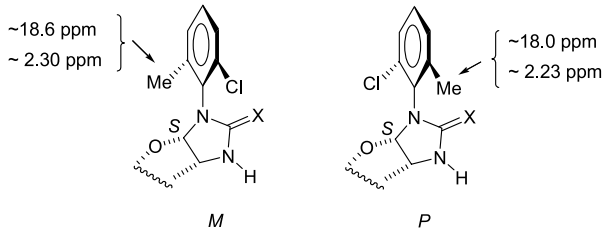
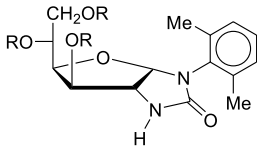


Figure 4. Average values of δ_{CH_3} (^{1}H and ^{13}C NMR).



38 R = H
39 R = Ac

2.3. Axial stereochemistry of 1-arylimidazolidine-2-ones and 2-thiones derived from 2-alkyl-6-methylphenyl isocyanates and isothiocyanates

Selected NMR data for compounds **26–28** and **29–31**, derived from 2-ethyl-6-methylphenyl, 2-isopropyl-6-methyl, and 2-*tert*-butyl-6-methylphenyl isocyanates, and their thioanalogues **36** and **37** are collected in Table 3.

Because of *M* and *P* rotamers for **26**, **27** and **36** are obtained in a similar ratio, their relative abundance cannot be used to make any correlation with the *M* and *P* rotamers of their acetyl derivatives **29**, **30** and **37**.

However, chemical shifts for the methyl groups on the aromatic ring should behave as those of compounds **1–4**. The presence of an ethyl group instead of a chlorine atom has no effect on the sequence rules established by the Cahn–Ingold–Prelog system. This does mean that the NMR signals at higher field for this methyl group should reasonably be attributed to *P* atropisomers (Fig. 5).

Spectroscopic data for methylene and methine hydrogens in ethyl and isopropyl groups also reflect the soundness of the assigned stereochemical configurations. Thus, as expected, the chemical shifts for such methylene and methine groups follow the opposite order than that observed for the methyl group.

The coupling pattern observed for the methylene protons in ethyl groups also reinforces our stereochemical assignment. These protons are diastereotopics resonating at different chemical shifts, and we have observed that such a methylene group in *P* atropisomers always undergoes a larger diamagnetic anisotropy. Probably, the ethyl groups in *P* atropisomers have less conformational freedom than their *M* counterparts, as depicted in Figure 5. The ethyl group in *P* atropisomers is located in the concavity formed

Table 3. Selected NMR data for compounds **26–31**, **36** and **37**

Comp.	^{1}H NMR				^{13}C NMR				Axial stereochem
	$ArCH_3$		$ArCHRR'$		$ArCH_3$		$ArCCH_3$		
	δ_{CH_3}[a]	$\Delta\delta_{CH_3}$	δ_{CHR}	$\Delta\delta_{CHR}$	δ_{CH_3}[a]	$\Delta\delta_{CH_3}$	δ_{CH_3}[b]	$\Delta\delta_{CH_3}$	
26[c]	2.17	0.07	2.45	−0.12	18.6	0.7	15.0	0.3	*M*
	2.10		2.57		17.9		14.7		*P*
27[c]	2.17	0.07	2.83	−0.31	18.7	0.7	24.9/23.5	0.2	*M*
	2.10		3.14		18.0		24.7/23.7		*P*
28[c]	2.17	—	1.30		19.2	—	32.4	—	*M*
29[c]	2.32	0.13	2.49	−0.20	18.4	0.4	14.6	−0.1	*M*
	2.19		2.69		18.0		14.7		*P*
30[d]	2.32	0.14	2.77	−0.45	18.6	0.4	24.9/23.6	0.2	*M*
	2.18		3.22		18.2		24.7/23.6		*P*
31[d]	2.31	—	1.40	—	19.1		32.6	—	*M*
36[d]	2.23	0.14	2.41	−0.18	18.8	1.0	14.7	0.6	*M*
	2.09		2.59		17.8		14.1		*P*
37[d]	2.34	0.16	2.46	−0.22	18.6	0.7	14.4	0.2	*M*
	2.18		2.68		17.9		14.2		*P*

[a] δ_{ArCH_3}.
[b] δ_{CH_3} for Et, ^{i}Pr or ^{t}Bu groups.
[c] In DMSO-d_6.
[d] In $CDCl_3$.

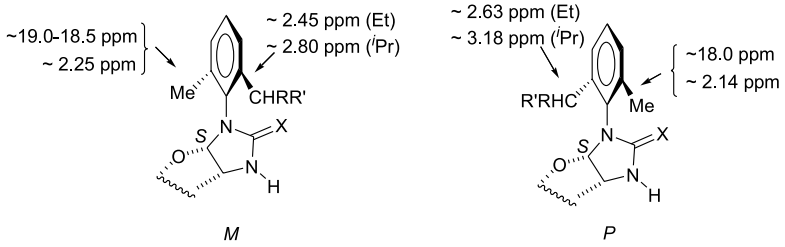


Figure 5. δ_{CH_3} and δ_{CH} average values (^{1}H and ^{13}C NMR).

by the glucofuranose and imidazolidine rings, while that ethyl in *M* atropisomers is situated on the convex area and has more mobility.

For compound **28**, only one rotamer could be isolated. Chemical shifts for the methyl group bound to the aromatic ring are consistent with *M* configurations, a fact also supported by the X-ray diffraction analysis of rotamer **28*M***. Probably, the *P* atropisomer is not formed at all or formed in tiny amounts due to the large steric effect caused by the *tert*-butyl group in the cavity formed by the two fused heterocyclic rings.

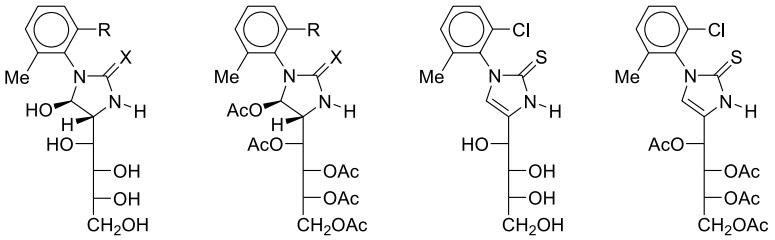
2.4. Axial stereochemistry in monocyclic *o*,*o*′-disubstituted 1-arylimidazolidine-2-thiones and imidazoline-2-thiones

Some selected NMR data for compounds **5**–**8**,[1] **22**, **25**, and **35** are collected in Tables 4 and 5.

It is worth pointing out that compound **5** is a precursor of **3** as outlined in Scheme 4. On considering the mechanism for this cyclisation reaction, there are two possibilities. It has been previously suggested[7,9] a S_Ni mechanism assisted by acid catalysis (**40**). Were this hypothesis correct, the cyclisation would have no effect on the axial stereochemistry of **5**, that is, the configuration would be the same as in compound **3**. In other words, the *M* atropisomers of **5** would be stereospecifically transformed into the *M* atropisomers of **3**. Similarly, **3*P*** would stem from **5*P***.

Therefore, like **3**, the major atropisomer of **5** would be (*P*)-configured and, the acetylation reaction will equally produce the major atropisomer of **6**, also with *P* chirality. In this way, the axial stereochemistry for **5** and **6** can easily be established.

By considering a S_N1 mechanism, one can arrive at the same conclusions (Scheme 4). In this case, the formation of the



5 R = Cl, X = S
22 R = Et, X = O
35 R = Et, X = S

6 R = Cl, X = S
25 R = Et, X = O

7

8

Table 4. Selected NMR data for compounds **5**–**8**

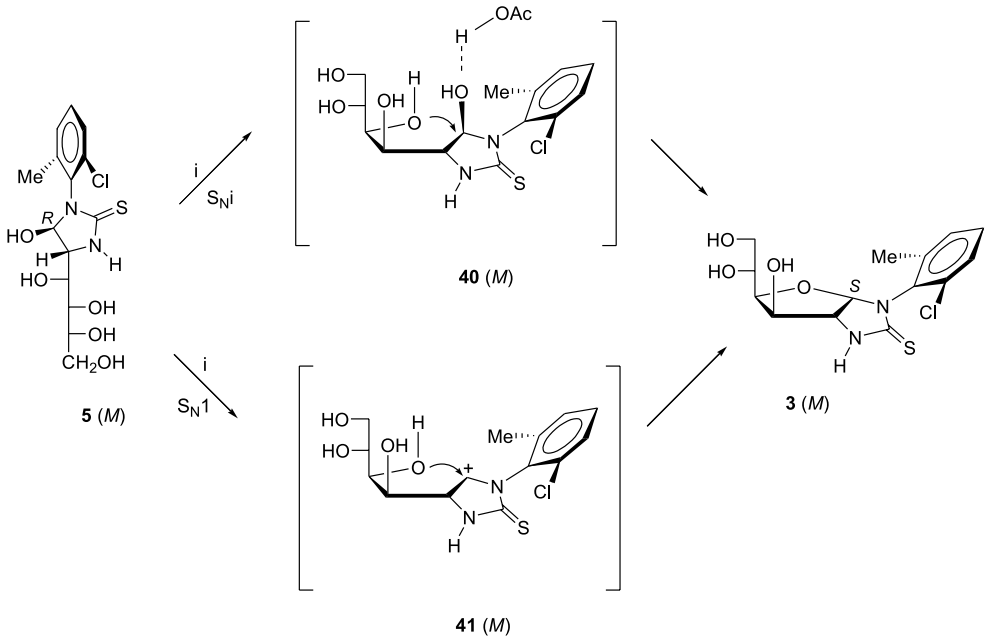
Comp.	^{1}H NMR			^{13}C NMR		Abund.[b]	Axial stereochem.
	δ_{CH_3}[a]	$\Delta\delta_{CH_3}$	$\delta_{H\text{-}5}$	δ_{CH_3}	$\Delta\delta_{CH_3}$		
5[c]	2.36	0.08	6.79	19.3	1.2	+	*P*
	2.18		6.61	18.1		−	*M*
6[d]	2.37	0.04	6.58	18.7		+	*P*
	2.33		6.41			−	*M*
7[c]	2.08		6.82	18.3		+	*P*
			6.62			−	*M*
8[d]	2.18		6.64	18.3		+	*P*

[a] δ_{ArCH_3}.
[b] Symbols +/− indicate major/minor atropisomers.
[c] In DMSO-d_6.
[d] In $CDCl_3$.

Table 5. Selected NMR data for compounds **22**, **25**, and **35**

Comp.	^{1}H NMR					^{13}C NMR				Axial stereochem.
	δ_{CH_3}[a]	$\Delta\delta_{CH_3}$	$\delta_{H\text{-}5}$	δ_{CH_2}	$\Delta\delta_{CH_2}$	δ_{CH_3}[a]	$\Delta\delta_{CH_3}$	δ_{Et}[b]	$\Delta\delta_{Et}$	
22[c]	2.25	0.12	6.29	2.48	−0.19	18.8	0.6	15.3	0.4	*P*
	2.13		6.28	2.69		18.2		14.9		*M*
25[d]	2.31	0.04	6.31	2.59	−0.09	18.4	0.3	14.6	0.2	*P*
	2.27		6.27	2.68		18.1		14.4		*M*
35[c]	2.30	0.18	5.21	2.50	−0.18	21.2	1.9	15.0	0.4	*P*
	2.12		5.17	2.68		19.3		14.6		*M*

[a] δ_{ArCH_3}.
[b] δ_{CH_3}.
[c] In DMSO-d_6.
[d] In $CDCl_3$.



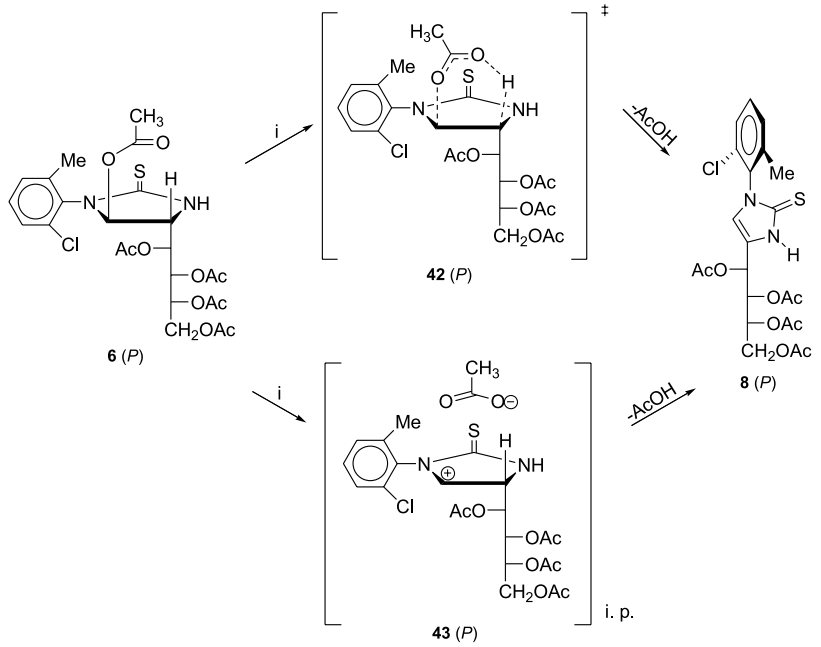
Scheme 4. (i) AcOH 30%, △.

intermediate carbocation **41** does not imply the loss of axial stereochemistry as the planar geometry of that carbocation must be analogous to that of C-5 in compound **7**, for which room temperature stable *M* and *P* atropisomers were found. Accordingly, were a carbocationic intermediate involved, the process would also be stereospecific, from the viewpoint of the axial chirality.

In a further extension, the stereochemistry for atropisomers of **7** and **8** can be assigned, because like **5** and **6**, the major atropisomer will have *P* chirality. It is interesting to point out that the latter conclusion can be reached regardless of the elimination mechanism converting **6** into **8**. If the process corresponds to a pericyclic mechanism (**42**), the axial stereochemistry of the atropisomers will not be affected. In contrast, if elimination proceeds via an E1 mechanism (**43**), the above discussion about the intermediate carbocation will be valid (Scheme 5).

Remarkably, comparisons of data from Table 4 with those of Table 2 evidence that the sequence of chemical shifts for the methyl group is reversed. Thus, the *P* atropisomers of **5** and **6** now exhibit more deshielded signals (Fig. 6).

Nevertheless, this change is totally justified considering that configuration at C-5 is inverted when going from **5** (5*R*) to **3**



Scheme 5. (i) $KHCO_3$, C_6H_6, △.

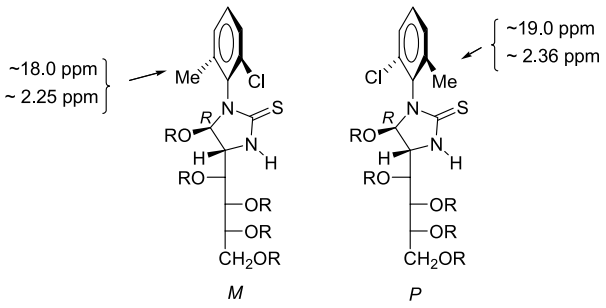


Figure 6. Average values of δ_{CH_3} (^{1}H and ^{13}C NMR).

(5*S*) and, consequently, the spatial interaction between the methyl group on the aromatic ring and the oxygenated substituent at C-5 is also exchanged.

The axial stereochemistry for atropisomers of **22**, **25** and **35** can equally be assigned by comparison of their spectroscopic data with those of **5** and **6** (Tables 4 and 5, Fig. 7).

3. Conclusions

A new family of nonbiaryl atropisomers, generated by reaction of protected aminosugars with *o*,*o*′-disubstituted aryl isocyanates and isothiocyanates, have been prepared and fully characterized, including X-ray diffraction analyses of some individual rotamers. The axial stereochemistry of these substances can be established by means of their ^{1}H and ^{13}C NMR data in solution. Furthermore, we have suggested a series of mechanistic insights that are consistent with the observed stereochemical outcome.

4. Experimental

4.1. General methods

General methods have been described in a previous paper.[1] High resolution mass spectra (chemical ionisation) were recorded on a Micromass Autospec spectrometer by Unidade de Masas da Universidade de Santiago de Compostela (Spain). Compounds **10**–**12** and **32** were purchased from Lancaster and used as received.

4.1.1. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[3-(2-ethyl-6-methylphenyl)ureido]-β-D-glucopyranose (13). To a solution of $NaHCO_3$ (3.13 g, 37.3 mmol) in water (150 mL) was added under stirring 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride[4] **9**, (11.9 g, 31.0 mmol) and dichloromethane (150 mL), keeping the stirring for 30 min. The organic layer was separated and the aqueous phase was extracted with dichloromethane (50 mL). The combined organic fractions were washed with water and dried over anhydrous magnesium sulfate and concentrated to approx. 50 mL. 2-Ethyl-6-methylphenyl isocyanate (5.0 g, 31.0 mmol) was then added and the reaction mixture was left at room temperature for 24 h. Compound **13** crystallized as a white solid, which was filtered and washed with cold diethyl ether, (84%), mp 230–232 °C (dec), $[\alpha]_D$ +13.5° (*c* 1.0, $CHCl_3$); ν_{max} 3350 (NH), 1730 (C=O), 1660 (C=O), 1520 (NH), 1210 (C–O–C), 1060, 1025 (C–O), 780 cm^{-1} (aromatic); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.09 (br s, 3H, Ar), 6.92 (br s, 1H, ArNH), 5.77 (d, $J_{1,2}$=8.7 Hz, 1H, H-1), 5.26 (t, $J_{2,3}$=$J_{3,4}$=9.5 Hz, 1H, H-3), 5.04 (t, $J_{3,4}$=$J_{4,5}$=9.5 Hz, 1H, H-4), 4.23 (dd, $J_{5,6}$=4.5 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6), 4.07 (d, $J_{6,6'}$=12.2 Hz, 1H, H-6′), 4.00 (m, 1H, H-2), 3.84 (m, 1H, H-5), 2.51 (m, 2H, CH_2CH_3), 2.18 (s, 3H, CH_3), 2.09 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.15 (t, *J*=7.5 Hz, 3H, CH_2CH_3); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 170.6 (CH_3–*C*O), 170.5 (CH_3–*C*O), 169.3 (CH_3–*C*O), 169.2 (CH_3–*C*O), 156.0 (NH–*C*O–NH), 142.4, 136.9, 132.2, 128.4 (2C), 126.6 (aromatics), 92.4 (C-1), 72.3 (2C, C-3, C-5), 68.3 (C-4), 61.7 (C-6), 53.6 (C-2), 24.5 (CH_2CH_3), 20.7 (CH_3–CO), 20.6 (CH_3–CO), 20.5 (CH_3–CO), 20.4 (CH_3–CO), 17.8 (CH_3), 14.4 (CH_2CH_3). Anal. Calcd for $C_{24}H_{32}N_2O_{10}$: C, 56.67; H, 6.34; N, 5.51. Found: C, 56.80; H, 6.30; N, 5.55.

4.1.2. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[3-(2-isopropyl-6-methylphenyl)ureido]-β-D-glucopyranose (14). Following the procedure described for **13** and using 2-isopropyl-6-methylphenylisocyanate, compound **14** crystallized as a white solid, which was filtered and washed with cold diethyl ether, (73%), mp 229–231 °C, $[\alpha]_D$ +7.3° (*c* 0.5, $CHCl_3$); ν_{max} 3381 (NH), 1749 (C=O), 1668 (C=O), 1543 (NH), 1219 (C–O–C), 1045 (C–O), 601 cm^{-1} (aromatic); ^{1}H NMR (400 MHz, $CDCl_3$, 318 K) δ 7.26–7.08 (m, 3H, Ar), 6.03 (br s, 1H, ArNH), 5.72 (d, $J_{1,2}$=8.6 Hz, 1H, H-1), 5.20 (t, $J_{2,3}$=$J_{3,4}$=9.4 Hz, 1H, H-3), 5.04 (t, $J_{3,4}$=$J_{4,5}$=9.4 Hz, 1H, H-4), 4.24 (dd, $J_{5,6}$=4.7 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6), 4.10 (dd, $J_{5,6'}$=2.3 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6′), 4.07 (m, 1H, H-2), 3.80 (m, 1H, H-5), 3.12 (m, *J*=6.9 Hz,1H, CH), 2.20 (s, 3H, CH_3), 2.10 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.18 (d, *J*=7.5 Hz, 3H, CH_3CH), 1.17 (d, *J*=6.9 Hz, 3H, CH_3CH); ^{13}C NMR

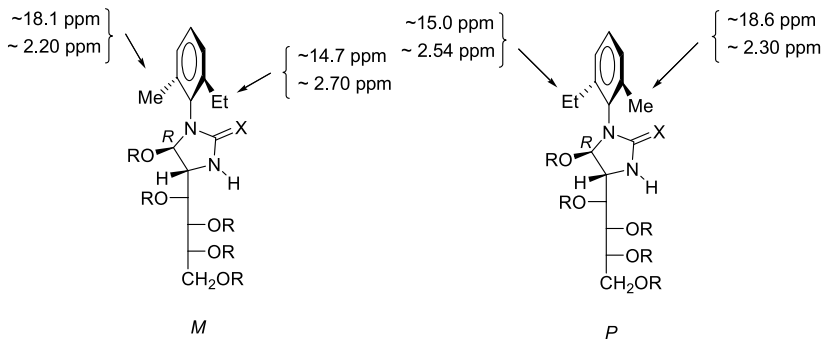


Figure 7. Average values of δ_{CH_3} and δ_{CH_2} (^{1}H and ^{13}C NMR).

(100 MHz, $CDCl_3$, 298 K) δ 170.7 (2C, CH_3–*C*O), 169.4 (CH_3–*C*O), 169.2 (CH_3–*C*O), 155.9 (NH–*C*O–NH), 147.2, 137.1, 131.7, 128.5 (2C), 124.2 (aromatics), 92.4 (C-1), 72.3 (2C, C-3, C-5), 68.3 (C-4), 61.6 (C-6), 53.4 (C-2), 28.3 (CH_3*CH*), 24.2 (*CH*$_3$CH), 22.7 (*CH*$_3$CH), 20.9 (*C*H_3–CO), 20.7 (2C, *C*H_3–CO), 20.5 (*C*H_3–CO), 17.9 (CH_3). Anal. Calcd for $C_{25}H_{34}N_2O_{10}$: C, 57.46; H, 6.56; N, 5.36. Found: C, 57.26; H, 6.33; N, 5.27.

4.1.3. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[3-(2-*tert*-butyl-6-methylphenyl)ureido]-β-D-glucopyranose (15). Following the procedure described for **13** and using 2-*tert*-butyl-6-methylphenylisocyanate, a mixture of rotamers of **15** crystallized as a white solid, which was filtered and washed with cold diethyl ether, (67%), mp 203–205 °C, $[\alpha]_D$ +11.2° (*c* 0.5, $CHCl_3$); ν_{max} 3331 (NH), 1757 (C=O), 1641 (C=O), 1562 (NH), 1229 (C–O–C), 1041 (C–O), 781 cm^{-1} (aromatic); ^1H NMR (400 MHz, $CDCl_3$) δ 7.33–7.09 (m, 7H, Ar and NH), 6.35 (s, 1H, NH), 6.09 (s, 1H, NH), 6.05 (s, 1H, NH), 5.71 (d, $J_{1,2}$=8.5 Hz, 1H, H-1), 5.59 (d, $J_{1,2}$=8.5 Hz, 1H, H-1), 5.26 (t, $J_{2,3}$=$J_{3,4}$=9.1 Hz, 1H, H-3), 5.17 (t, $J_{2,3}$=$J_{3,4}$=9.1 Hz, 1H, H-3), 5.08 (m, 2H, H-4), 4.29 (m, 2H, H-6), 4.08 (m, 4H, H-6′, H-2), 3.82 (m, 2H, H-5), 2.19 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 2.14 (s, 3H, OAc), 2.10 (s, 3H, OAc) 2.07 (s, 6H, OAc), 2.04 (s, 3H, OAc), 1.98 (s, 6H, OAc), 1.97 (s, 3H, OAc) 1.34 (s, 18H, 2 (CH_3)$_3$C); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (2C, CH_3–*C*O), 170.5 (2C, CH_3–*C*O), 169.6 (2C, CH_3–*C*O), 169.3 (2C, CH_3–*C*O), 156.1 (NH–*C*O–NH), 156.0 (NH–*C*O–NH), 149.0 (2C), 139.2, 138.8 (2C), 132.7, 129.6 (2C), 128.7 (2C), 125.6 (2C) (aromatics), 92.4 (C-1), 92.2 (C-1), 72.6 (C-3), 72.1 (3C, C-3, C-5), 68.8 (2C, C-4), 61.6 (2C, C-6), 53.0 (C-2), 52.9 (C-2), 35.4 (C), 35.0 (C), 30.9 (3C, (CH_3)$_3$C), 30.7 (3C, (CH_3)$_3$C), 21.0 (*C*H_3–CO), 20.9 (*C*H_3–CO), 20.8 (2C, *C*H_3–CO), 20.7 (2C, *C*H_3–CO), 20.6 (2C, *C*H_3–CO), 18.3 (CH_3), 17.9 (CH_3). Anal. Calcd for $C_{26}H_{36}N_2O_{10}$: C, 58.20; H, 6.76; N, 5.22. Found: C, 57.60; H, 6.28; N, 5.01.

4.1.4. 2-[3-(2-Ethyl-6-methylphenyl)ureido]-2-deoxy-α-D-glucopyranose (19) and (4*R*,5*R*)-1-(2-ethyl-6-methylphenyl)-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)-imidazolidine-2-one (22). To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-ethyl-6-methylphenyl)ureido]-β-D-glucopyranose, **13**, (10.0 g, 19.7 mmol) in methanol (310 mL), was added a saturated solution of ammonia in methanol (315 mL). The reaction was controlled by TLC (chloroform–methanol, 3:1) and left for 24 h at room temperature. The mixture was then evaporated to dryness and the crude crystallized from 96% ethanol. Several fractions were collected as mixtures of **19** and **22** in different proportions. The third fraction resulted to be compound **19** (0.25 g, 7%), mp 188–190 °C (dec), $[\alpha]_D$ +40.0° (*c* 0.5, *N*,*N*-dimethylformamide); ν_{max} 3500–3100 (OH, NH), 1600 (C=O, urea), 1560 (NH), 1065, 1015 (C–O), 760 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 7.63 (s, 1H, ArNH), 7.04 (m, 3H, Ar), 6.54 (d, $J_{1,OH}$=4.0 Hz, 1H, C1–OH), 4.98 (t, $J_{1,2}$=$J_{1,OH}$=3.6 Hz, 1H, H-1), 4.89 (d, $J_{3,OH}$=5.4 Hz, 1H, C3–OH), 4.74 (d, $J_{4,OH}$=5.3 Hz, 1H, C4–OH), 4.42 (t, $J_{6,OH}$=$J_{6',OH}$=5.8 Hz, 1H, C6–OH), 3.60 (m, 2H, H-5, H-6), 3.48 (m, 2H, H-2, H-6′), 3.35 (m, 1H, H-4), 3.14 (m, 1H, H-3), 2.53 (c, *J*=7.4 Hz, 2H, CH_2CH_3), 2.15 (s, 3H, CH_3), 1.09 (t, *J*=7.5 Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.8 (C=O), 141.6, 136.4, 135.6, 127.8, 126.1 (2C), (aromatics), 91.5 (C-1), 72.4 (C-5), 71.9 (C-4), 71.4 (C-3), 61.4 (C-6), 54.9 (C-2), 24.6 (CH_2CH_3), 18.6 (CH_3), 15.0 (CH_2CH_3). Anal. Calcd for $C_{16}H_{24}N_2O_6\cdot 3/2H_2O$: C, 52.31; H, 7.41; N, 7.62. Found: C, 52.28; H, 7.13; N, 7.71.

Recrystallization of the second fraction (0.7 g) from ethanol gave compound **22** as mixture of rotamers (~1:1), (0.23 g, 6.5%): mp 115–117 °C, $[\alpha]_D$ +26.0° (*c* 0.5, *N*,*N*-dimethylformamide), ν_{max} 3600–3000 (OH, NH), 1680 (C=O), 1050, 1020 (C–O); ^1H NMR (400 MHz, DMSO-d_6) δ 7.19–7.07 (m, 6H, Ar, *M* and *P*), 6.29 (d, $J_{5,OH}$=5.7 Hz, 1H, C5–OH, *P*), 6.28 (d, $J_{5,OH}$=6.5 Hz, 1H, C5–OH, *M*), 6.21 (d, *J*=6.9 Hz, 1H, ethanol), 5.06 (d, $J_{5,OH}$=6.4 Hz, 1H, H-5, *P*), 5.01 (dd, $J_{4,5}$=3.0 Hz, $J_{5,OH}$=6.8 Hz, 1H, H-5, *M*), 4.65–4.36 (m, 8H, C1′–OH, C2′–OH, C3′–OH, C4′–OH, *M* and *P*), 3.70–3.37 (m, 12H, H-4, H1′, H-2′, H-3′, H-4′ and H-4″, *M* and *P*), 2.75 (m, J_{gem}=22.3 Hz, *J*=7.6 Hz, 1H, CH_2CH_3, *M*), 2.60 (m, J_{gem}=22.2 Hz, *J*=7.8 Hz, 1H, CH_2CH_3, *M*), 2.48 (m, 2H, CH_2CH_3, *P*), 2.25 (s, 3H, CH_3, *P*), 2.13 (s, 3H, CH_3, *M*), 1.11 (t, *J*=7.5 Hz, 3H, CH_2CH_3, *P*), 1.07 (t, *J*=6.5 Hz, 3H, CH_2CH_3, *M*), 1.05 (t, *J*=7.0 Hz, 3H, ethanol); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.6 (C=O, *P*), 158.4 (C=O, *M*), 145.6, 143.3, 139.9, 137.3 (2C), 134.8, 134.6, 128.1, 127.9, 127.8, 126.4, 126.2 (aromatics, *M* and *P*), 84.6 (C-5, *P*), 84.1 (C-5, *M*), 71.5 (C-1′, *M*), 71.4 (C-1′, *P*), 71.3 (C-2′, *M* and *P*), 69.9 (C-3′, *P*), 69.8 (C-3′, *M*), 63.6 (C-4′, *M* and *P*), 62.3 (C-4, *M* and *P*), 56.3 (CH_2 ethanol), 24.2 (CH_2CH_3, *M*), 23.9 (CH_2CH_3, *P*), 19.1 (ethanol), 18.8 (CH_3, *P*), 18.2 (CH_3, *M*), 15.3 (CH_2CH_3, *P*), 14.9 ($CH_2'CH_3$, *M*). Anal. Calcd for $C_{16}H_{24}N_2O_6\cdot C_2H_5OH\cdot H_2O$: C, 53.46; H, 7.97; N, 6.93. Found: C, 53.24; H, 8.20; N, 7.43.

4.1.5. (4*R*,5*R*)-4-(1,2,3,4-Tetra-*O*-acetyl-D-*arabino*-tetritol-1-yl)-5-acetoxy-1-(2-ethyl-6-methylphenyl)imidazolidine-2-one (25). To a solution of (4*R*,5*R*)-1-(2-ethyl-6-methylphenyl)-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)imidazolidine-2-thione (**22**), (0.080 g, 0.24 mmol), in pyridine (1 mL) cooled at −20 °C for 15 min, was added acetic anhydride (1.2 mL) and the reaction mixture was kept at that temperature for 24 h. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water, affording **25** (0.08 g, 59%) as mixture of rotamers in ~2:3 ratio, mp: 123–125 °C, $[\alpha]_D$ +58.5° (*c* 0.5, $CHCl_3$), ν_{max} 3360 (NH), 1740 (C=O, ester), 1540 (NH), 1260, 1220 (C–O–C, ester), 1480, 780 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.08 (m, 6H, Ar, *M* and *P*), 6.31 (s, 1H, H-5, *P*), 6.27 (s, 2H, H-5, *M* and NH, *P*), 6.20 (s, 1H, NH, *M*), 5.53 (dd, $J_{1',2'}$=2.5 Hz, $J_{4,1'}$=7.4 Hz, 1H, H-1′, *M*), 5.51 (dd, $J_{1',2'}$=2.4 Hz, $J_{4,1'}$=8.6 Hz, 1H, H-1′, *P*), 5.36 (dd, $J_{1',2'}$=2.4 Hz, $J_{2',3'}$=8.2 Hz, 1H, H-2′, *P*), 5.34 (dd, $J_{1',2'}$=2.5 Hz, $J_{2',3'}$=8.5 Hz, 1H, H-2′, *M*), 5.03 (m, 2H, H-3′, *P* and *M*), 4.23 (dd, $J_{3',4'}$=2.4 Hz, $J_{4',4''}$=12.6 Hz, 2H, H-4′, *P* and *M*), 4.17 (dd, $J_{3',4''}$=4.2 Hz, $J_{4',4''}$=12.6 Hz, 2H, H-4″, *P* and *M*), 3.80 (bd, $J_{4,1'}$=7.5 Hz, 1H, H-4, *M*), 3.78 (bd, $J_{4,1'}$=9.5 Hz, 1H, H-4, *P*), 2.76 (m, J_{gem}=22.5 Hz, *J*=7.5 Hz, 1H, CH_2CH_3, *M*), 2.62 (m, J_{gem}=22.5 Hz, *J*=7.5 Hz, 1H, CH_2CH_3, *M*), 2.59 (m, 2H, CH_2CH_3, *P*), 2.31 (s, 3H, CH_3, *P*), 2.27 (s, 3H CH_3, *M*), 2.11 (s, 6H, OAc, *M*), 2.10 (s, 3H, OAc, *P*), 2.09 (s, 3H, OAc, *M*), 2.08 (s, 3H, OAc, *P*), 2.06 (s, 3H, OAc, *M*), 2.04

(s, 6H, OAc, *P* and *M*), 1.99 (s, 3H, OAc, *M*), 1.98 (s, 3H, OAc, *P*), 1.25 (t, $J=7.2$ Hz, 3H, CH_2CH_3, *P*), 1.23 (t, $J=7.5$ Hz, 3H, CH_2CH_3, *M*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (CH_3–*C*O), 170.0 (CH_3–*C*O), 169.9 (CH_3–*C*O), 169.7 (CH_3–*C*O, *P* and *M*), 158.6 (C=O, *P* and *M*), 144.4, 142.5, 138.7, 136.5, 131.7, 131.6, 128.9, 128.8 (2C), 128.5, 126.6 (2C) (aromatics, *P* and *M*), 84.4 (C-5, *M*), 83.8 (C-5, *P*), 69.3 (C-2′, *P*), 69.2 (C-2′, *M*), 68.5 (C-1′, *P* and *M*), 68.4 (C-3′, *P* and *M*), 61.2 (C-4′, *P* and *M*), 57.5 (C-4, *P*), 57.4 (C-4, *M*), 24.3 (CH_2CH_3, *P*), 23.6 (CH_2CH_3, *M*), 20.8 (CH_3–CO), 20.7 (CH_3–CO), 20.6 (CH_3–CO), 20.4 (CH_3–CO), *P* and *M*, 18.4 (CH_3, *M*), 18.1 (CH_3, *P*), 14.6 (CH_2CH_3, *M*), 14.4 (CH_2CH_3, *P*). Anal. Calcd for $C_{26}H_{34}N_2O_{11}$: C, 56.72; H, 6.23; N, 5.09. Found: C, 57.00; H, 6.20; N, 5.34.

4.1.6. 1-(2-Ethyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (26). *Procedure A*. To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-ethyl-6-methylphenyl)ureido]-β-D-glucopyranose, **13**, (2.0 g, 3.9 mmol) in methanol (70 mL), was added a saturated solution of ammonia in methanol (80 mL). The reaction was controlled by TLC (chloroform–methanol 3:1) and after 48 h it was evaporated to dryness. The resulting residue was treated with acetic acid (72 mL) and heated at ~100 °C (external bath), for 30 min. After evaporating to dryness, the solid was crystallized from 96% ethanol, affording **26** (0.11 g, 13%), as mixture of rotamers in ~1:1 ratio.

Procedure B. A mixture of **19** and **22** (1.2 g, 3.5 mmol) dissolved in acetic acid (44 mL) was heated at ~100 °C (external bath), for 30 minutes. After evaporating to dryness, the solid was crystallized from 96% ethanol, affording a mixture of rotamers of **26** (0.6 g, 66%) in ~1:1 ratio, mp 207–209 °C (dec), $[\alpha]_D$ +91.4° (*c* 0.5, *N*,*N*-dimethylformamide); ν_{max} 3500–3000 (OH, NH), 1690 (C=O), 1470 (NH), 1080, 1020 (C–O), 1590, and 780 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.23–7.08 (m, 8H, Ar and NH *M* and *P*), 5.60 (d, $J_{1,2}=6.3$ Hz, 2H, H-1 *M* and *P*), 5.21 (d, $J_{3,OH}=4.8$ Hz, 2H, C3–OH *M* and *P*), 4.74 (d, $J_{5,OH}=6.9$ Hz, 1H, C5–OH *M*), 4.72 (d, $J_{5,OH}=6.7$ Hz, 1H, C5–OH *P*), 4.46 (t, $J_{6,OH}=J_{6',OH}=5.6$ Hz, 1H, C6–OH *P*), 4.45 (t, $J_{6,OH}=J_{6',OH}=5.4$ Hz, 1H, C6–OH *M*), 4.10 (m, 4H, H-2, H-3 *M* and *P*), 3.86 (dd, $J_{3,4}=2.0$ Hz, $J_{4,5}=8.6$ Hz, 1H, H-4 *M*), 3.81 (dd, $J_{3,4}=2.1$ Hz, $J_{4,5}=8.7$ Hz, 1H, H-4 *P*), 3.71 (m, 2H, H-5 *M* and *P*), 3.55 (m, 2H, H-6 *M* and *P*), 3.33 (m, 2H, H-6′ *M* and *P*), 2.61 (m, $J_{gem}=22.1$ Hz, $J=7.5$ Hz, 1H, CH_2CH_3 *P*), 2.51 (m, $J_{gem}=22.1$ Hz, $J=7.5$ Hz, 1H, CH_2CH_3 *P*), 2.45 (c, $J=7.5$ Hz, 2H, CH_2CH_3 *M*), 2.17 (s, 3H, CH_3 *M*), 2.10 (s, 3H, CH_3 *P*), 1.11 (t, $J=7.5$ Hz, 3H, CH_2CH_3 *M*), 1.10 (t, $J=7.5$ Hz, 3H, CH_2CH_3 *P*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.9 (C=O *M*), 158.6 (C=O *P*), 144.7, 142.6, 139.2, 136.9, 135.3, 134.8, 128.2 (2C), 128.1 (2C), 126.5, 126.2 (aromatics *M* and *P*), 91.8 (C-1 *P*), 91.4 (C-1 *M*), 79.9 (C-4 *M*), 79.7 (C-4 *P*), 74.5 (C-3 *M*), 74.4 (C-3 *P*), 68.7 (C-5 *M* and *P*), 64.3 (C-6 *M*), 64.2 (C-6 *P*), 61.9 (C-2 *P*), 61.8 (C-2 *M*), 23.8 (CH_2CH_3 *M* and *P*), 18.6 (CH_3 *M*), 17.9 (CH_3 *P*), 15.0 (CH_2CH_3 *M*), 14.7 (CH_2CH_3 *P*). Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.62; H, 6.88; N, 8.69. Found: C, 59.52; H, 6.93; N, 8.55.

Procedure C. The rotamer of low R_f, **26*P***, was obtained pure by preparative TLC separation of the mixture of acetylated rotamers of **29**, due to deacetylation of **29*P*** in methanol/silica gel (see procedure for **29**), mp 198–200 °C (dec), $[\alpha]_D$ +83° (*c* 0.5, *N*,*N*-dimethylformamide); ν_{max} 3600–3200 (OH, NH), 1650 (C=O), 1475 (NH), 1080, 1030 (C–O), 1590, 790, and 730 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.23–7.09 (m, 4H, Ar and NH), 5.60 (d, $J_{1,2}=6.4$ Hz, 1H, H-1), 5.27 (d, $J_{3,OH}=3.9$ Hz, 1H, C3–OH), 4.80 (d, $J_{5,OH}=4.2$ Hz, 1H, C5–OH), 4.46 (t, $J_{6,OH}=J_{6',OH}=5.5$ Hz, 1H, C6–OH), 4.09 (m, 2H, H-2, H-3), 3.81 (dd, $J_{3,4}=2.2$ Hz, $J_{4,5}=8.7$ Hz, 1H, H-4), 3.72 (m, 1H, H-5), 3.54 (m, 1H, H-6), 3.32 (m, 1H, H-6′), 2.61 (m, $J_{gem}=26.1$ Hz, $J=7.5$ Hz, 1H, CH_2CH_3), 2.51 (m, 1H, CH_2CH_3), 2.10 (s, 3H, CH_3), 1.11 (t, $J=7.5$ Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.9 (C=O), 144.6, 136.8, 135.3, 128.2, 128.0, 126.1, (aromatics), 91.4 (C-1), 79.7 (C-4), 74.5 (C-3), 68.7 (C-5), 64.3 (C-6), 61.9 (C-2), 23.7 (CH_2CH_3), 17.8 (CH_3), 14.7 (CH_2CH_3). Anal. Calcd for $C_{16}H_{22}N_2O_5\cdot 3/2H_2O$: C, 55.00; H, 7.21; N, 8.02. Found: C, 55.14; H, 7.19; N, 8.04.

4.1.7. 1-(2-Isopropyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (27). To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-isopropyl-6-methylphenyl)ureido]-β-D-glucopyranose, **14**, (4.0 g, 7.7 mmol) in methanol (120 mL) was added a saturated solution of ammonia in methanol (120 mL). The reaction was controlled by TLC (chloroform–methanol 3:1) and after 48 h it was evaporated to dryness. The resulting residue was treated with aqueous acetic acid (137 mL) and heated at ~100 °C (external bath) for 30 min. After evaporating to dryness, the solid was purified by column chromatography affording **27** (1.27 g, 50%) as mixture of rotamers (~1:1), mp 108–110 °C, $[\alpha]_D$ +59.6° (*c* 0.5, *N*,*N*-dimethylformamide); ν_{max} 3500–3000 (OH, NH), 1695 (C=O), 1472 (NH), 1080, 1022 (C–O), 789 cm^{-1} (aromatics); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24–7.07 (m, 8H, Ar and NH *P* and *M*), 5.59 (d, $J_{1,2}=6.4$ Hz, 1H, H-1 *P*), 5.52 (d, $J_{1,2}=6.2$ Hz, 1H, H-1 *M*), 5.24 (d, $J_{3,OH}=3.1$ Hz, 2H, C3–OH *P* and *M*), 4.75 (br s, 2H, C5–OH *P* and *M*), 4.47 (t, $J_{6,OH}=J_{6',OH}=5.3$ Hz, 2H, C6–OH *P* and *M*), 4.13 (m, 2H, H-2 *P* and *M*), 4.09 (m, 2H, H-3 *P* and *M*), 3.86 (dd, $J_{3,4}=2.3$ Hz, $J_{4,5}=8.6$ Hz, 1H, H-4 *M*), 3.78 (dd, $J_{3,4}=2.1$ Hz, $J_{4,5}=8.6$ Hz, 1H, H-4 *P*), 3.71 (m, 2H, H-5 *P* and *M*), 3.55 (m, 2H, H-6 *P* and *M*), 3.33 (m, 2H, H-6′ *P* and *M*), 3.14 (sept, $J=6.9$ Hz, 1H, $CH(CH_3)_2$ *P*), 2.83 (sept, $J=6.8$ Hz, 1H, $CH(CH_3)_2$ *M*), 2.17 (s, 3H, CH_3 *M*), 2.10 (s, 3H, CH_3 *P*), 1.21 (d, $J=6.9$ Hz, 3H, $CH(CH_3)_2$ *M*), 1.15 (d, $J=6.8$ Hz, 3H, $CH(CH_3)_2$ *M*), 1.07 (d, $J=6.9$ Hz, 3H, $CH(CH_3)_2$ *P*), 1.04 (d, $J=6.7$ Hz, 3H, $CH(CH_3)_2$ *P*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.2 (C=O *M*), 158.7 (C=O *P*), 149.5, 139.1, 133.9, 128.4, 128.0, 123.9 (aromatics, *M*), 147.5, 136.7, 134.4, 128.3, 127.9, 124.0, (aromatics *P*), 92.6 (C-1 *M*), 91.5 (C-1 *P*), 80.0 (C-4 *P*), 79.9 (C-4 *M*), 74.6 (C-3 *P*), 74.5 (C-3 *M*), 68.9 (C-5 *M*), 68.8 (C-5 *P*), 64.5 (C-6 *P*), 64.2 (C-6 *M*), 61.9 (C-2 *P*), 61.8 (C-2 *M*), 27.9 ($CH(CH_3)_2$ *M*), 27.8 ($CH(CH_3)_2$ *P*), 24.9 ($CH(CH_3)_2$ *M*), 24.7 ($CH(CH_3)_2$ *P*), 23.7 ($CH(CH_3)_2$ *P*), 23.5 ($CH(CH_3)_2$ *M*), 18.7 (CH_3 *M*), 18.0 (CH_3 *P*). Anal. Calcd for $C_{17}H_{24}N_2O_5\cdot 3/2H_2O$: C, 56.14; H, 7.43; N, 7.71. Found: C, 56.04; H, 7.10; N, 7.70.

4.1.8. 2-[3-(2-*tert*-Butyl-6-methylphenyl)ureido]-2-deoxy-α-D-glucopyranose (21) and (*M*)-1-(2-*tert*-butyl-6-

methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (28*M*). To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-*tert*-butyl-6-methylphenyl)ureido]-β-D-glucopyranose, **15**, (4.0 g, 7.5 mmol) in methanol (118 mL) was added a saturated solution of ammonia in methanol (118 mL). The reaction was controlled by TLC (chloroform–methanol, 3:1) and left for 24 h at room temperature. The mixture was then evaporated to dryness and the resulting residue was treated with aqueous acetic acid (120 mL) and heated at ~100 °C (external bath), for 30 min. After evaporating to dryness, the solid was crystallized from 96% ethanol and water, affording **21** (0.43 g, 16%) as mixture of rotamers (~1:1), mp 195–197 °C (dec), $[\alpha]_D$ +34.6° (*c* 0.5, *N*,*N*-dimethylformamide); ν_{max} 3500–3100 (OH, NH), 1618 (C=O, urea), 1570 (NH), 1072, 1026 (C–O), 775 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 7.48 and 7.46 (s, 1H, NH *M* and *P*), 7.17–7.05 (m, 6H, Ar *M* and *P*), 6.56 (br s, 2H, C1–OH), 6.12 (br s, 2H, NH *M* and *P*), 5.01 and 4.93 (br s, 1H, H-1 *M* and *P*), 4.88 (d, $J_{3,OH}$=3.6 Hz, 1H, C3–OH *M* and *P*), 4.72 (d, $J_{4,OH}$=4.5 Hz, 1H, C4–OH), 4.67 (d, $J_{4,OH}$=4.1 Hz, 1H, C4–OH), 4.42 (t, $J_{6,OH}=J_{6',OH}$=5.8 Hz, 2H, C6–OH *M* and *P*), 3.64 (m, 2H, H-5, H-6), 3.48 (m, 3H, H-2, H-4, H-6′), 3.12 (m, 1H, H-3), 2.13 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 1.31 (s, 18H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.9 (C=O), 157.1 (C=O) 147.8 (2C), 139.4, 139.2, 136.2, 136.1, 128.3, (2C), 126.6 (2C), 124.01 (2C), (aromatics), 91.8 (C-1), 91.7 (C-1), 72.4 (2C, C-5), 71.9 (2C, C-4), 71.2 (2C, C-3), 61.4 (2C, C-6), 55.0 (C-2), 54.9 (C-2), 35.1 (2C, C), 31.1 (6C, $(CH_3)_3C$), 18.7 (2C, CH_3). Anal. Calcd for $C_{18}H_{28}N_2O_6$: C, 58.68; H, 7.66; N, 7.60. Found: C, 58.31; H, 7.64; N, 7.49.

When the mother liquors were treated with water, a small amount of compound **28*M*** spontaneously crystallized (0.105 g, 4%), mp 240–242 °C, $[\alpha]_D$ +70.6° (*c* 0.5, *N*,*N*-dimethylformamide); ν_{max} 3388, 2965 (OH, NH), 1467 (NH), 1670 (C=O), 1043 (C–O), 887, 785 cm^{-1} (aromatics); ^1H NMR (400 MHz, DMSO-d_6) δ 7.38–7.10 (m, 3H, Ar), 7.26 (s, 1H, NH), 5.60 (d, $J_{1,2}$=5.8 Hz, 1H, H-1), 5.22 (d, $J_{3,OH}$=5.0 Hz, 1H, C3–OH), 4.72 (d, $J_{5,OH}$=5.8 Hz, 1H, C5–OH), 4.44 (t, $J_{6,OH}=J_{6',OH}$=5.4 Hz, 1H, C6–OH), 4.10 (d, $J_{3,4}$=2.7 Hz, 1H, H-3), 4.04 (d, $J_{1,2}$=5.8 Hz, 1H, H-2), 3.89 (d, $J_{4,5}$=8.6 Hz, 1H, H-4), 3.68 (m, 1H, H-5), 3.54 (m, 1H, H-6), 3.32 (m, 1H, H-6′), 2.17 (s, 3H, CH_3), 1.30 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.8 (C=O), 148.7, 140.8, 134.0, 128.6, 127.9, 126.7, (aromatics), 93.6 (C-1), 80.5 (C-3), 74.0 (C-4), 68.8 (C-5), 64.1 (C-6), 61.6 (C-2), 36.0 (C), 32.4 (3C, $(CH_3)_3C$), 19.2 (CH_3). Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.47; H, 7.45; N, 7.99.

4.1.9. 1-(2-Ethyl-6-methylphenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (29). To a solution of 1-(2-ethyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one, **26**, (0.4 g, 1.2 mmol) in pyridine (3.0 mL), cooled at −20 °C, was added acetic anhydride (3.1 mL) and the reaction mixture was kept at that temperature overnight. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water and identified as mixture of rotamers of **29** (0.4 g, 73%), in ~1:1 ratio. Both rotamers were isolated by preparative TLC (diethyl ether). Silica-gel was extracted with ethyl acetate and the residue was crystallized from 96% ethanol.

Compound **29*P***. R_f=0.5, mp 174–176 °C, $[\alpha]_D$ +106.0° (*c* 0.1, $CHCl_3$), ν_{max} 3500–3100 (H_2O, NH)[12], 1750, 1720 (C=O), 1690 (NC=O) 1240 (C–O–C), 1040 (C–O), 1590, 1570, 790 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.11 (m, 3H, Ar), 5.77 (d, $J_{1,2}$=6.3 Hz, 1H, H-1), 5.49 (s, 1H, NH), 5.33 (d, $J_{3,4}$=2.8 Hz, 1H, H-3), 5.23 (m, 1H, H-5), 4.64 (dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6), 4.55 (dd, $J_{3,4}$=2.8 Hz, $J_{4,5}$=9.2 Hz, 1H, H-4), 4.26 (dd, $J_{2,NH}$=2.3 Hz, $J_{1,2}$=6.4 Hz, 1H, H-2), 3.99 (dd, $J_{5,6'}$=4.8 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6′), 2.74 (m, J_{gem}=22.3 Hz, J=7.5 Hz, 1H, CH_2CH_3), 2.61 (m, J_{gem}=22.3 Hz, J=7.5 Hz, 1H, CH_2CH_3), 2.18 (s, 3H, CH_3), 2.10 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.25 (t, J=7.5 Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 169.9 (CH_3–*C*O), 169.7 (CH_3–*C*O), 159.0 (C=O), 144.5, 136.1, 133.4, 128.9, 128.6, 126.7 (aromatics), 92.4 (C-1), 75.9 (C-3), 75.7 (C-4), 67.7 (C-5), 62.8 (C-6), 60.4 (C-2), 24.1 (CH_2CH_3), 20.7 (3C, *C*H_3–CO), 18.0 (CH_3), 14.7 (CH_2CH_3). Anal. Calcd for $C_{22}H_{28}N_2O_8$=H_2O: C, 56.64; H, 6.48; N, 6.01. Found: C, 56.45; H, 5.86; N, 6.28.

Compound **29*M***. R_f=0.6, mp 163–165 °C, $[\alpha]_D$+101.5° (*c* 0.4, $CHCl_3$); ν_{max} 3500–3000 (H_2O, NH)[12], 1750, 1740 (C=O), 1680 (NC=O), 1230 (C–O–C), 1040 (C–O), 1590, 790 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.13 (m, 3H, Ar), 5.81 (d, $J_{1,2}$=6.3 Hz, 1H, H-1), 5.49 (d, $J_{2,NH}$=1.8 Hz, 1H, NH), 5.35 (d, $J_{3,4}$=2.8 Hz, 1H, H-3), 5.22 (m, 1H, H-5), 4.61 (dd, $J_{3,4}$=2.8 Hz, $J_{4,5}$=9.6 Hz, 1H, H-4), 4.58 (dd, $J_{5,6}$=2.2 Hz, $J_{6,6'}$=12.1 Hz, 1H, H-6), 4.32 (dd, $J_{2,NH}$=2.3 Hz, $J_{1,2}$=6.3 Hz, 1H, H-2), 4.04 (dd, $J_{5,6'}$=4.4 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6′), 2.49 (m, 2H, CH_2CH_3), 2.32 (s, 3H, CH_3), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.22 (t, J=7.6 Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 169.9 (CH_3–*C*O), 169.7 (CH_3–*C*O), 158.7 (C=O), 142.0, 138.7, 133.0, 128.9, 128.7, 126.7 (aromatics), 92.8 (C-1), 75.8 (C-3), 75.6 (C-4), 67.6 (C-5), 62.8 (C-6), 60.3 (C-2), 24.1 (CH_2CH_3), 20.8 (2C, *C*H_3–CO), 20.7 (*C*H_3–CO), 18.4 (CH_3), 14.6 (CH_2CH_3). Anal. Calcd for $C_{22}H_{28}N_2O_8 \cdot 1/2H_2O$: C, 57.76; H, 6.39; N, 6.12. Found: C, 57.87; H, 6.65; N, 6.56.

The rotamer **26*P***, was obtained pure by preparative TLC separation of the mixture of acetylated rotamers of **29**, due to deacetylation of **29*P*** in methanol silica gel (see above procedure for **26**).

4.1.10. 1-(2-Isopropyl-6-methylphenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (30). To a solution of 1-(2-isopropyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one, **27**, (0.3 g, 0.89 mmol) in pyridine (2.3 mL), cooled at −20 °C, was added acetic anhydride (2.3 mL) and the reaction mixture was kept at that temperature overnight. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water and identified as mixture of rotamers (0.33 g, 79%), in a 60:40 ratio (*P*:*M*). Both rotamers were isolated by preparative TLC (diethyl ether). Silica-gel was extracted with ethyl acetate and the residue was crystallized from 96% ethanol.

Major rotamer, **30*P***. R_f=0.2 (diethyl ether), mp 221–223 °C (dec), $[\alpha]_D$ +85.0° (*c* 0.3, $CHCl_3$), ν_{max} 3242, 2973 (NH), 1753 (C=O), 1242 (C–O–C), 1040 (C–O), 1445, 796 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.10 (m, 3H, Ar), 5.75 (d, $J_{1,2}$=6.4 Hz, 1H, H-1), 5.34 (d, $J_{3,4}$=2.8 Hz, 1H, H-3), 5.26 (m, 1H, H-5), 5.18 (d, $J_{2,NH}$=1.8 Hz, 1H, NH), 4.71 (dd, $J_{5,6}$=2.4 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6), 4.55 (dd, $J_{3,4}$=2.9 Hz, $J_{4,5}$=9.0 Hz, 1H, H-4), 4.32 (dd, $J_{2,NH}$=2.3 Hz, $J_{1,2}$=6.4 Hz, 1H, H-2), 3.97 (dd, $J_{5,6'}$=5.6 Hz, $J_{6,6'}$=12.3 Hz, 1H, H-6′), 3.22 (sept, J=6.9 Hz, 1H, $CH(CH_3)_2$), 2.18 (s, 3H, CH_3), 2.09 (s, 6H, OAc), 2.03 (s, 3H, OAc), 1.23 (d, J=6.9 Hz, 3H, $CH(CH_3)_2$), 1.22 (d, J=6.9 Hz, 3H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 170.0 (CH_3–*C*O), 169.7 (CH_3–*C*O), 159.1 (C=O), 149.3, 135.9, 132.6, 129.2, 128.5, 124.4 (aromatics), 92.5 (C-1), 76.2 (C-3), 75.7 (C-4), 67.8 (C-5), 63.0 (C-6), 60.3 (C-2), 28.4 ($CH(CH_3)_2$), 24.7 ($CH(CH_3)_2$), 23.6 $CH(CH_3)_2$), 20.7 (3C, CH_3–CO), 18.2 (CH_3). Anal. Calcd for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.69; H, 6.41; N, 5.97.

Minor rotamer, **30*M***. R_f=0.3 (diethyl ether), mp 168–170 °C, $[\alpha]_D$ +68.8° (*c* 0.3, $CHCl_3$); ν_{max} 2953 (NH), 1745 (C=O), 1232 (C–O–C), 1030 (C–O), 1458, 793 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.11 (m, 3H, Ar), 5.75 (d, $J_{1,2}$=6.3 Hz, 1H, H-1), 5.34 (d, $J_{3,4}$=2.8 Hz, 1H, H-3), 5.23 (m, 1H, H-5), 5.17 (d, $J_{2,NH}$=1.8 Hz, 1H, NH), 4.61 (dd, $J_{3,4}$=2.9 Hz, $J_{4,5}$=9.2 Hz, 1H, H-4), 4.60 (dd, $J_{5,6}$=2.4 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6), 4.34 (dd, $J_{2,NH}$=2.3 Hz, $J_{1,2}$=6.3 Hz, 1H, H-2), 4.04 (dd, $J_{5,6'}$=4.3 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6′), 2.77 (sept, J=6.9 Hz, 1H, $CH(CH_3)_2$), 2.32 (s, 3H, CH_3), 2.10 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.28 (d, J=6.9 Hz, 3H, $CH(CH_3)_2$), 1.16 (d, J=6.8 Hz, 3H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 169.9 (CH_3–*C*O), 169.6 (CH_3–*C*O), 158.6 (C=O), 146.9, 138.5, 132.1, 129.2, 128.5, 124.3 (aromatics), 93.5 (C-1), 75.8 (C-3), 75.7 (C-4), 67.6 (C-5), 62.8 (C-6), 60.2 (C-2), 28.6 ($CH(CH_3)_2$), 24.9 ($CH(CH_3)_2$), 23.6 $CH(CH_3)_2$), 20.7 (3C, CH_3–CO), 18.6 (CH_3). Anal. Calcd for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.33; H, 6.22; N, 5.95.

4.1.11. (*M*)-1-(2-*tert*-Butyl-6-methylphenyl)-(3,5,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one (31*M*). To a solution of (*M*)-1-(2-*tert*-butyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-one, **28*M***, (0.05 g, 0.14 mmol) in pyridine (0.36 mL), cooled at −20 °C, was added acetic anhydride (0.36 mL) and the reaction mixture was kept at that temperature overnight. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water and identified as rotamer **31*M*** (0.03 g, 45%), mp 196–199 °C (dec), $[\alpha]_D$ +76.0° (*c* 0.25, $CHCl_3$), ν_{max} 3366 (NH), 1750, (C=O), 1442, 1373, 1229 (C–O–C), 1035 (C–O), 790, 718 cm^{-1} (aromatics); ^1H NMR (400 MHz, $CDCl_3$) δ 7.41–7.13 (m, 3H, Ar), 5.86 (d, $J_{1,2}$=5.9 Hz, 1H, H-1), 5.49 (d, $J_{2,NH}$=1.5 Hz, 1H, NH), 5.37 (d, $J_{3,4}$=2.7 Hz, 1H, H-3), 5.18 (m, 1H, H-5), 4.63 (dd, $J_{3,4}$=2.8 Hz, $J_{4,5}$=9.3 Hz, 1H, H-4), 4.58 (dd, $J_{5,6}$=2.2 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6), 4.28 (dd, $J_{2,NH}$=2.1 Hz, $J_{1,2}$=5.9 Hz, 1H, H-2), 4.03 (dd, $J_{5,6'}$=4.5 Hz, $J_{6,6'}$=12.4 Hz, 1H, H-6′), 2.31 (s, 3H, CH_3), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.40 (s, 9H, $(CH_3)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.7 (CH_3–*C*O), 159.0 (C=O), 148.4, 140.1, 132.1, 129.3, 128.7, 127.2 (aromatics), 94.5 (C-1), 76.4 (C-3), 75.2 (C-4), 67.7 (C-5), 62.8 (C-6), 60.0 (C-2), 36.3 (C), 32.6 (3C, $(CH_3)_3C$), 20.7 (3C, CH_3–CO), 19.1 (CH_3). Anal. Calcd for $C_{24}H_{32}N_2O_8$: C, 60.49; H, 6.77; N, 5.88. Found: C, 60.00; H, 6.60; N, 5.60.

4.1.12. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[3-(2-ethyl-6-methylphenyl)thioureido]-β-D-glucopyranose (33). Following the procedure described for **13** and using 2-ethyl-6-methylphenyl isothiocyanate, compound **33** (13%) was obtained as a mixture of atropisomers (~1:1), mp 173–175° C, $[\alpha]_D$ −10.8° (*c* 0.5, $CHCl_3$); ν_{max} 3300, 3190, 1530 (NH), 1740 (C=O), 1210 (C–O–C), 1060, 1030 (C–O), 780 cm^{-1} (aromatic); ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 2H, NH–Ar), 7.32–7.14 (m, 6H, Ar), 5.55 (d, $J_{1,2}$=8.6 Hz, 1H, H-1), 5.52 (d, $J_{1,2}$=8.9 Hz, 1H, H-1), 5.28 (d, $J_{2,NH}$=9.5 Hz, 2H, NH), 5.18 (c, $J_{1,2}$=$J_{2,NH}$=$J_{2,3}$=9.3 Hz, 2H, H-2), 5.13 (t, $J_{4,5}$=$J_{3,4}$=9.3 Hz, 2H, H-4), 4.99 (t, $J_{2,3}$=$J_{3,4}$=9.0 Hz, 1H, H-3), 4.97 (t, $J_{2,3}$=$J_{3,4}$=9.0 Hz, 1H, H-3), 4.23 (dd, $J_{5,6}$=4.3 Hz, $J_{6,6'}$=12.5 Hz, 2H, H-6), 4.08 (bd, $J_{6,6'}$=12.2 Hz, 2H, H-6′), 3.70 (ddd, $J_{5,6'}$=2.2 Hz, $J_{4,5}$=9.6 Hz, $J_{5,6}$=4.1 Hz, 2H, H-5), 2.52 (m, J_{gem}=22.0 Hz, J=7.2 Hz, 2H, CH_2CH_3), 2.41 (m, J_{gem}=23.0 Hz, J=7.3 Hz, 2H, CH_2CH_3), 2.17 (s, 3H, CH_3), 2.15 (s, 6H, OAc), 2.14 (s, 3H, CH_3), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.18 (t, J=7.7 Hz, 3H, CH_2CH_3), 1.16 (t, J=7.7 Hz, 3H, CH_2CH_3); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 182.3 (C=S), 170.7 (2C, CH_3–*C*O), 169.2 (2C, CH_3–*C*O), 136.9, 131.3 (2C), 129.7, 129.1, 127.2 (aromatics), 92.3 (C-1), 72.4 (C-3), 72.2 C-5), 67.9 (C-4), 61.5 (C-6), 57.2 (C-2), 24.2 (CH_2CH_3), 20.9 (CH_3–CO), 20.7 (2C, CH_3–CO), 20.5 (CH_3–CO), 17.7 (CH_3), 14.4 (CH_2CH_3). Anal. Calcd for $C_{24}H_{32}N_2O_9S$: C, 54.95; H, 6.15; N, 5.34; S, 6.11. Found: C, 54.50; H, 6.08; N, 5.37; S, 6.00.

4.1.13. (4*R*,5*R*)-1-(2-Ethyl-6-methylphenyl)-5-hydroxy-4-(D-*arabino*-tetritol-1-yl)imidazolidine-2-thione (35). To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-ethyl-6-methylphenyl)thioureido]-β-D-glucopyranose, **33**, (0.12 g, 0.25 mmol) in methanol (4.0 mL), under vigorous stirring, was added a saturated solution of ammonia in methanol (4.0 mL). The process was followed by TLC (chloroform–methanol, 3:1) and after 12 h at room temperature, the reaction crude was evaporated to dryness yielding an oily compound (0.089 g, 99%), as mixture of atropisomers (~1:1); ν_{max} 3500–3000 (OH, NH), 1650 (C=O), 1450 (NH), 1100, 1050, 1020 (C–O) and 780 cm^{-1} (aromatic); ^1H NMR (400 MHz, DMSO-d_6) δ 8.05 (s, 1H, NH, *P*), 8.03 (s, 1H, NH, *M*), 7.22–7.07 (m, 6H, Ar, *P* and *M*), 6.72 (d, $J_{5,OH}$=6.9 Hz, 1H, C5–OH, *P*), 6.69 (d, $J_{5,OH}$=7.4 Hz, 1H, C5–OH, *M*), 5.21 (d, $J_{5,OH}$=6.5 Hz, 1H, H-5, *P*), 5.17 (d, $J_{5,OH}$=6.8 Hz, 1H, H-5, *M*) 4.81 (br s, 2H, C1′–OH, *M* and *P*), 4.60 (m, 4H, C2′–OH, C3′–OH, *M* and *P*), 4.43 (br s, 2H, C4′–OH, *M* and *P*), 3.78–3.35 (m, 12H, H-4, H-1′, H-2′, H-3′, H-4′, H-4″, *P* and *M*), 2.68 (m, J_{gem}=21.8 Hz, J=6.9 Hz, 1H, CH_2CH_3, *M*), 2.50 (m, 2H, CH_2CH_3, *P*), 2.45 (m, J_{gem}=22.8 Hz, J=7.5 Hz, 1H, CH_2CH_3, *M*), 2.30 (s, 3H, CH_3, *P*), 2.12 (s, 3H, CH_3, *M*), 1.17 (t, J=7.6 Hz, 3H, CH_2CH_3, *P*), 1.14 (t, J=7.6 Hz, 3H, CH_2CH_3, *M*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1

(C=S, *P*), 180.9 (C=S, *M*), 145.0, 142.5, 140.0, 136.7, 135.7, 135.5, 128.1 (2C), 128.0 (2C), 126.3, 125.9 (aromatics *M* and *P*), 88.5 (C-5, *P*), 87.7 (C-5, *M*), 71.4 (C-1′, 2C, *M* and *P*), 71.2 (C-2′, *M*), 71.1 (C-2′, *P*), 69.9 (C-3′, *P*), 69.8 (C-3′, *M*), 66.0 (C-4, *M*), 65.9 (C-4, *P*), 63.5 (C-4′, *M* and *P*), 23.9 (CH_2CH_3, *M*), 23.5 (CH_2CH_3, *P*), 21.2 (CH_3, *P*), 19.3 (CH_3, *M*), 15.0 (CH_2CH_3, *P*), 14.6 (CH_2CH_3, *M*). HRMS: Calcd for $M^+ + 1$ ($C_{16}H_{25}N_2O_5S$): 357.1484. Found: 357.1483.

4.1.14. 1-(2-Ethyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thione (36). *Procedure A*. To a solution of 2-amino-2-deoxy-D-glucopyranose hydrochloride (2.44 g, 11.3 mmol) in water (14 mL) was added $NaHCO_3$ (1.04 g, 12.4 mmol), 2-ethyl-6-methylphenyl isothiocyanate (2.0 g, 11.3 mmol) and ethanol (72 mL). The mixture was stirred vigorously and heated to 80 °C for 2.5 h. The reaction was followed by TLC (chloroform–methanol 3:1). The solution was evaporated to dryness and the dark residue obtained was dissolved in 30% aqueous acetic acid (100 mL) and heated to 100 °C (external bath) for 30 min. Then the reaction mixture was evaporated to dryness and treated with 96% ethanol, affording a solid (0.64 g, 25%), identified as a mixture of rotamers of **36** in a ratio ~1:1, which was purified by flash chromatography, (chloroform–methanol, 4:1), mp 243–245 °C (dec), $[\alpha]_D$ +120° (*c* 0.5, *N*,*N*-dimethylformamide), ν_{max} 3460, 3420, 3320 (OH, NH), 1480 (NH), 1030 (C–O), 770 cm^{-1} (aromatic); ^1H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H, NH, *P*), 9.05 (s, 1H, NH, *M*), 7.24–7.11 (m, 6H, Ar, *P* and *M*), 5.78 (d, $J_{1,2}=6.9$ Hz, 1H, H-1, *P*), 5.76 (d, $J_{1,2}=8.1$ Hz, 1H, H-1, *M*), 5.38 (d, $J_{3,OH}=4.7$ Hz, 2H, C3–OH, *P* and *M*), 4.77 (d, $J_{5,OH}=5.2$ Hz, 1H, C5–OH, *P*), 4.76 (d, $J_{5,OH}=5.9$ Hz, 1H, C5–OH, *M*), 4.48 (t, $J_{6,OH}=J_{6',OH}=5.5$ Hz, 2H, C6–OH, *P* and *M*), 4.30 (d, $J_{1,2}=7.2$ Hz, 1H, H-2, *M*), 4.28 (d, $J_{1,2}=7.2$ Hz, 1H, H-2, *P*), 4.17 (m, 2H, H-3, *P* and *M*), 3.77 (m, 4H, H-4, H-5, *P* and *M*), 3.55 (m, 2H, H-6, *P* and *M*), 3.34 (m, 2H, H-6′, *P* and *M*), 2.59 (m, 2H, CH_2CH_3, *P*), 2.41 (m, 2H, CH_2CH_3, *M*), 2.23 (s, 3H, CH_3, *M*), 2.09 (s, 3H, CH_3, *P*), 1.17 (t, $J=7.2$ Hz, 3H, CH_2CH_3, *P*), 1.16 (t, $J=7.1$ Hz, 3H, CH_2CH_3,*M*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 185.2 (C=S, *M*), 182.4 (C=S, *P*), 144.0, 141.9, 136.3, 136.2, 135.9, 128.3 (2C), 128.1 (2C), 126.3, 125.7 (2C) (aromatics), 95.5 (C-1, *M*), 94.9 (C-1, *P*), 80.3 (C-4, *P* and *M*), 73.9 (C-3, *P* and *M*), 68.6 (C-5, *P* and *M*), 66.0 (C-2, *P*), 65.9 (C-2, *M*), 64.1 (C-6, *P*), 64.0 (C-6, *M*), 23.4 (CH_2CH_3, *P* and *M*), 18.8 (CH_3, *M*), 17.8 (CH_3, *P*), 14.7 (CH_2CH_3, *M*), 14.1 (CH_2CH_3, *P*). Anal. Calcd for $C_{16}H_{22}N_2O_4S$: C, 56.79; H, 6.55; N, 8.28; S, 9.47. Found: C, 56.69; H, 6.53; N, 8.27; S, 9.45.

Recrystallization from 96% ethanol gave pure material **36*P***, with mp 245–247 °C (dec), $[\alpha]_D$ +122° (*c* 0.5, *N*,*N*-dimethylformamide), ν_{max} 3460, 3320 (OH, NH), 1480 (NH), 1030 (C–O), 790 cm^{-1} (aromatic); ^1H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H, NH), 7.22–7.10 (m, 3H, Ar), 5.78 (d, $J_{1,2}=6.9$ Hz, 1H, H-1), 5.38 (d, $J_{3,OH}=4.8$ Hz, 2H, C3–OH), 4.78 (d, $J_{5,OH}=5.4$ Hz, 1H, C5–OH), 4.49 (t, $J_{6,OH}=J_{6',OH}=5.6$ Hz, 1H, C6–OH), 4.28 (d, $J_{1,2}=6.7$ Hz, 1H, H-2), 4.17 (d, $J_{3,4}=3.3$ Hz, 1H, H-3), 3.74 (m, 2H, H-4, H-5), 3.54 (m, 1H, H-6), 3.33 (m, 1H, H-6′), 2.58 (m, $J_{gem}=21.6$ Hz, $J=7.5$ Hz, 2H, CH_2CH_3), 2.09 (s, 3H, CH_3), 1.17 (t, $J=7.5$ Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4 (C=S), 144.0, 136.3, 135.9, 128.3, 128.1, 125.7 (aromatics), 94.9 (C-1), 80.3 (C-4), 73.9 (C-3), 68.6 (C-5), 66.0 (C-2), 64.1 (C-6), 23.4 (CH_2CH_3), 17.8 (CH_3), 14.1 (CH_2CH_3). Anal. Calcd for $C_{16}H_{22}N_2O_4S$: C, 56.79; H, 6.55; N, 8.28; S, 9.47. Found: C, 56.71; H, 6.45; N, 8.23; S, 9.41.

Procedure B. A solution of **35** (0.040 g, 0.112 mmol) in 30% aqueous acetic acid (1.4 mL) was heated at 100 °C for 30 min. After that time the mixture was evaporated to dryness and the crude obtained characterized as compound **36** (0.037 g, 98%).

4.1.15. 3,5,6-Tri-*O*-acetyl-1-(2-ethyl-6-methylphenyl)-(1,2-dideoxy-α-D-glucofurano)[2,1-*d*]imidazolidine-2-thione (37). To a solution of **36** (3.0 mmol) in pyridine (10.0 mL), cooled at −20 °C for 15 min, was added acetic anhydride (6.0 mL) and the reaction mixture was kept at that temperature for 24 h. Then it was poured into ice-water and the resulting solid was filtered and washed with cold water affording the title compound in 75% yield, as mixture of rotamers (~1:1). ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.13 (m, 8H, Ar and NH, *P* and *M*), 5.93 (d, $J_{1,2}=6.5$ Hz, 1H, H-1, *M*), 5.90 (d, $J_{1,2}=6.6$ Hz, 1H, H-1, *P*), 5.39 (d, $J_{3,4}=2.8$ Hz, 2H, H-3, *P* and *M*), 5.23 (m, 2H, H-5, *P* and *M*), 4.68 (dd, $J_{5,6}=2.3$, $J_{6,6'}=12.3$ Hz, 1H, H-6, *P*), 4.61 (dd, $J_{5,6}=2.3$ Hz, $J_{6,6'}=10.8$ Hz, 1H, H-6, *M*), 4.56 (dd, $J_{3,4}=2.8$ Hz, $J_{4,5}=9.3$ Hz, 2H, H-4, *P* and *M*), 4.47 (d, $J_{1,2}=4.7$ Hz, 1H, H-2, *M*), 4.45 (dd, $J_{2,NH}=0.9$ Hz, $J_{1,2}=5.9$ Hz, 1H, H-2, *P*), 4.02 (dd, $J_{5,6'}=4.3$, $J_{6,6'}=12.4$ Hz, 1H, H-6′, *M*), 3.97 (dd, $J_{5,6'}=5.0$, $J_{6,6'}=12.4$ Hz, 1H, H-6′, *P*), 2.74 (m, $J_{gem}=22.6$ Hz, $J=7.5$ Hz, 1H, CH_2CH_3, *P*), 2.62 (m, $J_{gem}=22.6$ Hz, $J=7.5$ Hz, 1H, CH_2CH_3, *P*), 2.49 (m, $J=7.5$ Hz, 1H, CH_2CH_3, *M*), 2.43 (m, $J_{gem}=22.5$ Hz, $J=7.5$ Hz, 1H, CH_2CH_3, *M*), 2.34 (s, 3H, CH_3, *M*), 2.18 (s, 3H, CH_3, *P*), 2.13 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 6H, OAc), 1.29 (t, $J=7.6$ Hz, 3H, CH_2CH_3, *P*), 1.27 (t, $J=7.6$ Hz, 3H, CH_2CH_3, *M*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.2 (C=S, *P*), 183.5 (C=S, *M*), 170.5 (CH_3–*C*O), 169.7 (CH_3–*C*O), 169.6 (CH_3–*C*O), 149.2, 143.9, 141.2, 135.5, 134.8, 134.5, 129.2 (2C), 128.7, 128.6, 126.5, 126.4 (aromatics), 96.3 (C-1, *M*), 95.7 (C-1, *P*), 76.3 (C-3, *P* and *M*), 75.2 (C-4, *P*), 75.1 (C-4, *M*), 67.5 (C-5, *P*), 67.4 (C-5, *M*), 64.1 (C-2, *P*), 64.0 (C-2, *M*), 62.6 (C-6, *P* and *M*), 23.7 (CH_2CH_3, *P* and *M*), 20.7 (6C,CH_3–CO), 18.6 (CH_3, *M*), 17.9 (CH_3, *P*), 14.4 (CH_2CH_3, *M*), 14.2 (CH_2CH_3, *P*).

The rotamer **37*P*** was isolated by recrystallization from 96% ethanol (50%), $R_f=0.5$, mp 180–182 °C, $[\alpha]_D$ +147° (*c* 0.5, $CHCl_3$), ν_{max} 3440 (NH), 1740 (C=O), 1450, 1350, 1240 (C–O–C), 1040 (C–O), 780 cm^{-1} (aromatic); ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.13 (m, 3H, Ar), 6.81 (s, 1H, NH), 5.90 (d, $J_{1,2}=6.6$ Hz, 1H, H-1), 5.38 (d, $J_{3,4}=2.9$ Hz, 1H, H-3), 5.24 (m, 1H, H-5), 4.68 (dd, $J_{5,6}=2.5$ Hz, $J_{6,6'}=12.3$ Hz, 1H, H-6), 4.56 (dd, $J_{3,4}=3.0$ Hz, $J_{4,5}=9.1$ Hz, 1H, H-4), 4.45 (dd, $J_{2,NH}=1.5$ Hz, $J_{1,2}=6.8$ Hz, 1H, H-2), 3.97 (dd, $J_{5,6'}=4.9$ Hz, $J_{6,6'}=12.3$ Hz, 1H, H-6′), 2.74 (m, $J_{gem}=22.6$, $J=7.6$ Hz, 1H, CH_2CH_3), 2.62 (m, $J_{gem}=22.6$, $J=7.6$ Hz, 1H, CH_2CH_3), 2.18 (s, 3H, CH_3), 2.15 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.29 (t, $J=7.6$ Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.2 (C=S), 170.5 (CH_3–*C*O), 169.8 (CH_3–*C*O), 169.6

(CH_3–*C*O), 143.9, 135.5, 134.8, 129.2, 128.6, 126.4 (aromatics), 95.8 (C-1), 75.2 (C-3, C-4), 67.6 (C-5), 64.1 (C-2), 62.6 (C-6), 23.7 (*C*H_2CH$_3$), 20.7 (*C*H_3–CO), 20.6 (2C, *C*H_3–CO), 17.9 (CH_3), 14.2 (CH$_2$*C*H_3). Anal. Calcd for $C_{22}H_{28}N_2O_7S$: C, 56.88; H, 6.08; N, 6.03; S, 6.90. Found: C, 56.64; H, 6.14; N, 6.18; S, 7.26.

Acknowledgements

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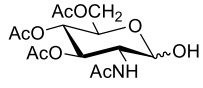
5. When α-anomer of **9** is utilized as starting material, variable amounts of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-gluco-pyranose (**38**) are formed.[1]



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6. Equation $\Delta G^{\ddagger}(\text{cal mol}^{-1}) = 1.987T_c[22.961 + \ln(T_c/\Delta\nu)]$ was used for this calculation: Sandström, J. *Dynamic NMR Spectroscopy*; Academic: London, 1982.
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10. The authors have deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. Crystal data for **29*M***, (CCDC-245086), $C_{18}H_{26}N_2O_5$, $M_r = 350.41$, orthorhombic, $P2_12_12_1$ $a = 8.013(5)$, $b = 16.480(5)$, $c = 26.449(5)$ Å, $V = 3493(3)$ Å^3, $Z = 8$ (2 molecules), $D_{calcd} = 1.333$ g cm^{-3}, λ(Mo Kα) = 0.71073 Å, $\mu = 0.097$ mm^{-1}, $F(000) = 1504$, $T = 150(2)$ K, Goo$F^2 = 1.026$, independent reflections = 61,758 [$R_{int} = 0.0580$] of a total of 21035 collected reflections, $R(F)$ obeying $F^2 > 2\sigma(F^2) = 0.0514$, $wR(F^2) = 0.1087$, R(all data) = 0.0842, $wR(F^2) = 0.1223$.
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14. The authors have deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. Crystal data for **38*P***, (CCDC-241005), $C_{22}H_{28}N_2O_7S$, $M_r = 464.52$, orthorhombic, $P2_12_12_1$ $a = 11.8399(3)$, $b = 11.9763(3)$, $c = 16.5247(4)$ Å, $V = 2343.17(10)$ 3Å, $Z = 4$, $D_{calcd} = 1.317$ g cm^{-3}, λ(Mo Kα) = 0.71073 Å, $\mu = 0.183$ mm^{-1}, $F(000) = 984$, $T = 150(2)$ K, Goo$F^2 = 1.074$, independent reflections = 4139 [$R_{int} = 0.0643$] of a total of 24,350 collected reflections, $R(F)$ obeying $F^2 > 2\sigma(F^2) = 0.0383$, $wR(F^2) = 0.0920$, R(all data) = 0.0495, $wR(F^2) = 0.0979$.
15. Compounds: **23** ($\Delta G^{\neq} > 20.84$ kcal mol^{-1}), **26** ($\Delta G^{\neq} > 21.76$ kcal mol^{-1}), **27** ($\Delta G^{\neq} > 21.62$ kcal mol^{-1}), **30** ($\Delta G^{\neq} > 21.18$ kcal mol^{-1}), **36** ($\Delta G^{\neq} > 20.50$ kcal mol^{-1}), **37** ($\Delta G^{\neq} > 20.71$ kcal mol^{-1}), **38** ($\Delta G^{\neq} > 20.60$ kcal mol^{-1}), and **31** ($\Delta G^{\neq} > 20.71$ kcal mol^{-1}).
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Stereoselective synthesis of optically active mono and diaminoalcohols

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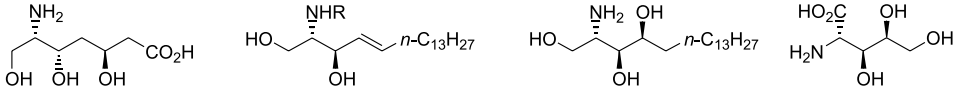
Abstract—Several optically active mono and diaminopolyols have been synthesized starting from the octadienedioate **1**, by regio- and stereo selective azidation of the corresponding alcohol by Mitsunobu/S_N2 substitution.

1. Introduction

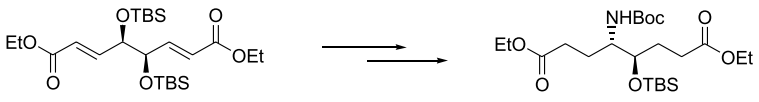
Chiral amino polyols are constituents of several compounds and are of major importance as partial structures of biologically active compounds covering a wide range of biological activities from antibiotic to immunosuppressive properties. For example, (Fig. 1) D-*erythro*-sphingosine and ceramides[1c–e] have been shown to exhibit potent inhibitory activity against protein kinase C. Polyhydroxylated amino acids, like galantinic acid[2] and polyoxamic acid,[3] are components of important biologically active substances such as the complex peptide antibiotic galantin I, which exhibits powerful antibacterial properties and polyoxins (antifungal antibiotics).

Within the scope of our studies on the potential of diversely substituted octadienedioates such as **1** derived from D-mannitol, as building blocks[4a] for the synthesis of polyhydroxylated amino acids and alkaloids,[4b] we have explored a versatile route to functionalised chiral mono and



Galantinic acid | D-*erythro*-sphingosine; R=H ceramide: R=$CO(CH_2)_{14\text{-}22}Me$ | L-Lyxo-sphingosine | Polyoxamic acid

Figure 1.



1 **2**

Scheme 1.

Keywords: Aminoalcohols; Stereoselective; Optically active.
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diamino polyols. Herein, we describe the synthesis of new chiral protected amino alcohols in a highly enantio- and diastereoselective fashion.

We have previously reported[4b] the preparation of the amino diester **2** in five steps starting from the protected diene dioate **1** (Scheme 1). We wished to test the scope of this approach to aminoalcohols and to prepare some diamino-alcohols stereo-and regioselectively.

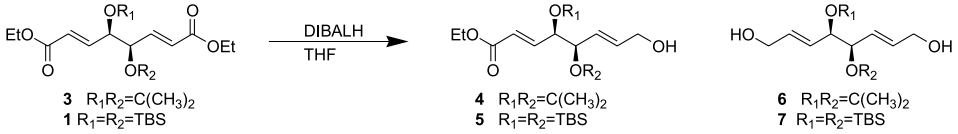
2. Results and discussion

We envisaged the preparation of analogous derivatives where one or both of the ester groups would be reduced to an alcohol. The reduction of the ester groups on the *O*-isopropylidene protected diol dienedioate **3** with DIBALH (*x* equiv) in THF (Scheme 2) afforded the desired allylic mono and diol **4** and **6** in 48 and 32% yield, respectively. The monoreduced compound **4**, obtained as a by-product during the reduction of the 2 ester groups could be prepared up to 50% yield by quenching the reaction after 15 min. However, the lability of the *O*-isopropylidene protecting group prohibited an acidic work-up, making tedious the extraction of the final product. Disilylated analogue **1** (Scheme 2) undergoes the same reactions but the product is more stable to acid and subsequent functionalisations of the double bonds of **5** and **7** were more diastereoselective. By adjusting the experimental conditions (time, temperature and stoechiometry of DIBALH used (see Table 1) it was possible to obtain the monoreduced compound **5** in moderate yield, while the tetrol **7** could be obtained in excellent yield (Table 1).

The selective monoazidation (Scheme 3) of **7** under Mitsunobu conditions using 1.2 equiv of reagents led to the mono azide **8** and the diazide **9** that were readily isolated by chromatography, respectively, in 52 and 19% yield. The two silyl ether groups could be removed by treatment with TBAF in THF. The resulting highly polar triol **10** was benzoylated (**11**, 87%) in order to characterise it. Tosylation with *p*-toluenesulfonic anhydride of all three hydroxyls of **10** was equally achieved in good yield (86%) to give the rather unstable azide **12**.

Finally the *N*-Boc protected amino alcohol **13** was obtained in 56% yield from **8** by the two-step sequence catalytic hydrogenation/N-protection (Scheme 4).

By using 3 equiv of reagent, the diazidation of **7** was achieved in 78% yield (Scheme 5) and following the same sequence as for **8**, the protected bisamine was obtained in good yield. The deprotection of the hydroxyl functions on **14** with TBAF in THF cleanly afforded the diamino diol **15**.

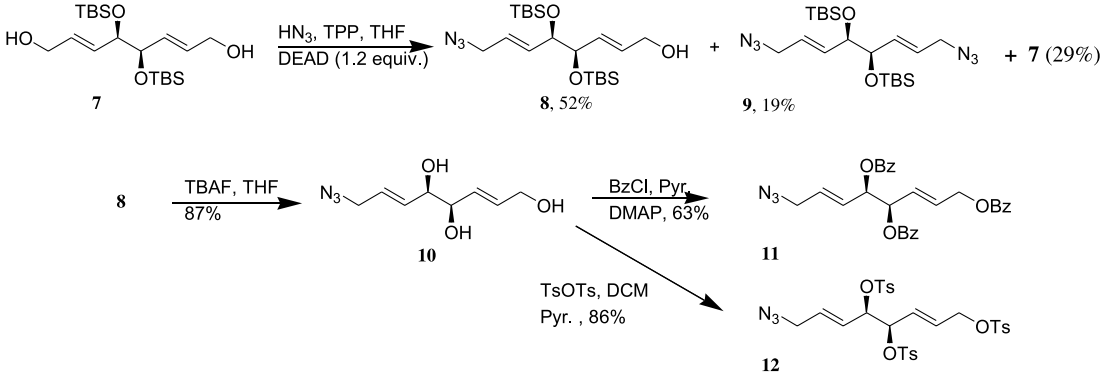


Scheme 2.

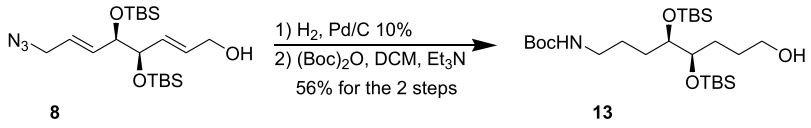
Table 1. DIBAL reduction of diesters **1** and **3**

Experiment[a]	Equiv of DIBALH	Reaction time (min)	% **1** recovered	% **5**	% **5** from unreacted **1**	% **7**
1	2.5	10	72	19	69	2
2	2.5	75	67	13	39	4.6
3	2.5	130	57	25	58	16
4	5.0	45	30	30	47	40
5[b]	5.5	315	0	12	12	66
6[b] (−50 °C)	6.0	60	0	0	0	97

[a] At −78 °C except where indicated.
[b] Acidic work-up.

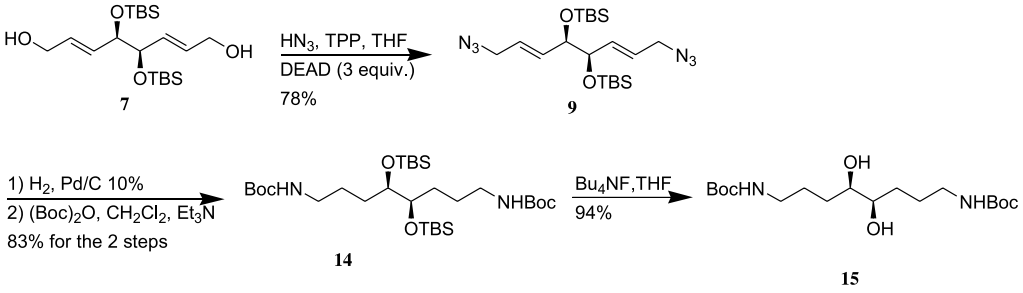


Scheme 3.



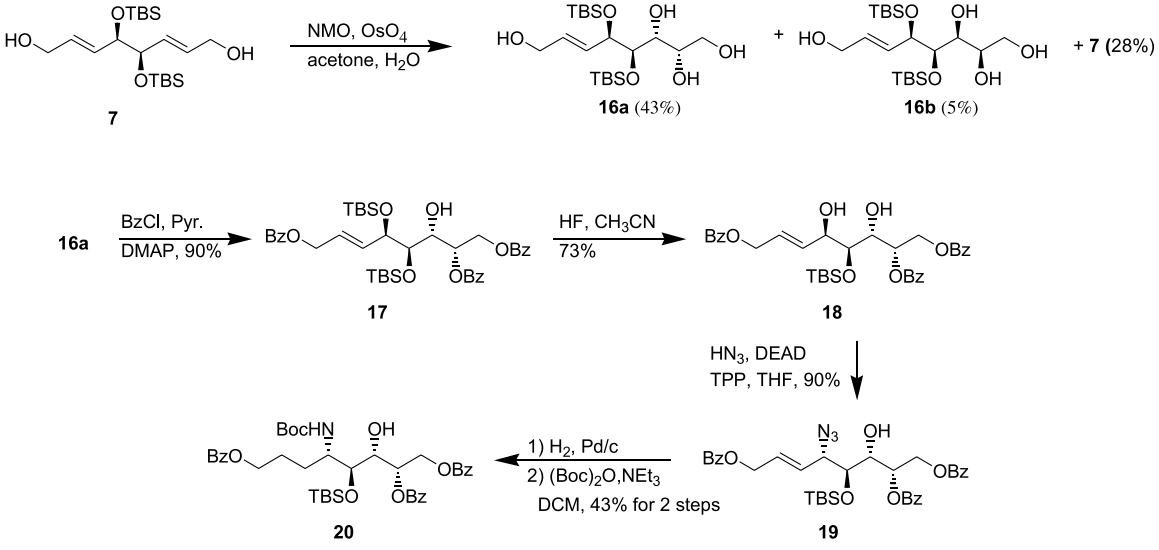
8 13

Scheme 4.



7 9 14 15

Scheme 5.

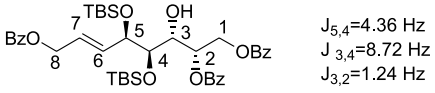


7 16a (43%) 16b (5%) 17 18 20 19

Scheme 6.

We then turned to the preparation of more highly oxygenated compounds (Scheme 6).

The dihydroxylation of one of the two C=C double bond on **7** was performed as described for the dienedioate **1**.[4a] The reaction was stopped before completion as we started to observe dihydroxylation of the second double bond. A slightly lower diastereoselectivity than for **1**[4a] was observed and an easily separable mixture of the diastereoisomers **16a** (majoritary) and **16b** as well as traces of the octitol **16c** was obtained. The stereochemistry of the thus two newly created stereocenters on **16a** was acertained after partial protection of its four free hydroxyl functions: **16a** was selectively benzoylated by treatment with benzoyl chloride in pyridine at rt in the presence of catalytic DMAP, affording tribenzoate **17**.



Scheme 7.

The complete attribution of the signal of the ^{1}H NMR spectrum of **17** was achieved by 2D (Scheme 7 for numbering) was confirmed by the large coupling constant $J_{3,4}=8.72$ Hz while the *syn* relationship between H-5 and H-4 on one hand, H-3 and H-2 on the other hand, was proved by the smaller coupling constants $J_{5,4}=4.36$ Hz, $J_{3,2}=1.24$ Hz, respectively. The silyl protected allylic secondary alcohol on **17** was selectively liberated in good yield by treatment with HF, giving **18**. An azido group was selectively introduced at this allylic position in 90% yield by Mitsunobu reaction. The inversion of configuration on C-5 bearing the azido group was attested by the coupling constants $J_{4,5}=J_{4,3}=5.60$ Hz in the NMR signal of H-4 that appears as a double triplet. The resulting azido compound **19** was lightly contaminated by an unidentified rearrangement product. The two steps sequence hydrogenation/N-protection on **19** led to the expected amino polyol **20** in moderate yield.

3. Conclusions

A concise stereo selective route has been developed for the

synthesis of optically active polyhydroxy amino alcohols and diamino alcohols from the readily available D-mannitol. They are promising key intermediates in the synthesis of attractive and potent biological units and for the synthesis of new unnatural amino-acids; that work being in course in our laboratory.

4. Experimental

4.1. General

Melting points were determined on a Büchi 530 apparatus and are uncorrected. Infrared spectra were recorded on a Mattson 7000 FTIR spectrometer. Optical rotations were recorded at 20 °C on an Optical activity AA 1000 polarimeter using a 0.5 dm cell. Concentrations are given in g/100 ml. NMR spectra (^{1}H: 400 MHz; ^{13}C: 100 MHz) were recorded on a Brüker ARX 400 spectrometer in $CDCl_3$ using Me_4Si (^{1}H) and the solvent peak (^{13}C) at δ 77.0 ppm as an internal reference. Chemical shifts are expressed in parts per million downfield. Medium pressure column chromatography were performed on MN Silica gel 60M. Preparative thin-layer chromatography was performed on MN Silica gel G/UV 254 with fluorescent indicator. Elemental analysis were performed by the Micro analytical Laboratory, operated by the Department of Analysis at Instituto Superior Técnico (Lisbon, Portugal).

4.1.1. (4*R*,5*R*)-4,5-Bis-(*tert*-butyldimethylsilanyloxy)-8-hydroxy-octa-2(*E*),6(*E*)-dienoic acid ethyl ester, 5. Compound **1** (1.550 g, 3.18 mmol) was dissolved under argon in 20 ml of dry THF. DIBALH (8 ml of 1 M sol. in THF, 2.5 equiv) was added at −78 °C and the mixture was stirred for 2 h. A saturated solution of NH_4Cl (25 ml) was then added and the mixture was stirred for 20 min and allowed to reach rt. The crude product was dissolved in AcOEt, filtered and the gel was washed with AcOEt. The organic layer was separated and dried with Na_2SO_4, evaporated and the crude product separated by flash column chromatography (Hex/AcOEt 4:1 then 3:2) to give 0.358 g (25%) of **5**, 0.876 g (57%) of unreacted **1** and 0.207 g (16%) of the di reduced compound **7**. Compound **5**: $[\alpha]_D$ +68.05 (*c* 1.55, $CHCl_3$); IR (neat, cm^{-1}, ν): 3330 (OH); 1722 (C=O); ^{1}H NMR: 7.00 (dd, 1H, J=15.6, 3.6 Hz, H-3); 5.94 (dd, 1H, J=15.6, 1.6 Hz, H-2); 5.78 (dtd, 1H, J=15.6, 4.8, 4.8, 0.8 Hz, H-7); 5.63 (dd, 1H, J=15.6, 4.8 Hz, H-6); 4.29 (m, 1H J=2.0, 3.2, 3.6, 1.6 Hz, H-4); 4.20–4.13 (m, 3H, O*C*H_2CH$_3$, H-5); 4.08 (d, 2H, H-8,8′); 1.81 (sl, 1H, OH); 1.27 (t, 3H, OCH$_2$C*H*$_3$); 0.07, 0.06, 0.04, 0.036 (4s, 12H, SiCH$_3$); ^{13}C NMR: 166.6 (C-1); 147.5 (C-3), 131.2 (C-6); 129.7 (C-7); 121.4 (C-2); 75.0 (C-4); 74.8 (C-5); 63.1 (C-8); 60.3 (O–*C*H$_2$CH$_3$); 25.8 (*t*Bu); 18.2 (*t*Bu quat.); 14.3 (OCH$_2$*C*H$_3$); −4.5, −4.8, −4.8, SiCH$_3$. Anal. Calcd for $C_{22}H_{44}O_5Si_2$: C, 59.41; H, 9.97. Found: C, 59.60; H, 10.29. Compound **7**: mp 58–60 °C. $[\alpha]_D$ +79.44 (*c* 0.79, $CHCl_3$); IR (KBr, cm^{-1}, ν): 3330 (OH); ^{1}H NMR: 6.97 (m, 2H, J= 15.6 Hz, H-3, H-6); 5.97 (d, 2H, J=15.6 Hz, H-2, H-7); 4.37 (m, 2H, H-4, H-5); 4.25–4.12 (m, 4H, O*C*H_2CH$_3$); 1.31–1.26 (m, 6H, OCH$_2$C*H*$_3$); 0.94 (s, 18H, *t*Bu); 0.10 (s, 6H, Si(CH$_3$)$_2$); 0.08 (s 6H, Si(CH$_3$)$_2$)); ^{13}C NMR: 130.9 (C-3, C-6), 130.1 (C-2, C-7); 75.2 (C-4, C-5); 63.1 (C-1, C-8); 25.9 (*t*Bu); 18.2 (*t*Bu quat.); −4.5, −4.7 (SiMe). Anal. Calcd for $C_{20}H_{42}O_4Si_2$: C, 59.65; H, 10.51. Found: C, 59.20; H, 10.38.

4.1.2. 3-[(4*R*,5*R*)-5-(3-Hydroxy-1-(*E*)-propen-1-yl)-2,2-dimethyl-[1,3]dioxolan-4-yl]-2-(*E*)-acrylic acid ethyl ester, 4 and 3-[(4*R*,5*R*)-5-(3-hydroxy-1-(*E*)-propen-1-yl)-2,2-dimethyl-[1,3]dioxolan-4-yl]-prop-2-(*E*)-en-1-ol, 6. To a solution of **3** (0.513 g, 1,7 mmol) in dry THF (5 ml) under argon was added 8.59 ml of 1 M solution of DIBALH in THF at −78 °C. After stirring for 0.5 h, the reaction was quenched with 10 ml of a saturated solution of NH_4Cl and allowed to stir for 20 min. After filtration and extraction of the gel with ethyl acetate, the combined organic phase was dried on Na_2SO_4 and evaporated. Purification by preparative TLC of the crude product offered 0.058 g (11%) of unreacted **3**, 0.186 g (48%) of the monoreduced product **4** and 0.106 g (32%) of the desired **6**. Compound **4**; ^{1}H NMR: 6.75 (ddd, 1H, $J_{5,6}$=15.56 Hz, $J_{3,2}$=15.64 Hz, $J_{3,4}$= 5.20 Hz, H-3); 6.02 (dd, 1H, $J_{2,3}$=15.64 Hz, $J_{2,4}$= 0.84 Hz, H-2); 5.89 (dt, 1H, $J_{7,6}$=15.56 Hz, $J_{7,8}$= 4.64 Hz, H-2″); 5.63 (dd, 1H, $J_{6,7}$=15.56 Hz, $J_{6,5}$= 7.36 Hz, H-1″); 4.20–4.00 (m, 6H, O*C*H_2CH$_3$, H-3″, H-3″, H-4′, H-5′); 2.98 (sl, 1H, OH); 1.35 (s, 3H, Me); 1.34 (s, 3H, Me); 1.20 (t, 3H, O–CH$_2$C*H*$_3$); ^{13}C NMR: 165.9 (C-1); 142.7 (C-3); 135.3 (C-1″); 125.3 (C-2″); 122.6 (C-2); 109.7 (quat., C-2′); 81.4 (C-4′); 79.8 (C-5′); 61.9 (C-3″); 60.5 (O–*C*H$_2$CH$_3$); 26.8, 26.6 (2CH$_3$); 14.07 (OCH$_2$*C*H$_3$). Compound **6**: $[\alpha]_D$ −13.66 (*c* 0.41, $CHCl_3$); IR (neat, cm^{-1}, ν): 3401 (OH); ^{1}H NMR: 5.89 (dt, 2H, $J_{5,6}$= 15.60 Hz, J=15.60 Hz, J=5.00 Hz, H-2, H-2″); 5.62 (m, 2H, J=15.60 Hz, J=3.24 Hz, H-3, H-1″); 4.08 (m, 6H, H-1, H-1, H-3″, H-3″, H-4′, H-5′); 3.31 (s, 2H, OH); 1.40 (s, 6H, 2CH$_3$); ^{13}C NMR: 134.5 (C-3, C-1″); 126.2 (C-2, C-2″); 109.1 (quat., C-2′); 81.4 (C-4′, C-5′); 62.2 (C-1, C-3″); 27.0 (2Me). Anal. Calcd for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.73; H, 8.59.

4.1.3. (4*R*,5*R*)-4,5-Bis(*tert*-butyldimethylsilanyloxy)-octa-2-(*E*),6-(*E*)-diene-1,8-diol, 7. To a solution of **1** (1.206 g, 2.48 mmol) in dry THF (8 ml) at −78 °C was added 14.75 ml (6 equiv) of a 1 M solution of DIBALH in THF. The temperature was allowed to reach −50 °C and the mixture was stirred for 2 h (TLC Hex/EtOAc 1:4). The reaction was quenched with 30 ml of a saturated solution of NH_4Cl and stirred for 20 min. The mixture was diluted with AcOEt and carefully brought to pH=6 by addition of 1 N HCl until dissolution of the gel and clear separation of the 2 phases. The aqueous phase was extracted with AcOEt (5× 50 ml), The combined organic phase were dried on Na_2SO_4. Evaporation of the solvent gave 1.052 g of crude product that was purified by flash chromatography (Hex/EtOAc 1:4) to give 0.997 g (97%) of **7** (see above for characterization).

4.1.4. (4*R*,5*R*)-8-Azido-4,5-bis-(*tert*-butyldimethylsilanyloxy)-octa-2-(*E*),6-(*E*)-dien-1-ol, 8. To 0.281 g (0.70 mmol) of **7** dissolved in 12 ml of dry THF was added triphenylphosphine (TPP) (0.220 g, 1.2 equiv). The mixture was stirred for 20 min then HN_3 (0.655 ml of a 1.28 M solution in benzene, 1.2 equiv) was added, followed by DEAD (0.182 g in 1.5 ml of THF, dropwise). The mixture was stirred at rt for 1 h. The solvent was evaporated; the crude product was taken first in CH_2Cl_2 then AcOEt/ Hexane 1:7, filtered and the filtrate evaporated and purified

by flash chromatography to give successively 0.059 g (19%) of **9**, 0.156 g (52, 74% from reacted **7**) of **8** and 0.085 g (29%) of unreacted **7**. Compound **8**: $[\alpha]_D$ +68.4 (*c* 0.614, $CHCl_3$); IR (neat, cm^{-1}, ν): 3350, 2099; ^1H NMR: 5.85–5.65 (m, 4H, H-2,3,6,7); 4.19–4.12 (m, 4H, H-4, H-5, H-1,1′); 3.73 (AB part of ABX system, $J_{1,1'}=13.60$ Hz); 1.58 (sl, 1H, OH); 0.92 (s, 18H, *t*Bu); 0.84, 0.08, 0.06, 0.06 (s, 3H each, $SiCH_3$); ^{13}C NMR: 135.12 (C-3), 130.64 (C-6), 130.08 (C-7), 123.68 (C-2), 74.91, 74.81 (C-4, C-5), 63.35 (C-1), 52.54 (C-8), 25.90 (*t*Bu), 18.22 (*t*Bu quat.), −4.52, −4.64, −4.76 ($SiCH_3$). Compound **9**; IR (neat, cm^{-1}, ν): 2104 (N_3); ^1H NMR: 5.81 (dd, 2H, $J=15.60$, 1.60 Hz, H-3, H-6); 5.71 (td, 2H, $J=15.60$, 6.40 Hz, H-2, H-7); 4.20 (sl, 2H, H-4, H-5); 3.73 (m, 4H, H-1, H-1′, H-8, H-8′); 0.93 (s, 18H, *t*Bu); 0.10 (s, 6H, $SiCH_3$); 0.07 (s, 6H, $SiCH_3$); ^{13}C NMR: 134.5 (C-3, C-6), 124.2 (C-2, C-7), 74.6 (C-4, C-5), 52.5 (C-1, C-8), 25.9 (*t*Bu), 18.2 (*t*Bu quat.), −4.6, −4.8 ($SiCH_3$).

4.1.5. (4*R*,5*R*)-1,8-Diazido-4,5-bis-(*tert*-butyldimethylsilanyloxy)-octa-2-(*E*),6-(*E*)-diene, 9. To 0.205 g (0.5 mmol) of **7** dissolved in 10 ml of dry THF were added 0.400 g (3 equiv) of TPP. The mixture was stirred for 20 min then 1 ml of a 1.58 M solution of HN_3 in benzene was added, followed by DEAD (0.265 g, 3 equiv in 2 ml of THF, dropwise). Stirring was continued for 1.25 h. The solvent was evaporated and the crude product purified by medium pressure column chromatography (Hex/AcOEt 9:1) to yield 0.179 g (78%) of **9** as a colourless oil that was used immediately for the next step (see upper for characterisation).

4.1.6. (4*R*,5*R*)-8-Azido-octa-2-(*E*),6-(*E*)-diene-1,4,5-triol, 10. The azide **8** (0.088 g, 0.21 mmol) in 4 ml of anhydrous THF was stirred at rt for 45 min with 0.412 ml (2 equiv) of a 1 M solution of Bu_4NF in THF. Evaporation of the solvent followed by flash chromatography (AcOEt) gave **10** (0.034 g, 82%) as a viscous syrup. The product has been characterized as its tribenzoate.

4.1.7. (4*R*,5*R*)-8-Azido-1,4,5-tribenzoyloxy-octa-2-(*E*),6-(*E*)-diene, 11. Compound **10** (0.042 g, 0.21 mmol) was dissolved in 1.5 ml of pyridine. BzCl (0.141 g, 4.5 equiv) and a catalytic amount of DMAP were added The mixture was stirred for 0.5 h at rt and the reaction was quenched with 8 ml of a saturated sol. of $NaHCO_3$. The product was extracted with DCM (3×15 ml) and the organic phase was dried on Na_2SO_4. The crude was purified by preparative TLC (Hex/AcOEt 7:1) to give 0.068 g of **11** as an oil. $[\alpha]_D$ +29.3 (*c* 1.37, $CHCl_3$); IR (neat, cm^{-1}, ν): 2102 (N_3), 1723 (C═O); ^1H NMR: 8.15–7.98 (m, 6H, arom.); 7.62–7.34 (m, 9H, arom.); 6.18 (dt, 1H, $J=5.60$, 5.60, 14.96 Hz, H-2); 6.06–5.87 (m, 5H, H-3, H-4, H-5, H-6, H-7); 4.85 (d, 2H, $J=5.60$ Hz, H-1, H-1′); 3.78 (d, 2H, $J=5.60$ Hz, H-8, H-8′); ^{13}C NMR: 166.1, 165.7 (C═O), 133.7, 133.3, 133.1, 130.2, 129.9, 129.7, 129.7, 129.7, 129.0, 128.5, 128.4, 128.3, 127.4 (C-2, C-3, C-6, C-7, arom.); 73.9 (C-4, C-5); 64.0 (C-1); 51.8 (C-8).

4.1.8. (4*R*,5*R*)-8-Azido-1,4,5-tri-*O*-tosyl-octa-2-(*E*),6-(*E*)-diene, 12. 0.055 g (0.28 mmol) of **10** were dissolved in 3 ml of dry DCM. TsOTs (0.543 g, 6 equiv) and pyridine (0.135 ml, 6 equiv) were added at 0 °C. The mixture was stirred for 30 min at rt then HCl 1 N (2 ml) was added. The organic phase was decanted and the aqueous phase extracted with CH_2Cl_2. The combined organic phase were washed with saturated $NaHCO_3$, dried on Na_2SO_4. The crude product (0.254 g) was purified by preparative TLC (Hex/AcOEt 6:4) to yield 0.158 g (86%) of **12**. $[\alpha]_D$ +3.3 (*c* 0.484, $CHCl_3$); IR (neat, cm^{-1}, ν): 2104 (N_3); 1364, 1176 (OTs); ^1H NMR: 7.76–7.67 (m, 6H, arom. tosyl); 7.38–7.29 (m, 6H, arom. tosyl); 5.69–5.60 (m, 2H, H-2, H-7); 5.57–5.46 (m, 2H, $J=15.40$, 6.28 Hz, H-3, H-6); 4.94 (m, 2H, H-4, H-5); 4.34 (d, 2H, $J=5.12$ Hz, H-1, H-1′); 3.64 (d, 2H, $J=5.40$ Hz, H-8, H-8′); 2.46 (s, 9H, Me); ^{13}C NMR: 145.5, 145.5, 145.2 (quat. tosyl); 133.3, 133.24 (quat. tosyl); 131.1, 129.5, 126.3, 125.3 (C-2, C-3, C-6, C-7); 130.0, 128.1, 128.0 (arom. tosyl); 79.6, 79.3 (C-4, C-5); 68.5 (C-1); 51.5 (C-8); 21.8 (Me Tosyl).

4.1.9. (4*R*,5*R*)[4,5-Bis-(*tert*-butyl-dimethylsilanyloxy)-8-hydroxy-octyl]-carbamic acid *tert*-butyl ester, 13. 0.131 g (0.30 mmol) of **8** were dissolved in 6 ml of absolute ethanol and submitted to a pressure of 15 psi of hydrogen in the presence of 32 mg of Pd/C 10% for 45 min, then 50 psi for more 2.25 h. The catalyst was filtered off and washed with EtOH then AcOEt; the solvent was evaporated in vacuo to yield 0.132 g of crude product that was dissolved in 5 ml of dry DCM. $(Boc)_2O$ (0.080 g, 2.2 equiv) was added at rt and the mixture was stirred for 1 h. The reaction was quenched with HCl 1 N (12 ml); the organic phase was decanted, washed successively with saturated $NaHCO_3$ then water, dried on Na_2SO_4 and the solvent was evaporated in vacuo. Purification by medium pressure column chromatography (Hex/AcOEt 5:1) yielded 0.087 g (56%) of **13** as a viscous oil. $[\alpha]_D$ +34.54 (*c* 1.60, $CHCl_3$); IR (neat, cm^{-1}, ν): 3356 (OH); 1694 (C═O); ^1H NMR: 4.54 (br s, 1H, NH); 3.60 (m, 2H, H-4, H-5); 3.52 (m, 2H, H-8, H-8′); 3.07 (m, 2H, H-1, H-1′); 2.01–1.20 (m, 26H, H-3,3′; H-6,6′; H-2,2′; H-7,7′, OH, 2Boc); 0.85 0.84 (2s, 18H, Si*t*Bu); 0.01 (s, 12H, $SiCH_3$); ^{13}C NMR: 156.2 (C═O), 75.3, 75.2 (C-4, C-5); 63.2 (C-8); 40.7 (C-1); 30.1 (C3, C-6); 28.3 (Boc); 27.3, 26.3 (C-2, C-7); 25.8 (Si*t*Bu); 17.9 (*t*Bu quat.); −4.2, −4.3, −4.7 ($SiCH_3$). Anal. Calcd for $C_{22}H_{55}NO_5Si_2$: C, 59.36; H, 10.96; N, 2.77. Found: C, 59.48; H, 11.04; N, 2.66.

4.1.10. (4*R*,5*R*) [8-*tert*-Butoxycarbonylamino-4,5-bis-(*tert*-butyldimethylsilanyloxy)-octyl]-carbamic acid *tert*-butyl ester, 14. 0.179 g (0.40 mmol) of **9** dissolved in 12 ml of absolute ethanol were hydrogenated for 30 min at 15 psi in the presence of 43 mg of Pd/C 10%, then for 3 h at 50 psi. The mixture was filtered on celite and concentrated in vacuo. The crude product (0.191 g) was dissolved in 6 ml of dry DCM. Et_3N (0.25 ml, 4.4 equiv) followed by $(Boc)_2O$ (0.196 g, 2.2 equiv) was added. The mixture was stirred for 1.5 h at rt and quenched with 12 ml of HCl (1 N). The organic phase was decanted and the aqueous phase extracted with CH_2Cl_2 (2×20 ml). The combined extracts were washed with saturated $NaHCO_3$ then water, dried on Na_2SO_4 and concentrated in vacuo. Medium pressure column chromatography (2×35 cm, eluent Hex/AcOEt 8:1) of the crude product (0.267 g) afforded **14** (0.179 g, 83%) as a white solid. Mp 99–101 °C. $[\alpha]_D$ +34.0 (*c* 0.31, $CHCl_3$); IR (KBr, cm^{-1}, ν): 3357 (NH), 1694 (C═O); ^1H NMR: 4.52 (br s, 2H, NH); 3.50 (bd, 2H, $J=8.24$ Hz, H-4, H-5); 3.08 (br s, 4H, H-1, H-1′, H-8, H-8′); 1.67–1.18 (m,

26H, H-3,3′; H-6,6′; H-2,2′; H-7,7′, 2Boc); 0.85 (s, 18H, Si*t*Bu); 0.03 (s, 12H, $SiCH_3$); ^{13}C NMR: 156.1 (C=O), 75.2 (C-4, C-5); 40.7 (C-1, C-8); 28.4 (Boc); 27.2 (C-2, C-3, C-6, C-7); 25.8 (Si*t*Bu); 17.9 (*t*Bu quat.); −4.3, −5.0 ($SiCH_3$). Anal. Calcd for $C_{30}H_{64}N_2O_6Si_2$: C, 59.56; H, 10.66; N, 4.63. Found: C, 59.91; H, 10.75; N, 4.50.

4.1.11. (4*R*,5*R*) (8-*tert* Butoxycarbonylamino-4,5-dihydroxy-octyl)-carbamic acid *tert*-butyl ester, 15. Compound **14** (0.133 g, 0.22 mmol) was dissolved in 5 ml of dry THF. Then TBAF (0.115 g, 2 equiv) was added at rt. The mixture was stirred for 5 h. Evaporation of the solvent gave 0.275 g of a crude product that was purified by medium pressure column chromatography (Hex/AcOEt 6:1 then AcOEt) to yield 0.078 g (94%) of **15** as a colourless viscous syrup that crystallized on standing in fridge. Mp 81–82 °C. $[\alpha]_D$ +14.61 (*c* 0.96, $CHCl_3$); IR (KBr, cm^{-1}, *ν*): 3386, 3365 (OH, NH), 1687.80 (C=O); ^1H NMR: 4.92 (br s, 2H, NH); 3.51 (br s, 2H, OH); 3.37 (br s, 2H, H-4, H-5); 3.10 (m, 4H, H-1, H-1′, H-8, H-8′); 1.72–1.35 (m, 26H, H-3,3′; H-6,6′; H-2,2′; H-7,7′, OH, 2Boc); ^{13}C NMR: 156.6 (C=O), 79.2 (Boc, quat.); 74.1 (C-4, C-5); 40.3 (C-1, C-8); 30.3 (C3, C-6); 28.3 (Boc); 26.3 (C-2, C-7). Anal. Calcd for $C_{18}H_{36}N_2O_6$: C, 57.42; H, 9.64; N, 7.44. Found: C, 57.49; H, 9.71; N, 7.43.

4.1.12. (2*S*,3*S*,4*R*,5*R*)-4,5-Bis-(*tert*-butyldimethylsilanyloxy)-oct-6-(*E*)-ene-1,2,3,8-tetraol, 16a. To 0.807 g (2.0 mmol) of **7** dissolved in 10 ml of acetone and cooled by an ice bath were added 0.406 g (1.5 equiv) of NMO in 0.8 ml of H_2O and 5 drops of a solution of OsO_4 in acetonitrile. After 0.5 h, the stirring was continued at rt for 3 h then solid $K_2S_2O_3$ was added an the mixture was stirred for more 0.5 h, extracted with AcOEt, dried on Na_2SO_4 and concentrated in vacuo. Purification of the crude by flash chromatography gave 0.330 g (40%) of unreacted **7**, 0.336 g (39%) of **16a** and 0.043 g (5%) of **16b**. Compound **16a**: $[\alpha]_D$ +57.68 (*c* 0.99, $CHCl_3$); IR (neat, cm^{-1}, *ν*): 3326 (OH); ^1H NMR: 5.94 (m, 2H, H-6, H-7); 4.40 (m, 1H); 4.21 (m, 2H); 3.83 (dd, 1H, *J*=4.36, 8.76 Hz, H-4);3.78–3.63 (m, 4H); 2.81 (br s, 4H, OH); 0.93 (s, 9H, Si*t*Bu); 0.90 (s, 9H, Si*t*Bu); 0.16, 0.14, 0.13, 0.11 (s, 3H each, $SiCH_3$); ^{13}C NMR: 131.19 (C-6); 128.15 (C-7); 75.72, 74.22, 71.12, 69.05, 65.53, 63.08 (C-8, C-5, C-4, C-3, C-2, C-1); 25.84, 25.80 (Si*t*Bu); 18.12, 18.02 (Si*t*Bu quat.); −4.34, −4.78, −4.92, −5.06 ($SiCH_3$). Anal. Calcd for $C_{20}H_{44}O_6Si_2$: C, 55.00; H, 10.16. Found: C; H; %. Compound **16b**: $[\alpha]_D$ +41.56 (*c* 0.90, $CHCl_3$); ^1H NMR: 5.84 (m, 2H, H-6, H-7); 4.20 (m, 1H); 4.10 (m, 2H); 3.75 (dd, 1H, *J*=3.12, 5.00 Hz, H-4); 3.71–3.54 (m, 4H); 3.15 (br s, 4H, OH); 0.92 (s, 9H, Si*t*Bu); 0.91 (s, 9H, Si*t*Bu); 0.15, 0.14, 0.08, 0.06 (s, 3H each, $SiCH_3$); ^{13}C NMR: 130.6 (C-6); 129.4 (C-7); 74.3, 73.7, 72.6, 69.6, 64.0, 62.8 (C-8, C-5, C-4, C-3, C-2, C-1); 25.9 (Si*t*Bu); 18.2, 18.1 (Si*t*Bu quat.); −4.2, −4.6, −4.7, −4.8 ($SiCH_3$).

4.1.13. (2*S*,3*S*,4*R*,5*R*)-1,2,8-Tri-benzoyloxy-4,5-bis(*tert*-butyldimethylsilanyloxy)-oct-6-(*E*)-ene-3-ol, 17. To 0.321 g (0.74 mmol) of **16a** dissolved in 5 ml of dry pyridine at 0 °C were added 0.52 ml (4.4 mmol, 6 equiv) of BzCl and a catalytic amount of DMAP. The mixture was stirred at rt for 1.5 h (TLC Hex/AcOEt 4:1). The reaction was quenched with a saturated solution of $NaHCO_3$. The product was extracted with DCM (4×20 ml), the organic phase dried on Na_2SO_4. Evaporation of the solvent and purification of the crude product by flash chromatography afforded **17** (0.495 g, 90%). $[\alpha]_D$ +32.15 (*c* 2.42, $CHCl_3$); IR (neat, cm^{-1}, *ν*): 3483 (OH), 1724 (C=O); ^1H NMR: 8.11 (d, 2H, *J*=7.36 Hz, arom. *ortho*); 8.06 (d, 2H, *J*=7.48 Hz, arom. *ortho*); 8.0 (d, 2H, *J*=7.40 Hz, arom. *ortho*); 7.60–7.34 (m, 9H, arom. *meta*, *para*); 6.20 (ddt, 1H, *J*=15.60, 4.36, 1.84 Hz, H-6); 6.06 (ddt, 1H, *J*=15.60, 4.56, 1.24 Hz, H-7); 5.57 (dt, 1H, *J*=6.24, 6.24, 1.24 Hz, H-2); 4.92 (AB part of ABX system, *J*=13.64 Hz H-8, H-8′); 4.66 (, 2H, *J*=6.36 Hz, H-1, H-1′); 4.52 (m, 1H, *J*=4.36, 1.88 Hz, H-5); 4.19 (br s, 1H, OH); 4.03 (dd, 1H, *J*=8.72, 1.24 Hz, H-3); 3.88 (dd, 1H, *J*=4.36, 8.72 Hz, H-4); 0.98 (s, 9H, *t*Bu); 0.88 (s, 9H, *t*Bu); 0.15 (s, 3H, $SiCH_3$); 0.14 (s 3H, $SiCH_3$); 0.03 (s, 3H, $SiCH_3$); −0.04 (s, 3H, $SiCH_3$); ^{13}C NMR: 166.20, 165.85 (C=O); 133.10, 133.03, 132.89 (arom. *para*); 130.4 (C-7); 130.3, 130.2, 130.1 (arom. quat.); 129.8, 129.8, 129.7 (arom. *ortho*); 128.4, 128.3 (arom. *meta*); 126.3 (C-6); 75.5 (C-2); 72.5 (C-3); 71.0 (C-5, C-4); 64.6 (C-8); 63.4 (C-1); 25.8, 25.8 (*t*Bu); 18.2, 18.0 (*t*Bu quat.); −3.8, −4.7, −5.2 ($SiCH_3$). Anal. Calcd for $C_{41}H_{56}O_9Si_2$: C, 65.47; H, 7.54. Found: C, 65.73; H, 7.53.

4.1.14. (2*S*,3*S*,4*R*,5*R*)-1,2,8-Tri-benzoyloxy-4-*tert*-butyldimethylsilanyloxy-oct-6-(*E*)-ene-3,5-diol, 18. Compound **17** (0.263 g, 0.35 mmol) was dissolved in 7 ml of acetonitrile. HF (40% in water, 0.216 ml) was added and the mixture was stirred at rt for 30 min (A longer reaction time resulted in lower yield in expected product) until apparition of a more polar compound (checked by TLC Hex/AcOEt 4:1). The reaction was quenched with saturated $NaHCO_3$, extracted with DCM, and the organic phase dried on Na_2SO_4. Evaporation of solvent and purification of the crude product by preparative TLC gave 0.053 g (22%) of **17** and 0.174 g (73%) of **18**. $[\alpha]_D$ +12.31 (*c* 0.93, $CHCl_3$); IR (neat, cm^{-1}, *ν*): 3456 (OH); 1723 (C=O); ^1H NMR: 8.08 (dd, 2H, *J*=8.76, 1.28 Hz, arom. *ortho*); 8.02 (dd, 2H, *J*=8.12, 1.28 Hz, arom. *ortho*); 7.97 (dd, 2H, *J*=8.08, 1.24 Hz, arom. *ortho*); 7.60–7.50 (m, 3H, arom *para*); 7.47–7.35 (m, 6H, arom. *meta*); 6.05 (AB part of ABMX system, J_{AB}=15.60 Hz, H-6, H-7); 5.49 (dt, 1H, *J*=6.24, 1.88 Hz, H-2); 4.83 (AB part of ABX system, *J*=12.76 Hz, H-8, H-8′); 4.76 (dd, 1H, *J*=11.24, 6.24 Hz, H-1); 4.60 (dd, 1H, *J*=11.24, 6.24 Hz, H-1′); 4.43 (m, 1H, H-5); 3.96 (AB part of ABMX system, H-3, H-4, J_{AB}=7.48 Hz); 3.20 (br s, 2H, OH; 0.85 (s, 9H, Si*t*Bu); 0.03 (s, 3H, $SiCH_3$); −0.01 (s 3H, $SiCH_3$); ^{13}C NMR: 166.6, 166.3, 166.0 (C=O); 133.4, 133.3, 133.0 (arom. *para*); 132.6 (C-6); 130.2 (arom. quat.); 130.0, 129.8, 129.7 (arom. *ortho*); 129.5 (arom. quat.); 128.6; 128.5, 128.4 (arom. *meta*); 125.9 (C-7); 73.4 (C-2); 72.8 (C-3); 72.4 (C-5); 71.3 (C-4); 64.8 (C-8); 62.7 (C-1); 25.9 (*t*Bu); 18.0 (*t*Bu quat.); −4.0, −4.9 ($SiCH_3$). Anal. Calcd for $C_{35}H_{42}O_9Si$: C, 66.22; H, 6.67. Found: C, 66.50; H, 6.50.

4.1.15. (2*S*,3*S*,4*R*,5*R*)-5-Azido-1,2,8-tri-benzoyloxy-4-*tert*-butyldimethylsilanyloxy-oct-6-(*E*)-ene-3-ol, 19. To 0.296 g (0.47 mmol) of **18** dissolved in 9 ml of dry THF was added TPP (0.186 g, 1.5 equiv), followed by 0.400 ml (1.5 equiv) of a 1.7 M solution of hydrazoic acid in benzene. The mixture was stirred at rt for 20 min then DEAD (0.124 g in 1.7 ml of dry THF) was added dropwise. After 30 min of stirring, the solvent was evaporated and the crude product

was purified by preparative TLC (Hex/EtOAc 4:1) to give 0.277 g (90%) of **19** as a colourless syrup. $[\alpha]_D$ −13.36 (*c* 1.05, $CHCl_3$); IR (neat, cm^{-1}, ν): 3475 (OH), 2106 (N_3), 1724 (C=O); ^1H NMR: 8.08–8.03 (m, 4H, arom. *ortho*); 8.00–7.97 (m, 2H, arom. *ortho*); 7.59–7.50 (m, 3H, arom. *para*); 7.47–7.37 (m, 6H, arom. *meta*); 6.06 (ddd, 1H *J*= 15.60, 6.24, 1.24 Hz, H-6); 5.81 (ddd, 1H, *J*=15.60, 6.24, 1.24 Hz, H-7); 5.69 (ddd, 1H, *J*=6.24, 5.00, 2.48 Hz, H-2); 4.63 (m, 2H, AB part of ABX system, J_{AB}=11.84 Hz, H-8, H-8′); 4.45 (dd, 1H, *J*=10.60, 3.12 Hz, H-1); 4.39 (dt, 1H, *J*=5.62, 1.24 Hz, H-4); 4.36–4.27 (m, 2H, H-1′ and H-3 or H-5); 3.87 (m, 1H, *J*=5.60, 3.12 Hz, H-5 or H-3); 2.30 (br s, 1H, OH); 0.82 (s, 9H, *t*Bu); 0.01, −0.01, −0.04 (3s, 3H each, $SiCH_3$); ^{13}C NMR: 166.3, 166.2, 165.6 (C=O); 135.4 (C-6), 133.3, 133.3, 133.2 (arom. *para*); 129.9; 129.9; 129.8 (arom. *meta*); 129.5 (arom. quat.); 128.5, 128.5 (arom. *ortho*); 126.7 (C-7); 73.83, 73.22, 70.51, 66.12; 63.61 (C-8); 61.74 (C-1); 25.77 (*t*Bu); 18.06 (Si*t*Bu quat.); −4.13, −5.00 ($SiCH_3$).

4.1.16. (2*S*,3*S*,4*R*,5*R*)-5-*tert*-Butoxycarbonylamino-1,2,8-tri-benzoyloxy-4-*tert*-butyldimethylsilanyloxy-oct-6-(*E*)-ene-3-ol, 20. 0.167 g (0.25 mmol) of **19** in 10 ml of EtOH were submitted to a pressure of 15 psi of hydrogen in the presence of 30 mg of 10% Pd/C for 0.5 h then to 55 psi for 3 h. The catalyst was filtered off and the solvent evaporated in vacuo. The crude product was taken in DCM (5 ml) then Et_3N (0.1 ml, 2.2 equiv) was added at 0 °C followed by $(Boc)_2O$ (63 mg, 1.1 equiv) then the mixture was stirred at rt for 2 h. The reaction was quenched with HCl 1 N, the product was extracted with DCM, washed with $NaHCO_3$ sat. and dried on Na_2SO_4. The solvent was evaporated in vacuo and the crude product was purified by preparative TLC (Hex/AcOEt 3:1) to give 0.080 g (43%) of **20** as a colourless viscous oil. $[\alpha]_D$ +9.7 (*c* 1.53, $CHCl_3$); IR (neat, cm^{-1}, ν): 3444, 3379 (OH, NH), 1722, 1713 (C=O); ^1H NMR: 8.07–7.95 (m, 6H, arom. *ortho*); 7.56–7.48 (m, 3H, arom. *para*); 7.42–7.32 (m, 6H, arom. *meta*); 4.89–4.20 (m, 7H); 1.38 (s, 9H, *t*Bu Boc); 0.88 (s, 9H, Si*t*Bu); 0.06 (s, 3H, $SiCH_3$); 0.03 (s 3H, $SiCH_3$); ^{13}C NMR: 166.54, 166.51, 166.08 (C=O Bz); 155.58 (C=O Boc); 133.49, 133.30, (arom. *para*); 129.94; 129.86; 129.75 (arom. *meta*); 128.54, 128.42 (arom. *ortho*); 79.6 (quat. Boc); 73.30; 73.12 (C-1, C-8); 68.27, 66.72, 65.78 (C-2, C-4, C-3); 49.56 (C-5); 30.22 (C-6); 28.11 (*t*Bu, Boc); 27.32 (C-7); 25.55 (Si*t*Bu); 17.69 (Si*t*Bu quat.); −4.76, −5.11 ($SiCH_3$). Anal. Calcd for $C_{40}H_{53}NO_{10}Si$: C, 65.28; H, 7.26; N, 1.90. Found: C, 64.99; H, 7.29; N, 1.85.

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Synthesis of photolabile *o*-nitroveratryloxycarbonyl (NVOC) protected peptide nucleic acid monomers

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Abstract—The chemical synthesis of peptide nucleic acid (PNA) monomers was accomplished using various combinations of the *o*-nitroveratryloxycarbonyl (NVOC) group (*N*-aminoethylglycine backbone) and base labile acyl-type nucleobase protecting groups (anisoyl for adenine and cytosine; isobutyryl for guanine), thus offering a photolithographic solid-phase PNA synthetic strategy compatible with photolithographic oligonucleotide synthesis conditions and allowing the in situ synthesis of PNA microarrays in an essentially neutral medium, by avoiding the use of the commonly used deprotection reagents such as trifluoroacetic acid or piperidine. Convenient methods were also explored to prepare 1-(carboxymethyl)-4-*N*-(4-methoxybenzoyl)cytosine and 9-(carboxymethyl)-2-*N*-(isobutyryl)guanine with good yields.

1. Introduction

Peptide nucleic acids (PNAs) are DNA analogs in which the normal phosphodiester backbone is replaced by 2-aminoethyl glycine linkages.[1] PNAs recognize their complementary DNAs, RNAs or PNAs obeying the Watson–Crick base-pairing rules with a remarkably high specificity and affinity, and are resistant to nucleases and proteases. Accordingly, since their invention, there have been many reports on PNAs in such diverse fields as chemistry, biochemistry, biotechnology, and medicine.[2–5] More important is that surface-attached PNAs have been shown to retain the unique and efficient hybridization properties, which have been reported in solution studies of regular PNAs.[6] PNA recognition layers thus offer significant advantages over their DNA counterparts in the realm of sequence-specific DNA biosensors. These advantages include significantly higher sensitivity and specificity (including greater discrimination against single-base mismatches), faster hybridization at room and elevated temperatures, minimal dependence on ionic strength, and the use of shorter probes. Accordingly, the utilization of peptide nucleic acids as arrayed probe molecules could offer an alternative to DNA microarrays affording superior performance.[7–11]

In fact, PNA-arrays have come a long way since their initial discovery a few years ago.[12] As with DNA arrays, there are two approaches that can be used for the preparation of PNA arrays. In the first approach pre-fabricated PNA molecules are spotted onto an appropriate support medium. This method can produce PNA arrays of high quality if HPLC-purified molecules are used. Ole Brandt et al.[13] successfully employed a modified spotting method to prepare PNA microarrays for the hybridization of unlabelled DNA samples. However, PNA chemistry is not widely used and is therefore rather expensive. Additionally, the scale of the standard synthesis is about 2 μmol, which is much more than is needed for microarray production, thus increasing the cost. Also, the standard HPLC purification of many oligomers would be cost-intensive and time-consuming. As an alternative to the spotting method, PNAs can be synthesized in situ in a parallel manner on an appropriate substrate.[12,14–17] This procedure permits the simultaneous synthesis of many different molecules and has the advantage of consuming only very small amounts of the reagents per oligomer. Using this technique, PNA macroarrays have been developed on porous support media and silicon or glass slides using fluorenylmethoxycarbonyl (FMOC) chemistry. Porous membranes, however, have several inherent

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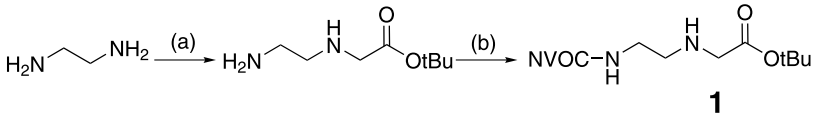

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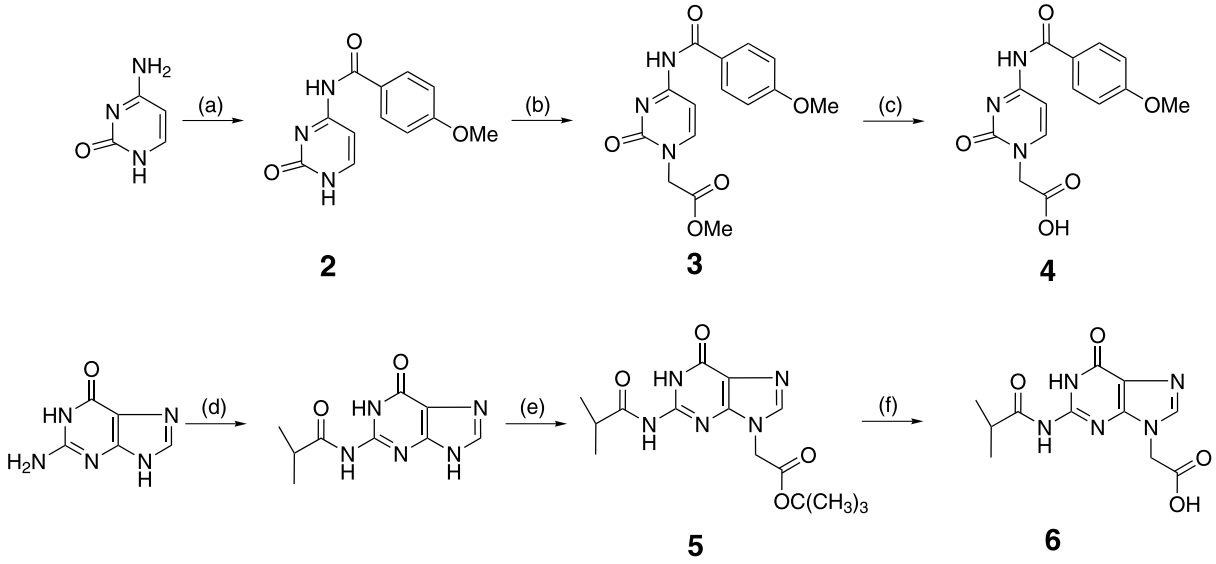
Scheme 1. Synthesis of *tert*-butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxy carbonyl)aminoethyl] glycinate. Reagents and conditions: (a) *tert*-butyl bromoacetate, dichloromethane; (b) *o*-nitroveratryloxycarbonyl chloride, DIEA.

disadvantages that are detrimental to their routine application. They lack mechanical stability, for example, which makes them difficult to handle. In addition, they are rather inflexible with respect to the mode of labeling and detection. As for the in situ synthesis on glass or silicon slides, only a few nanoliters of activated monomer are consumed per oligomer. However, the spotting device needs to be highly accurate, since the monomers have to be delivered to exactly the same slide coordinates many times over. Otherwise, a large percentage of truncated sequences will be created. Therefore, the in situ synthesis of high-density PNA microarrays will only be possible once appropriately adapted hardware or PNA chemistry becomes available, a problem which will most likely be addressed using photolithography. The combinatorial photolithographic process was first described by Fodor et al. for peptide array synthesis[18] and then successfully applied to the synthesis of DNA microarrays.[19,20] If we examine this issue from a different viewpoint, PNA chemistry is basically peptide chemistry combined with the simplicity of DNA chemistry. In other words, if appropriate PNA monomers can be synthesized, light-directed PNA array synthesis will become possible. Herein, we describe the efficient synthesis of PNA monomers, using *o*-nitroveratryloxycarbonyl (NVOC) (backbone)/acyl (anisoyl for cytosine and adenine; isobutyryl for guanine) protecting-group combinations, which are compatible with photolithographic solid phase PNA synthesis, DNA synthesis and the assembly of DNA–PNA chimeras.

2. Results and discussion

The chemical synthesis of PNA monomers relies on the assembly of the protected *N*-(2-aminoethyl)glycine backbone and protected nucleobase-substituted acetic acid structural units.[5] In the case of light-directed PNA synthesis, photolabile protecting groups are needed which are stable to the different chemical steps involved in usual peptide coupling chemistry, but which are photolytically cleaved with high efficiency by irradiation at a wavelength which does not damage the basic nucleosides structure. Hence, we chose *o*-nitroveratryloxycarbonyl (NVOC = 4,5-dimethoxy-2-nitrobenzyloxycarbonyl) as the photolabile protecting group, because of its established use in peptide synthesis and its *o*-nitrobenzyl moiety which is generally removed by irradiation at wavelengths >300 nm without damaging the nucleic acids.[18,21,22] The key intermediate in our synthetic route to the PNA building blocks is *tert*-butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]glycinate (**1**), which was synthesized according to the route outlined in Scheme 1. *tert*-Butyl *N*-(2-aminoethyl)glycinate was obtained according to the method previously reported in the literature.[23] The selective reaction of the primary amine of *tert*-butyl *N*-(2-aminoethyl)glycinate with *o*-nitroveratryloxycarbonyl chloride gave the oily product **1** after purification with flash chromatography.

The nucleobases have exocyclic amino functions, which can be protected by base-labile acyl protecting groups. Protection at these sites not only blocks any undesirable side reactions, but also increases the solubility of the monomers, as well as their compatibility with the mild conditions used for DNA synthesis.[24–28] 1-*N*-Carboxymethylthymine was synthesized according to the procedure of Kosynkina et al.[29] 9-(Carboxymethyl)-6-*N*-(4-methoxybenzoyl)adenine was synthesized as described by Uhlmann



Scheme 2. Synthesis of acyl-protected carboxymethyl nucleobases. Reagents and conditions: (a) anisoyl chloride, pyridine; (b) methyl bromoacetate, NaH, DMF; (c) NaOH, H_2O/dioxane; (d) isobutyric acid anhydride, DMF; (e) *tert*-butyl bromoacetate, NaH, DMF; (f) TFA, triethylsilane dichloromethane.

et al.[24,25] 1-(Carboxymethyl)-4-*N*-(4-methoxybenzoyl)cytosine (**4**) was synthesized as described by Timár and Uhlmann et al. with a modification designed to improve the yield.[25,27] As shown in Scheme 2, cytosine was acylated with anisoyl chloride in pyridine to give **2** with high yield. Compound **2** was then converted to its sodium salt using NaH in DMF and alkylated using methyl bromoacetate. The resulting methyl ester **3** was saponified using NaOH in a dioxane–water mixture. The desired product **4** was isolated by pH dependent precipitation with $KHSO_4$ solution. The synthesis of 9-(carboxymethyl)-2-*N*-(isobutyryl)guanine was relatively difficult, because the substitution of guanine is notorious for giving *N*9/*N*7 regioisomers requiring a labor-consuming separation process. Fortunately, it was found that lowering the temperature favored the formation of the desired *N*9-regioisomer.[25,27] 2-*N*-Isobutyrylguanine was synthesized according to the procedure of Jenny et al.[30] This was then alkylated using NaH/*tert*-butyl bromoacetate at low temperature to give the desired *N*9-alkylated derivative **5** with good yield along with other regioisomers,[27] which could be separated through simple recrystallization from ethyl acetate. Removal of the *tert*-butyl ester using TFA in dichloromethane in the presence of triethylsilane gave acid **6** in quantitative yield.

The general synthetic routes toward the monomers are outlined in Scheme 3. The carboxymethylated nucleobases were coupled to the *tert*-butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]glycinate backbone unit **1** using several standard methods. The simplest and most effective reagents were found to be propylphosphonic anhydride for the pyrimidines and BOP reagent/HOBT for the purines. As shown in Scheme 3, coupling of the base acetic acid to the backbone was followed by removal of the *tert*-butyl ester under standard conditions.

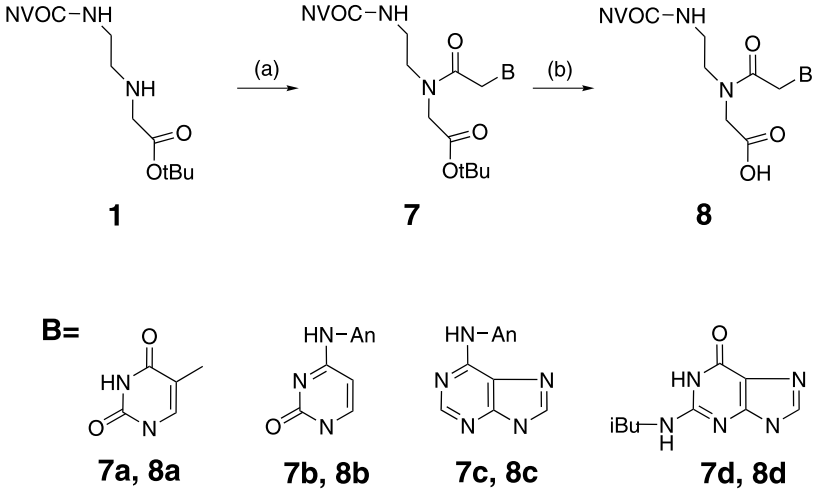
In conclusion, in this study we described the synthetic route to photolabile NVOC protected PNA monomers. Further studies relating to the use of the above monomers in the preparation of PNA oligomers using the Micro Array Mirror system developed in our laboratory[31–33] and our quest to synthesize the PNA backbones in high yield are in progress and will be reported on in due course.

3. Experimental

3.1. General

The following abbreviations are employed: *N*,*N*-dimethylformamide (DMF); *o*-nitroveratryloxycarbonyl (NVOC); 4-methoxybenzoyl (An); isobutyryl (iBu); trifluoroacetic acid (TFA); *N*,*N*-diisopropylethylamine (DIEA); triethylamine (TEA); *O*-benzotriazol-l-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (BOP reagent); 1-hydroxybenzotri-azole (HOBT). All reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Reactions involving air- and/or moisture-sensitive reagents were conducted under an atmosphere of dry nitrogen. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM). Melting points were obtained with electrothermal 9300 apparatus without correction. ^{1}H and ^{13}C NMR spectra were obtained in the solvents indicated at 300 and 75 MHz, respectively. Chemical shifts are reported in parts per million downfield relative to the internal standard, tetramethylsilane. Coupling constants are reported in hertz (Hz). Spectral patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Mass spectra were recorded using fast atom bombardment (FAB). Elemental analyses were recorded using Perkin–Elmer CHN analyzer model 2400.

3.1.1. *tert*-Butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]glycinate (1). To a solution of *tert*-butyl *N*-(2-aminoethyl)glycinate[23] (1.75 g, 10.0 m mol) in dichloromethane (100 ml) was added DIEA (1.69 ml, 9.5 mmol) with mechanical stirring. A solution of 4,5-dimethoxy-2-nitrobenzylchloroformate (2.62 g, 9.5 mmol) in dichloromethane (10 ml) was added dropwise at 0 °C over a period of 2 h. The resulting solution was allowed to warm to room temperature and stirring was continued for another 2 h at this temperature. The reaction mixture was then washed sequentially with 1 M aq. HC1, sat. aq. $NaHCO_3$ (3×20 ml) and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography eluting with 10% methanol in ethyl acetate gave **1** as a green oily product with a yield of 1.51 g (38%). R_f=0.37 (ethyl



Scheme 3. Synthesis of NVOC-protected PNA monomers. Reagents and conditions: (a) B–CH_2–COOH + propylphosphonic anhydride for **7a** and **7b**; B–CH_2–COOH + BOP reagent, HOBT, DIEA for **7c** and **7d**; (b) TFA, dichloromethane.

acetate/methanol, 9:1); MS (FAB): 414.1872 ($C_{18}H_{27}N_3O_8$+H requires 414.1876); ^{1}H NMR ($CDCl_3$) δ 7.71 (s, 1H), 7.04 (s, 1H), 5.52 (s, 2H), 3.99 (s, 3H), 3.96 (s, 3H), 3.32 (t, J=5.5 Hz, 2H), 3.28 (s, 2H), 2.78 (t, J=5.6 Hz, 2H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.6, 155.9, 153.3, 147.7, 139.4, 128.3, 109.8, 107.9, 81.2, 63.2, 56.2, 51.0, 48.4, 40.6, 27.9.

3.1.2. 4-*N*-(4-Methoxybenzoyl)cytosine (2). To a suspension of cytosine (2.5 g, 22.5 mmol) in pyridine (100 ml) was added 4-methoxybenzoyl chloride (5.76 g, 33.8 mmol) at room temperature. The reaction mixture was stirred in an oil-bath at 80 °C. The cytosine rapidly dissolved and then the product precipitated from the solution. After 2 h, the precipitate was filtered off, washed with methanol and dried in vacuo to afford **2** as a white powder with a yield of 4.5 g (81%). ^{1}H NMR (d_6-DMSO) δ 8.00 (d, J=8.9 Hz, 2H), 7.84 (d, J=7.0 Hz, 1H), 7.22 (s, br, 1H), 7.02 (d, J=8.9 Hz, 2H), 3.84(s, 3H). This compound was previously obtained by Timár et al., but without detailed NMR characterization.[27]

3.1.3. 4-*N*-(4-Methoxybenzoyl)-1-(methoxycarbonylmethyl)cytosine (3). To a suspension of **2** (3.68 g, 15.0 mmol) in dry DMF (300 ml) was added NaH (0.36 g; 15.0 mmol) at 0 °C and the mixture was stirred for 1 h. Methyl bromoacetate (2.32 g; 15.2 mmol, in 5 ml of dichloromethane) was added dropwise at 0 °C using a syringe with stirring over a period of 30 min. The resulting solution was then allowed to warm to room temperature. Stirring was continued for 1.5 h at room temperature, and methanol (10 ml) was then added. The solvent was removed in vacuo, and the residue was partitioned between dichloromethane and water. The organic phase was washed with water, dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting crude product was recrystallized from methanol (200 ml) to give **3** as a white needle-like crystalline solid with a yield of 4.1 g (86%). Mp=218–220 °C; MS (FAB): 318.1098 ($C_{15}H_{15}N_3O_5$+H requires 318.1098); R_f=0.56 (ethyl acetate/methanol, 9:1); ^{1}H NMR (d_6-DMSO) δ 11.12 (s, 1H), 8.08 (d, J=7.3 Hz, 1H), 8.00 (d, J=9.0 Hz, 2H), 7.32 (d, J=7.0 Hz, 1H), 7.02 (d, J=9.0 Hz, 2H), 4.66 (s, 2H), 3.77 (s, 3H), 3.54 (s, 3H); ^{13}C NMR (d_6-DMSO) δ 168.5, 166.5, 164.0, 162.9, 155.3, 150.4, 130.7, 125.1, 113.7, 96.2, 55.5, 52.3, 50.7. Anal. Calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24. Found: C, 56.58; H, 4.82; N, 13.12.

3.1.4. 1-(Carboxymethyl)-4-*N*-(4-methoxybenzoyl)cytosine (4). To a solution of **3** (3.62 g, 24 mmol) in a mixture of dioxane (50 ml) and water (25 ml) was added a 2 M aq. NaOH solution dropwise with stirring at room temperature (pH 11–12). When hydrolysis of the methyl ester was complete (traced by TLC), the pH of the reaction solution was adjusted to **3** using 2 M $KHSO_4$ solution. The precipitate which separated out was filtered off. This crude product was dissolved in aq. $NaHCO_3$ solution and reprecipitated by adding 2 M $KHSO_4$ solution. The product was filtered off, washed with a small amount of water and dried in vacuo to give the product as a white solid with a yield of 3.31 g (95%). R_f=0.52 (*n*-butanol/acetic acid/H_2O, 3:1:1); ^{1}H NMR (d_6-DMSO) δ 8.09 (d, J=7.3 Hz, 1H), 8.02 (d, J=8.8 Hz, 2H), 7.31 (d, J=7.5Hz, 1H), 7.03 (d, J=8.6 Hz, 2H), 4.47 (s, 2H), 3.85(s, 3H). These values were well matched with those of a previous report.[27]

3.1.5. 2-*N*-(Isobutyryl)-9-(*tert*-butyloxycarbonylmethyl) guanine (5). To a suspension of 2-*N*-isobutyrylguanine[30] (6.63 g, 30.0 mmol) in anhydrous DMF (250 ml) was added NaH (0.72 g, 30.0 mmol) at 0 °C. The mixture was stirred for 1 h and then *tert*-butyl bromoacetate (6.44 g, 33.0 mmol) was added dropwise over a period of 30 min at 0 °C. The reaction was stopped after 2 h by the addition of a small amount of solid CO_2 and methanol (5 ml). The reaction mixture was evaporated in vacuo and the residue was treated with dichloromethane and water. The water phase was re-extracted with dichloromethane. The dichloromethane fractions were combined and concentrated in vacuo to give the crude product. The resulting crude product was recrystallized from ethyl acetate to give the desired *N*9-isomer product **5** with a yield of 6.46 g (64%). Mp= 204–205 °C (decomp.); R_f=0.13 (dichloromethane/methanol, 9.5:0.5); ^{1}H NMR ($CDCl_3$) δ 12.06 (s, 1H), 8.87 (s, 1H), 7.88 (s, 1H), 4.72 (s, 2H), 2.76 (m, 1H); 1.28 (d, J=6.8 Hz, 6H). Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.88. Found: C, 53.88; H, 6.47; N, 20.75. These values were well matched with those of a previous report.[27]

3.1.6. 9-(Carboxymethyl)-2-*N*-(isobutyryl)guanine (6). To a suspension of **5** (2.0 g, 6 mmol) in anhydrous dichloromethane (10 ml) was added triethylsilane (4.8 ml, 30 mmol). The mixture was cooled to 0 °C and TFA (15 ml) was then added over a period of 5 min. After 30 min the reaction was allowed to warm to room temperature. Upon completion of the reaction (traced by TLC), the mixture was concentrated in vacuo to give a foam which was stirred with diethyl ether, filtered, washed with diethyl ether, and dried in vacuo to afford the desired product **6** with a yield of 1.67 g (100%). R_f=0.44 (*n*-butanol/acetic acid/H_2O, 3:1:1); ^{1}H NMR (d_6-DMSO) δ 12.07 (s, 1H), 11.69 (s, 1H), 7.94 (s, 1H), 4.87 (s, 2H), 2.75 (q, 1H), 1.11 (d, J=6.8 Hz, 6H). The NMR values were well matched with those of a previous report.[24]

3.1.7. *tert*-Butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-[(thymin-l-yl)acetyl]glycinate (7a). To a solution of **1** (4.13 g, 10.0 mmol) in anhydrous DMF (50 ml) was added the acid 1-*N*-carboxymethylthymine[29] (1.84 g, 10.0 mmol), and the mixture was stirred until most of the acid was dissolved. Propylphosphonic anhydride (50% solution in ethyl acetate) (10.5 ml; 10.0 mmol) and DIEA (3.44 ml; 20 mmol) were added at room temperature. The mixture was stirred at room temperature for 2 h and then poured into a stirred mixture of ice water (200 ml) and sat. $NaHCO_3$ solution (15 ml). The mixture was shaken vigorously for several minutes, which led to the precipitation of fine pale yellow solids. The solids were collected by filtration, stirred with water (15 ml), filtered, washed with cold water, and dried in a vacuum. Purification by flash chromatography eluting with 10% methanol in $CHCl_3$ gave **7a** in the form of pale green solids with a yield of 5.68 g (98%). R_f=0.50 (ethyl acetate/methanol, 9:1); MS (FAB): 580.2251 ($C_{25}H_{33}N_5O_{11}$+H requires 580.2254); ^{1}H NMR ($CDCl_3$) (two rotamers) δ 8.00 (s, 1H), 7.97 (s, 1H), 7.70 (s, 1H), 7.04 and 7.02 (rotamer, 2×s, 1H), 7.00 (s, 1H), 5.49 (s, 2H), 4.52 and 4.39 (rotamer,

2×s, 2H), 4.09 and 3.99 (rotamer, 2×s, 2H), 3.98 (s, 3H), 3.96 (s, 3H), 3.57 (t, J=6.0 Hz, 2H), 3.41 (t, J=6.8 Hz, 2H), 1.92 and 1.91 (rotamer, 2×s, 3H), 1.50 and 1.46 (rotamer, 2×s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.8, 168.5, 168.1, 167.3, 164.1, 156.2, 156.1, 153.5, 153.4, 151.1, 151.0, 148.4, 148.0, 141.2, 140.9, 140.1, 139.7, 128.1, 127.1, 111.4, 110.8, 110.5, 110.2, 108.2, 108.1, 83.7, 82.7, 64.1, 63.6, 56.5, 56.4, 51.1, 49.6, 48.7, 47.8, 39.2, 39.1, 28.0, 27.9, 12.3.

3.1.8. *N*-[2-*N*-(4,5-Dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-[(thymin-1-yl)acetyl]glycine (8a). To a suspension of **7a** (10 mmol) in dichloromethane (30 ml) was added TFA (45 ml). A clear solution was formed which was stirred at room temperature for 2 h (traced by TLC). The reaction mixture was dried in a vacuum and residual TFA was removed by co-evaporation twice with toluene. The residue was triturated with diethyl ether and filtered. Purification by recrystallization from acetone gave **8a** as pale green solids with a yield of 5.68 g (93%). Mp=182 °C (decomp.); R_f=0.52 (*n*-butanol/acetic acid/H_2O, 3:1:1); MS (FAB): 524.1631 ($C_{21}H_{25}N_5O_{11}$+H requires 524.1628); ^{1}H NMR (d_6-DMSO) (two rotamers) δ 11.28 and 11.27 (rotamer, 2×s, 1H), 8.0 (br, 1H), 7.92 (s, 1H), 7.70 (s, 1H), 7.30 and 7.26 (rotamer, 2×s, 1H), 7.20 (s, 1H), 5.35 and 5.33 (rotamer, 2×s, 2H), 4.65 and 4.47 (rotamer, 2×s, 2H), 4.18 and 3.99 (rotamer, 2×s, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.46–2.99 (br, CH_2+H_2O), 1.73 (s, 3H); ^{13}C NMR (d_6-DMSO) δ 171.1, 170.7, 167.9, 167.4, 164.6, 156.1, 155.9, 153.5, 153.4, 151.1, 147.9, 147.8, 142.2, 142.1, 139.4, 139.3, 128.0, 127.6, 110.9, 110.5, 108.4, 108.3, 108.1, 62.8, 62.6, 56.3, 56.1, 49.4, 48.7, 47.9, 47.1, 38.8, 38.1, 12.0. Anal. Calcd for $C_{21}H_{25}N_5O_{11}$: C, 48.19; H, 4.81; N, 13.38. Found: C, 48.29; H, 4.78; N, 13.24.

3.1.9. *tert*-Butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-{[4-*N*-(4-methoxybenzoyl)-cytosin-1-yl]acetyl}glycinate (7b). To a solution of **1** (4.13 g, 10.0 mmol) in ethyl acetate (50 ml) were added the acid **4** (3.33 g, 11.0 mmol) and TEA (2.77 ml; 20.0 mmol). Propylphosphonic anhydride (50% solution in ethyl acetate) (9.55 ml; 15.0 mmol) was added at room temperature. The mixture was adjusted to pH 8 by the addition of TEA and stirred for a further 1 h, after which the mixture was evaporated in vacuo. The residue was taken up in dichloromethane (100 ml), and the resulting solution was washed with water (3×20 ml). The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. Purification by flash chromatography eluting with 5% methanol in dichloromethane gave **7b** in the form of pale green solids with a yield of 6.09 g (87%). R_f=0.50 (ethyl acetate/methanol, 9:1); MS (FAB): 699.2619 ($C_{32}H_{38}N_6O_{12}$+H requires 699.2625); ^{1}H NMR ($CDCl_3$) (two rotamers) δ 7.85 (d, J=8.4 Hz, 2H), 7.71 (s, 1H), 7.69(s, 1H), 7.61 and 7.58 (rotamer, 2×s, 1H), 7.10 and 7.04 (rotamer, 2×s, 1H), 6.98(d, J=8.8 Hz, 2H), 5.51 and 5.50 (rotamer, 2×s, 2H), 4.75 and 4.46 (rotamer, 2×s, 2H), 4.24 and 4.02 (rotamer, 2×s, 2H), 3.99 and 3.98 (rotamer, 2×s, 3H), 3.95 and 3.92 (rotamer, 2×s, 3H), 3.89 (s, 3H), 3.66 and 3.60 (rotamer, 2×t, J=6.0, 6.3 Hz, 2H), 3.48 and 3.41 (rotamer, 2×t, J=6.0, 6.1 Hz, 2H), 1.51 and 1.45 (rotamer, 2×s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.6, 168.5, 168.0, 167.1, 163.4, 162.9, 156.2, 156.1, 155.6, 153.6, 153.5, 150.2, 148.1, 147.8, 139.7, 139.5, 129.8, 128.4, 127.6, 125.0, 114.1, 114.0, 110.8, 110.0, 108.0, 107.9, 96.6, 83.5, 82.4, 63.9, 63.4, 56.6, 56.5, 56.3, 56.2, 55.5, 51.4, 49.7, 49.4, 48.8, 48.6, 39.1, 39.0, 27.9.

3.1.10. *N*-[2-*N*-(4,5-Dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-{[4-*N*-(4-methoxybenzoyl)-cytosin-1-yl]acetyl}glycine (8b). To a suspension of **7b** (6.98 g, 10 mmol) in dichloromethane (30 ml) was added TFA (30 ml). A clear solution was formed which was stirred at room temperature for 3 h (traced by TLC). The reaction mixture was dried in vacuo and residual TFA was removed by co-evaporation twice with toluene. The residue was triturated with diethyl ether and filtered. Purification by recrystallization from acetone gave **8b** as pale green solids with a yield of 5.91 g (92%). Mp=204 °C (decomp.); R_f=0.69 (*n*-butanol/acetic acid/H_2O, 3:1:1); MS (FAB): 665.1991 ($C_{28}H_{30}N_6O_{12}$+H requires 643.1999); ^{1}H NMR (d_6-DMSO) (two rotamers) δ 8.00(d, J=8.8 Hz, 2H), 7.93 and 7.60 (rotamer, 2×d, J=7.7, 5.5 Hz, 1H), 7.69 and 7.55 (rotamer, 2×s, 2H), 7.43 and 7.27 (rotamer, 2×d, J=5.9, 6.9 Hz, 1H), 7.21 and 7.17 (rotamer, 2×s, 2H), 7.01(d, J=9.0 Hz, 2H), 5.35 and 5.32 (rotamer 2×s, 2H), 4.86 and 4.68 (rotamer s, 2H), 4.27 and 4.01 (rotamer s, 2H) 3.89 and 3.88 (rotamer s, 3H), 3.85 and 3.84 (rotamer s, 3H), 3.83 (s, 3H), 3.48–3.13 (br, CH_2+H_2O); ^{13}C NMR (d_6-DMSO) δ 170.9, 170.5, 167.6, 167.1, 163.5, 162.8, 156.0, 155.8, 155.2, 153.4, 153.3, 150.9, 147.7, 147.6, 139.3, 139.2, 130.6, 127.9, 127.6, 125.2, 113.7, 110.8, 110.4, 108.0, 95.8, 62.7, 62.5, 56.2, 56.0, 55.5, 50.9, 49.6, 49.1, 47.7, 46.9, 38.7, 38.0. Anal. Calcd for $C_{28}H_{30}N_6O_{12}$: C, 52.34; H, 4.71; N, 13.08. Found: C, 52.46; H, 4.77; N, 13.05.

3.1.11. *tert*-Butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-{[6-*N*-(4-methoxybenzoyl) adenin-9-yl]acetyl}glycinate (7c). To a solution of **1** (4.13 g, 10 mmol) in anhydrous DMF (50 ml) were added 9-*N*-(carbonylmethyl)-6-*N*-(4-methoxybenzoyl)adenine[24,25] (3.27 g, 10 mmol), BOP reagent (6.62 g, 15 mmol), HOBT (2.02 g, 15 mmol) and DIEA (4.30 ml, 26 mmol). After stirring at room temperature for 5 h (traced by TLC), the mixture was concentrated in vacuo and the residue was partitioned between ethyl acetate (100 ml) and half saturated brine (100 ml), followed by extraction of the aqueous phase with ethyl acetate (3×100 ml). The combined organic extract was washed with 1 M aq. HC1 (2×50ml), sat. aq. $NaHCO_3$ (2×50 ml), brine (1×50 ml), and then dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography eluting with 5% methanol in dichloromethane gave **7c** in the form of pale green solids with a yield of 6.29 g (87%). R_f=0.31 (ethyl acetate/methanol, 9:1); MS (FAB): 723.2735 ($C_{33}H_{38}N_8O_{11}$+H requires 723.2738); ^{1}H NMR ($CDCl_3$) (two rotamers) δ 8.90(s, 1H), 8.71 and 8.69 (rotamer, 2×s, 1H), 8.14 and 8.13 (rotamer, 2×s, 1H), 7.98 (d, J=8.6 Hz, 2H), 7.69 and 7.67 (rotamer, 2×s, 1H), 7.02 and 7.01 (rotamer, 2×s, 1H), 6.98 (d, J=2.4 Hz, 2H), 5.51 and 5.47 (rotamer, 2×s, 2H), 5.16 and 5.02 (rotamer, 2×s, 2H), 4.22 and 3.98 (rotamer, 2×s, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.70 and 3.60 (rotamer, 2×t, J=6.0, 6.1 Hz, 2H), 3.50 and 3.40 (rotamer, 2×t, J=5.5, 5.7 Hz, 2H), 1.47 and 1.45 (rotamer, 2×s, 6H). ^{13}C NMR ($CDCl_3$) δ 168.6, 168.2, 167.1, 166.4, 164.2, 164.1, 163.2, 163.1, 156.3, 156.1,

153.5, 153.4, 152.4, 152.3, 151.9, 151.8, 149.5, 149.4, 148.2, 147.9, 144.2, 144.0, 139.9, 139.5, 130.0, 129.9, 128.0, 127.1, 125.8, 125.7, 122.0, 121.9, 114.0, 113.9, 110.95, 110.8, 108.2, 108.0, 83.9, 82.7, 64.1, 63.6, 56.4, 56.3, 55.5, 51.0, 50.7, 48.8, 48.6, 39.2, 38.9, 34.6, 34.5, 27.9, 27.8.

3.1.12. *N*-[2-*N*-(4,5-Dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-{[6-*N*-(4-methoxybenzoyl)adenin-9-yl]acetyl}glycine (8c). To a suspension of **7c** (7.22 g, 10 mmol) in dichloromethane (30 ml) was added TFA (30 ml). A clear solution was formed which was stirred at room temperature for 2.5 h (traced by TLC). The reaction mixture was dried in a vacuum and residual TFA was removed by co-evaporation twice with toluene. The residue was triturated with diethyl ether and filtered. Purification by recrystallization from methanol gave **8c** as pale green solids with a yield of 6.33 g (95%). Mp = 200 °C (decomp.); R_f = 0.49 (*n*-butanol/acetic acid/H_2O, 3:1:1); MS (FAB): 667.2106 ($C_{29}H_{30}N_8O_{11}$ + H requires 667.2112); ^{1}H NMR (d_6-DMSO) (two rotamers) δ 11.0 (s, 1H), 8.65 and 8.62 (rotamer, 2×s, 1H), 8.31 (s, 1H), 8.04 (d, J = 8.8 Hz, 2H), 7.68 (s, 1H), 7.21 and 7.17 (rotamer, 2×s, 1H), 7.06 (*d*, J = 8.6 Hz, 2H), 5.39 and 5.35 (rotamer, 2×s, 2H), 5.15 (s, 2H), 3.97–3.84 (m, 11H), 3.41–3.17 (br, $CH_2 + H_2O$). ^{13}C NMR (d_6-DMSO) δ 170.9, 170.5, 167.1, 165.2, 162.7, 156.2, 155.9, 153.4, 153.3, 152.7, 151.5, 150.2, 147.8, 147.7, 145.4, 139.5, 139.3, 130.7, 127.9, 127.5, 125.6, 124.5, 113.7, 110.9, 110.5, 108.1, 62.8, 62.5, 56.2, 56.1, 55.5, 50.0, 49.2, 47.8, 47.0, 44.2, 44.0, 35.7. Anal. Calcd for $C_{29}H_{30}N_8O_{11}$: C, 52.25; H, 4.54; N, 16.81. Found: C, 52.28; H, 4.59; N, 16.79.

3.1.13. *tert*-Butyl *N*-[2-*N*-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-{[2-*N*-(isobutyryl)guanin-9-yl]acetyl}glycinate (7d). To a solution of **1** (4.13 g, 10 mmol) and **6** (3.27 g, 10 mmol) in anhydrous DMF (50 ml) were added BOP reagent (6.62 g, 12.5 mmol), HOBT (2.02 g, 12.5 mmol) and DIEA (4.30 ml, 15.0 mmol). After stirring at room temperature for 4.5 h (traced by TLC), the mixture was concentrated in vacuo and the residue was partitioned between dichloromethane (150 ml) and half saturated brine (150 ml), and the aqueous phase was extracted with ethyl acetate (3×100 ml). The combined organic extract was washed with 1 M aq. HC1 (2×100 ml), sat. aq. $NaHCO_3$ (2×100 ml), brine (1×100 ml), and then dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography eluting with 10% methanol in dichloromethane gave **2d** in the form of pale green solids with a yield of 5.46 g (81%). R_f = 0.11 (ethyl acetate/methanol, 9:1); MS (FAB): 675.2736 ($C_{29}H_{38}N_8O_{11}$ + H requires 675.2738); ^{1}H NMR ($CDCl_3$) (two rotamers) δ 12.11 and 12.05 (rotamer, 2×s, 1H), 7.96 and 7.84 (rotamer, 2×s, 1H), 7.66 and 7.63 (rotamer, 2×s, 1H), 7.07 and 6.97 (rotamer, 2×s, 1H), 5.53 and 5.48 (rotamer, 2×s, 2H), 5.01 and 4.98 (rotamer, 2×s, 2H), 4.02 and 3.97 (rotamer, 2×s, 2H), 3.94 (s, br, 6H should be two peaks but shielded by itself), 3.64 (m, 2H), 3.56 and 3.48 (rotamer, 2×t, J = 6.5, 7.0 Hz, 2H), 2.66 (rotamer, 2×q, J = 7.7, 7.0 Hz, 1H), 1.49 and 1.42 (rotamer, 2×s, 9H), 1.24 and 1.21 (rotamer, 2×d, J = 3.1, 3.1 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 179.5, 179.4, 168.6, 168.3, 167.3, 166.6, 156.6, 156.5, 155.5, 155.4, 153.7, 153.3, 148.8, 148.7, 148.2, 148.1, 140.6, 140.1, 139.9, 127.6, 127.5, 119.4, 119.3, 111.9, 110.5, 108.2, 108.1, 82.8, 82.6, 65.0, 64.0, 56.5, 56.4, 49.6, 49.5, 48.6, 48.3, 39.5, 39.3, 35.1, 34.9, 30.0, 29.7, 28.0, 27.9, 18.9, 18.8.

3.1.14. *N*-[2-*N*-(4, 5-Dimethoxy-2-nitrobenzyloxycarbonyl)aminoethyl]-*N*-{[2-*N*-(isobutyryl)guanin-9-yl]acetyl}-glycine (8d). To a suspension of **7d** (6.74 g, 10 mmol) in dichloromethane (20 ml) was added TFA (30 ml). A clear solution was formed which was stirred at room temperature for 3.5 h (traced by TLC). The reaction mixture was dried in a vacuum and residual TFA was removed by co-evaporation twice with toluene. The residue was triturated with diethyl ether and filtered. Purification by recrystallization from methanol gave **8d** as pale green solids with a yield of 5.57 g (90%). Mp = 227 °C (decomp.); R_f = 0.46 (*n*-butanol/acetic acid/H_2O, 3:1:1) MS (FAB), *m/z* 619.2110 ($C_{25}H_{30}N_8O_{11}$ + H requires 619.2112), ^{1}H NMR (d_6-DMSO) (two rotamers) δ 11.69 and 11.62 (rotamer, 2×s, 1H), 7.81 (s, 1H), 7.67 and 7.64 (rotamer, 2×s, 1H), 7.19 and 7.13 (rotamer, 2×s, 1H), 5.34 and 5.31 (rotamer, 2×s, 2H), 5.11 and 4.94 (rotamer, 2×s, 2H), 4.01 and 3.84 (rotamer, 2×s, 2H), 3.88 and 3.85 (2×s, 6H), 3.51 and 3.34 and 3.17 (3×br, $CH_2 + H_2O$), 2.74 (m, 1H), 1.08 (d, J = 6.8 Hz, 6H). ^{13}C NMR (d_6-DMSO) δ 180.2, 180.1, 171.1, 170.6, 167.1, 166.5, 156.1, 155.8, 155.0, 154.9, 153.4, 153.3, 149.3, 148.0, 147.9, 147.7, 140.6, 139.5, 139.2, 127.8, 127.3, 119.6, 111.1, 110.5, 108.1, 108.0, 62.8, 62.7, 56.2, 56.1, 49.6, 49.5, 47.1, 46.9, 44.1, 44.0, 34.8, 34.7, 31.0, 30.9, 18.9, 18.8. Anal. Calcd for $C_{25}H_{30}N_8O_{11}$: C, 48.54; H, 4.89; N, 18.12. Found: C, 48.58; H, 4.81; N, 18.08.

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Hydrogen-bonding-induced oligoanthranilamide foldamers. Synthesis, characterization, and complexation for aliphatic ammonium ions

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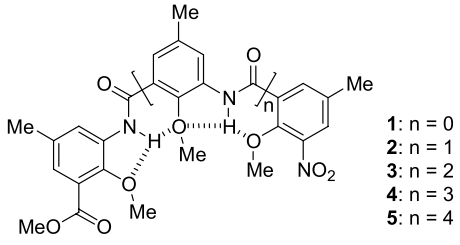
Abstract—The self-assembly of a novel series of intramolecular hydrogen bonding-driven foldamers have been described. Five linear aromatic amide oligomers **1**–**5**, which bear two to six repeating benzoyl amide subunits, respectively, have been prepared by continuous amide-coupling reactions. The existence of three-centered hydrogen bonds in the oligomers and consequently, the folding conformation of the oligomers in the solid state and solution have been proved by the X-ray analysis (for **2**) and the ^{1}H NMR and IR experiments. Molecular modeling reveals a planar and rigid conformation for the oligomers and a cavity of 0.86 nm in diameter for 6-mer **5**. Fluorescent and ^{1}H NMR experiments have demonstrated that the new aromatic oligo-amide foldamers can bind primary and secondary alkyl ammonium ions in chloroform and the associated binding constants have been determined. It is revealed that 5-mer **4** exhibits the largest binding ability. A face-to-face binding mode has been proposed for the complexes.

1. Introduction

Since, Perdesen's discovery of the complexing affinity of 18-crown-6 and analogs for alkaline ions in 1967,[1] a large number of crown ethers, cryptands and related macrocycles have been prepared for complexing discrete metal or ammonium ions.[2] Although early investigations have revealed that flexible oligo(ethylene glycols) with two terminal aromatic donors might also complex a cation by wrapping themselves around the guest, the negative entropy produced by the reduced conformational flexibility of the linear molecules upon complexation usually greatly decreases the binding ability of linear molecules.[3,4] Covalent bonded rigid folding or crescent receptors represent a new series of non-ring receptors for molecular recognition.[5] Nevertheless, the syntheses of these artificial receptors are usually time-consuming and over-rigidified shapes also make them generally lack the conformational adaptability to meet the specific features of discrete guests.



In the last decade, there has been increasing interest in utilizing hydrogen bonding to control the folding or helical conformation of unnatural organic molecules.[6] Recently, it has been revealed that several hydrogen bonding and hydrophobic interaction-driven folding molecules can promote or even catalyze oxidation or alkylation reaction of the pyridine nitrogen.[7] Previously, we had reported that intramolecular hydrogen bonding could be utilized to facilitate the self-assembly of a new series of metallomacrocycles from rigid aromatic oligoamides.[8] We also found that hydrogen bonding-driven aromatic oligohydrazide foldamers exhibit remarkably strong complexing ability for saccharide derivatives in chloroform.[9,10] The folding architectures represent a new generation of non-ring synthetic receptors, which possess recognizing ability comparable to those of cyclophane receptors.[11] In this paper, we report the synthesis and characterization of a new

Keywords: Hydrogen bonding; Foldamer; Aromatic amide; Molecular recognition; Alkyl ammonium ion.
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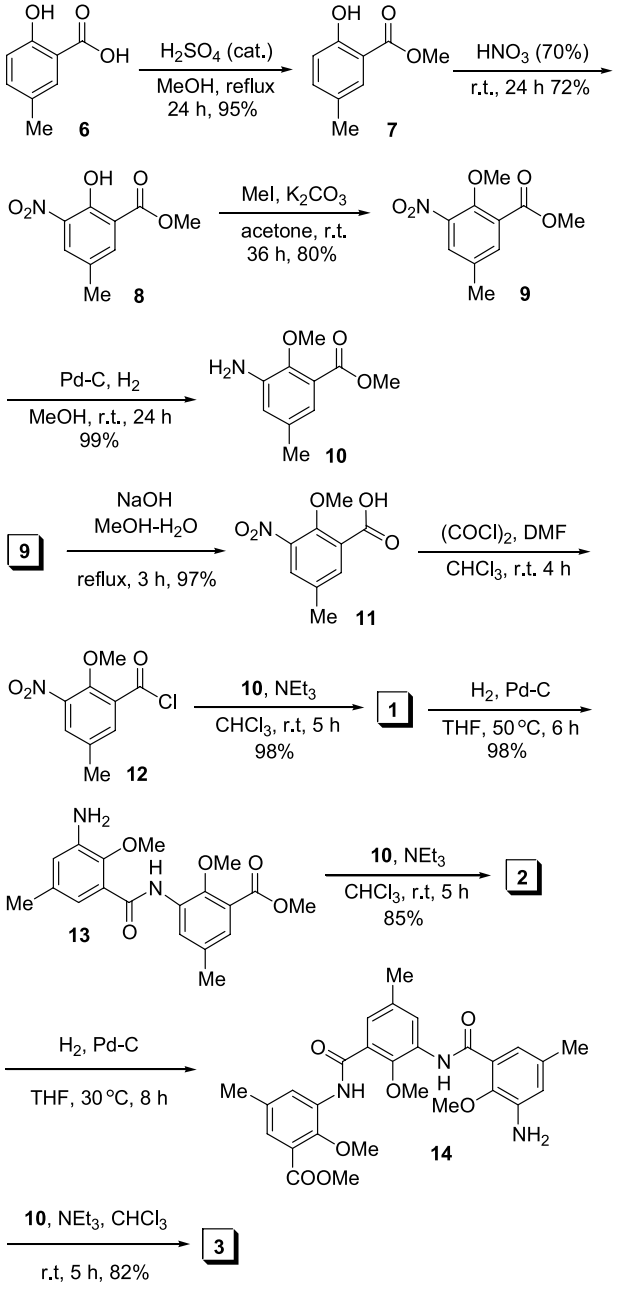
series of hydrogen bonded aromatic oligoamide foldamers **1**–**5** and their complexing behavior toward aliphatic ammonium ions in chloroform.

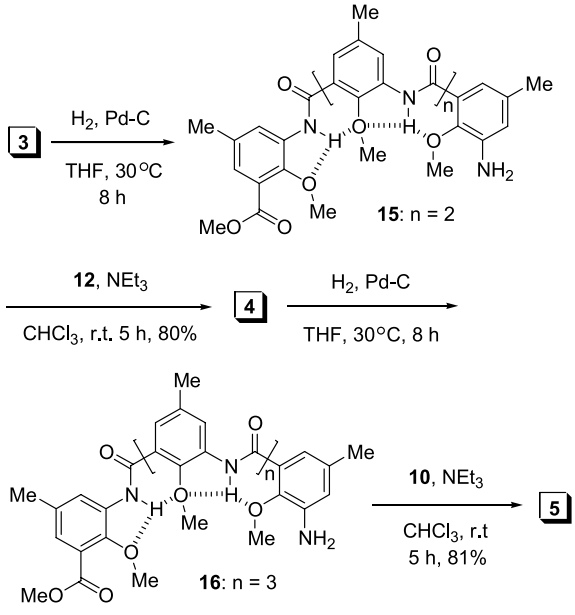
2. Results and discussion

Five aromatic oligoamides **1**–**5** have been designed, which contain two to six benzene rings. Previous studies have revealed that 2-methoxy-*N*-(2-methoxyphenyl)benzamide, the repeating subunit of the present oligomers, adopts a rigid and planar conformation as a result of the presence of two intramolecular hydrogen bonds.[8,12] It was envisioned that this subunit in longer oligomers of the same skeleton should also take up this conformation, which would led to the formation of crescent or helical structures for the longer oligomers.



Scheme 1.

The synthetic route for compounds **1**–**3** is shown in Scheme 1. Compound **7**[13] was first prepared from acid **6** in 95% yield in refluxing methanol catalyzed by sulfuric acid, and then treated with concentrated nitric acid at room temperature to afford compound **8** in 72% yield.[14] The latter was reacted with methyl iodide in acetone at room temperature with potassium carbonate as base to produce compound **9** in 80% yield. Palladium-catalyzed hydrogenation of **9** in methanol gave rise to intermediate **10** in quantitative yield, whereas its hydrolysis with sodium hydroxide in aqueous methanol gave another intermediate **11** in 97% yield. Reaction of compound **12**, obtained from reaction of **11** with oxalyl chloride in the presence of *N*,*N*-dimethyl formamide (DMF), with **10** in chloroform with triethylamine as base produced 2-mer **1** in 98% yield. Hydrogenation of **1** in THF gave **13** in 98% yield. The latter was then reacted with **10** to produce 3-mer **2** in 85% yield. By repeating the hydrogenation and coupling reactions, the longer oligomers **3**–**5** could be conveniently prepared starting from **2**. The reaction conditions for the preparation of **4** and **5** are presented in Scheme 2.



Scheme 2.

Single crystals of 2-mer **1** were grown by slow evaporation of the solution in chloroform at room temperature. The crystal structure had been determined by the X-ray analysis and is presented in Figure 1. As expected, the two benzene rings and the central amide subunit share a perfectly rigidified planar conformation because of the existence of the three-centered hydrogen bonds.[8,12] The NH···O distance is 1.98 and 2.17 Å for the six-numbered and five-numbered ring hydrogen bonds, respectively, whereas the NH···O angle is 141.2 and 112.4°, respectively, for both hydrogen bonds. No single crystals of longer oligomers have been obtained yet. Because the longer oligomers **2**–**5** are actually elongation of the same backbone in **1**, it is reasonable to

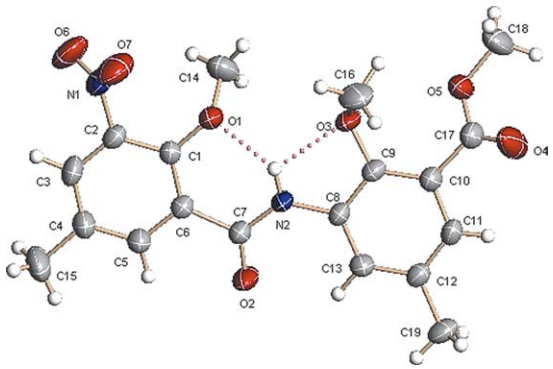


Figure 1. Crystal structure of 2-mer **1**, which reveals a rigid planar conformation due to the presence of two intramolecular hydrogen bonds.

assume that, without any important steric hindrance, similar three-centered hydrogen bonds should exist in the longer oligomers, which should make them take up a rigidified folding conformation, as shown in Figure 3 with **5** as example (vide infra).

Compounds **1**–**5** have been characterized by the ^{1}H and ^{13}C NMR and mass spectroscopy, and microanalysis. The ^{1}H NMR spectrum of the new oligomers in chloroform-d is presented in Figure 2. It can be found that all the amide protons of the oligomers are in the downfield area (10.35–9.80 ppm), indicating that three-centered hydrogen bonds also exist in these molecules in the solution. With the elongation of the oligomers, the chemical shift of the amide protons moves upfield. This is in accordance with the results observed for the aromatic hydrazide-based foldamers,[9] reflecting the decreased electron-donating ability of the methoxyl oxygen atoms for the formation of two hydrogen bonds simultaneously. The resolution of the spectrum of the longest 6-mer **5** (Fig. 2e) is not reduced greatly compared to that of the shorter oligomers, showing that no important intra- or intermolecular stacking occurs in **5** (infra vide). Additional evidence for the intramolecular hydrogen bonding came from 2D NOESY ^{1}H NMR experiments in chloroform-d, which revealed strong NOE connections

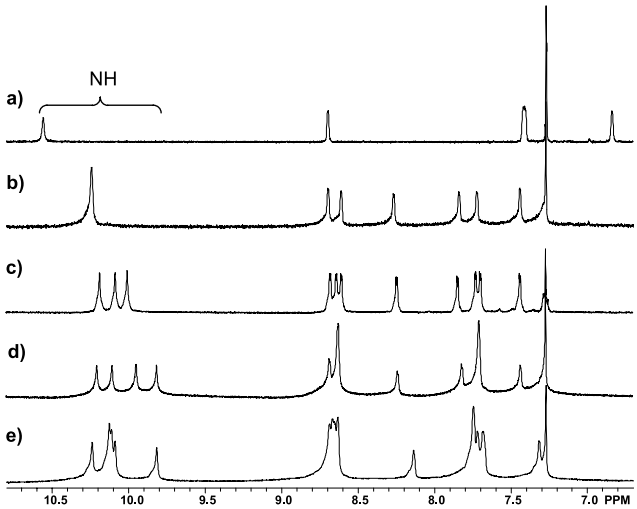


Figure 2. Partial ^{1}H NMR spectrum (400 MHz, 3 mM) of oligomers **1** (a), **2** (b), **3** (c), **4** (d), and **5** (e) in chloroform-d at 25 °C, highlighting the downfield chemical shift of the amide protons due to the existence of the three-centered hydrogen bonds.

between the amide protons and the neighboring methoxyl protons but not the neighboring benzene protons for all the oligomers. This result obviously supports the hydrogen bonded rigidified conformation but not a flexible, C–N bond-freely rotatable conformation for the amide subunits. The IR spectrum obtained in chloroform revealed the N–H stretching frequency of less than 3350 cm^{-1} for all the oligomers and the stretching frequency is independent on the concentration (within the range of 0.5–5 mM), which is also consistent with those of amides involved in intramolecular hydrogen bonds.[15]

Upon dilution from 35 to 0.5 mM, the signals of the NH and aromatic protons in the ^{1}H NMR spectrum of oligomers **1**–**5** in chloroform-d shift downfield only slightly. The largest change of the chemical shift has been observed for 6-mer **5** (0.007 ppm for one of the amide protons). In principle, the signals of hydrogen bonded amide protons would shift upfield upon dilution of the solution due to the weakening of the hydrogen bonding, this small downfield shifting should reflect that very weak intermolecular aggregation or π–π stacking exists at high concentration. Assuming a 1:1 binding mode for the intermolecular aggregation,[9,16] self-association constant of less than 8 M^{-1} was derived for oligomers **3**, **4**, and **5**, respectively, from the ^{1}H NMR dilution experiments.[17] The UV–vis spectrum of all the oligomers has similar shape with absorption maxima at 295 nm in chloroform. The shape of the spectrum of the oligomers is not sensitive to its concentrations, and Beer's law was observed for all oligomers at $\leq 1.0\times10^{-3}$ M as judged from the changes of their absorbance as a function of their concentration. This is consistent with the above ^{1}H NMR result and suggests that the intermolecular association can be ruled out in this concentration range.

Molecular modeling revealed that oligomers **2**–**5** adopt a rigid crescent conformation as a result of the existence of the intramolecular hydrogen bonds, which create a rigid cavity with all the methoxyl groups located to the center of the cavity. The distance between two cross-ring methoxyl oxygen is approximately 0.86 nm for 6-mer **5** (Fig. 3). Such a cavity size is comparable to that of the dibenzo[24]crown-8 ring,[18] while the arrangement of its six methoxyl oxygen atoms in the rigid conformation is similar to that in an assumedly rigidified planar 18-crown-6 or Cram's spherands,[19] with a difference of an increased spatial separation of the neighboring oxygen atoms and subsequently a larger cavity size in **5**. Therefore, the complexing behavior of this new series of folding oligomers towards

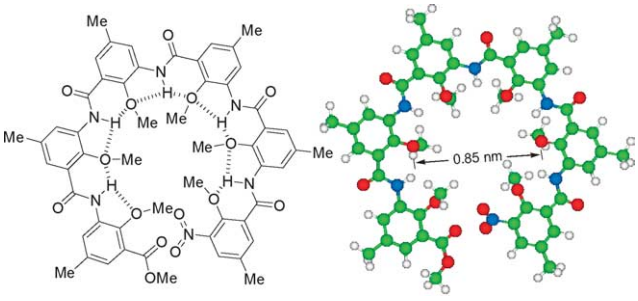
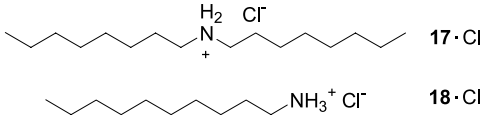


Figure 3. Hydrogen bonding-induced rigidified crescent conformation of 6-mer **5** and its energy-minimized structure.

alkylated ammonium ions **17**·Cl and **18**·Cl was investigated in less polar chloroform.



Adding compounds **17**·Cl or **18**·Cl to the solution of oligomers **1**–**5** in chloroform caused the fluorescent emission of these oligomers to increase substantially. In contrast, no obvious strengthening (<3%) was observed when tetrabutylammonium chloride of the identical concentration was added under the same experimental conditions, which ruled out the possibility of salt effect and supported that complexes were formed between the oligomers and the added ammonium ions in chloroform. Upon addition of 1 equiv of the oligomers to the solution of **17**·Cl or **18**·Cl in chloroform-d, the signals of the NH signal of the latter also shifted downfield (0.12 ppm for the case of **17**·Cl and **5** at 5 mM) significantly. These observations also indicate that the oligomers are able to bind the ammonium ions. Job's plot for the mixture of **5** and **17**·Cl in chloroform-d revealed the largest downfield shifting for the NH proton signal at the 1:1 ratio,[8c,20] which supports a 1:1 binding stroichiometry between the oligomer and the ammonium guest. The association constants (K_{assoc}) between the oligomer and ammonium ion had been determined by using an iterative least-square curve-fitting method of the fluorescent and ^{1}H NMR titration experiments,[9,21] and the corresponding free energy changes were calculated from the association constant using equation $\Delta G = -RT \ln K_a$. The results are listed in Table 1. As an example, Figure 4 presents the fluorescent spectra of **4** upon addition of **17**·Cl at different concentrations.

It can be found that, except 2-mer **1**, all other longer oligomers exhibit comparable complexing ability to the ammonium, although 5-mer **4** gives rise to the largest association constant for both ammonium ions. The existence of the methyl groups in the cavity obviously reduces the binding ability of the oligomers and, as a result, the binding may occur in a face-to-face mode,[22] in which the methyl groups in the cavity are located away from the bound ammonium ion. The association constant of the primary ammonium **18**·Cl is always larger than that of secondary ammonium **17**·Cl. This result may be rationalized by considering that the former ion has one more proton for the formation of more intermolecular hydrogen bonds and it also imposes smaller steric hindrance. Compared to oligomer **4**, the longest oligomer **5** display a reduced binding ability. This may reflect that the increase of the steric hindrance with the increasing methyl groups in the cavity may counteract the positive effect of the increased methoxy oxygen number in the longer oligomers for complexation with ammonium ion. Because all the methoxyl oxygen atoms are involved in intramolecular hydrogen bonding, the complexation of the foldaming oligomers with ammonium may be driven by the intermolecular cation–π interaction, together with the intermolecular C═O···H–N and MeO···H–N hydrogen bonding. The increase of the binding stability with the increase of the oligomer length may be caused by the statistic effect. However, at the present stage, we are not able to differentiate the discrete interactions.

Table 1. Association constants (K_{assoc}, M^{-1}) of the complexes between the folding oligomers and alkyl ammonium ions in $CDCl_3$ at 25 °C[a]

Complex	K_{assoc} (M^{-1})	ΔG (kJ)	Complex	K_{assoc} (M^{-1})	ΔG (kJ)
1·**17**·Cl	17	7.0	**4**·**17**·Cl	200	13.1
1·**18**·Cl	40	9.1	**4**·**18**·Cl	360	14.6
2·**17**·Cl	140	12.2	**5**·**17**·Cl	150	12.4
2·**18**·Cl	210	13.2	**5**·**17**·Cl[b]	140	12.2
3·**17**·Cl	160	12.6	**5**·**18**·Cl	200	13.1
3·**17**·Cl	260	13.8			

[a] With an error ≤20%.
[b] Obtained by ^{1}H NMR dilution method with the N*H* signal of the salt as probe.

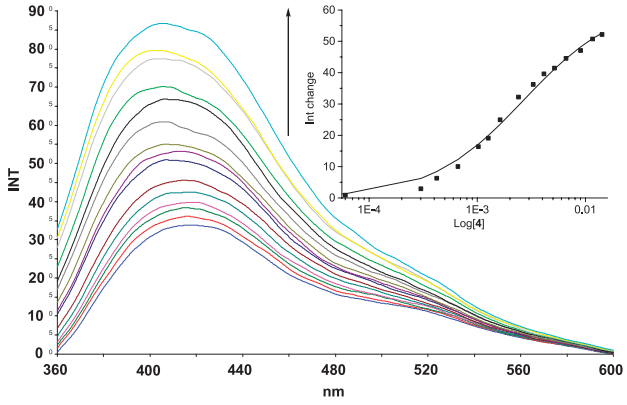


Figure 4. Partial fluorescent spectra of **4** (6.0×10^{-5} M, excitation wavelength = 345 nm) in chloroform at 25 °C, increased gradually with the incremental addition of **17**·Cl from 0 to 1.3 mM (inset: intensity change of **4** with log[**17**·Cl]).

3. Conclusion

In summary, we have reported the synthesis and characterization of a new series of aromatic amide oligomers, which adopt folding conformation due to the existence of intramolecular three-centered hydrogen bonds. The new foldamers display modest binding affinity toward primary and secondary alkyl ammonium ions in chloroform. A face-to-face binding mode has been proposed for the resulting complexes. In principle, one or two of the methoxyl groups in the oligomers can be replaced with phenoxyl anion without destroying the rigid folding conformation.[23] The resulting foldamers might display interesting binding ability

toward metal ions or ammonium ions due to increased electrostatic interaction, which is being investigated.

4. Experimental

4.1. General methods

Melting points are uncorrected. All reactions were performed under an atmosphere of dry nitrogen. The ^{1}H NMR spectra were recorded on 400 or 300 MHz spectrometers in the indicated solvents. Chemical shifts are expressed in parts per million (δ) using residual solvent protons as internal standards. Chloroform (δ 7.26 ppm) was used as an internal standard for chloroform-d. Elemental analysis was carried out at the SIOC analytical center. Unless otherwise indicated, all starting materials were obtained from commercial suppliers and were used without further purification. All solvents were dried before use following standard procedures. The methods for the determination of binding constants have been reported in a previous paper.[9]

4.1.1. Compound 7. To a solution of 2-hydroxy-5-methylbenzoic acid **6** (10.0 g, 65.8 mmol) in methanol (150 mL) was added dropwise concentrated sulfuric acid (2 mL), and the solution was heated under reflux for 24 h. Upon removal of the solvent under reduced pressure, the resulting residue was dissolved in ethyl acetate (100 mL) and the solution was washed with saturated sodium bicarbonate solution (25 mL×2), water (25 mL), brine (25 mL), and dried over sodium sulfate. After the solvent was evaporated in vacuo, the crude product was subjected to flash column chromatography (dichloromethane/ethyl acetate 10:1) to afford compound **7** as a colorless oil (10.4 g, 95%).[24] ^{1}H NMR (300 MHz, $CDCl_3$): 10.56 (s, 1H), 7.63 (s, 1H), 7.26 (d, J=8.1 Hz, 1H), 6.89 (d, J=8.1 Hz, 1H), 3.94 (s, 3H), 2.28 (s, 3H). ESI-MS: m/z 167 $[M+H]^+$.

4.1.2. Compound 8. A solution of compound **7** (10.3 g, 68.0 mmol) in water (110 mL) was cooled to 0 °C. To the solution was added slowly concentrated nitric acid (70%, 110 mL) in 30 min. The solution was then stirred at room temperature for 24 h and poured into ice water (250 mL). The mixture was extracted with ether (100 mL×3) and the combined organic phase was washed with aqueous sodium carbonate (1 M, 50 mL), water (50 mL×2), brine (50 mL), and dried over magnesium sulfate. After removal of the solvent under reduced pressure, the resulting residue was purified by column chromatography (petroleum ether/ethyl acetate 30:1) to give compound **8** as a yellow solid (9.45 g, 72%). Mp 140–142 °C [142–144 °C][14]. ^{1}H NMR (300 MHz, $CDCl_3$): 11.78 (s, 1H), 7.98 (d, J=4.8 Hz, 2H), 4.00 (s, 3H), 2.37 (s, 3H). ESI-MS: m/z 212 $[M+H]^+$.

4.1.3. Compound 9. A suspension of compound **8** (9.44 g, 45.0 mmol), methyl iodide (12.8 g, 90.0 mmol), potassium carbonate (19.9 g, 144 mmol), and 18-crown-6 (10 mg) in acetone (250 mL) was stirred at room temperature for 36 h and then the solvent was removed in vacuo. The resulting residue was triturated with ether (200 mL) and the organic phase was washed with diluted sodium bicarbonate (1 N, 50 mL×2), water (50 mL), brine (50 m) and dried over sodium sulfate. Upon evaporation of the solvent under reduced pressure, the crude product was purified by column chromatography (petroleum ether/ethyl acetate 10:1) to give compound **9** as a pale yellow solid (8.05 g, 80%). Mp 54–58 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.84 (s, 1H), 7.73 (s, 1H), 3.97 (s, 6H), 2.41 (s, 3H). EI-MS: m/z 225 $[M]^+$. Anal. Calcd for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.74; H, 5.04; N, 6.02.

4.1.4. Compound 10. A suspension of compound **9** (1.34 g, 5.96 mmol) and Pd–C (5%, 0.12 g) in methanol was stirred under 1 atm of hydrogen gas at room temperature for 24 h. The solid was filtered off over Celite and the filtrate concentrated. The resulting residue was subjected to flash chromatography (dichloromethane) to give the titled compound as colorless oil (1.15 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.01 (s, 1H), 6.73 (s, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 2.24 (s, 3H). EI-MS: m/z 195 $[M]^+$. Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.48; H, 6.86; N, 7.14.

4.1.5. Compound 11. To a stirred solution of compound **9** (7.16 g, 31.8 mmol) in methanol (100 mL) and water (50 mL) was added sodium hydroxide (2.67 g, 66.8 mmol) at room temperature, the solution was then heated under reflux for 3 h. Upon concentration under reduced pressure to about 15 mL, the solution was neutralized with diluted hydrochloric acid. The precipitate was formed, which was filtered and washed with cold water to give compound **11** as pale yellow powder (6.71 g, 97%). The product was further recrystallized from ethanol for microanalysis. Mp 181–183 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.12 (s, 1H), 7.87 (s, 1H), 4.06 (s, 3H), 2.46 (s, 3H). EI-MS: m/z 211 $[M]^+$. Anal. Calcd for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.43; H, 4.32; N, 6.52.

4.1.6. Compound 1. To a solution of compound **11** (1.70 g, 8.06 mmol) in chloroform (25 mL) were added oxalyl chloride (0.44 g, 5.80 mmol) and DMF (0.05 mL). The solution was stirred at room temperature for 4 h and then concentrated in vacuo to give compound **12** as yellow oil. This crude product was dissolved in chloroform (20 mL) and the solution was added to a solution of compound **10** (1.44 g, 7.38 mmol) and triethylamine (1.30 g, 1.30 mmol) in chloroform (25 mL). The mixture was stirred at room temperature for 5 h and then washed with dilute hydrochloric acid (1 N, 8 mL×2), aqueous sodium bicarbonate solution (1 N, 10 mmol), water (15 mL), brine (15 mL), and dried over sodium sulfate. The resulting residue was subjected to column chromatography (chloroform/ethyl acetate 20:1) to afford compound **1** as a yellow solid (2.81 g, 98%). Mp 160–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.35 (s, 1H), 8.63 (s, 1H), 8.24 (s, 1H), 7.80 (s, 1H), 7.27 (s, 1H), 4.05 (s, 3H), 3.13 (s, 6H), 2.47 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$): 166.1, 161.5, 149.3, 147.4, 144.1, 136.8, 135.3, 134.2, 132.4, 128.9, 128.7, 126.8, 125.3, 123.1, 64.4, 62.6, 52.3, 21.2, 20.7. IR ($CHCl_3$): ν 3320, 1710, 1676, 1531, 1471, 1251, 1198, 982 cm^{-1}. EI-MS: m/z 389 $[M+H]^+$. Anal. Calcd for $C_{19}H_{20}N_2O_7$: C, 58.76; H, 5.19; N, 7.21. Found: C, 58.89; H, 5.32; N, 6.94.

4.1.7. Compound 13. A suspension of compound **1** (2.80 g, 7.82 mmol) and Pd–C (5%, 0.20 g) in tetrahydrofuran

(35 mL) was stirred at 50 °C under 1 atm of hydrogen gas for 6 h. The solid was then filtered off over Celite. Upon removal of the solvent under reduced pressure, the resulting residue was subjected to flash chromatography (ethyl acetate/dichloromethane 1:10) to give compound **13** as a white powder (2.58 g, 98%). Mp 197–199 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.56 (s, 1H), 8.69 (s, 1H), 7.41 (s, 2H), 6.84 (s, 1H), 3.91 (s, 1H), 2.39 (s, 3H), 2.31 (s, 6H). EI-MS: *m/z* 358 $[M]^+$. Anal. Calcd for $C_{19}H_{22}N_2O_5$: C, 63.68; H, 6.19; N, 7.82. Found: C, 63.62; H, 6.23; N, 7.64.

4.1.8. Compounds 14, 15, and 16. These compounds were prepared from the Pd–C-catalyzed hydrogenation of compounds **2**–**4**, respectively, by using the reaction conditions described for **13**. These compounds were unstable in the air and used for the next step immediately after purification.

4.1.9. Compounds 2, 3, 4 and 5. These compounds were prepared from the reaction of compound **11** and the corresponding aniline **14**–**16** according to the experimental procedure described for compound **1**.

4.1.10. Compound 2. Solvent for column chromatography: chloroform, white solid in 85% yield. Mp 211–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.23 (s, 1H), 8.68 (s, 1H), 8.60 (s, 1H), 8.26 (s, 1H), 8.25 (s, 1H), 7.83 (s, 1H), 7.71 (s, 1H), 7.43 (s, 1H), 4.08 (s, 3H), 3.94 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 2.48 (s, 3H), 2.45 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.1, 163.0, 161.5, 149.1, 147.2, 145.3, 144.0, 136.9, 135.7, 135.6, 134.3, 132.7, 131.7, 129.1, 128.6, 127.0, 126.4, 126.1, 125.2, 125.2, 123.0, 64.4, 63.2, 62.5, 52.3, 21.4, 21.3, 20.7. IR ($CHCl_3$): ν 3335, 1676, 1533, 1465, 1340, 1264, 1246, 1200, 988 cm^{-1}. ESI-MS: *m/z* 552 $[M+H]^+$. Anal. Calcd for $C_{28}H_{29}N_3O_9$: C, 60.97; H, 5.30; N, 7.62. Found: C, 60.82; H, 5.52; N, 7.67.

4.1.11. Compound 3. Solvent for column chromatography: dichloromethane, white solid in 82% yield. Mp 216–218 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.20 (s, 1H), 10.09 (s, 1H), 10.01 (s, 1H), 8.68 (s, 1H), 8.64 (d, $J=1.5$ Hz, 1H), 8.60 (s, 1H), 8.24 (d, $J=2.4$ Hz, 1H), 7.85 (d, $J=2.7$ Hz, 1H), 7.73 (s, 1H), 7.69 (s, 1H), 7.44 (s, 1H), 4.09 (s, 3H), 3.95 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 2.49 (s, 3H), 2.46 (s, 3H), 2.45 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.1, 162.1, 162.1, 160.7, 148.1, 146.2, 144.2, 144.2, 143.2, 143.1, 135.8, 134.9, 134.7, 134.7, 133.3, 131.7, 130.9, 130.6, 128.1, 127.8, 126.0, 125.6, 125.2, 125.1, 124.5, 124.3, 124.3, 122.0, 63.4, 62.2, 62.1, 61.4, 51.3, 20.4, 20.2, 19.7. IR ($CHCl_3$): ν 3329, 1677, 1534, 1459, 1338, 1251, 980, 732 cm^{-1}. ESI-MS: *m/z* 715 $[M+H]^+$. Anal. Calcd for $C_{37}H_{38}N_4O_{11}$: C, 62.18; H, 5.36; N, 7.84. Found: C, 62.46; H, 5.68; N, 7.65.

4.1.12. Compound 4. Solvent for column chromatography: dichloromethane/ethyl acetate (40:1), white solid in 80% yield. Mp 225–226.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.20 (s, 1H), 10.10 (s, 1H), 9.94 (s, 1H), 9.80 (s, 1H), 8.74 (s, 1H), 8.68 (s, 1H), 8.65 (s, 1H), 8.62 (s, 1H), 8.23 (s, 1H), 7.81 (s, 1H), 7.70 (s, 3H), 7.43 (s, 1H), 4.08 (s, 3H), 3.97 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 2.47 (s, 9H), 2.45 (s, 3H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.8, 163.3, 163.3, 163.1, 161.7, 149.1, 147.2, 145.3, 145.2, 145.2, 143.9, 136.9, 135.9, 135.8, 135.7, 135.6, 134.3, 132.8, 131.9, 131.8, 131.6, 129.0, 128.8, 126.9, 126.7, 126.6, 126.4, 126.3, 126.1, 126.0, 125.7, 125.5, 125.5, 125.4, 122.8, 64.4, 63.2, 63.0, 62.3, 52.1, 21.4, 21.4, 21.2, 20.2, 14.2. IR ($CHCl_3$): ν 3338, 2946, 1726, 1677, 1533, 1460, 1423, 1247, 981, 732 cm^{-1}. MALDI-TOF-HRMS: Required for $C_{46}H_{47}N_5O_{13}Na$: 900.3014. Found: 900.3060.

4.1.13. Compound 5. Solvent for column chromatography: dichloromethane/ethyl acetate (35:1), pale yellow powder in 81% yield. Mp 232–234 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 10.23 (s, 1H), 10.12 (s, 1H), 10.10 (s, 1H), 10.08 (s, 1H), 9.81 (s, 1H), 8.68 (s, 1H), 8.66 (s, 1H), 8.65 (s, 1H), 8.62 (s, 2H), 8.13 (s, 1H), 7.73 (s, 2H), 7.71 (s, 1H), 7.67 (s, 2H), 7.31 (s, 1H), 4.04 (s, 3H), 3.99 (s, 3H), 3.96 (s, 6H), 3.92 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 2.47 (s, 9H), 2.41 (s, 3H), 2.39 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 165.5, 163.5, 163.2, 163.1, 163.0, 161.5, 148.8, 147.0, 145.3, 145.2, 145.0, 143.8, 136.7, 136.0, 135.9, 135.8, 135.6, 134.2, 132.8, 132.0, 131.9, 131.7, 128.9, 128.6, 126.9, 126.8, 126.6, 126.4, 126.2, 126.2, 126.1, 125.9, 125.9, 125.8, 125.5, 125.4, 125.3, 122.6, 64.3, 63.2, 63.0, 62.9, 62.2, 52.1, 21.4, 21.3, 20.7. IR ($CHCl_3$): ν 3342, 2947, 1677, 1533, 1459, 1423, 1246, 979, 732 cm^{-1}. MALDI-TOF-MS: *m/z* 1064 $[M+Na]^+$. Anal. Calcd for $C_{55}H_{56}N_6O_{15}$: C, 63.45; H, 5.42; N, 8.07. Found: C, 62.96; H, 5.48; N, 7.97.

4.2. Molecular mechanics calculation

The folding patterns of the oligomers were constructed by using the Builder program within the package HyperChem. Then they were optimized by the conjugate gradient with AccuModel software 2.1 using the MM3 force field. To explore lower energy conformation, molecular dynamics calculations were performed without constraints for the oligomers.

4.3. X-ray data

Empirical formula: $C_{19}H_{20}N_2O_7$. Space group Triclinic, *P*-1, $\alpha=7.5626(12)$ Å, $\alpha=107.155(3)°$. $\beta=11.4681(17)$ Å, $\beta=100.719(3)°$. $\gamma=11.7866(17)$ Å, $\gamma=100.902(3)°$. Volume 926.7(2) $Å^3$. Crystal size $0.382\times0.207\times0.138$ mm^3.

Acknowledgements

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Use of isopropyl alcohol as a solvent in Ti(O-*i*-Pr)$_4$-catalyzed Knöevenagel reactions

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Abstract—Knöevenagel reactions of aldehydes and ketones with malononitrile, isopropyl cyanoacetate and diisopropyl malonate catalyzed by Ti(O-*i*-Pr)$_4$ proceeded smoothly in *i*-PrOH to give the corresponding reaction products in good to high yield. 3-Substituted coumarins were prepared by the present method.

1. Introduction

The Knöevenagel reaction is a well-known classical reaction as a condensation between carbonyl compounds and activated methylene compounds catalyzed by amines.[1] As activated methylene compounds, alkyl acetoacetates and dialkyl malonates have often been used.

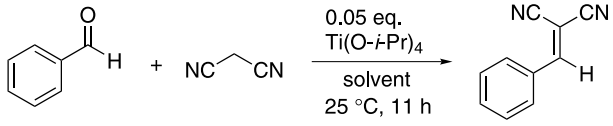
Recently, on the other hand, Knöevenagel reactions catalyzed by Lewis acids have been reported. The Venkataratnam[2] and Sandhu[3,4] groups reported reactions catalyzed by $ZnCl_2$, $BiCl_3$, and CdI_2, respectively. However, all of them required a high temperature (75–100 °C) under solvent-free conditions. Furthermore, the reaction in an ionic liquid was also reported.[5]

We recently reported Ti(O-*i*-Pr)$_4$-promoted Knöevenagel reaction of aldehydes with diketene.[6] During the course of this study, we observed that Ti(O-*i*-Pr)$_4$ promoted the reaction of aldehydes with isopropyl acetoacetate. Therefore, we examined the scope of Ti(O-*i*-Pr)$_4$-promoted Knöevegenal reaction of carbonyl compounds with activated methylene compounds.

2. Results and discussion

We first examined the reaction of benzaldehyde with malononitrile in the presence of Ti(O-*i*-Pr)$_4$ in a variety of solvents (Table 1).

Table 1. Solvent effect in the reaction of benzaldehyde with malononitrile



Entry	Solvent	Product (% yield[a])
1	*i*-PrOH	93
2	CH_3CN	72
3	CH_2Cl_2	66[b]
4	Toluene	24[b]

[a] Isolated yield by fractional recrystallizations unless otherwise noted.
[b] Isolated yield by silica-gel column chromatography.

As shown in Table 1, we found that the reaction using *i*-PrOH as a solvent was accelerated by 0.05 equiv of Ti(O-*i*-Pr)$_4$ to produce 2-(phenylmethylene)malononitrile in 93% yield at 25 °C for 11 h. Under identical conditions, a variety of aldehydes were reacted with malononitrile to afford 2-(arylidene)malononitrile in good to high yield (Table 2, entries 1–9). In the cases of ketones, larger amounts of Ti(O-*i*-Pr)$_4$ were required (0.5–1 equiv) to obtain the products in satisfactory yield (entries 10 and 11).

As for the reaction mechanism, the carboanion of malonate will attack the electrophilic carbonyl carbon coordinated by Ti(O-*i*-Pr)$_4$. The carboanionic character of malononitrile might be enhanced by the alcoholic solvent producing a keteneimine intermediate.[5a] After addition to carbonyl compounds, some kind of an elimination process may occur to produce 2-substituted methylenemalononitrile and to regenerate the Ti species those activate aldehydes.

We then examined the reactions of some aldehydes with

Keywords: Knöevenagel reaction; Titanium alkoxide; Isopropyl alcohol.
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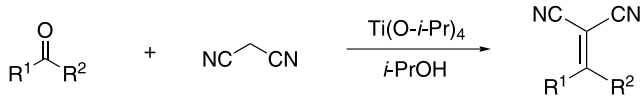
Table 2. Reactions of a variety of aldehydes and ketones with malononitrile promoted by Ti(O-*i*-Pr)$_4$



1a—1k

Entry	R^1	R^2	Ti(O-*i*-Pr)$_4$/equiv	Conditions		Product (% yield[a])
				Temperature/°C	Time/h	
1	Ph	H	0.05	25	11	93 (**1a**)
2	*p*-ClC_6H_4	H	0.05	22	12	97 (**1b**)
3	*p*-$MeOC_6H_4$	H	0.05	21	12	92 (**1c**)
4	*p*-BrC_6H_4	H	0.05	21	13	98 (**1d**)
5	*c*-C_6H_{11}	H	0.05	20	25	92[b] (**1e**)
6	$Ph(CH_2)_2$	H	0.05	20	3	74[b] (**1f**)
7	(*E*)-PhCH=CH	H	0.05	22	11	64 (**1g**)
8	$CH_3(CH_2)_2$	H	0.05	20	12	63[b] (**1h**)
9	*t*-Bu	H	0.05	20	24	60[b] (**1i**)
10	Ph	Me	1.0	70	18	61[b] (**1j**)
11	Ph	Ph	1.0	70	52	86 (**1k**)

[a] Isolated yield by fractional recrystallizations unless otherwise noted.
[b] Isolated yield by silica-gel column chromatography.

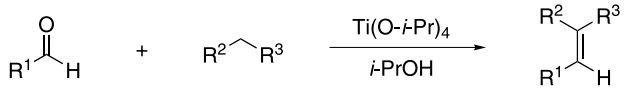
isopropyl cyanoacetate and diisopropyl malonate with the aid of Ti(O-*i*-Pr)$_4$ in *i*-PrOH (Table 3). The reactions of isopropyl cyanoacetate and diisopropyl malonate with aldehydes were carried out with the aid of 0.2–1.0 equiv of Ti(O-*i*-Pr)$_4$ in *i*-PrOH.

While 0.05 equiv of Ti(O-*i*-Pr)$_4$ was enough for the reaction of aldehydes with malononitrile, it was found that 0.2 equiv and 1 equiv of Ti(O-*i*-Pr)$_4$ were necessary in the cases of isopropyl cyanoacetate and diisopropyl malonate, respectively, for the completion of the reaction, probably due to the lower reactivity of activated methylene compounds. In the cases of entry 1 and 2, only (*E*)-isomers (**2a** and **2b**) were obtained, that was consistent with the results of the reported methods.[2,3,5]

We applied our method to the synthesis coumarin derivatives. Coumarins (2*H*-1-benzopyran-2-one) are important compounds found in natural and artificial compounds such as perfume, agricultural and pharmaceutical products. So far, there have been many reports for the synthesis of coumarin frameworks. There are fundamentally two methods, one is the so-called Pechmann condensation, that is, acid-mediated condensation of phenol with β-ketoesters to produce coumarins.[7] In these reactions, strong acids such as H_2SO_4 and $AlCl_3$ have been used. The other method is the Knöevenagel reaction between salicylaldehydes and dialkyl malonate. For example, solvent-free synthesis,[8] Montmorillonite KSF-catalyzed synthesis[9] and a solid-phase synthesis[10] for substituted coumarin-3-carboxylic acids derivatives have been recently reported.

We first examined the reaction of salicylaldehyde with malononitrile in the presence of 0.1 equiv of Ti(O-*i*-Pr)$_4$ in *i*-PrOH (Scheme 1). The reaction proceeded smoothly at room temperature to afford 3-cyanocoumarin (**4**) in 70% yield. The introduction of isopropyl carboxylate to the 3-position of coumarin was performed by using isopropyl cyanoacetate in the presence of 1 equiv of Ti(O-*i*-Pr)$_4$ in *i*-PrOH to give 3-isopropoxycarbonylcoumarin (**5**). The reaction of diisopropyl malonate was so sluggish that the yield of **5** was only moderate (37%). It should be mentioned that the reaction of salicylaldehyde with diketene in the

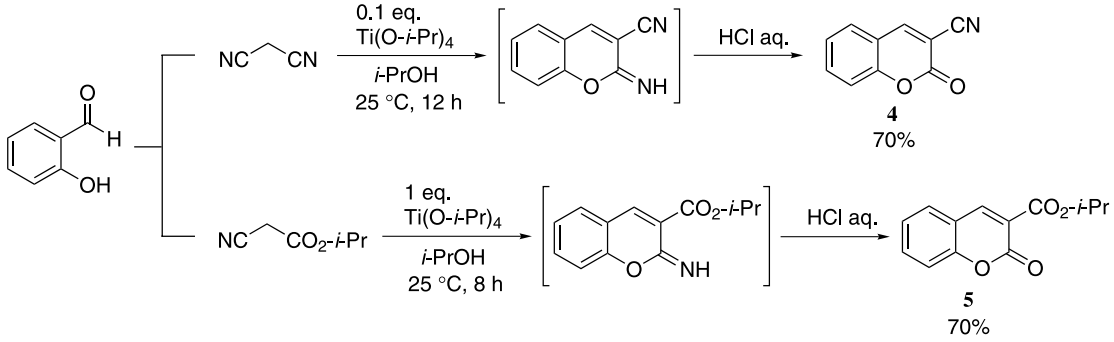
Table 3. Reactions of some aldehydes with isopropyl cyanoacetate and diisopropyl malonate promoted by Ti(O-*i*-Pr)$_4$



2a, **2b**, **3a**, **3b**

Entry	R^1	R^2	R^3	Ti(O-*i*-Pr)$_4$/equiv	Conditions		Product (% yield[a])
					Temperature/°C	Time/h	
1	Ph	CN	CO_2-*i*-Pr	0.2	70	25	78[b] (**2a**)
2	(*E*)-PhCH=CH	CN	CO_2-*i*-Pr	0.2	70	24	63[b,c] (**2b**)
3	Ph	CO_2-*i*-Pr	CO_2-*i*-Pr	1.0	22	50	93 (**3a**)
4	*c*-C_6H_{11}	CO_2-*i*-Pr	CO_2-*i*-Pr	1.0	20	51	97 (**3b**)

[a] Isolated yield by silica-gel column chromatography unless otherwise noted.
[b] Only (*E*)-isomer was obtained (determined by NOE experiments).
[c] Isolated yield by fractional recrystallizations.



Scheme 1.

presence of 2 equiv of Ti(O-*i*-Pr)$_4$ at 25 °C proceeded smoothly to produce 3-acetylcoumarin in 76% yield in CH_2Cl_2 and 59% yield in *i*-PrOH, respectively.

The present method has the following characteristic features: (1) Ti(O-*i*-Pr)$_4$-catalyzed Knöevegenal reaction of aldehydes and ketones is performed in non-toxic *i*-PrOH. (2) In the case of aldehydes, only a catalytic amount (0.05 equiv) of Ti(O-*i*-Pr)$_4$ was required for smooth reaction. (3) Coumarin derivatives are synthesized when salicylaldehyde is used.

3. Experimental

3.1. General procedure for Ti(O-*i*-Pr)$_4$-promoted Knöevenagel reaction

Malononitrile (330 mg, 5 mmol) and *i*-PrOH (Wako dehydrated grade) 6 mL were placed in a Schlenk tube under argon atmosphere. To this solution, aldehydes (5 mmol) then Ti(O-*i*-Pr)$_4$ 0.08 mL (0.025 mmol) were added and stirred at room temperature (20–25 °C) for 3–25 h. After confirmation of the completion of the reaction, the reaction mixture was poured into 1 N HCl and vigorously stirred at 0 °C for 0.5 h. It was extracted by ethyl acetate and the extract was washed with sodium bicarbonate and brine solution. The organic layer was dried with anhydrous sodium sulfate and evaporated. Purification of the residues by recrystallization or silica-gel column chromatography afforded Knöevenagel reaction products.

3.1.1. 2-(Phenylmethylene)malononitrile (1a). R_f=0.56 (3:1 hexane–ethyl acetate); mp 84–85 °C (lit.[11] 83 °C); IR (KBr, ν_{max} (cm^{-1})): 2223 (CN), 1591 (C=C); ^{1}H NMR: δ 7.91 (d, 2H, J=7.2 Hz), 7.78 (s, 1H), 7.64 (t, 1H, J=7.2 Hz), 7.55 (t, 2H, J=7.2 Hz); ^{13}C NMR: δ 160.1, 134.6, 131.0, 130.7, 129.6, 113.8, 112.7, 82.6; MS *m*/*z* (relative intensity): 154 (M$^+$, 100%), 127 (94%), 103 (70%), 76 (23%).

3.1.2. 2-[(4-Chlorophenyl)methylene]malononitrile (1b). R_f=0.54 (3:1 hexane–ethyl acetate); mp 167–168 °C (lit.[2] 165 °C); IR (KBr, ν_{max} (cm^{-1})): 2228 (CN), 1584 (C=C); ^{1}H NMR: δ 7.85 (d, 2H, J=8.4 Hz), 7.73 (s, 1H), 7.52 (d, 2H, J=8.4 Hz); ^{13}C NMR: δ 158.2, 141.2, 131.8, 130.1, 129.3, 113.4, 112.3, 83.4; MS *m*/*z* (relative intensity): 190 (^{37}Cl-M$^+$, 26%), 188 (^{35}Cl-M$^+$, 75%), 161 (29%), 153 (100%), 137 (22%), 126 (19%), 100 (12%), 75 (21%).

3.1.3. 2-[(4-Methoxyphenyl)methylene]malononitrile (1c). R_f=0.47 (2:1 hexane–ethyl acetate); mp 116–118 °C (lit.[2] 119 °C); IR (KBr, ν_{max} (cm^{-1})): 2225 (CN), 1560 (C=C); ^{1}H NMR: δ 7.91 (d, 2H, J=8.8 Hz), 7.65 (s, 1H,), 7.01 (d, 2H, J=8.8 Hz), 3.92 (s, 3H); ^{13}C NMR: δ 164.8, 158.8, 133.4, 124.0, 115.1, 114.4, 113.3, 78.7, 55.8; MS *m*/*z* (relative intensity): 184 (M$^+$, 100%), 169 (13%), 141 (28%), 114 (45%).

3.1.4. 2-[(4-Bromophenyl)methylene]malononitrile (1d). R_f=0.61 (3:1 hexane–ethyl acetate); mp 165–166 °C (lit.[11] 164 °C); IR (KBr, ν_{max} (cm^{-1})): 2225 (CN), 1558 (C=C); ^{1}H NMR: δ 7.77 (d, 2H, J=8.2 Hz), 7.72 (s, 1H), 7.69 (d, 2H, J=8.2 Hz); ^{13}C NMR: δ 158.4, 133.0, 131.8, 130.0, 129.6, 113.4, 112.3, 83.5; MS *m*/*z* (relative intensity): 235 (^{81}Br-M$^+$, 11%), 234 (88%), 233 (^{79}Br-M$^+$, 13%), 232 (89%), 153 (100%), 126 (79%), 100 (34%).

3.1.5. 2-(Cyclohexylmethylene)malononitrile (1e). R_f=0.54 (4:1 hexane–ethyl acetate); IR (KBr, ν_{max} (cm^{-1})): 2235 (CN), 1608 (C=C); ^{1}H NMR: δ 7.15 (d, 1H, J=10.4 Hz), 2.78–2.68 (m, 1H), 1.83–1.71 (m, 4H), 1.43–1.18 (m, 6H); ^{13}C NMR: δ 173.6, 112.2, 110.6, 87.8, 42.1, 30.9, 25.1, 24.6; MS *m*/*z* (relative intensity): 160 (M$^+$, 3%), 159 (9%), 145 (11%), 132 (14%), 118 (7%), 105 (18%), 82 (19%), 67 (52%), 56 (78%), 41 (100%). Anal. Calcd for $C_{10}H_{12}N_2$: C, 74.97; H, 7.55; N, 17.48. Found: C, 74.89; H, 7.59; N, 17.32.

3.1.6. 2-(3-Phenylpropylidene)malononitrile (1f). R_f=0.54 (3:1 hexane–ethyl acetate); IR (KBr, ν_{max} (cm^{-1})): 2236 (CN), 1601 (C=C); ^{1}H NMR: δ 7.34 (t, 2H, J=7.2 Hz), 7.30–7.27 (m, 2H), 7.17 (d, 2H, J=7.2 Hz), 2.96–2.86 (m, 4H); ^{13}C NMR: δ 168.3, 138.2, 136.9, 129.0, 128.3, 127.1, 114.9, 112.0, 110.4, 90.5, 34.2, 33.5; MS *m*/*z* (relative intensity): 182 (M$^+$, 40%), 91 (100%), 77 (25%), 65 (100%), 51 (59%). Anal. Calcd for $C_{12}H_{10}N_2$: C, 79.10; H, 5.53; N, 15.37. Found: C, 79.10; H, 5.56; N, 15.22.

3.1.7. 2-[(*E*)-3-Phenyl-2-propenylidene]malononitrile (1g). R_f=0.53 (3:1 hexane–ethyl acetate); mp 126–128 °C (lit.[12] 126 °C); IR (KBr, ν_{max} (cm^{-1})): 2224 (CN), 1609 (C=C), 1560 (C=C); ^{1}H NMR: δ 7.61–7.59 (m, 3H), 7.48–7.42 (m, 3H), 7.27 (d, 2H, J=10.4 Hz); ^{13}C NMR: δ 159.9, 150.3, 134.0, 132.1, 129.3, 128.9, 122.3, 113.5, 111.6; MS *m*/*z* (relative intensity): 180 (M$^+$, 99%), 179 (41%), 153 (100%), 115 (97%), 51 (40%).

3.1.8. 2-(Butylidene)malononitrile (1h). R_f=0.54 (3:1 hexane–ethyl acetate); IR (KBr, ν_{max} (cm^{-1})): 2239 (CN), 1607 (C=C); ^1H NMR: δ 7.33 (t, 1H, J=7.6 Hz), 2.58 (q, 2H, J=7.6 Hz), 1.62 (sextet, 2H, J=7.6 Hz), 1.02 (t, 3H, J=7.6 Hz); ^{13}C NMR: δ 169.4, 112.1, 90.2, 34.6, 21.1, 19.6, 13.5; MS m/z (relative intensity): 120 (M^+, 19%), 119 (21%), 105 (31%), 92 (33%), 79 (28%), 67 (46%), 55 (59%), 42 (100%), 41 (73%). Anal. Calcd for $C_7H_8N_2$: C, 69.97; H, 6.71; N, 23.32. Found: C, 69.93; H, 6.65; N, 23.23.

3.1.9. 2-(2,2-Dimethylpropylidene)malononitrile (1i). R_f=0.53 (3:1 hexane–ethyl acetate); mp 64–65 °C; IR (KBr, ν_{max} (cm^{-1})): 2233 (CN), 1606 (C=C); ^1H NMR: δ 7.26 (s, 1H), 1.32 (s, 9H); ^{13}C NMR: δ 177.4, 113.1, 111.1, 86.9, 37.0, 28.5; MS m/z (relative intensity): 133 (M^+-1, 8%), 119 (29%), 92 (63%), 73 (38%), 65 (53%), 57 (35%), 42 (100%). Anal. Calcd for $C_8H_{10}N_2$: C, 71.61; H, 7.51; N, 20.88. Found: C, 71.40; H, 7.47; N, 20.76.

3.1.10. 2-(1-Phenylethylidene)malononitrile (1j). R_f=0.42 (3:1 hexane–ethyl acetate); mp 94–96 °C (lit.[13] 92 °C); IR (KBr, ν_{max} (cm^{-1})): 2228 (CN), 1585 (C=C); ^1H NMR: δ 7.58–7.48 (m, 5H), 2.64 (s, 3H); ^{13}C NMR: δ 175.4, 135.9, 132.2, 129.1, 127.3, 112.7, 84.7, 24.2; MS m/z (relative intensity): 168 (M^+, 100%), 141 (64%), 140 (67%), 128 (58%), 114 (37%), 103 (30%), 77 (33%), 51 (49%).

3.1.11. 2-(Diphenylmethylene)malononitrile (1k). R_f=0.51 (3:1 hexane–ethyl acetate); mp 143–144 °C (lit.[14] 138 °C); IR (KBr, ν_{max} (cm^{-1})): 2223 (CN), 1530 (C=C); ^1H NMR: δ 7.58 (t, 2H, J=7.2 Hz), 7.51–7.42 (m, 8H); ^{13}C NMR: δ 175.0, 136.1, 132.7, 130.4, 128.9, 113.9, 81.7; MS m/z (relative intensity): 230 (M^+, 96%), 229 (75%), 203 (64%), 165 (100%), 88 (29%).

3.1.12. Isopropyl (*E*)-2-cyano-3-phenyl-2-propenoate (2a). R_f=0.63 (3:1 hexane–ethyl acetate); mp 76–79 °C; IR (KBr, ν_{max} (cm^{-1})): 2219 (CN), 1717 (C=O), 1607 (C=C); ^1H NMR: δ 8.24 (s, 1H), 7.99 (d, 2H, J=6.8 Hz), 7.58–7.48 (m, 3H), 5.26–5.16 (m, 1H), 1.38 (d, 6H, J=6.4 Hz); ^{13}C NMR: δ 161.9, 154.7, 133.1, 131.5, 130.9, 129.2, 115.4, 103.5, 70.7, 21.7; MS m/z (relative intensity): 215 (M^+, 32%), 173 (100%), 172 (100%), 156 (85%), 129 (78%), 128 (63%), 102 (58%), 101 (33%), 77 (57%), 43 (88%). Anal. Calcd for $C_{10}H_6N_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.52; H, 6.11; N, 6.46.

3.1.13. Isopropyl (2*E*,4*E*)-2-cyano-5-phenyl-2,4-pentadienoate (2b). R_f=0.69 (3:1 hexane–ethyl acetate); mp 104–106 °C; IR (KBr, ν_{max} (cm^{-1})): 2220 (CN), 1712 (C=O), 1612 (C=C), 1582 (C=C); ^1H NMR: δ 8.00 (dd, 1H, J=8.0, 2.4 Hz), 7.59 (dd, 2H, J=6.8, 2.4 Hz), 7.44–7.42 (m, 3H), 7.28 (d, 1H, J=8.0 Hz), 7.27 (d, 1H, J=2.4 Hz), 5.22–5.12 (m, 1H), 1.35 (d, 6H, J=6.0 Hz); ^{13}C NMR: δ 161.8, 155.1, 148.5, 134.7, 131.1, 129.1, 128.5, 123.1, 114.6, 105.2, 70.3, 21.7; MS m/z (relative intensity): 241 (M^+, 20%), 199 (74%), 182 (27%), 171 (58%), 155 (64%), 154 (100%), 127 (64%), 115 (55%), 77 (33%), 43 (69%). Anal. Calcd for $C_{10}H_6N_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.51; H, 6.28; N, 5.75.

3.1.14. Diisopropyl 2-(phenylmethylene)malonate (3a). R_f=0.41 (7:1 hexane–ethyl acetate); IR (KBr, ν_{max} (cm^{-1})): 1724 (C=O), 1631 (C=C); ^1H NMR: δ 7.69 (s, 1H), 7.48 (dd, 2H, J=6.8, 2.4 Hz), 7.41–7.34 (m, 3H), 5.29–5.21 (m, 1H), 5.19–5.11 (m, 1H), 1.31 (d, 6H, J=6.4 Hz), 1.29 (d, 6H, J=6.4 Hz); ^{13}C NMR: δ 166.3, 163.7, 141.4, 133.0, 130.4, 129.5, 128.7, 127.1, 69.3, 69.2, 21.8, 21.5; MS m/z (relative intensity): 276 (M^+, 9%), 217 (20%), 175 (66%), 174 (100%), 158 (27%), 146 (29%), 130 (24%), 102 (28%), 43 (98%). Anal. Calcd for $C_{10}H_6N_2$: C, 69.54; H, 7.30. Found: C, 69.54; H, 7.31.

3.1.15. Diisopropyl 2-(cyclohexylmethylene)malonate (3b). R_f=0.54 (6:1 hexane–ethyl acetate); IR (KBr, ν_{max} (cm^{-1})): 1718 (C=O), 1646 (C=C); ^1H NMR: δ 6.73 (d, 1H, J=10.8 Hz), 5.24–5.14 (m, 1H), 5.13–5.03 (m, 1H), 2.42–2.33 (m, 1H), 1.74–1.66 (m, 4H), 1.31 (d, 6H, J=6.4 Hz), 1.27 (d, 6H, J=6.8 Hz), 1.23–1.10 (m, 6H); ^{13}C NMR: δ 165.4, 163.8, 152.5, 127.6, 68.7, 68.6, 38.9, 31.7, 25.6, 25.2, 21.7; MS m/z (relative intensity): 180 (49%), 163 (55%), 162 (100%), 95 (33%), 43 (89%), 41 (72%). Anal. Calcd for $C_{10}H_6N_2$: C, 68.06; H, 9.28. Found: C, 68.13; H, 9.37.

3.1.16. 3-Cyanocoumarin (4). R_f=0.30 (2:1 hexane–ethyl acetate); mp 184–186 °C (lit.[15] 182–184 °C); IR (KBr, ν_{max} (cm^{-1})): 2229 (CN), 1728 (C=O), 1604 (C=C); ^1H NMR: δ 8.28 (s, 1H), 7.73 (t, 1H, J=8.0 Hz), 7.62 (dd, 1H, J=8.0, 1.6 Hz), 7.43 (t, 1H, J=8.0 Hz), 7.42 (t, 1H, J=8.0 Hz); ^{13}C NMR: δ 155.5, 154.0, 136.5, 130.8, 126.5, 118.5, 118.3, 117.9, 115.2, 103.8; MS m/z (relative intensity): 171 (M^+, 100%), 143 (99%), 115 (50%), 88 (27%), 63 (24%), 62 (23%). Anal. Calcd for $C_{10}H_6N_2$: C, 70.18; H, 2.94; N, 8.18. Found: C, 70.30; H, 2.94; N, 8.35.

3.1.17. 3-Isopropoxycarboxycoumarin (5). R_f=0.62 (3:1 hexane–ethyl acetate); mp 89–90 °C (lit.[16] 84–86 °C); IR (KBr, ν_{max} (cm^{-1})): 1750 (C=O), 1606 (C=C); ^1H NMR: δ 8.47 (s, 1H), 7.64 (t, 1H, J=8.0 Hz), 7.61 (dd, 1H, J=8.0, 1.6 Hz), 7.36 (d, 1H, J=8.0 Hz), 7.34 (t, 1H, J=8.0 Hz), 5.32–5.23 (m, 1H), 1.4 (d, 6H, J=6.0 Hz); ^{13}C NMR: δ 162.4, 156.7, 155.1, 148.0, 134.2, 129.4, 124.8, 118.7, 117.9, 116.8, 69.7, 21.8. MS m/z (relative intensity): 232 (M^+, 26%), 174 (35%), 173 (98%), 146 (100%), 118 (41%), 101 (22%), 89 (41%), 43 (49%). Anal. Calcd for $C_{13}H_{12}O_2$: C, 67.23; H, 5.21. Found: C, 67.23; H, 5.12.

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Synthesis of functionalized polyhedral oligomeric silsesquioxane (POSS) macromers by microwave assisted 1,3-dipolar cycloaddition

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Abstract—In this paper we report the first synthesis of isoxazolidine and isoxazoline-POSS macromers by 1,3-dipolar cycloaddition reactions of vinyl- and styryl-POSS with *N*-methyl-*C*-ethoxycarbonylnitrone and ethoxycarbonyl nitrile oxide, promoted by microwave irradiation. The nature of the resulting cycloadducts has been determined by NOE experiments and supported by computational studies at PM3 and DFT B3LYP/6-31G* levels.

1. Introduction

Polyhedral oligomeric silsesquioxane (POSS) $(RSiO_{1.5})_n$ with $n=6$, 8, 10, are compounds that embody a truly hybrid (inorganic–organic) architecture.[1] Their structure is particularly interesting because it contains an inner inorganic framework made up of silicone and oxygen $(SiO_{1.5})_x$, that is externally covered by organic substituents. These substituents can be totally hydrocarbon in nature, or they can embody a range of polar structures and functional groups, able to make the POSS nanostructure compatible with polymers, biological systems, or surfaces. In this way, POSS can be incorporated into linear or thermosetting polymers improving their thermal and oxidation resistance and reduced flammability. Furthermore, they can be used in biomedical applications as scaffolds for drug delivery, imaging reagents, and for combinatorial drug development.[2]

POSS with $n=8$ are the most representative members of this family (Fig. 1).

In particular, the opportune functionalization of the R groups leads to the introduction of nanoscaled POSS into organic polymers by polymerization at the reactive sites. These features have spurred a continuous interest in improving known synthetic methods and exploring new ones, targeted at the development of new modified nanocomposites. In this context, we envisaged that an useful functionalization of cubic silsesquioxanes, in order to generate new nanocomposites, could be performed through the insertion in the siloxane frame of an heterocyclic nucleus which, in turn, could be successfully manipulated in a series of new derivatives.[1,2]

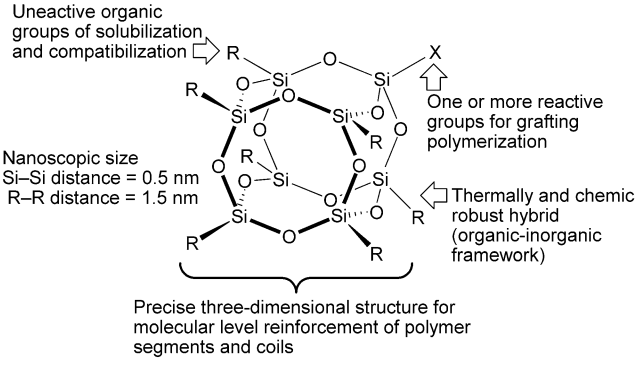

Figure 1. General structure of POSS with $n=8$.

Microwave irradiation is becoming an increasingly important method of heating which replaces the classical one: currently, more than 1000 papers, including several reviews,[3–11] have been published on this topic. The technique has been used in reactions of oxidation, reduction,

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alkylation, condensation, protection and deprotection, as well as in rearrangement, cycloaddition and many other processes of significance for organic chemistry.[9]

The key features in microwave-assisted reactions are represented by the enhanced selectivity, the improved reaction rates, the milder reactions, the formation of cleaner products with higher yields and minor wastes with respect to the classical method of heating.

In this paper, we report the conversion of vinyl- and styryl-POSS into isoxazole derivatives by the exploitation of microwave assisted 1,3-dipolar cycloaddition processes of nitrone and nitrile oxide: the synthesis of compounds of general formula $R_7R'Si_8O_{12}$ in which R is a cyclopentyl group while R′ is an isoxazole moiety, is described. The presence of the isoxazole ring can lead to an improvement of the properties of POSS giving access, through modification of the *N*,*O*-ring, to new functionalized derivatives that can be compatible with other materials. Furthermore, the importance of five membered nitrogen heterocycles, such as isoxazole derivatives, which possess important biological properties, or are precursors of polymeric compounds having thermal resistance is well known.[12]

The obtained derivatives represent the first example of this kind of compound which have not previously been reported in literature.

2. Results and discussion

The cycloaddition of *N*-methyl-*C*-ethoxycarbonyl nitrone **1**[13] with vinyl-POSS **2a** in toluene at 130 °C for 8 h in a sealed tube, using a 1:1 relative ratio of dipole to dipolarophile, produced a mixture of three isoxazolidines **3a**, **4a** and **5a** in ca. 3.5:19.8:1 ratio, with a total yield of 20%.

A sharp improvement of the cycloaddition process has been obtained under microwave irradiation. In fact, the reaction of nitrone **1** with **2a**, in toluene as solvent, under microwave irradiation within 90 min affords the 1,3-dipolar cycloadducts **3a**, **4a** and **5a** in the above reported relative ratio but with a total yield of 73%.

The molecular structure of the reaction products was assigned on the basis of analytical and spectroscopic data. As expected,[14] the pericyclic reaction showed a good control of regio- and stereoselectivity with the *trans* isomer **4a**, as the major product. The regiochemistry of the cycloaddition process was readily deduced from the ^1H NMR data in C_6D_6 for compounds **3a** and **4a**, and in $CDCl_3$ solution for compound **5a**. In the case of 5-regioisomers **3a** and **4a**, there was one proton signal in the range 4.32–4.15 δ which corresponds to the H_5 proton; on the contrary, the alternative 4-regioisomer **5a** shows the two diagnostic H_5 protons as doublet of doublets at 4.11 δ (J=9.4, 8.3 Hz) and 4.01 δ (J=9.4, 8.1 Hz), respectively.

The relative stereochemical assignments in compounds **3a**, **4a** and **5a** were attributed by NOE experiments. In particular, for *cis* epimer **3a**, a positive NOE effect has been detected between H_5, the upfield H_{4a} protons and H_3, thus indicating a *cis* topological relationship between these protons. For *trans* compound **4**, irradiation of H_5 at δ=4.15 resulted in a positive NOE effect on H_{4b} the downfield resonance of methylene protons at C_4 (δ=2.90), while irradiation of the H_{4a} (δ=2.70) gave rise to the enhancement of H_3 resonance (δ=3.2).

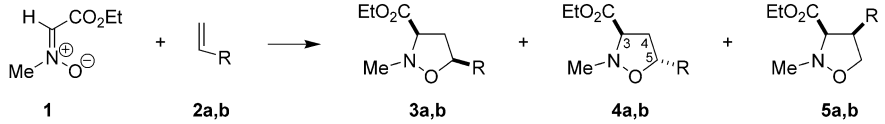
For *cis* compound **5a**, irradiation of H_3 at δ=3.41 resulted in a positive NOE effect on H_{5b} the downfield resonance of methylene protons at C_5 (δ=4.15), and H_4, while irradiation of the H_4 (δ=2.35) gave rise to the enhancement of H_3 resonance and H_{5b}.

A complete inversion of diastereoselectivity was observed in the reaction of nitrone **1** with styryl-POSS **2b**. Thus, the reaction performed under classical heating or microwave irradiation (see Table 1) gave rise to a mixture of isoxazolidines **3b** and **4b** in 20:1 relative ratio in which the *cis* isomer was the predominant compound. The relative stereochemistry was determined by NOE experiments.

Then, we turned our attention to the synthesis of 3-ethoxycarbonyl functionalized 4,5-dihydroisoxazoles **9** by 1,3-dipolar cycloaddition of nitrile oxide **6** with vinyl- **2a** and styryl-POSS **2b**.

Three different procedures have been applied: (a) reaction of oxime **6** with 1.0 equiv of *N*-bromosuccinimide in CH_2Cl_2 for 20 min followed of addition of 0.12 equiv of dipolarophile, and subsequent slow addition of triethylamine for 15 min, and overnight stirring of the resulting mixture;[15] (b) reaction of oxime **6** with 1.5-fold excess of dipolarophile and with sodium hypochlorite in CH_2Cl_2 solution at 0 °C for 3 h;[16] (c) reaction of 7.5 equiv of chloro

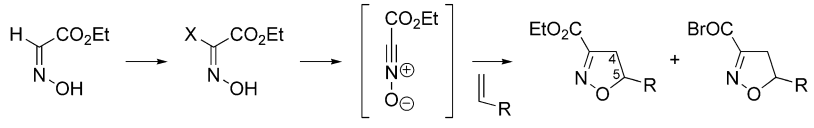
Table 1. Reaction of nitrone **1** with vinyl **2a** and styryl-POSS **2b** under microwave irradiation in 2 mL of toluene



Compound	R	Time/min[a]	Global yield (%)	Classical[b] heating (%)	Products ratio **3**:**4**:**5**
a	(c-C_5H_9)$_7Si_8O_{12}$	90	73.4	20	3.5:19.8:1
b	p-[(c-C_5H_9)$_7Si_8O_{12}$]–C_6H_4	90	90.0	30	20:1:0

[a] Irradiation at 100 W, the temperatures reached by the reaction mixture are in the range of 100–110 °C.
[b] Reaction performed in toluene at 130 °C in sealed tube for 6 h.

Table 2. Reaction of nitrile oxide **13** with vinyl- **2a** and styryl-POSS **2b**



6 **7** **8** **2a,b** **9a,b** **10a,b**

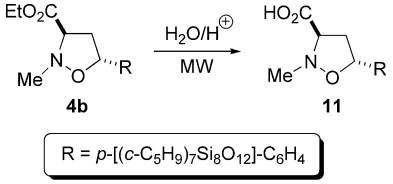
Entry	Compound	Method	X	R	Time/min	Total yield (%)	**9**:**10** ratio
1	**a**	A[a]	Br	$(c\text{-}C_5H_9)_7Si_8O_{12}$	1200	50	1:3.4
2	**b**	A[a]	Br	$p\text{-}[(c\text{-}C_5H_9)_7Si_8O_{12}]\text{–}C_6H_4$	1200	60	1:3.4
3	**a**	B[b]	H	$(c\text{-}C_5H_9)_7Si_8O_{12}$	180	15	1:0
4	**b**	B[b]	H	$p\text{-}[(c\text{-}C_5H_9)_7Si_8O_{12}]\text{–}C_6H_4$	180	15	1:0
5	**a**	C[c]	Cl	$(c\text{-}C_5H_9)_7Si_8O_{12}$	30	70	1:0
6	**b**	C[c]	Cl	$p\text{-}[(c\text{-}C_5H_9)_7Si_8O_{12}]\text{–}C_6H_4$	30	75	1:0

[a] Reaction performed at rt.
[b] Reaction performed with sodium hypochlorite in dichloromethane solution at 0 °C.
[c] Irradiation at 80 W with 2 mL of $CHCl_3$; alumina was used as support for dehydrohalogenation of chloro oxime.[18]

oxime **7**,[17] with 1.0 equiv of dipolarophile in the presence of 0.2 g of Al_2O_3 under microwave assisted conditions (see Table 2). Structures of the obtained cycloadducts were assigned on the basis of analytical and spectroscopic data. The ^1H NMR spectra of compounds **9** showed the diagnostic resonances of H_5 protons in the range 5.79–4.20 ppm as doublet of doublets, while methylene protons at C_4 appear as two doublet of doublets at 3.65–3.70 and 3.22–3.19 ppm. For compounds **10** the H_5 protons appear in the range 5.16–4.22 ppm as a doublet of doublets, while the H_4 protons resonate in the range 4.1–3.97 ppm as multiplets. The investigated 1,3-dipolar cycloaddition proceeds regiospecifically and always affords 5-substituted isoxazolines. The formation of compounds **10**, can be easily explained on the basis of acylic nucleophilic substitution of the initial cycloadduct **9** with the triethylammonium bromide, formed during dehydrobromination of bromo oxime by reaction with triethylamine.

As reported in Table 2, the best results were obtained when the cycloaddition reaction was performed under microwave irradiation (entries 5 and 6). In this case, the reaction time was dramatically reduced and the yield of compounds **9** increased to 70–75%.

Our final goal, directed towards the design of a new synthetic approach to suitable substituted POSS containing a carboxylic group at C_3 of the heterocyclic moiety **11** has been reached by the hydrolysis of esters **4b** under microwave irradiation at 150 W in a CEM apparatus for 30 min (Scheme 1).



4b **11**

R = $p\text{-}[(c\text{-}C_5H_9)_7Si_8O_{12}]\text{-}C_6H_4$

Scheme 1.

3. Computational studies

3.1. Prediction of regiochemistry

The dipolarophilic reactivity of substituted-POSS towards nitrone **1** and nitrile oxides **8**, rationalized in terms of Frontier Molecular Orbital (FMO) theory using PM3 methods, is consistent with the dominant interaction HOMO (dipolarophile)–LUMO (dipole) but the regiochemistry cannot be clearly deduced because the values of atomic coefficients, in the reactive orbitals, are almost identical.

Therefore, in consideration of the interest emerged towards the prediction of regiochemistry, in a wide series of pericyclic reactions, by more sophisticated and rigorous approach that overcome the failure of FMO theory, we decided to study the reactions at hand using the DFT-based reactivity indexes,[19] that had already performed very well for several 1,3-dipolar cycloaddition[20] and Diels–Alder reactions.[21] So, the global and local reactivity indexes were evaluated at the ground state of reactants making use of the B3LYP functional in combination with the standard 6-31G* basis set,[22] utilizing the Gaussian 03W set of programs,[23] and are reported in Table 3. The prediction of regioselectivity was assessed utilizing the local counterpart of the Pearson's hard and soft acids and bases principle, stating that interacting sites of a Lewis acid and base will have local softnesses that are as close as possible. As such, it can be rationalized that two molecules containing two possible interaction sites A and B, and C and D, respectively, will

Table 3. Global properties (electronic chemical potential μ, chemical hardness η and chemical softness S values are in a.u.; electrophilicity power ω values are in eV) of nitrone **1**, vinyl-POSS **2a**, styryl-POSS **2b**, and nitrile oxide **8**

	μ	η	S	ω
1	−0.15623	0.16990	2.94	1.95
2a	−0.14339	0.26626	1.88	1.05
2b	−0.13494	0.18476	2.71	1.34
8	−0.17372	0.22027	2.27	1.86

Table 4. Reverse energy gaps (eV) between molecular orbitals for the reaction of **1** and **8** with **2a** and **2b**

	$LUMO_{dipole}$–$HOMO_{dipolarophile}$					
	NBO		MK		Mulliken	
	Δ_4	Δ_5	Δ_4	Δ_5	Δ_4	Δ_5
1+**2a**	0.500	0.511	1.270	1.249	0.379	0.393
1+**2b**	0.193	0.255	0.795	0.596	0.238	0.302
8+**2a**	0.216	0.235	0.480	0.492	0.331	0.393
8+**2b**	0.031	0.142	0.152	0.263	0.218	0.217

preferentially interact yielding the smallest of the two Δ values:[24]

$$\Delta_{CD}^{AB} = (s_A - s_C)^2 + (s_B - s_D)^2 \quad (1)$$

where A and B are the atoms of one molecule involved, in this specific case, in the formation of a cycloadduct with atoms C and D of another molecule, and s_i's are the appropriate type of atomic softnesses (if s_A and s_B are electrophilic then s_C and s_D are obviously nucleophilic).

The local softness s was calculated as a product $f \cdot S$, where f is the condensed form of Fukui functions calculated as reported elsewhere.[25] The main advantage of these condensed Fukui functions is the fact that they can be easily used, whereas the interpretation of the local Fukui functions is in some cases less straightforward and more subtle. The main drawback of their usage is that an adequate condensation scheme has to be chosen and that, in some cases, site selectivity may change when using two different population analysis schemes. Since there is not a rational method to choose the best condensation scheme to apply, the criterion usually used is based on 'personal preference'; so, we have elected to calculate the Fukui functions upon three population analysis (PA) schemes named, natural PA, Merz-Singh-Kollman PA and Mulliken PA, in order to determine which provide the best results and to produce a contribute to this problematic tool. All Δ values, calculated utilizing the NBO, MK and Mulliken derived charges, are reported in Table 4.

The electronic chemical potential, μ, of the two dipolarophiles **2** is higher than those for the dipoles **1** and **8**. Therefore the charge transfer at these 1,3-dipolar cycloaddition reactions will take place from the dipolarophile to the dipole, in complete agreement with the HOMO (dipolarophile)–LUMO (dipole) interaction predicted by FMO theory.

Moreover, the two dipoles present high electrophilicity values and, according to the absolute scale of electrophilicity based on the ω index,[20c,21b] they can be classified as strong electrophiles; on the contrary, the two dipolarophiles, using the same considerations are only moderately electrophiles. Thus, the more favourable interaction will take place between the vinyl-POSS **2a**, the less electrophilic species, and nitrone **1**, even though compound **2a** will react well with nitrile oxide **8**, too. Conversely, styryl-POSS **2b** will be less reactive. Moreover, the electrophilicity differences $\Delta\omega$ between dipoles **1** and **8** and dipolarophiles **7** are in the range of 0.52–0.90 and indicates a lower polar character for these cycloadditions, characteristic of non-polar (pericyclic) reactions.[20c,21b]

In all the 1,3-dipolar cycloadditions studied we should consider only the Δ values for a $LUMO_{dipole}$–$HOMO_{dipolarophile}$ approach, in accord to global parameter analysis. From inspection of Table 4, it emerges that the approach to regioselectivity based upon NBO charge predicts, in all the cases, the prevalent or exclusive formation of the 4-adduct, in contrast with experimental results. Instead, the analysis conducted by MK charges, correctly predicts the prevalent formation of 5-adduct, in the case of the reaction between **1** and **2a**, and the exclusive formation of the 5-regioisomer for the 1,3-DC of **1** with **2b** while the same analysis fails for nitrile oxide cycloadditions. Finally, the Mulliken scheme gives almost equal results of NBO.

Therefore, the regiochemistry prediction based on local properties such as softness, established on HSAB principle, is strongly influenced by the type of PA selected to obtain charge distribution: in this case MK derived charges predict the regiochemistry well for nitrone cycloadditions whereas none of the three schemes reproduce the correct regiochemistry for nitrile oxide cycloadditions; probably, in this last case, the steric effects of the POSS prevailed and the 5-regioisomer is favoured.

To overcome the low prediction power of the DFT approach based on the global properties of reagents at their ground state, we have calculated the formation enthalpies for the

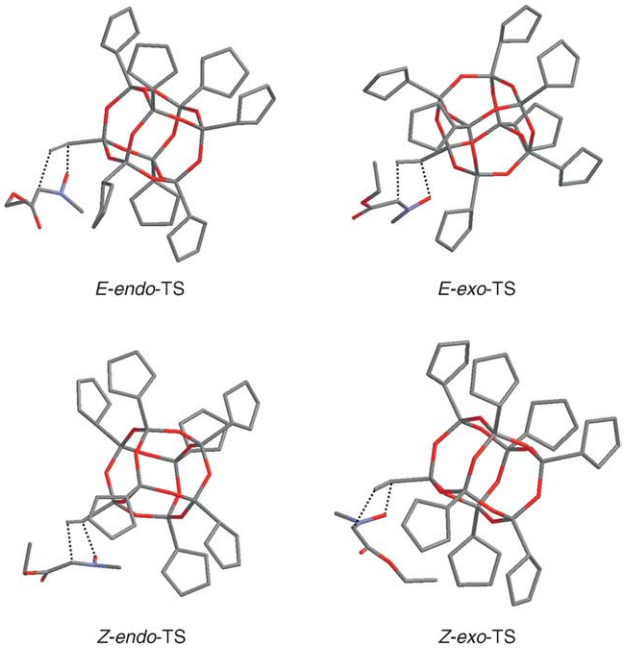


Figure 2. Transition state structures for compounds **3a** and **4a**.

Table 5. PM3 formation enthalpies for transition states leading to compounds **3** and **4**

Compound	TS	ΔH_f[a]	$\Delta\Delta H_f$	Calculate %	Ratio	*cis*/*trans* calculated ratio	*cis*/*trans* observed ratio
3a	*E-endo*	−1509.15	0.99	13.54	1.00	1.00	1.00
	Z-exo	−1503.12	7.02	0.00	0.00		
4a	*E-exo*	−1510.14	0.00	71.24	5.26	6.38	5.66
	Z-endo	−1509.22	0.92	15.22	1.12		
3b	*E-endo*	−1486.60	0.00	97.57	102.70	104.25	20.00
	Z-exo	−1484.10	2.50	1.48	1.55		
4b	*E-exo*	−1483.84	2.76	0.95	1.00	1.00	1.00
	Z-endo	−1480.01	6.59	0.00	0.00		

[a] All values are in kcal/mol.

transition states involved in the cycloaddition process by the aid of semiempirical PM3 calculations.

Thus, we studied the diastereoselectivity for the reaction of (*E*)- and (*Z*)-**1** with vinyl-POSS **2a** and styryl-POSS **2b** considering all transition states deriving from an *endo* or *exo* approach. The formation of 3,5-disubstituted isoxazolidines (compounds **3** and **4**) was considered, according to other theoretical studies on this topics.[26]

Consequently, four transition states, leading to two cycloadducts, have been located for each dipolarophile (Fig. 2), and the obtained results are summarized in Table 5.

The *E-exo*-TS was the most stable in the case of reaction of nitrone **1** with **2a**, whereas, the *E-endo*-TS was preferred for the reaction of **1** with **2b**. The relative ratio between compounds **3** and **4**, in *cis* and *trans* configuration, respectively, obtained according to the Boltzmann equation are also reported in Table 5. These are correctly predicted by calculations and are completely in accord with experimental results.

Concerning the cycloaddition reaction of nitrile oxide **8** with vinyl and styryl-POSS, we have examined the possibility to obtain both regioisomers, e.g. the formation of compounds **9** and **12** (Fig. 3).

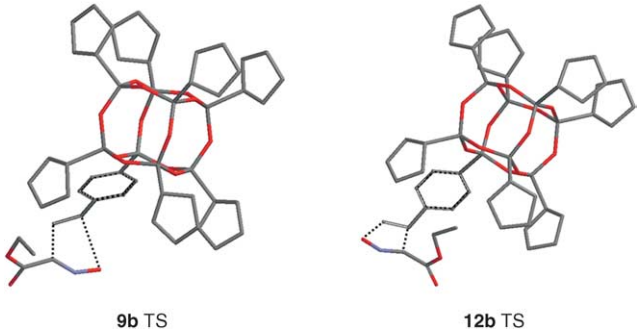


Figure 3. Transition state structures for compounds **9b** and **12b**.

Table 6 summarizes the obtained results for the reaction of nitrile oxide **8** with compounds **2**. In both cases the regioisomeric transition states leading to compounds **9** are the more stable of transition states leading to compounds **12** and the values of the regioisomeric ratio, calculated by the Boltzman equation, in accord with the difference in formation enthalpies ($\Delta\Delta H_f$TS) of regioisomeric transition states, agree very well with the experimentally observed ones.

4. Conclusion

In conclusion, we have shown that the general features of microwave chemistry, faster and cleaner reactions, can be successfully applied to the preparation of functionalized POSS derivatives by 1,3-dipolar cycloadditions of *C*-ethoxycarbonyl nitrone or ethoxycarbonyl nitrile oxide, affording cycloadducts generally in higher yields than under conventional heating. These results, then, open the way to a high number of opportunities, including the preparation of nanocomposites. More work on other types of cycloadditions involving modified ethenyl-POSS and the influence of irradiation power on the nature of the resulting products will be the target of further studies.

Moreover, the introduction of such nanoscaled POSS macromers into an organic polymer by polymerization at the single reactive site (one of eight corner groups in a POSS macromer) can lead to the design of new POSS-modified polymers with improved properties.

5. Experimental

5.1. General information

NMR spectra were recorded on a Varian Unity Inova instrument (^{1}H 500 MHz, ^{13}C 125.67 MHz, ^{29}Si 99.32 MHz) in $CDCl_3$ or C_6D_6 as solvents. Chemical shifts are in ppm (δ) from TMS as internal standard. NOE difference spectra were obtained by subtracting

Table 6. PM3 formation enthalpies for transition states leading to **9** and **12** for the 1,3-DC of nitrile oxide **8** with compounds **2**

TS Regioisomer	ΔH_f[a]	$\Delta\Delta H_f$	Calculated %	**9/12** calculated ratio	**9/12** observed ratio
9a	−1489.44	0.00	99.94	16656	1
12a	−1485.00	4.44	0.06	1	0
9b	−1462.99	0.00	99.54	216	1
12b	−1459.79	3.20	0.46	1	0

[a] All values are in kcal/mol.

alternatively right-off-resonance free induction decays (FIDS) from right-on-resonance-induced FIDS. MS spectra were performed on a Hewlett-Packard 1100 chromatograph equipped with on-line diode array detector and an Agilent MSD supplied with API-ES (atmospheric pressure ionization-electrospray) interface. The reactions under microwave irradiations were carried out using a CEM Corporation Focused Microwave System, Model Discover. Elemental analyses were performed on a Perkin–Elmer 240B microanalyzer. Merck silica gel 60H was used for preparative short-column chromatography. Vinyl and styryl-POSS have been purchased from Aldrich Co.

5.2. Preparation of isoxazolidinyl-POSS 3, 4 and 5a under microwave irradiation

General procedure. A mixture of 1.7 mmol (223 mg) of nitrone **1**, and 0.2 mmol of vinyl-POSS **2a** or styryl-POSS **2b**, dissolved in toluene (2 mL) in a pressure tube equipped with a stirrer bar, was inserted into the cavity of a Discover Microwave System apparatus and heated at 100 W for 90 min (internal temperature 100–110 °C). The mixture was evaporated and the resulting solid was purified by flash chromatography on silica gel with cyclohexane/ethyl acetate (80:20) as eluent to give isoxazolines **3a**–**5a** from **2a** and isoxazolines **3b** and **4b** from **2b**, respectively.

5.2.1. Reaction of nitrone 1 with vinyl-POSS 2a. The first eluted product was ethyl (3*RS*,5*SR*)-5-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.1^{3,9}.1^{5,15},1$^{7.13}$]octasilox-1-yl)-2-methylisoxazolidine-3-carboxylate **4a**. (59.8%, 63.23 mg), white solid, mp >300 °C; δ_H (500 MHz, C_6D_6) 0.89 (t, 3H, J=7.1 Hz, –CH_2–CH_3), 0.91–1.01 (m, 7-CH cyclopentyl), 1.42–1.58 (m, 14H, –CH_2 cyclopentyl), 1.62–1.70 (m, 14H, –CH_2 cyclopentyl), 1.71–1.82 (m, 14H, –CH_2 cyclopentyl), 1.83–1.95 (m, 14H, –CH_2 cyclopentyl), 2.71 (ddd, 1H, J=9.6, 10.7, 11.9 Hz, H_{4a}), 2.82 (s, 3H, N–CH_3), 2.91 (ddd, 1H, J=5.3, 7.9, 11.9 Hz, H_{4b}), 3.20 (dd, 1H, J=5.3, 9.6 Hz, H_3), 3.91 (q, 2H, J=7.1 Hz, –CH_2–CH_3), 4.15 (dd, 1H J=7.9, 10.7 Hz, H_5); δ_C (125 MHz, $CDCl_3$) 14.1, 22.1, 26.9, 27.0, 27.3, 36.3, 45.5, 61.2, 66.3, 69.3, 171.0. δ_{Si} (99.9 MHz, $CDCl_3$) −65.99, −66.56 (3:4, cyclopentyl-Si), −77.41 (isoxazolidinyl-Si). Anal. Calcd for $C_{42}H_{75}NO_{15}Si_8$: C, 47.65; H, 7.14; N, 1.32. Found: C, 47.81; H, 7.22; N, 1.39. MS (ESI +ve ion): m/z=1059.7 $[M+H]^+$.

The second eluted product was ethyl (3*RS*,4*RS*)-4-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.1^{3,9}.1^{5,15},1$^{7.13}$]-octasilox-1-yl)-2-methylisoxazolidine-3-carboxylate **5a**. (3.0%, 3.17 mg), white solid, mp >300 °C; δ_H (500 MHz, $CDCl_3$) 0.87 (t, 3H, J=7.1 Hz, –CH_2–CH_3), 0.92–1.02 (m, 7-CH cyclopentyl), 0.93–1.78 (m, 42H, –CH_2 cyclopentyl), 1.81–1.94 (m, 14H, –CH_2 cyclopentyl), 2.35 (dd, 1H, J=8.5, 9.4 Hz, H_4), 2.74 (s, 3H, N–CH_3), 3.41 (d, 1H, J=8.5 Hz, H_3), 4.01 (dd, 1H J=8.1, 9.4 Hz, H_{5a}), 4.11 (dd, 1H J=8.3, 9.4 Hz, H_{5b}), 4.15 (q, 2H, J=7.1 Hz, –CH_2–CH_3); δ_C (125 MHz, $CDCl_3$). 14.1, 21.7, 22.5, 22.6, 26.9, 31.9, 44.9, 61.4, 68.1, 70.8, 170.8. δ_{Si} (99.9 MHz, $CDCl_3$) −65.76, −66.19 (3:4, cyclopentyl-Si), −71.34 (isoxazolidinyl-Si). Anal. Calcd for $C_{42}H_{75}NO_{15}Si_8$: C, 47.65; H, 7.14; N, 1.32. Found: C, 47.83; H, 7.25; N, 1.37. MS (ESI +ve ion): m/z=1059.7 $[M+H]^+$.

The third eluted product was ethyl (3*RS*,5*RS*)-5-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.1^{3,9}.1^{5,15},1$^{7.13}$]-octasilox-1-yl)-2-methylisoxazolidine-3-carboxylate **3a**. (10.6%, 11.21 mg), white solid, mp >300 °C; δ_H (500 MHz, C_6D_6) 097 (t, 3H, J=7.1 Hz, –CH_2–CH_3), 0.94–1.03 (m, 7-CH cyclopentyl), 1.44–1.56 (m, 14H, –CH_2 cyclopentyl), 1.64–1.74 (m, 14H, –CH_2 cyclopentyl), 1.68–1.79 (m, 14H, –CH_2 cyclopentyl), 1.85–1.96 (m, 14H, –CH_2 cyclopentyl), 2.54 (ddd, 1H, J=7.3, 11.9, 12.2 Hz, H_{4a}), 2.84 (s, 3H, N–CH_3), 3.10 (ddd, 1H, J=6.2, 8.0, 12.2 Hz, H_{4b}), 3.46 (dd, 1H, J=7.3, 8.0 Hz, H_3), 3.91 (q, 2H, J=7.1 Hz, –CH_2–CH_3), 4.27 (dd, 1H J=6.2, 11.9 Hz, H_5); δ_C (125 MHz, $CDCl_3$) 14.2, 22.2, 26.9, 27.0, 27.3, 37.0, 44.7, 61.2, 63.2, 69.3, 171.1;. δ_{Si} (99.9 MHz, $CDCl_3$) −71.06, −71.67 (3:4, cyclopentyl-Si), −81.92 (isoxazolidinyl-Si). Anal. Calcd for $C_{42}H_{75}NO_{15}Si_8$: C, 47.65; H, 7.14; N, 1.32. Found: C, 47.91; H, 7.19; N, 1.43. MS (ESI +ve ion): m/z=1059.7 $[M+H]^+$.

5.2.2. Reaction of nitrone 1 with styryl-POSS 2b. The first eluted product was ethyl (3*RS*,5*RS*)-5-[4-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.1^{3,9}.1^{5,15},1$^{7.13}$]octasilox-1-yl)phenyl]-2-methylisoxazolidine-3-carboxylate **4b**. (85.9%, 97.3 mg), white solid, mp >300 °C; δ_H (500 MHz, $CDCl_3$) 0.94–1.04 (m, 7-CH cyclopentyl), 1.32 (t, 3H, J=7.1 Hz, –CH_2–CH_3), 1.45–1.57 (m, 42H, –CH_2 cyclopentyl), 1.78–1.92 (m, 14H, –CH_2 cyclopentyl), 2.48 (ddd, 1H, J=8.1, 9.5, 12.5 Hz, H_{4a}), 2.91 (s, 3H, N–CH_3), 2.92 (ddd, 1H, J=6.0, 7.4, 12.5 Hz, H_{4b}), 3.94 (dd, 1H, J=6.0, 9.5 Hz, H_3), 4.26 (q, 2H, J=7.1 Hz, –CH_2–CH_3), 5.13 (dd, 1H J=7.4, 8.1 Hz, H_5), 7.36 (d, 2H, J=8.1 Hz, aromatic protons), 7.65 (d, 2H, J=8.1 Hz, aromatic protons); δ_C (50 MHz, $CDCl_3$), 14.2, 22.2, 26.9, 27.0, 27.3, 41.1, 45.3, 61.5, 69.8, 79.0, 125.7, 132.0, 134.3, 141.4, 169.7; δ_{Si} (99.32 MHz, $CDCl_3$), −66.03, −66.36 (3:4, cyclopentyl-Si), −79.84 (isoxazolidinyl-Si). Anal. Calcd for $C_{48}H_{79}NO_{15}Si_8$: C, 50.80; H, 7.02; N, 1.23. Found: C, 50.87; H, 6.92; N, 1.25 MS (ESI +ve ion): m/z=1135.8 $[M+H]^+$.

The second eluted product was ethyl (3*RS*,5*SR*)-5-[4-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.1^{3,9}.1^{5,15},1$^{7.13}$]-octasilox-1-yl)phenyl]-2-methylisoxazolidine-3-carboxylate **3b**. (4.1%, 4.6 mg), white solid, mp >300 °C; δ_H (500 MHz, $CDCl_3$) 0.80–0.97 (m, 7-CH cyclopentyl), 1.30 (t, 3H, J=7.1 Hz, –CH_2–CH_3), 1.43–1.70 (m, 42H, –CH_2 cyclopentyl), 1.70–1.81 (m, 14H, –CH_2 cyclopentyl), 2.60 (ddd, 1H, J=5.2, 7.8, 12.8 Hz, H_{4a}), 2.88 (s, 3H, N–CH_3), 3.02 (ddd, 1H, J=68, 8.2, 12.8 Hz, H_{4b}), 3.64 (dd, 1H, J=5.2, 6.8 Hz, H_3), 4.22 (q, 2H, J=7.1 Hz, –CH_2–CH_3), 5.23 (dd, 1H, J=7.8, 8.2 Hz, H_5), 7.42 (d, 2H, J=8.1 Hz, aromatic protons), 7.75 (d, 2H, J=8.1 Hz, aromatic protons); δ_C (125 MHz, $CDCl_3$) 14.1, 22.3, 26.9, 27.0, 27.3, 41.3, 45,2, 61.4, 72.2, 78.7, 125.8, 131.9, 134.3, 142.7, 170.2; δ_{Si} (99.32 MHz, $CDCl_3$), −66.03, −66.36 (3:4, cyclopentyl-Si), −79.84 (isoxazolidinyl-Si). Anal. Calcd for $C_{48}H_{79}NO_{15}Si_8$: C, 50.80; H, 7.02; N, 1.23. Found: C, 50.84; H, 6.88; N, 1.21. MS (ESI +ve ion): m/z=1135.8 $[M+H]^+$.

5.3. Synthesis of 2-isoxazolines

5.3.1. Method A. *N*-Bromosuccinimide (NBS, 300 mg, 1.7 mmol) was stirred in a flask containing dry

dichloromethane (2 mL). The oxime **6** (200 mg, 1.7 mmol) was added in one portion at 25 °C. The bromination was usually over ca. 20 min, as observed by the disappearance of the suspended NBS. The vinyl- **2a** or styryl-POSS **2b** (0.2 mmol) was added at 25 °C and the triethylamine (182 mg, 1.8 mmol) in dichloromethane (2 mL) was added dropwise over ca. 15 min. The reaction mixture was stirred at room temperature overnight under nitrogen atmosphere. The solution was washed with water (3×4 mL), dried and evaporated in vacuum. The resulting solid was purified by flash chromatography on silica gel with cyclohexane/ethyl acetate (85:15) as eluent to give the pure 2-isoxazolines **9a**, **10a**, and **9b**, **10b**, respectively.

5.4. Reaction of oxime 6 with NBS and vinyl-POSS 2a

The first eluted product was 5-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.13,9.15,15.17,13]octasilox-1-yl)-4,5-dihydroisoxazole-3-carbonylbromide **10a** (38.64%, 83.27 mg), white solid, mp > 300 °C; δ_H (200 MHz, $CDCl_3$) 0.92–1.03 (m, 7-CH cyclopentyl), 1.35–1.68 (m, 42H, –CH_2 cyclopentyl), 1.69–1.77 (m, 14H, –CH_2 cyclopentyl), 3.33–3.47 (m, 2H, H_{4a} and H_{4b}), 4.22 (dd, 1H, J=5.2, 6.4 Hz, H_5); δ_C (50 MHz, $CDCl_3$), 23.3, 26.9, 27.2, 28.1, 36.1, 73.6, 159.9, 163.4. δ_{Si} (99.32 MHz, $CDCl_3$), −65.70, −66.26 (3:4, cyclopentyl-Si), –78.34 (isoxazolidinyl-Si). Anal. Calcd for $C_{39}H_{66}BrNO_{14}Si_8$: C, 43.47; H, 6.17; Br, 7.42; N, 1.30. Found: C, 43.75; H, 6.12; Br, 7.43, N, 1.25. MS (ESI +ve ion): m/z=1078.5 $[M+H]^+$.

The second eluted product was ethyl 5-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.13,9.15,15.17,13]octasilox-1-yl)-4,5-dihydroisoxazole-3-carboxylate **9a** (11.36%, 23.69 mg). White solid, mp > 300 °C; δ_H (200 MHz, $CDCl_3$) 0.88 (t, 3H, J=7.1 Hz, –CH_2–CH_3), 0.91–1.01 (m, 7-CH cyclopentyl), 1.29–1.37 (m, 42H, –CH_2 cyclopentyl), 1.39–1.45 (m, 14H, –CH_2 cyclopentyl), 3.19 (dd, 1H, J=9.0, 14.8 Hz, H_{4a}), 3.37 (dd, 1H, J=12.8, 14.8 Hz, H_{4b}), 4.20 (dd, 1H, J=9.0, 12.8 Hz, H_5), 4.34 (q, 2H, J=7.1 Hz, –CH_2–CH_3); δ_C (50 MHz, $CDCl_3$), 14.1, 21.9, 22.0, 22.1, 26.9, 36.2, 72.5, 151.1, 160.9. δ_{Si} (99.32 MHz, $CDCl_3$), −66.56, −66.37 (3:4, cyclopentyl-Si), −78.61 (isoxazolidinyl-Si). Anal. Calcd for $C_{41}H_{71}NO_{15}Si_8$: C, 47.23; H, 6.86; N, 1.34. Found: C, 47.78; H, 6.76; N, 1.35. MS (ESI +ve ion): m/z=1043.7 $[M+H]^+$.

5.5. Reaction of oxime 6 with NBS and styryl-POSS 2b

The first eluted product was 5-[4-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.13,9.15,15.17,13]octasilox-1-yl)phenyl]-4,5-dihydroisoxazole-3-carbonylbromide **10b** (46.36%, 108.8 mg). White solid, mp > 300 °C; δ_H (200 MHz, $CDCl_3$) 0.98–1.07 (m, 7-CH cyclopentyl), 1.45–1.56 (m, 42H, –CH_2 cyclopentyl), 1.74–1.83 (m, 14H, –CH_2 cyclopentyl), 4.00–4.14 (m, 2H, H_{4a} and H_{4b}), 5.16 (dd, 1H, J=5.5, 10.5 Hz, H_5), 7.42 (d, 2H, J=8.0 Hz, aromatic protons), 7.70 (d, 2H, J=8.0 Hz, aromatic protons); δ_C (50 MHz, $CDCl_3$), 22.2, 27.0, 27.2, 27.3, 26.9, 34.8, 50.8, 126.8, 133.6, 134.6, 140.3, 159.2, 162.3. δ_{Si} (99.32 MHz, $CDCl_3$), −66.07, −66.46 (3:4, cyclopentyl-Si), −80.40 (isoxazolidinyl-Si). Anal. Calcd for $C_{45}H_{70}BrNO_{14}Si_8$: C, 46.85; H, 6.12; Br, 6.93, N, 1.21. Found: C, 46.78; H, 6.73; N, 1.35. MS (ESI +ve ion): m/z=1154.6 $[M+H]^+$.

The second eluted product was ethyl 5-[4-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.13,9.15,15.17,13]octasilox-1-yl)phenyl]-4,5-dihydroisoxazole-3-carboxylate **9b** (13.64%, 30.21 mg). White solid, mp > 300 °C; δ_H (200 MHz, $CDCl_3$) 0.98–1.08 (m, 7-CH cyclopentyl), 1.42–1.60 (m, 42H, –CH_2 cyclopentyl), 1.70–1.86 (m, 14H, –CH_2 cyclopentyl), 4.01–4.11 (m, 2H, H_{4a} and H_{4b}), 5.16 (dd, 1H, J=5.5, 10.5 Hz, H_5), δ_C (125 MHz, $CDCl_3$), 14.1, 22.2, 26.9, 27.0, 27.3, 41.5, 62.2, 84.8, 125.0, 132.9, 134.6, 141.4, 151.1, 160.6 δ_{Si} (99.32 MHz, $CDCl_3$), −66.56, −66.37 (3:4, cyclopentyl-Si), −78.61 (isoxazolidinyl-Si). Anal. Calcd for $C_{47}H_{75}NO_{15}Si_8$: C, 50.46; H, 6.76; N, 1.25. Found: C, 50.78; H, 6.73; N, 1.22. MS (ESI +ve ion): m/z=1119.8 $[M+H]^+$.

5.5.1. Method B. A mixture of 1.7 mmol (200 mg) of oxime **6**, and 0.2 mmol of vinyl- **2a** or styryl-POSS **2b**, was dissolved in 30 mL of dichloromethane. To this solution was added dropwise 5 mL of sodium hypochlorite (7% solution) at 0–5 °C. The mixture was slowly allowed to warm to 25 °C with vigorous stirring over a period of 3 h. The aqueous layer was extracted with 15 mL of methylene chloride, and the organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by flash chromatography on a silica gel with cyclohexane/ethyl acetate (85:15) as eluent to give the 2-isoxazolines **9a**, and **9b** in 15% yield, respectively.

5.5.2. Method C. To a mixture of 1.7 mmol (259 mg) of oxime **7**, and 0.2 mmol of vinyl- **2a** or styryl-POSS **2b**, dissolved in chloroform (2 mL) in a pressure tube equipped with a stir bar, was added 200 mg of dry Al_2O_3. The tube was, then, inserted into the cavity of a Discover Microwave System apparatus and heated at 80 W for 30 min (internal temperature 95 °C). The mixture was filtered, the organic layer was evaporated and the resulting solid was purified by flash chromatography to give compound **9a** (70%) and **9b** (75%), respectively.

5.5.3. Preparation of ethyl (3*RS*,5*RS*)-5-[4-(3,5,7,9,11,13,15-heptacyclopentylpentacyclo[9.5.1.1^{3,9}.1^{5,15},1$^{7.13}$]-octasilox-1-yl)phenyl]-2-methylisoxazolidine-3-carboxylic acid 11. To a mixture of 0.1 mmol (113 mg) of **4b**, in a pressure tube equipped with a stir bar, was added 2 mL of water and 100 μL of acetic acid glacial. The tube was, inserted into the cavity of a Discover Microwave System apparatus and heated at 150 W for 30 min (internal temperature 140 °C). The aqueous layer was extracted with 15 mL of methylene chloride, and the organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. The residue was purified by flash chromatography on a silica gel with cyclohexane/ethyl acetate (80:20) as eluent to give **11**, (15%, 16.6 mg). White solid, mp > 300 °C; δ_H (200 MHz, $CDCl_3$) 0.94–1.06 (m, 7H, –CH cyclopentyl), 1.45–1.55 (m, 42H, –CH_2 cyclopentyl), 1.71–1.89 (m, 14H, –CH_2 cyclopentyl), 2.51 (ddd, 1H, J=8.1, 9.5, 12.5 Hz, H_{4a}), 2.94 (s, 3H, N–CH_3), 2.93 (ddd, 1H, J=6.0, 7.4, 12.5 Hz, H_{4b}), 3.43 (dd, 1H, J=6.0, 9.5 Hz, H_3), 5.16 (dd, 1H J=7.4, 8.1 Hz, H_5), 7.78–7.90 (m, 4H, aromatic protons); δ_C (50 MHz, $CDCl_3$), 14.1, 22.2, 26.9,

27.0, 27.3, 41.1, 72.0, 79.0, 125.7, 132.0, 134.3, 141.4, 172.7; δ_{Si} (99.32 MHz, $CDCl_3$), −66.31, −66.39 (3:4, cyclopentyl-Si), −79.81 (isoxazolidinyl-Si). Anal. Calcd for $C_{46}H_{75}NO_{15}Si_8$: C, 49.92; H, 6.83; N, 1.27. Found: C, 49.85; H, 6.79; N, 1.23. MS (ESI +ve ion): m/z=1107.8 $[M+H]^+$.

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Asymmetric transfer hydrogenation using amino acid derivatives; further studies and a mechanistic proposal

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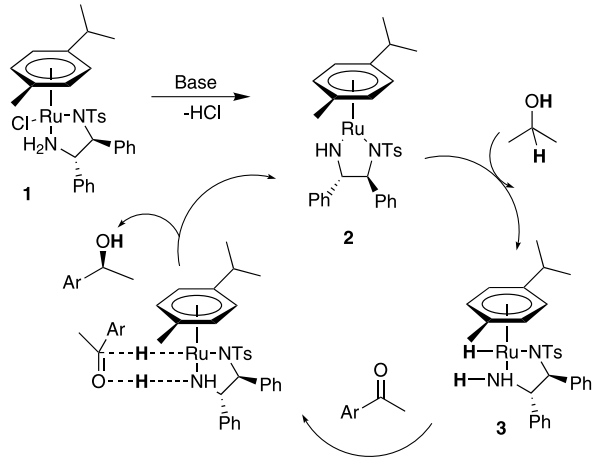
Abstract—A series of investigations into the use of amino acid derivatives for the asymmetric catalysis of the transfer hydrogenation of ketones are presented. Based on the results observed, a mechanistic suggestion for the origin of the enantioselective induction is proposed.

1. Introduction

In recent years, a variety of ligands have been prepared and their effectiveness in Ru(II) catalysed asymmetric transfer hydrogenation (ATH) evaluated.[1] Amongst the best ligands are β-amino alcohols and monoarylsulfonylated 1,2-diamines.[1–7]Applications include α- and β-substituted substrates,[5] medicinally important targets[6] and unsaturated ketones.[7] A mixture of *N*-tosyl-(1*S*,2*S*)-diphenylethylene-diamine (TsDPEN), $[RuCl_2(\eta^6\text{-mesitylene})]_2$ and TsDPEN when heated at 80 °C for 20 min gave the catalyst **1**. Addition of acetophenone in isopropanol to **1** and KOH led to the formation of (*S*)-1-phenylethanol with a yield of 95% (97% ee) after 15 h.[3]

The mechanism for the catalytic cycle of transfer hydrogenation is illustrated in Scheme 1. A ruthenium hydride **3** is formed, via the reaction of 16-electron intermediate **2** with the hydrogen donor (isopropanol or formic acid). The hydritic Ru–H and the protic N–H are transferred simultaneously to the C=O functional group via a six-membered transition state.[2–4] Noyori has isolated and characterised intermediates, **1**, **2**, and **3** by X-ray crystallography.[3a]

Amino alcohols represent good ligands for this application. A combination of (1*S*,2*S*)-*N*-methyl-1,2-diphenylethanol **4** and $[RuCl_2(\eta^6\text{-hexamethylbenzene})]_2$ formed a catalyst, which afforded (*S*)-1-phenylethanol in 94% yield and 92% ee after 1 h at 28 °C.[2a] Work in our group revealed



Scheme 1. Asymmetric reduction by Ru/TsDPEN catalysts.

that (1*R*,2*S*)-*cis*-aminoindan-2-ol was also an excellent ligand.[4a]

Despite the fact that they exist naturally in abundance, are readily accessible, and relatively inexpensive, amino acids and peptides have not been widely applied to asymmetric transfer hydrogenation.[8a] α-Amino acidate ruthenium catalyst complexes have been synthesised by reaction of α-amino acidate anion with $[RuCl_2(arene)]_2$.[8b,c] The catalyst complex derived from L-proline **5** gave (*R*)-1-phenylethanol in 72% yield and 81% ee (Scheme 2).

Chung[8d] reported the use of aminoamides derived from proline as chiral ligands. Ligand **6** catalysed the reduction of acetophenone in 97% conversion with an 80% ee in 7 h.

Keywords: Asymmetric; Transfer; Hydrogenation; Ketones; Aminoacids; Amino alcohols; Reduction.
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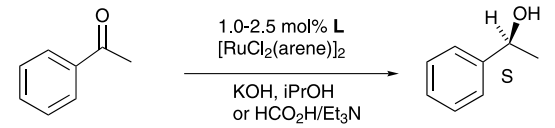
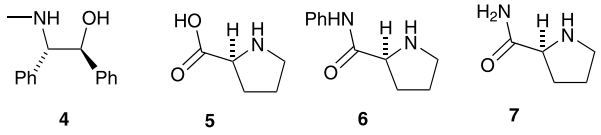


(**4**) 94% yield, 92% e.e. (S) (arene = HMB)
(**5**) 72% yield, 81% e.e. (R) (arene = p-cymene)
(**6**) 97% yield, 79% e.e. (R) (arene = p-cymene)
(**7**) 90% yield, 79% e.e. (R) (arene = p-cymene)



4 **5** **6** **7**

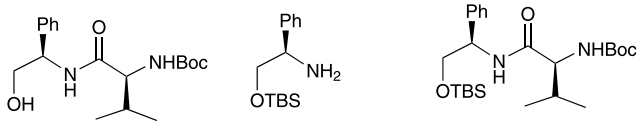
Scheme 2. Ketone reduction using aminoalcohol and peptide ligands.

Faller[8e] et al. also researched chiral ligands derived from L-prolinamide **7** for ruthenium catalysed asymmetric transfer hydrogenation. In 20 h at −24 °C, **7** reduced acetophenone to give (*R*)-1-phenylethanol in 90% yield and 79% ee. The asymmetric reduction of other substrates proceeded smoothly to afford moderate to good yields and ees. Adolfsson[9] reported the use of **8**, a dipeptide analogue, as a chiral ligand. In the asymmetric reduction of a series of ketones; (*S*)-1-phenylethanol was obtained in 78% conversion and 95% ee. Other ketones were also reduced in good conversions and ees. It was found that, although, the chirality of the amino alcohol contributed to enhancing the ee of the reduction product, its configuration was primarily determined by the stereocenter of the amino acid part of the ligand. Adolfsson also noted that the *t*-Boc protecting group was critical for the reaction as, without it, no conversion was observed.

Since, we have published in the area of asymmetric transfer hydrogenation, we chose to undertake a short investigation into this practical and effective class of ligands. Following the conclusion of our research, Adolfsson has published further full papers[10] describing his detailed research on dipeptide anologues in enantioselective reduction of ketones.

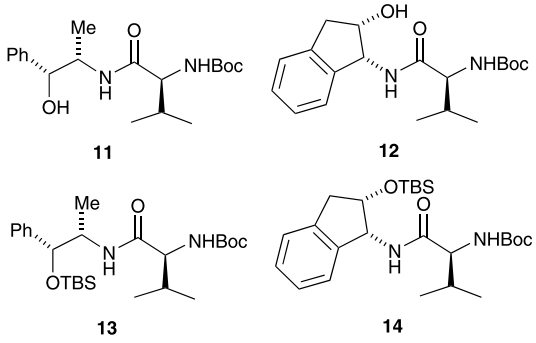
2. Results and discussion

In order to confirm that our practical technique was adequate, ligand **8** was examined first. The synthesis of **8** was achieved using EDCI/HOBt coupling of *O*-*t*-butyldimethylsilyl protected *R*-phenylglycine **9** with *S*-*N*-*t*Boc-valine to give **10**, followed by desilylation. Subsequently, it was discovered that the synthesis of similar ligands could in some cases be reduced to just the coupling step; the protection of the free hydroxy was not always necessary. Ligand **8** was applied to the reduction of acetophenone, using the conditions reported by Adolfsson, that is, 3 mol% of **8**, 0.5 mol% [$RuCl_2$(*p*-cymene)], 5 mol% 0.2 M NaOH, the reduction was run for 2 h at room temperature under nitrogen. Analysis of the product by ^{1}H NMR and HPLC showed that (*S*)-1-phenylethanol was formed in 90% conversion and 98% ee.



8 **9** **10**

We wished to examine whether the amino alcohol part of the dipeptide ligand could contain a 1,2-disubstituted system. Towards this end, norephedrine and *cis*-amino indanol were investigated in place of phenylglycinol. These amino alcohols have been used successfully in ATH. Ligands **11** and **12** were prepared by the coupling of the appropriate TBS-protected amino alcohols with *S*-*N*-*t*Boc-valine to give **13** and **14**, respectively, followed by desilylation. Attempted application of both ligands **11** and **12** in ATH of acetophenone was not successful. ^{1}H NMR spectra showed no formation of phenylethanol. This clearly demonstrated that a 1,2-disubstitution pattern in the amino alcohol is detrimental to the activity of the ligand.



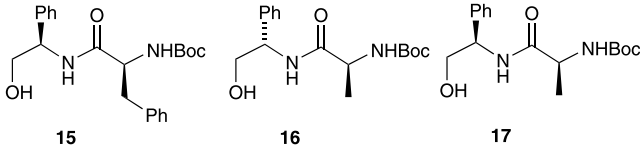
11 **12** **13** **14**

Boc-L-alanine and Boc-L-phenylalanine were selected to replace Boc-L-valine in the ligand. These were selected in order to examine the effects of smaller and larger amido substitutents on the selectivity of the ligands. Compound **15** was anticipated to be the matched version of the dipeptide analogue ligand, whereas **16** the mismatched, on the basis of analogy with Adolfsson's results. Both ligands were prepared via peptide couplings to give **18a** and **18b**, respectively, followed by desilylation. The other diastereoisomer of the alanine compound, **17** (matched), was synthesised using the one-step coupling method between *R*-phenylalanine and *S*-*N*-*t*Boc-valine. The yield for this product was 76% after purification by flash column chromatography. Adolfsson has since, reported the use of the same ligands in ATH. Ligands **15**–**17** were applied to the Ru(II)-catalysed asymmetric transfer hydrogenation of a series of ketones. In all cases, the ligand loading was 3 mol%, and the Ru dimer 0.5 mol% representing a threefold excess of ligand relative to Ru(II) atoms. The reactions were carried out for 2 h at room temperature under nitrogen and the products analysed by GC or HPLC after isolation. The results for these asymmetric reductions are summarised in Table 1.

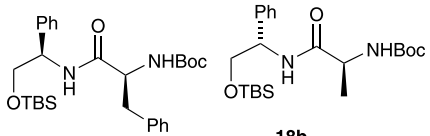
Table 1. Asymmetric reduction of ketones using ligands **15–17**

Substrate	Ligand					
	15 Conv%	**15** ee/%	**16** Conv%	**16** ee/%	**17** Conv%	**17** ee/%
Acetophenone	31	91 *S*	76	88 *S*	80	90 *S*
4-Chloroacetophenone	60	89 *S*	93	88 *S*	17	62 *S*
4-Fluoroacetophenone	34	90 *S*	27	89 *S*	84	74 *S*
4-Methoxyacetophenone	11	82 *S*	35	80 *S*	55	89 *S*
4-Acetonapthone	35	65 *S*	76	96 *S*	86	87 *S*

Ketone:ligand:$[RuCl_2(p\text{-cymene})]_2$: NaOH = 100:3:0.5:1, 2 h.



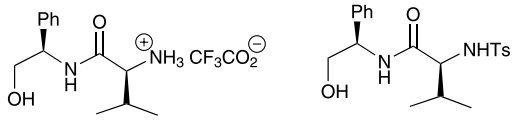
15 **16** **17**



18a **18b**

Comparisons of results between matched alanine and matched phenylalanine ligands (**15** and **17**) indicated that ligands with the smaller *R* group on the amino acid residue gave better conversions. Enantiomeric excesses for both these ligands are good and lie within the 60–90% region. The major enantiomer formed by all three ligands was *S*, confirming that the amino acid has the dominant directing effect.

The importance of the *N* termini of the amino acid part of the ligand has been noted.[9] The possibility of replacing the Boc group with other protecting groups was investigated. The *t*-Boc group was first replaced by a tosyl protecting group. Carbamate **8** was deprotected with 50% TFA/DCM to give **19**, which was tosylated directly with TsCl and Et_3N in DCM to give **20**. As the dipeptide ligands derived from alanine in place of valine were effective in the reduction of ketones under catalytic ATH conditions, ligand **21**, with a dibenzyl protecting group in place of the *t*-Boc group was synthesised. L-alanine was refluxed with benzyl bromide and K_2CO_3 in $EtOH/H_2O$ to give **22**, which was then converted to **21** via carboxyl deprotection, and coupling to phenylglycinol.

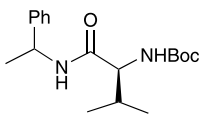


19 **20**

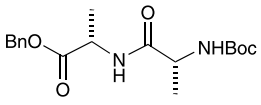
21 **22**

Ligands **20** and **21** were applied to the Ru(II)-catalysed asymmetric transfer hydrogenation of acetophenone (3 mol% ligand, 0.5 mol% Ru(II) dimer). Neither were effective ligands and analysis of these reactions by ^1H NMR showed no presence of phenylethanol even when the reactions were heated at 80 °C for 18 h. This shows that the presence of a carbamate may be essential for the catalyst to be effective. This has since been substantiated by Adolfsson.[10]

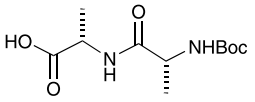
In order to investigate the importance of the presence of a hydroxy group in the amino alcohol part of the ligand, **23** and **24** were synthesised. The construction of these ligands were carried out via the EDCI/HOBt coupling method from Boc-L-valine and *S*- and *R*-α-methylbenzylamine, respectively. Purification of the compounds by recrystallisation afforded **23** and **24** in 74 and 32% yield. In addition, **18b**, an intermediate in the synthesis of **16**, was used as a ligand in the reduction of acetophenone to test whether the hydroxy group has to be unprotected, or if an alkoxy group at this position was an essential requirement. Ligands **23**, **24** and **18b** were applied to the Ru(II)-catalysed reduction of acetophenone. None of these were effective ligands and analysis of these reactions by ^1H NMR showed no presence of phenylethanol. This confirms that the presence of a OH group is vital for the reduction.



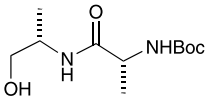
23 *SS*
24 *RS*



25



26



27

We wished to examine whether the terminal OH group of the ligand could be replaced by a carbonyl group such as an ester or a carboxylic acid. The synthesis of **25** was carried out via EDCI/HOBt coupling of benzyl ester L-alanine tosylate and Boc-D-alanine. Compound **25** was then deprotected to **26** using Pd/C-catalysed hydrogenation. Compound **27** (i.e., the equivalent alcohol) was synthesised via EDCI/HOBt coupling of *S*-alinol and Boc-D-alanine.

Neither **25** nor **26** were effective ligands for the asymmetric reduction of acetophenone, however, **27** worked well. The conversions and ees of the reduction of various ketones are summarised in Table 2. Although the conversions are low, several ees are reasonable.

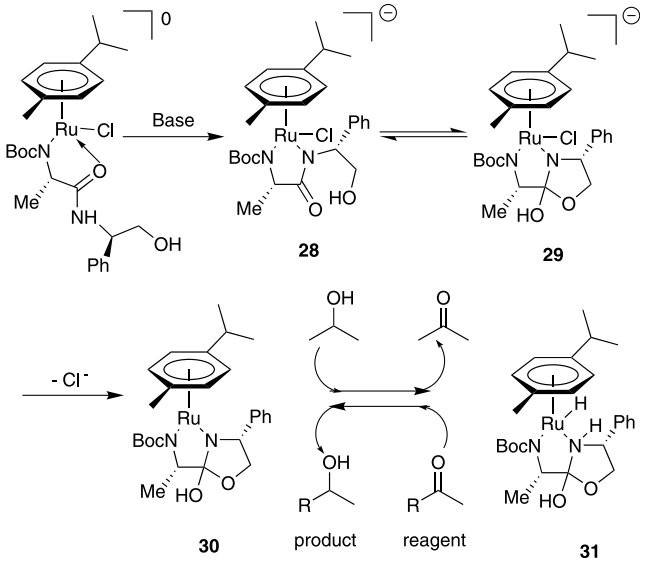
Table 2. Asymmetric reduction of ketones using **27**

Entry	Substrate	Conv/%	ee/%
1	Acetophenone	81	80 *R*
2	4-Chloroacetophenone	39	76 *R*
3	4-Fluoroacetophenone	39	83 *R*
4	4-Methoxyacetophenone	25	93 *R*
5	2′-Acetonaphthone	55	77 *R*

Substrate:ligand **27**:$[RuCl_2(p\text{-cymene})]_2$: NaOH = 100:3:0.5:1, 2 h.

In view of the results, a mechanism for reduction using compounds of type **27**, and **15–17**, is proposed. Initial complexation involves the oxygen of the amide coordinating to ruthenium. Adolfsson has obtained evidence to support this.[10]

Upon treatment with base, the amide may be deprotonated and an N–Ru bond may form to give a negatively charged complex **28**. This has the potential to exist in equilibrium with a cyclic form **29**. Loss of a chloride from **29** could give a neutral form **30**. Since, one nitrogen atom in **30** is now sp^3 hybridised, this represents a complex equivalent to the 16 electron species **2** in the TsDPEN cycle. This could enter the catalytic cycle by abstraction of 2 hydrogen atoms from isopropanol to give hydride **31**, which then returns the hydrogens to the substrate to yield the product and propagate the catalytic cycle (Scheme 3).



Scheme 3. Proposed reduction mechanism.

Since the proposed metallocycle is formed by the amino acid residue, this may explain why it acts as the dominant stereocentre in the reaction. However, the hydroxy group is essential in order to convert **28** to **29** and therefore, generate the sp^3 hybridised nitrogen atom. This may explain why it is essential to have an OH group at that position.

The hydride transfer could take place from **31** via the transition state illustrated in Figure 1. In this process, it is known that α-amino alcohol and TsDPEN diamine ligands favour formation of a complex, in which the group adjacent to O or NTs is *anti* to the Ru–H bond. In **31** this corresponds to an *anti* arrangement of Me (*) to the Ru–H bond. If the

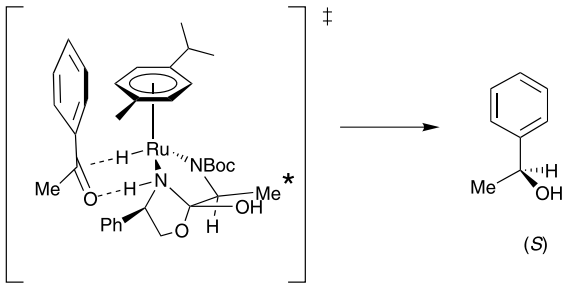


Figure 1. Stereochemical control in the reduction.

favoured diastereoisomer of **31** is assumed to be that depicted in Figure 1, hydrogen will be transferred to the face which gives the *S* product, which matches that observed. This mechanism would also account for the moderating effect (on yield and ee) of the group in the amino alcohol part of the ligand, as its position would influence the stability and conformation of **31** during the catalytic process.

In conclusion, application of the various dipeptide analogue ligands to the Ru(II)-catalysed ATH of simple aromatic ketones demonstrated that smaller *R* group substituents on the amino acid part of the ligand give better conversions. Matched versions of the ligands give higher conversions compared to the mismatched versions. NTs protected and NBn_2 ligands are ineffective suggesting that a carbamate group is necessary at this position. The deletion of the terminal hydroxy group from the ligands rendered them ineffective. The inactiveness of the norephedrine and *cis*-amino indanol substituted valine ligands suggest that the presence of a primary alcohol is desirable. The ineffectiveness of a carboxylic acid ligand also leads to the conclusion that a hydroxy group is specifically necessary for the reduction, not simply an ionisable one. Only an OH group can form the bicyclic intermediate **29** proposed in the catalytic cycle. The ees of products formed using the alaninol-derived ligands were comparable to those from the phenylgylcinol ligands but the conversions were lower.

3. Experimental

Unless otherwise stated, all reactions were run under an atmosphere of nitrogen in flame or oven dried glassware (round bottomed flask or Schlenk tubes). Room temperature refers to ambient room temperature (20–22 °C). Heated experiments were conducted using thermostatically controlled oil baths. Reactions were monitored by thin-layer chromatography (TLC) using aluminium backed silica gel 60 (F_{254}) plates, visualised using $UV_{254\ nm}$ and PMA. Flash column chromatography was routinely carried out using 60 Å silica gel (Merck). Elemental analyses were performed using the Exeter Analytical Model CE440. Melting points were recorded on a Stuart Scientific SMP1 instrument and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared (IR) spectra were recorded on a Nicolet Model Avatar 320 FTIR instrument. Nuclear magnetic resonance (NMR) spectra were recorded on either a Bruker DPX-300 (300 MHz) or a DPX-400 (400 MHz) spectrometer. Chemical shifts are reported in δ

units, parts per million (ppm). Coupling constants (J) are measured in Hz. Mass spectra were recorded on a 7070E VG mass spectrometer. Enantiomeric excesses (ee) were determined by high pressure liquid chromatography (HPLC) analysis (using a Merck-Hitachi L-6200 A intelligent pump, Merck-Hitachi L-4000 UV detector, Axiom chromatography data system and controller model 727 and a Daicel Chiralcel OD 4.6×250 mm column) or Gas Chromatography (GC) analysis (Hewlett Packard 5890A gas chromatography, Hewlett Packard 3396A integrator and Supelco BETA Dex™ 120 fused silica capillary column 30 m×0.25 mm×0.25 μm film thickness) as stated. All compounds were purchased from Aldrich, Avocado, Strem and NovabioChem.

3.1. General procedure 1 for the EDCI coupling of peptides and peptide analogues[11]

To a stirred solution of acid (1.0 equiv) and amine (1.5 equiv) in dry THF (10 vol) was added HOBt (1.5 equiv). The mixture was stirred for 30 min after, which EDCI (1.5 equiv) and DIPEA (5.0 equiv) were added. Stirring was continued for a further 18 h at room temperature and solvent evaporated in vacuo. EtOAc was added and the organic layer was washed with 5% citric acid (2×), saturated $NaHCO_3$ (2×), NaCl (concn) and H_2O. The organic layer was then dried with Na_2SO_4 or $MgSO_4$, filtered and concentrated in vacuo. Purification of crude product by recrystallisation or flash column chromatography afforded the product.

3.1.1. Preparation of (*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylamine, 9.[12] To a stirred solution of *R*-phenylglycinol (4.20 g, 30.6 2 mmol, 1.0 equiv) in dry THF (40 mL) was added TBSCl (5.54 g, 36.74 mmol, 1.2 equiv) and imidazole (5.21 g, 76.54 mmol, 2.5 equiv). The reaction mixture was refluxed for 18 h and cooled to room temperature. EtOAc (40 mL) was added and the organic layer was washed with 0.75 M HCl (2×20 mL) dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by recrystallisation (EtOAc/hexane) afforded the product as a white solid (6.63 g, 84%). Mp 228–230 °C; $[\alpha]_D^{24}$ −16.9 (*c* 1.00, EtOH); IR (neat, cm^{-1}) 2925, 2854, 1491, 1463, 1251, 1101, 835, 763 and 698; δ_H (400 MHz; CD_3OD) 7.36–7.23 (5H, m, Ar*H*), 4.07 (1H, dd, J=4.5, 7.0 Hz, ArC*H*), 3.80 (1H, dd, J=4.5, 10.3 Hz, OC*H*CH), 3.71 (1H, dd, J=7.0, 10.3 Hz, OCHC*H*), 0.85 (9H, s, SiC(CH_3)$_3$), −0.02 (3H, s, Si$CH_3$$CH_3$) and −0.04 (3H, s, Si$CH_3$$CH_3$); δ_C (100 MHz; CD_3OD) 141.1, 130.0, 129.5, 128.6, 69.0, 58.7, 26.7, 19.6, −4.9 and −4.9; *m/z* (FAB) 252 ($M+H^+$, 100%), 235 (37). Found: $M+H^+$, 252.1779. $C_{14}H_{26}NOSi$ requires 252.1778.

3.1.2. Preparation of {(*S*)-1-[(*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylcarbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester, 10. Following general procedure 1 for EDCI coupling, (*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylamine, **9** (1.28 g, 5.10 mmol, 1.0 equiv), Boc-L-valine (1.28 g, 5.87 mmol, 1.5 equiv), HOBt (1.03 g, 7.64 mmol, 1.5 equiv), EDCI (1.46 g, 7.64 mmol, 1.5 equiv), DIPEA (4.3 mL, 25.45 mmol, 5.0 equiv) in dry THF (15 mL) gave, after purification by flash column chromatography, the product **10** as a colourless oil (1.30 g, 58%). $[\alpha]_D^{24}$ −1.5 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3303, 2956, 2928, 1682, 1650, 1519, 1247, 1171, 1123, 834, 776 and 697; δ_H (400 MHz; $CDCl_3$; Si$(CH_3)_4$) 7.31–7.21 (5H, m, Ar*H*), 6.73 (1H, d, J=7.0 Hz, ArCHN*H*), 5.12–5.09 (1H, m, $(CH_3)_2$CHCHN*H*), 5.01–4.97 (1H, m, ArC*H*), 3.96–3.94 (1H, m, $(CH_3)_2$CHC*H*), 3.90–3.86 (1H, m, OC*H*H), 3.78–3.75 (1H, m, OCH*H*), 2.17–2.10 (1H, m, $(CH_3)_2$C*H*), 1.44 (9H, s, OC(CH_3)$_3$), 0.98 (3H, d, J=6.7 Hz, (CH_3), 0.94 (3H, d, J=6.5 Hz, (CH_3), 0.85 (9H, s, SiC(CH_3)$_3$), −0.05 (3H, s, Si(CH_3)) and −0.08 (3H, s, Si(CH_3)); δ_C (100 MHz; $CDCl_3$; Si$(CH_3)_4$) 171.3, 156.2, 140.4, 128.7, 127.7, 127.3, 80.1, 66.5, 60.5, 54.9, 31.3, 28.7, 26.3, 19.7, 18.6, 18.0 and −5.3; *m/z* (FAB) 451 ($M+H^+$, 86%), 395 (100), 337 (16), 235 (18), 136 (6). Found: $M+H^+$, 451.2991. $C_{24}H_{43}N_2O_4Si$ requires 451.2992.

3.1.3. Preparation of [(*S*)-1-((*R*)-2-hydroxy-1-phenyl-ethylcarbamoyl)-2-methyl-propyl]-carbamic acid *tert*-butyl ester, 8. To a solution of {(*S*)-1-[(*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylcarbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester, **10** (800 mg, 1.78 mmol, 1.0 equiv) in THF (10 mL) was added 1 M TBAF/THF (3.6 mL, 3.55 mmol, 2.0 equiv). The reaction mixture was stirred at room temperature for 18 h and the solvent evaporated in vacuo. The mixture was then diluted with EtOAc (10 mL) and washed with H_2O (10 mL). The aqueous layer was extracted with EtOAc (3×10 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by recrystallisation (EtOAc/hexane) yielded the product **8** as a white solid (506 mg, 84%). Mp 132–134 °C. (Found: C, 64.18; H, 8.38; N, 8.21. $C_{18}H_{28}N_2O_4$ requires C, 64.26; H, 8.39; N, 8.33%); $[\alpha]_D^{24}$ +6.8 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3304, 2968, 2870, 1686, 1651, 1519, 1336, 1300, 1244, 1170, 1041 and 699; δ_H (400 MHz; $CDCl_3$; Si$(CH_3)_4$) 7.28–7.25 (5H, m, Ar*H*), 5.37 (1H, d, J=7.0 Hz, $(CH_3)_2$CHCHN*H*), 5.00 (1H, br s, ArC*H*), 3.92–3.90 (1H, m, $(CH_3)_2$CHC*H*), 3.77–3.75 (2H, m, OCH_2), 3.47 (1H, br s, ArCHN*H*), 2.05–2.03 (1H, m, $(CH_3)_2$C*H*), 1.41 (9H, s, C(CH_3)$_3$), 0.96 (3H, d, J=6.0 Hz, (CH_3)) and 0.92 (3H, d, J=5.3 Hz, (CH_3)); δ_C (100 MHz; $CDCl_3$; Si$(CH_3)_4$) 172.7, 156.5, 139.4, 129.0, 127.9, 127.1, 80.4, 66.3, 61.0, 56.1, 30.9, 28.7, 19.7 and 18.6; *m/z* (FAB) 337 ($M+H^+$, 80%), 307 (21), 281 (74), 237 (35), 154 (100), 136 (77), 121 (24). Found: $M+H^+$, 337.2126. $C_{18}H_{29}N_2O_4$ requires 337.2127.

3.1.4. Preparation of (1*S*,2*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-indan-2-ylamine. To a stirred solution of (1*R*-2*S*)-*cis*-amino indan-2-ol (2.00 g, 13.42 mmol, 1.0 equiv) in dry THF (20 mL) was added TBSCl (2.42 g, 16.11 mmol, 1.2 equiv) and imidazole (2.28 g, 33.56 mmol, 2.5 equiv). The reaction mixture was refluxed overnight and cooled to room temperature. EtOAc (20 mL) was added and the organic layer was washed with 0.75 M HCl (2×20 mL) dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography afforded the product as a pale orange oil (3.01 g, 86%). $[\alpha]_D^{25}$ −34.4 (*c* 0.80, $CHCl_3$); IR (neat, cm^{-1}) 2927, 2855, 1472, 1361, 1254, 1110, 1067 and 632; δ_H (400 MHz; $CDCl_3$; Si$(CH_3)_4$) 7.38–7.36 (1H, m, Ar*H*), 7.22–7.18 (3H, m, Ar*H*), 5.36 (1H, br s, N*H*), 4.47 (1H, q, J=5.3 Hz, C*H*O), 4.15 (1H, d, J=5.3 Hz, C*H*N), 3.02 (1H, dd, J=5.9, 15.8 Hz, C*H*HCHO), 2.89 (1H, dd, J=5.9, 15.8 Hz, CH*H*CHO), 0.91 (9H, s,

$C(CH_3)_3$) and 0.12 (6H, s, $Si(CH_3)_2$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 144.8, 140.6, 128.0, 127.1, 125.3, 125.0, 75.7, 60.0, 39.6, 26.3, 18.6, −4.1 and −4.4; *m/z* (EI) 264 ($M+H^+$, 4%), 206 (73), 189 (15), 130 (41), 115 (27), 70 (100). Found: $M+H^+$, 264.1775. $C_{15}H_{26}NOSi$ requires 264.1778.

3.1.5. Preparation of {(*S*)-1-[(1*S*,2*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-indan-2-ylcarbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester, 14. Following general procedure 1 for EDCI coupling, Boc-L-valine (858 mg, 3.95 mmol, 1.0 equiv), (1*S*,2*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-indan-2-ylamine (1.56 g, 5.91 mmol, 1.5 equiv), HOBt (798 mg, 5.91 mmol, 1.5 equiv), EDCI (1.13 g, 5.91 mmol, 1.5 equiv), DIPEA (3.4 mL, 19.74 mmol, 5.0 equiv) in dry THF (20 mL) gave, after purification by flash column chromatography, the product **14** as a beige solid (1.10 g, 60%). Mp 90–92 °C; $[\alpha]_D^{21}$ +20.8 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3306, 2957, 2928, 2856, 1699, 1651, 1504, 365, 1250, 1161, 1070, 1002, 835, 776 and 739; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.18–7.16 (1H, m, Ar*H*), 7.09–7.04 (3H, m, Ar*H*), 6.59 (1H, d, *J*=8.0 Hz, N*H*CO), 5.29–5.26 (1H, m, C*H*O), 5.07 (1H, d, *J*=9.0 Hz, N*H*COO), 4.52–4.51 (1H, m, C*H*NHCO), 3.98–3.95 (1H, m, C*H*NHCOO), 3.01–2.97 (1H, m, OCHC*H*H), 2.83–2.79 (1H, d, *J*=16.1 Hz, OCHCH*H*), 2.17–2.13 (1H, m, COC*H*), 1.34 (9H, s, $OC(CH_3)_3$), 0.90 (3H, d, *J*=6.9 Hz, CH_3), 0.86 (3H, d, *J*=7.0 Hz, CH_3), 0.78 (9H, s, $SiC(CH_3)_2$) and 0.00 (6H, s, $Si(CH_3)_2$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 169.7, 154.0, 139.5, 137.8, 125.9, 125.0, 122.9, 122.8, 79.6, 73.9, 60.0, 56.7, 40.4, 30.7, 28.2, 25.7, 19.4, 18.0 and 17.4; *m/z* (CI) 463 ($M+H^+$, 77%), 407 (49), 363 (39), 348 (53), 247 (59), 199 (61), 69 (100). Found: $M+H^+$, 463.2982. $C_{25}H_{43}O_4N_2Si$ requires 463.2987.

3.1.6. Preparation of {(*S*)-1-[(1*S*,2*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-methyl-2-phenyl-ethylcarbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester, 13. Following general procedure 1 for EDCI coupling, Boc-L-valine (3.31 g, 1.52 mol, 1.0 equiv), (1*S*,2*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-methyl-2-phenyl-ethylamine (6.06 g, 2.28 mol, 1.5 equiv), HOBt (3.08 g, 2.28 mol, 1.5 equiv), EDCI (4.37 g, 2.28 mol, 1.5 equiv), DIPEA (12.9 mL, 7.61 mol, 5.0 equiv) in dry THF (100 mL) gave, after purification by flash column chromatography, the product **13** as a white solid (4.94 g, 70%). Mp 165–167 °C. (Found: C, 64.76; H, 9.55; N, 6.01. $C_{25}H_{44}N_2O_4Si$ requires C, 64.61; H, 9.54; N, 6.03%); $[\alpha]_D^{24}$ −52.5 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3318, 2859, 2929, 1683, 1645, 1524, 1249, 1170, 1079, 1064, 834, 775 and 695; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.21–7.20 (5H, m, Ar*H*), 6.05 (1H, d, *J*=8.5 Hz, N*H*CO), 4.95 (1H, d, *J*=9.0 Hz, N*H*COO), 4.75–4.74 (1H, m, ArC*H*), 4.18–4.10 (1H, m, CH_3CHN), 3.89–3.86 (1H, m, COC*H*), 2.17–2.12 (1H, m, $(CH_3)CH$), 1.42 (9H, s, $OC(CH_3)_3$), 0.97 (3H, d, *J*=6.8 Hz, CH_3CHN), 0.93–0.89 (12H, m, $OSiC(CH_3)_3$ and $C(CH_3CH_3)$), 0.84 (3H, d, *J*=7.0 Hz, $C(CH_3CH_3)$), 0.03 (3H, s, $Si(CH_3)$ and −0.19 (3H, s, $Si(CH_3)$; δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 171.1, 156.4, 141.9, −128.4, 127.8, 126.8, 80.3, 60.4, 51.8, 30.9, 28.7, 26.3, 19.9, 18.6, 17.7, 14.7, −4.2, −4.7; *m/z* (CI) 465 ($M+H^+$, 38%), 409 (29), 351 (36), 333 (71), 259 (44), 221 (100). Found: $M+H^+$, 465.3138. $C_{25}H_{45}N_2O_4Si$ requires 465.3143.

3.1.7. Preparation of [(*S*)-1-((1*S*,2*R*)-1-hydroxy-indan-2-ylcarbamoyl)-2-methyl-propyl]-carbamic acid *tert*-butyl ester, 12. To a stirred solution of {(*S*)-1-[(1*S*,2*R*)-1-(*tert*-butyl-dimethyl-silanyloxy)-indan-2-ylcarbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester, **14** (765 mg, 1.29 mmol, 1.0 equiv) in THF (5 mL) was added 1 M TBAF/THF (1.5 mL, 3.87 mmol, 3.0 equiv) The reaction mixture was stirred for 18 h. EtOAc (5 mL) was added and the organic layer was washed with H_2O (2×5 mL). The aqueous layer was extracted with EtOAc (3×5 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography (EtOAc/hexane) afforded the product **12** as a white solid (365 mg, 59%). Mp 148–151 °C; $[\alpha]_D^{23}$ −24.6 (*c* 0.50, $CHCl_3$); IR (neat, cm^{-1}) 3317, 2969, 2901, 1665, 1638, 1516, 1247, 1166, 1049 and 732; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.23–7.20 (4H, m, Ar*H*), 6.76 (1H, br s, N*H*CO), 5.39–5.35 (1H, m, C*H*O), 5.13 (1H, br s, N*H*COO), 4.56–4.54 (1H, m, C*H*NHCO), 3.99–3.96 (1H, m, C*H*NHCOO), 3.12 (1H, dd, *J*=5.0, 16.6 Hz, OCHC*H*H), 2.91 (1H, d, *J*=16.6 Hz, OCHCH*H*), 2.24–2.20 (1H, m, COC*H*), 1.43 (9H, s, $OC(CH_3)_3$), 1.04–1.02 (3H, m, CH_3) and 0.98 (3H, d, *J*=6.8 Hz, CH_3); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 172.5, 156.7, 140.9, 140.7, 128.6, 127.5, 125.7, 124.8, 80.9, 73.7, 61.1, 57.8, 40.0, 30.8, 28.7, 19.8 and 18.7; *m/z* (EI) 349 ($M+H^+$, 4%), 256 (44), 148 (28), 131 (56), 116 (74), 80 (46), 68 (100). Found: M^+, 348.2064. $C_{19}H_{28}N_2O_4$ requires 348.2049.

3.1.8. Preparation of [(*S*)-1-((1*S*,2*R*)-2-hydroxy-1-methyl-2-phenyl-ethylcarbamoyl)-2-methyl-propyl]-carbamic acid *tert*-butyl ester, 11. To a stirred solution of {(*S*)-1-[(1*S*,2*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-methyl-2-phenyl-ethylcarbamoyl]-2-methyl-propyl}-carbamic acid *tert*-butyl ester, **13** (1.94 g, 4.17 mmol, 1.0 equiv) in THF (20 mL) was added 1 M TBAF/THF 12.5 mL, 12.52 mmol, 3.0 equiv) The reaction mixture was stirred for 18 h. EtOAc (20 mL) was added and the organic layer was washed with H_2O (2×20 mL). The aqueous layer was extracted with EtOAc (3×20 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by recrytallisation (EtOAc/hexane) afforded the product **11** as a white solid (1.22 g, 83%). Mp 144–148 °C. (Found: C, 65.29; H, 8.69; N, 8.02. $C_{19}H_{30}N_2O_4$ requires C, 65.12; H, 8.63; N, 7.99%); $[\alpha]_D^{24}$ −58.6 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3298, 2970, 1684, 1650, 1515, 1366, 1244, 1170, 1041 and 698; δ_H (400 MHz; $CDCl_3$; TMS) 7.27–7.23 (5H, m, Ar*H*), 6.48 (1H, d, *J*=8.3 Hz, N*H*COO), 5.23 (1H, d, *J*=8.5 Hz, N*H*CO), 4.84 (1H, d, *J*=3.26 Hz, ArC*H*), 4.32–4.24 (1H, m, CH_3CH), 3.79 (1H, br s, COC*H*), 2.10–2.03 (1H, m, COCHC*H*), 1.42 (9H, s, $C(CH_3)_3$), 1.00 (3H, d, *J*=6.8 Hz, CH_3CH), 0.94 (3H, d, *J*=6.8 Hz, $CH_3$and 0.90 (3H, d, *J*=6.8 Hz, CH_3); δ_C (100 MHz; $CDCl_3$; TMS) 172.2, 156.5, 141.3, 80.4, 76.3, 60.7, 51, 31.2, 28.7, 19.7 and 14.6; *m/z* (EI) 351 ($M+H^+$, 9%), 244 (33), 200 (46), 144 (44), 116 (76), 79 (56), 57 (100). Found: $M+H^+$, 351.2271. $C_{19}H_{31}N_2O_4$ requires 351.2284.

3.1.9. Preparation of (*S*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylamine 9.[13] To a stirred solution of *S*-phenylglycinol (1.94 g, 14.16 mmol, 1.0 equiv) in dry THF (15 mL) was added TBSCl (2.56 g, 17.00 mmol,

1.2 equiv) and imidazole (2.41 g, 35.39 mmol, 2.5 equiv).The reaction mixture was refluxed for 18 h and cooled to room temperature. EtOAc (15 mL) was added and the organic layer was washed with 0.75 M HCl (2×15 mL) dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by recrystallisation (EtOAc/hexane) afforded the product as a white solid (2.87 g, 64%). Mp 221–225 °C; $[\alpha]_D^{24}+179.6$ (*c* 0.50, $CHCl_3$); IR (neat, cm^{-1}) 2925, 2363, 1592, 1252, 1103, 1074, 838, 764 and 699; δ_H (400 MHz; CD_3OD) 7.40–7.37 (5H, m, Ar*H*), 4.35 (1H, dd, *J*=4.5, 6.5 Hz, ArC*H*), 3.96 (1H, dd, *J*=4.5, 11.0 Hz, C*H*HOH), 3.88 (1H, dd, *J*=6.5, 11.0 Hz, CH*H*OH), 0.85 (9H, s, $C(CH_3)_3$), 0.00 (3H, s, $SiCH_3CH_3$) and −0.02 (3H, s, $SiCH_3CH_3$); δ_C (100 MHz; CD_3OD) 136.3, 130.7, 130.6, 128.9, 66.5, 58.3, 26.7, 19.7, −5.1 and −5.2; *m/z* (FAB) 252 ($M+H^+$, 100%), 235 (25), 138 (24), 136 (18), 121 (11). Found: $M+H^+$, 252.1777. $C_{14}H_{26}NOSi$ requires 252.1784.

3.1.10. Preparation of {(*S*)-1-[(*S*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylcarbamoyl]-ethyl}-carbamic acid *tert*-butyl ester, 18b. Following general procedure 1 for EDCI coupling, Boc-L-alanine (1.00 g, 2.65 mmol, 1.0 equiv), (*S*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylamine, **9** (500 mg, 3.98 mmol, 1.5 equiv), HOBt (537 mg, 3.98 mmol, 1.5 equiv), EDCI (762 mg, 3.98 mmol, 1.5 equiv), DIPEA (2.25 mL, 13.26 mmol, 5.0 equiv) in dry THF (15 mL) gave, after purification by flash column chromatography, the product **18b** as a colourless oil (700 mg, 63%). $[\alpha]_D^{24}-15.9$ (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3303, 2928, 2856, 1655, 1495, 1365, 1249, 1164, 1094, 834, 775 and 698; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.26–7.21 (5H, m, Ar*H*), 6.96 (1H, br s, ArCHN*H*), 5.01–4.97 (2H, m, ArC*H* and CH_3CHNH), 4.23 (1H, br s, CH_3CH), 3.90–3.86 (1H, m, OC*H*H), 3.80–3.76 (1H, m, OCH*H*), 1.44 (9H, s, $OC(CH_3)_3$), 1.37–1.34 (3H, m, CH_3CH), 0.84 (9H, s, $SiC(CH_3)_3$), −0.05 (3H, s, $SiCH_3$) and −0.10 (3H, s, $SiCH_3$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 172.2, 156.1, 137.4, 128.7, 127.7, 127.2, 80.5, 66.6, 54.0, 50.3, 28.7, 26.3, 18.6 and 18.4; *m/z* (FAB) 423 ($M+H^+$, 56%), 36 (100), 309 (23), 235 (60), 154 (18), 120 (23). Found: $M+H^+$, 423.2667. $C_{22}H_{39}N_2O_4Si$ requires 423.2679.

3.1.11. Preparation of [(*S*)-1-((*S*)-2-hydroxy-1-phenyl-ethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester, 16. To a stirred solution of {(*S*)-1-[(*S*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylcarbamoyl]-ethyl}-carbamic acid *tert*-butyl ester, **18b** (471 mg, 1.12 mmol, 1.0 equiv) in THF (20 mL) was added 1 M TBAF/THF (3.4 mL, 3.35 mmol, 3.0 equiv) The reaction mixture was stirred for 18 h. EtOAc (20 mL) was added and the organic layer was washed with H_2O (2×20 mL). The aqueous layer was extracted with EtOAc (3×20 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column recrytallisation (EtOAc/hexane) afforded the product **16** as a white solid (250 mg, 72%). Mp 105–108 °C; $[\alpha]_D^{25}-13.6$ (*c* 0.50, $CHCl_3$); IR (neat, cm^{-1}) 3333, 2934, 1683, 1655, 1518, 1316, 1238, 1159, 1041, 703 and 653; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.35–7.26 (5H, m, Ar*H*), 7.01 (1H, br s, N*H*CO), 5.07–5.02 (1H, m, ArC*H*), 4.98 (1H, d, *J*=6.8 Hz, N*H*COO), 4.19–4.18 (1H, COC*H*), 3.91–3.81 (2H, m, CH_2OH), 2.73 (1H, br s, O*H*), 1.43 (9H, s, $C(CH_3)_3$) and 1.39–1.36 (3H, m, CH_3CH); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 173.2, 157.1, 139.2, 129.2, 128.2, 127.1, 80.9, 66.8, 56.2, 50.9, 28.7 and 18.0; *m/z* (FAB) 309 ($M+H^+$, 27%), 289 (13), 253 (29), 154 (100), 136 (68). Found: $M+H^+$, 309.1819. $C_{16}H_{25}N_2O_4$ requires 309.1814.

3.1.12. Preparation of [(*S*)-1-((*R*)-2-hydroxy-1-phenyl-ethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester, 17.[14,15] Following general procedure 1 for EDCI coupling, *R*-phenylgylcinol (1.51 g, 10.93 mmol, 1.00 equiv), Boc-L-alanine (2.4 g, 12.57 mmol, 1.15 equiv), HOBt (1.71 g, 12.57 mmol, 1.15 equiv), EDCI (2.43 g, 12.57 mmol, 1.15 equiv), DIPEA (2.78 mL, 16.41 mmol, 1.5 equiv) in dry THF (40 mL) gave, after purification by flash column chromatography, the product **17** as a white solid (2.54 g, 76%). Mp 90–92 °C; $[\alpha]_D^{25}-57.1$ (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3352, 3326, 1686, 1656, 1534, 1515, 1316, 1155, 1063 and 703; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.27–7.23 (5H, m, Ar*H*), 5.41 (1H, d, *J*=7.5 Hz, COON*H*), 5.09–5.04 (1H, m, ArC*H*), 4.26–4.23 (1H, m, CH_3CH), 3.86–3.76 (2H, m, CH_2OH), 3.44 (1H, br s, CON*H*), 1.43 (9H, s, $C(CH_3)_3$) and 1.34 (3H, d, *J*=7.0 Hz, CH_3CH); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 173.3, 156.0, 138.9, 128.7, 127.7, 126.7, 80.3, 66.1, 55.6, 50.4, 28.3 and 18.3; *m/z* (FAB) 309 ($M+H^+$, 63%), 289 (13), 253 (55), 209 (15), 154 (100), 136 (70). Found: $M+H^+$, 309.1813. $C_{16}H_{25}N_2O_4$ requires 309.1814.

3.1.13. Preparation of {(*S*)-1-[(*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylcarbamoyl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester, 18a. Following general procedure 1 for EDCI coupling, Boc-L-phenylalanine (703 mg, 2.64 mmol, 1.0 equiv), (*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylamine, **9** (1 g, 3.98 mmol, 1.5 equiv), HOBt (537 mg, 3.98 mmol, 1.5 equiv), EDCI (762 mg, 3.98 mmol, 1.5 equiv), DIPEA (2.25 mL, 13.26 mmol, 5.0 equiv) in dry THF (20 mL) gave, after purification by flash column chromatography, the product **18** as a colourless oil (1.04 g, 79%). $[\alpha]_D^{25}-1.8$ (*c* 0.67, $CHCl_3$); IR (neat, cm^{-1}) 3277, 2928, 2856, 1686, 1647, 1522, 1248, 1169, 1114, 834, 775 and 696; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.26–7.18 (10H, m, Ar*H*), 6.62 (1H, d, *J*=7.0 Hz, ArCHN*H*), 5.01 (1H, br s, $ArCH_2CHNH$), 4.91 (1H, br s, ArC*H*), 4.42–4.40 (1H, m, $ArCH_2CH$), 3.69–3.67 (2H, m, $ArCHCH_2$), 3.13–3.02 (2H, m, $ArCH_2$), 1.42 (9H, s, $OC(CH_3)_3$), 0.80 (9H, s, $SiC(CH_3)_3$), −0.12 (3H, s, $SiCH_3$) and −0.16 (3H, s, $SiCH_3$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$); 170.4, 155.5, 139.9, 136.9, 129.4, 128.7, 128.2, 126.7, 80.1, 66.1, 55.9, 54.5, 38.5, 28.3, 25.8, 18.2, −5.6 and −5.7; *m/z* (FAB) 499 ($M+H^+$, 99%), 443 (100), 399 (28), 385 (20), 311 (23), 235 (31), 154 (20). Found: $M+H^+$, 499.3005. $C_{28}H_{43}N_2O_4Si$ requires 499.2992. Note: ^{13}C NMR shows coincident peaks in the aromatic region. Therefore, only 5 instead of 6 aromatic peaks are observed.

3.1.14. Preparation of [(*S*)-1-((*R*)-2-hydroxy-1-phenyl-ethylcarbamoyl)-2-phenyl-ethyl]-carbamic acid *tert*-butyl ester, 15.[14] To a stirred solution of {(*S*)-1-[(*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-1-phenyl-ethylcarbamoyl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester, **18a** (971 mg, 1.95 mmol, 1.0 equiv) in THF (20 mL) was added 1 M TBAF/THF (5.8 mL, 5.84 mmol, 3.0 equiv) The

reaction mixture was stirred for 18 h. EtOAc (20 mL) was added and the organic layer was washed with H_2O (2× 20 mL). The aqueous layer was extracted with EtOAc (3× 20 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography (EtOAc/hexane) afforded the product **15** as a white solid (400 mg, 53%). Mp 114–116 °C; $[\alpha]_D^{25}$ −8.9 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3337, 3300, 1687, 1651, 1520, 1170, 1045, 1028, 755 and 698; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.22–7.14 (10H, m, Ar*H*), 6.41 (1H, d, *J*=7.0 Hz, ArCHN*H*), 5.10–5.01 (1H, m, $ArCH_2CHNH$), 4.99–4.94 (1H, m, ArC*H*), 4.37–4.35 (1H, m, $ArCH_2CH$), 3.72–3.64 (2H, m, $ArCHCH_2$), 3.12 (1H, dd, *J*=13.6, 6.4 Hz, ArC*H*H), 3.02 (1H, dd, *J*=13.6, 7.8 Hz, ArCH*H*), 2.34 (1H, br s, O*H*) and 1.70 (9H, s, $OC(CH_3)_3$); δ_C (100 MHz; $CDCl_3$; TMS) 171.7, 156.9, 138.9, 137.1, 129.8, 129.2, 129.2, 128.2, 127.5, 127.1, 80.9, 66.5, 56.7, 56.2, 38.9 and 28.7; *m/z* (FAB) 385 ($M+H^+$, 71%), 329 (52), 307 (26), 285 (35), 154 (100), 126 (73). Found: $M+H^+$, 385.2140. $C_{22}H_{29}N_2O_4$ requires 385.2127.

3.1.15. Preparation of (*S*)-2-amino-*N*-((*R*)-2-hydroxy-1-phenyl-ethyl)-3-methyl-butyramide trifluoromethyl acetate, 19. To a stirred solution of [(*S*)-1-((*R*)-2-hydroxy-1-phenyl-ethylcarbamoyl)-2-methyl-propyl]-carbamic acid *tert*-butyl ester, **8** (823 mg, 2.45 mmol) in DCM (5 mL) was added dropwise TFA (5 mL). The reaction mixture was stirred at room temperature for 18 h and the solvent evaporated in vacuo. The product **19** was obtained as a brown oil (1.67 g, quantitative). $[\alpha]_D^{24}$ −20.5 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}); 3092, 2975, 1778, 1666, 1552, 1143, 798 and 699; δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.68–7.21 (5H, m, Ar*H*), 5.34–5.28 (1H, m, ArC*H*), 4.60–4.50 (2H, m, CH_2OH), 4.17–4.15 (1H, m, COC*H*), 2.19–2.08 (1H, m, $CH(CH_3)_2$) and 0.96–0.91 (6H, m, $C(CH_3)_2$); δ_C (75 MHz; $CDCl_3$; $Si(CH_3)_4$) 168.9, 161.7 (q, *J*=41.0 Hz), 135.7, 129.7, 129.6, 127.0, 113.6 (q *J*=8.6 Hz), 68.7, 59.7, 53.5, 30.9, 18.1 and 17.7; *m/z* (CI, NH_3) 237 ($M+H^+$, 100%), 122 (10), 205 (8), 166 (2), 128 (7), 117 (9), 106 (17), 72 (49). Found: $M+H^+$, 237.1596. $C_{13}H_{21}N_2O_2$ requires 237.1598.

3.1.16. Preparation of (*S*)-*N*-((*R*)-2-hydroxy-1-phenyl-ethyl)-3-methyl-2-(toluene-4-sulfonylamino)-butyramide, 20.[16] To a stirred solution of (*S*)-2-amino-*N*-((*R*)-2-hydroxy-1-phenyl-ethyl)-3-methyl-butyramide trifluoromethyl acetate, **19** (1.30 g, 3.71 mmol, 1.0 equiv) and Et_3N (1.55 mL, 11.13 mmol, 3.0 equiv) in DCM (10 mL) was added TsCl (707 mg, 3.71 mmol, 1.0 equiv). The reaction mixture was stirred for 18 h at room temperature. The solution was concentrated in vacuo and EtOAc (10 mL) added. The organic layer was washed with H_2O (2× 10 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography afforded the product **20** as a colourless oil (186 mg, 13%). $[\alpha]_D^{24}$ −46.8 (*c* 0.50, $CHCl_3$); IR (neat, cm^{-1}) 3317, 3259, 1640, 1547, 1318, 1159, 986, 698 and 666; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.75 (2H, d, *J*=8.3 Hz, Ar*H*SO_2), 7.37–7.22 (7H, m, Ar*H*SO_2 and Ar*H*), 6.54 (1H, d, *J*=7.0 Hz, CON*H*), 5.19 (1H, d, *J*=8.0 Hz, N*H*SO_2), 4.92–4.88 (1H, m, ArC*H*), 3.71–3.69 (2H, m, CH_2OH), 3.53–3.50 (1H, m, COC*H*), 2.43 (3H, s, ArCH_3), 2.14–2.05 (1H, m, $CH(CH_3)_2$), 0.84 (3H, d, *J*=6.8 Hz, $C(CH_3)$) and 0.81 (3H, d, *J*=7.0 Hz, $C(CH_3)$; δ_C (100 MHz; CD_3OD) 173.0, 144.8, 141.0, 139.2, 130.7, 129.4, 128.4, 128.3, 128.2, 65.7, 63.6, 56.9, 32.9, 21.5, 19.7 and 18.3; *m/z* (FAB) 391 ($M+H^+$, 33%), 359 (3), 307 (29), 289 (15), 226 (14), 154 (100), 136 (73), 120 (13). Found: $M+H^+$, 391.1688. $C_{20}H_{27}N_2O_4S$ requires 391.1692.

3.1.17. Preparation of (*S*)-2-dibenzylamino-propionic acid benzyl ester, 22.[17] To a stirred solution of L-alanine (1.50 g, 16.84 mmol, 1.0 equiv) and K_2CO_3 (11.63 g, 84.18 mmol, 5.0 equiv) in EtOH (42 mL) and H_2O (8 mL) was added benzyl bromide (14.4 g, 84.18 mmol, 5.0 equiv) in EtOH (8 mL). The reaction mixture was refluxed for 18 h and the solvent evaporated. H_2O (20 mL) was added and the aqueous layer was extracted with EtOAc (3×20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by distillation using Kugel Rohr afforded the product **22** as a yellow oil (3.48 g, 58%). (Found: C, 79.97; H, 7.02; N, 3.86. $C_{24}H_{25}NO_2$ requires C, 80.19; H, 7.01; N, 3.90%); $[\alpha]_D^{23}$ −89.3 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3027, 1728, 1493, 1454, 1187, 1136, 730 and 694; δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.27–7.17 (15H, m, Ar*H*), 5.38 (1H, d, *J*=12.4 Hz, ArCH*H*), 5.22 (1H, d, *J*=12.4 Hz, ArC*H*H), 3.82 (2H, d, *J*=13.9 Hz, C*H*HNC*H*H), 3.64–3.51 (3H, m, CH*H*NCH*H* and COC*H*) and 1.34 (3H, d, *J*=7.2 Hz, $COCCH_3$); δ_C (75 MHz; $CDCl_3$; $Si(CH_3)_4$) 174.0, 140.2, 136.6, 129.3, 129.1, 129.0, 128.7, 128.4, 127.4, 66.4, 56.6, 54.8 and 15.4; *m/z* (FAB) 360 ($M+H^+$, 32%), 282 (5), 224 (100), 181 (5). Found: $M+H^+$, 360.1977. $C_{24}H_{26}NO_2$ requires 360.1964.

3.1.18. Preparation of (*S*)-2-dibenzylamino-propionic acid.[18] A solution of (*S*)-2-dibenzylamino-propionic acid benzyl ester, **22** (3.48 g, 9.68 mmol) in dioxane/MeOH/KOH (2 M) (18:3:9 mL) was stirred for 18 h at room temperature. The reaction mixture was acidified with 6 M HCl and extracted with Et_2O (3×30 mL) and EtOAc (3× 30 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by distillation using Kugel Rohr afforded the product as a yellow oil (2.45 g, 94%). $[\alpha]_D^{22}$ −13.2 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3292, 2918, 2852, 1657, 1533, 1443, 1252, 1104, 828 and 740; δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 10.92 (1H, br s, O*H*), 7.53–7.50 (4H, m, Ar*H*), 7.39–7.26 (6H, m, Ar*H*), 4.30 (2H, d, *J*=13.1 Hz, C*H*HNC*H*H), 4.15 (2H, d, *J*=13.1 Hz, CH*H*NCH*H*) 3.77 (1H, q, *J*=7.1 Hz, COC*H*), 1.60 (3H, d, *J*=7.4 Hz, $COCCH_3$); δ_C (75 MHz; $CDCl_3$; $Si(CH_3)_4$) 172.3, 132.4, 130.1, 128.9, 128.8, 58.4, 54.2, 12.4; *m/z* (FAB) 270 ($M+H^+$, 100), 224 (21), 196 (1), 180 (6), 154 (11), 134 (12).

3.1.19. Preparation of (*S*)-2-dibenzylamino-*N*-((*R*)-2-hydroxy-1-phenyl-ethyl)-propionamide, 21. Following general procedure 1 for EDCI coupling, (*S*)-2-dibenzyl-amino-propionic acid (670 mg, 2.49 mmol, 1.0 equiv), *R*-phenylglycinol (392 mg, 2.86 mmol, 1.15 equiv), HOBt (386 mg, 2.86 mmol, 1.15 equiv), EDCI (548 mg, 2.86 mmol, 1.15 equiv), DIPEA (0.63 mL, 3.73 mmol, 1.5 equiv) in dry THF (10 mL) gave, after purification by flash column chromatography, the product **21** as a white crystals (532 mg, 55%). Mp 92–96 °C; $[\alpha]_D^{24}$ −20.0 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3376, 2839, 2812, 1657 1493, 1453, 1115, 749 and 699; δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$)

7.33–7.13 (15H, m, Ar*H*), 5.02–4.92 (1H, m, ArC*H*), 3.84–3.68 (4H, m, O*H*, C*H*HOH and C*H*HNC*H*H), 3.45–3.36 (4H, m, CH*H*OH, CH_3CH and CH*H*NCH*H*) and 1.34–1.29 (3H, m, CH_3CH); δ_C (75 MHz; $CDCl_3$; $Si(CH_3)_4$) 174.0, 138.8, 138.5, 128.8, 128.5, 127.5, 127.2, 126.7 and 126.4; *m/z* (FAB) 389 ($M+H^+$, 100%), 307 (19), 289 (9), 224 (75), 154 (67), 136 (52), 120 (10). Found: $M+H^+$, 389.2245. $C_{25}H_{29}N_2O_2$ requires 389.2229.

3.1.20. Preparation of [(*S*)-2-methyl-1-((*R*)-1-phenyl-ethylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester, 23. Following general procedure 1 for EDCI coupling, Boc-L-valine (500 mg, 2.30 mmol, 1.0 equiv), *R*-α-methyl-benzylamine (0.44 mL, 3.45 mmol, 1.5 equiv), HOBt (466 mg, 3.45 mmol, 1.5 equiv), EDCI (662 mg, 3.45 mmol, 1.5 equiv), DIPEA (1.95 mL, 3.45 mmol, 5.0 equiv) in dry THF (10 mL) gave, after purification by recrystallisation (EtOAc/hexane), the product **23** as a beige solid (236 mg, 32%). Mp 135–137 °C; $[\alpha]_D^{25}+21.9$ (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3278, 2968, 1681, 1648, 1521, 1365, 1247, 1169, 1021, 794 and 698; δ_H (400 MHz; $CDCl_3$; TMS) 7.32–7.23 (5H, m, Ar*H*), 6.41 (1H, br s, ArCN*H*), 5.13–5.08 (2H, m, ArC*H* and $(CH_3)_2CHCHN$*H*), 3.88–3.84 (1H, m, $(CH_3)_2CHCH$), 2.17–2.08 (1H, m, $(CH_3)_2CH$), 1.47 (3H, d, $J=7.0$ Hz, $ArCCH_3$), 1.42 (9H, s, $C(CH_3)_3$), 0.96 (3H, d, $J=6.8$ Hz, $C(CH_3)$) and 0.92 (3H, d, $J=5.3$ Hz, $C(CH_3)$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 171.1 156.4 143.4 129.0 127.7 126.5, 80.3, 60.6, 49.1, 31.0, 28.7, 22.3, 19.7 and 18.4; *m/z* (FAB) 321 ($M+H^+$, 78%), 265 (100), 221 (35), 172 (13), 154 (51), 136 (40). Found: $M+H^+$, 321.2189. $C_{18}H_{29}N_2O_3$ requires 321.2178.

3.1.21. Preparation of [(*S*)-2-methyl-1-((*S*)-1-phenyl-ethylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester, 24. Following general procedure 1 for EDCI coupling, Boc-L-valine (500 mg, 2.30 mmol, 1.0 equiv), *S*-α-methyl-benzylamine (0.44 mL, 3.45 mmol, 1.5 equiv), HOBt (466 mg, 3.45 mmol, 1.5 equiv), EDCI (662 mg, 3.45 mmol, 1.5 equiv), DIPEA (1.95 mL, 3.45 mmol, 5.0 equiv) in dry THF (10 mL) gave, after purification by recrystallisation (EtOAC/hexane), the product **24** as a white solid (549 mg, 74%). Mp 140–143 °C. (Found: C, 67.31; H, 8.69; N, 8.96. $C_{18}H_{28}N_2O_3$ requires C, 67.47; H, 8.81; N, 8.74%); $[\alpha]_D^{25}-59.6$ (*c* 0.51, $CHCl_3$); IR (neat, cm^{-1}) 3301, 3245, 2972, 1681, 1644, 1520, 1356, 1248, 1172, 1044, 1015 and 696; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.32–7.23 (5H, m, Ar*H*), 6.32 (1H, d, $J=6.5$ Hz, ArCN*H*), 5.12 (2H,br s, ArC*H* and $(CH_3)_2CHCHN$*H*), 3.87–3.83 (1H, m, $(CH_3)_2CHCH$), 2.08 (1H, br s, $(CH_3)_2CH$), 1.47 (3H, d, $J=6.8$ Hz, $ArCCH_3$), 1.42 (9H, s, $C(CH_3)_3$) and 0.89–0.86 (6H, m, $C(CH_3)_2$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 171.1, 156.4, 143.4, 129.0, 127.7, 126.5, 80.3, 60.6, 49.1, 31.2, 28.7, 22.0, 19.8 and 18.2; *m/z* (FAB) 321 ($M+H^+$, 51%), 307 (23), 265 (49), 221 (14), 154 (100), 136 (73). Found: $M+H^+$, 321.2181. $C_{18}H_{29}N_2O_3$ requires 321.2178.

3.1.22. Preparation of (*S*)-2-((*R*)-2-*tert*-butoxycarbonyl-amino-1-hydroxy-propylamino)-propionic acid benzyl ester, 25. Following general procedure 1hr for EDCI coupling, benzyl ester L-alanine tosylate (3.50 g, 10.0 mmol, 1.0 equiv), Boc-D-alanine (2.83 g, 14.9 mmol, 1.5 equiv), HOBt (2.02 g, 14.9 mmol, 1.5 equiv), EDCI (2.86 g, 14.9 mmol, 1.5 equiv), DIPEA (8.45 mL, 49.8 mmol, 5.0 equiv) in dry THF (50 mL) gave, after purification by flash column chromatography, the product **25** as a white solid (2.38 g, 68%). Mp 72–74 °C. (Found: C, 61.66; H, 7.43; N, 7.89. $C_{18}H_{26}N_2O_5$ requires C, 61.70; H, 7.48; N, 7.99%); $[\alpha]_D^{24}+25.4$ (*c* 0.50, $CHCl_3$); IR (neat, cm^{-1}) 3320, 2982, 2935, 1737, 1651, 1522, 1268, 1154, 1051, 845, 728 and 648; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.40–7.31 (5H, m, ArH), 6.79 (1H, br s, $ArCH_2$-OCOCHN*H*), 5.17 (2H, q, $J=12.3$ Hz, $ArCH_2$), 5.00 (1H, br s, $(CH_3)_3COCONH$), 4.64–4.57 (1H, m, $ArCH_2$-OCOC*H*), 4.20 (1H, br s, $(CH_3)_3COCONHCH$), 1.44 (9H, s, $C(CH_3)_3$), 1.41 (3H, d, $J=7.03$ Hz, $ArCH_2OCOCHCH_3$) and 1.34 (3H, d, $J=7.0$ Hz, $(CH_3)_3COCONHCHCH_3$); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 173.0, 172.6, 157.9, 135.7, 129.0, 128.8, 128.5, 80.6, 67.5, 50.4, 48.5, 28.7, 18.6 and 18.6; *m/z* (FAB) 351 ($M+H^+$, 68%), 295 (100), 251 (46), 154 (11). Found: $M+H^+$, 351.1918. $C_{18}H_{27}N_2O_5$ requires 351.1920.

3.1.23. Preparation of (*S*)-2-((*R*)-2-*tert*-butoxycarbonyl-amino-1-hydroxy-propylamino)-propionic acid 26.[20] To a stirred solution of (*S*)-2-((*R*)-2-*tert*-butoxycarbonylamino-1-hydroxy-propylamino)-propionic acid benzyl ester, **25** (1.30 g, 3.70 mmol) in MeOH (15 mL) was added Pd/C (5 mol%, 1.30 g). The reaction was hydrogenated at room temperature for 18 h. The solution was filtered through a plug of Celite and concentrated in vacuo. After drying under vacuum, the product **26** was obtained as a white solid (1.12 g, quantitative). Mp 146–150 °C; $[\alpha]_D^{24}+15.3$ (*c* 1.00, EtOH); IR (neat, cm^{-1}) 322, 3262, 2976, 1712, 1676, 1633, 1562, 1364, 1248, 1162, 1073 and 925; δ_H (400 MHz; CD_3OD) 4.77 (1H, br s, N*H*) 4.22–4.17 (1H, m, HOCOC*H*), 3.98–3.91 (1H, m, $(CH_3)_3COCONHCH$), 1.26 (9H, s, $C(CH_3)_3$), 1.21 (3H, d, $J=7.3$ Hz, $HOCOCHCH_3$) and 1.12 (3H, d, $J=7.3$ Hz, $(CH_3)_3COCONHCHCH_3$); δ_C (100 MHz; CD_3OD) 176.1, 175.9, 158.0, 81.1, 58.8, 52.0, 29.1, 18.8 and 18.3; *m/z* (FAB) 261 ($M+H^+$, 74%), 233 (13), 205 (100), 161 (33), 154 (41), 137 (30). Found: $M+H^+$, 261.1449. $C_{11}H_{21}N_2O_5$ requires 261.1450.

3.1.24. Preparation of [(*R*)-2-hydroxy-2-((*S*)-2-hydroxy-1-methyl-ethylamino)-1-methyl-ethyl]-carbamic acid *tert*-butyl ester, 27.[15] A solution of *S*-alaninol (1.0 g, 13.31 mmol, 1.0 equiv) in dry THF (10 mL) was stirred for 1 h. To that was added dropwise over 30 min a solution of Boc-D-alanine (2.9 g, 15.31 mmol, 1.15 equiv), HOBt (2.1 g, 15.31 mmol, 1.15 equiv) and EDCI (2.94 g, 15.31 mmol, 1.15 equiv) in dry THF (30 mL), which was stirred for 1 h prior. After stirring for another hour, DIPEA (3.39 mL, 19.97 mmol, 1.5 equiv) was added dropwise. Stirring was continued for 18 h at room temperature after which the solvent was evaporated in vacuo. EtOAc (30 mL) was added and the organic layer was washed with 5% citric acid (2×30 mL), saturated $NaHCO_3$ (2×30 mL), concn NaCl (30 mL) and H_2O (30 mL). The organic layer was then dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography afforded the product **27** as a yellow oil (593 mg, 18%). $[\alpha]_D^{24}+3.0$ (*c* 1.00, EtOH); IR (neat, cm^{-1}) 3288, 2976, 2933, 1646, 1520, 1365, 1246, 1161 and 1048; δ_H (400 MHz; $CDCl_3$; $Si(CH_3)_4$) 6.87 (1H, br s, $OHCH_2CHN$*H*), 5.62 (1H, d, $J=5.5$ Hz, N*H*COO), 4.18–4.13 (1H, m, COC*H*), 4.11–4.04 (1H, m, $OHCH_2CH$), 3.91 (1H, br s, O*H*), 3.66–3.63 (1H, m,

OHC*H*H), 3.49–3.45 (1H, m, OHCH*H*), 1.44 (9H, s, C(CH_3)$_3$), 1.35–1.34 (3H, m, COCCH_3) and 1.17–1.15 (3H, m, OHCH$_2$CCH_3); δ_C (100 MHz; $CDCl_3$; $Si(CH_3)_4$) 173.8, 156.2, 80.4, 66.4, 50.7, 47.9, 28.7, 18.9, 17.2 and 15.6; *m/z* (FAB) 247 (M+H$^+$, 92%), 219 (7), 191 (99), 173 (11), 154 (100), 137 (72), 120 (14). Found: M+H$^+$, 247.1650. $C_{11}H_{23}O_4N_2$ requires 247.1652.

3.2. General procedure 2 for ketone reduction

A solution of [$RuCl_2$(*p*-cymene)]$_2$ (0.005 mmol, 0.5 mol%), ligand (0.03 mmol, 3 mol%) and 0.2 M NaOH (5 mol%) in isopropanol (5 mL) was stirred at room temperature for 15 min under nitrogen. The ketone (1 mmol, 100 mol%) was then added and the reaction mixture stirred at room temperature under nitrogen for 2 h. The solution was then filtered through a pad of silica and washed through with EtOAc (20 mL) and the filtrate was concentrated in vacuo. The reaction mixture was analysed by either by GLC (Supelco BETA Dex™) or ^{1}H NMR and HPLC.

3.2.1. 1-Phenylethanol. δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.41–7.26 (5H, m, ArH), 4.90 (1H, q, J=6.4 Hz, CH), 1.96 (1H, br s, OH) and 1.51 (3H, d, J=6.4 Hz, CH_3); (*R*-isomer) $[\alpha]_D^{24}$+45.2 (*c* 1.00, DCM), (lit.[21] $[\alpha]_D$+48.6 (*c* 1.00, DCM), (*S*-isomer) $[\alpha]_D^{24}$−44.0 (*c* 1.00, DCM); Daicel Chiralcel OD, 5% EtOH/hexane, 1 mL min^{-1}, UV 254 nm, t_R=9.5 (*S*-isomer) and 8.2 min (*R*-isomer); Supelco BETA Dex™, 115 °C, t_R=8.9 (*R*-isomer) and 9.3 min (*S*-isomer).

3.2.2. 1-(4-Chlorophenyl)ethanol. δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.33–7.28 (2H, m, Ar*H*), 6.94–7.01 (2H, m, Ar*H*), 4.89 (1H, q, J=6.4 Hz, C*H*), 1.92 (1H, br s, O*H*) and 1.46 (3H, d, J=6.4 Hz, CH_3); (*R*-isomer) $[\alpha]_D^{24}$+37.4 (*c* 1.00, Et_2O), (lit.[21] $[\alpha]_D$+48.6 (*c* 0.9–1.1, Et_2O), (*S*-isomer) $[\alpha]_D^{24}$−56.2 (*c* 1.00, Et_2O), (lit.[22] $[\alpha]_D^{22}$−49.6 (*c* 1.8, Et_2O); Supelco BETA Dex™, 140 °C, t_R=10.9 (*R*-isomer) and 11.3 min (*S*-isomer).

3.2.3. 1-(4-Fluorophenyl)ethanol. δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.36–7.31 (2H, m, Ar*H*), 6.97–6.92 (2H, m, Ar*H*), 4.84 (1H, q, J=6.4 Hz, C*H*), 1.90 (1H, br s, O*H*) and 1.47 (3H, d, J=6.4 Hz, CH_3); (*R*-isomer) $[\alpha]_D^{24}$+45.8 (*c* 1.00, Et_2O), (*S*-isomer) $[\alpha]_D^{24}$−51.6 (*c* 1.00, Et_2O) (lit.[23] $[\alpha]_D^{25}$ 37.7 (*c* 0.93, MeOH); Supelco BETA Dex™, 115 °C, t_R= 9.75 (*R*-isomer) and 10.35 min (*S*-isomer).

3.2.4. 1-(4-Methoxyphenyl)ethanol. δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.31–7.25 (2H, m, Ar*H*), 6.89–6.85 (2H, m, Ar*H*), 4.85 (1H, q, J=6.4 Hz, C*H*), 3.81 (3H, s, OCH_3), 1.81 (1H, br s, O*H*) and 1.49 (3H, d, J=6.4 Hz, CH_3); (*R*-isomer) $[\alpha]_D^{24}$+42.7 (*c* 1.00, $CHCl_3$), (lit.[19] $[\alpha]_D$ +47.3 (*c* 0.90–1.1, $CHCl_3$), (*S*-isomer) $[\alpha]_D^{24}$−36.5 (*c* 1.00, $CHCl_3$) (lit.[24] $[\alpha]_D$ −51.9 (*c* 1.04, $CHCl_3$); Supelco BETA Dex™, 125 °C, t_R= 23.8 (*R*-isomer) and 24.6 min (*S*-isomer).

3.2.5. 1-(2-Naphthyl)ethanol. δ_H (300 MHz; $CDCl_3$; $Si(CH_3)_4$) 7.83–7.79 (4H, m, Ar*H*), 7.51–7.47 (3H, m, Ar*H*0, 5.05 (1H, q, J=6.4 Hz, C*H*), 1.95 (1H, br s, O*H*) and 1.57 (3H, d, J=6.4 Hz, CH_3); (*R*-isomer) $[\alpha]_D^{24}$+35.7 (*c* 5.00, EtOH), (*S*-isomer) $[\alpha]_D$ −22.6 (*c* 5.00, EtOH) (lit.[25] $[\alpha]_D$ −41.9 (*c* 4.92, $CHCl_3$); Daicel Chiralcel OD, 5% EtOH/hexane, 1 mL min^{-1}, UV 254 nm, t_R =12.5 (*S*-isomer) and 13.9 min (*R*-isomer).

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On the enzymatic hydrolysis of methyl 2-fluoro-2-arylpropionates by lipases

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Abstract—The enzymatic hydrolysis of methyl 2-fluoro-2-arylpropionates was performed using lipases from *Candida rugosa* and *Candida cylindracea* (OF-360). A careful analysis of the reaction products revealed that racemic 2-hydroxy-2-arylpropionic acid and traces of 2-arylacrylic acid are formed, in addition to the expected 2-aryl-2-fluoropropionic acid. The presence of powerful electron-releasing groups in the aromatic ring of the substrate increase the amount of 2-hydroxypropionic acid. A mechanistic hypothesis has been formulated according to which the enzyme facilitates the elimination of fluoride ion from the hydrolysed acid with the formation of an α-carboxy-stabilized carbocation which provides 2-hydroxypropionic acids by nucleophilic attack of H_2O and 2-arylacrylic acids by a β-elimination process.

1. Introduction

The insertion of fluorine atoms into organic molecules can cause profound and often unexpected effects on their activities ensuing from the peculiar properties of this element. Its high electronegativity frequently causes such dramatic electronic changes that it can also significantly affect the reactivity, stability and acidity/basicity of the neighbouring groups.[1]

Besides its ability to mimic the hydroxyl functionality and to act as a hydrogen-bond acceptor,[2] fluorine often causes remarkable conformational changes in biologically active compounds resulting in major pharmacological effects such as an increase in both activity and selectivity. This is why the synthesis of regio- and stereoselectively fluorinated molecules is popular with many researchers, especially during the last few decades.[3,4]

In this context, fluorinated non-steroidal anti-inflammatory drugs (NSAID), especially those belonging to the 'profen' family, have received particular attention. Among them, fluorinated surrogates such as flurbiprofen[5] and fluroxaprofen[6] are two of the most successful NSAIDs. Given that C–F bonds are stronger than C–H bonds, replacing the 2-hydrogen of a 2-arylpropionic acid with the quasi-isosteric fluorine conveys a higher configurational stability to the chiral carbon,[7] thus allowing the drug pharmacodynamics, as well as the stereochemical matching with the receptor, to be investigated.

Until now, only a limited number of methods have been reported for the synthesis of side-chain fluorinated 2-arylpropionic acids, and to our knowledge the synthesis of both (*R*)- and (*S*)-2-fluoro-2-(4-isobutylphenyl) propionic acid (α-fluoroibuprofen) reported by Schlosser and co-workers in 1996 is the only example of chiral α-fluoroprofen known to date.[8]

Although optically active cyanohydrins are readily available, potentially allowing the stereoselective synthesis of this compound,[9,10] resolution of racemic α-fluoroibuprofen by enzymatic hydrolysis of its methyl ester was preferred because it allows the enantiomers to be recovered in satisfactory yields, as well as the optical purity. Lipase from *Candida cylindracea* (OF-360) was found to be the best catalyst to perform the desired transformation.

During our research on a novel synthetic approach to both nucleus and side-chain fluorinated 2-arylpropionic acid,[11] we needed to resolve the racemic mixture of 2-fluoro-2-naphthylpropionic acids. Unexpectedly, all attempts to resolve racemic α-fluoronaproxen by enzymatic hydrolysis of its methyl ester using different lipases were unsuccessful, leading to everything but optically pure α-fluoronaproxen.

Keywords: Lipases; Enzymatic hydrolysis; Fluorinated compounds; Profens; α-hydroxyacids.
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Nevertheless, we felt that this intriguing process was worthy of being studied in depth. The results of this investigation are reported in this paper.

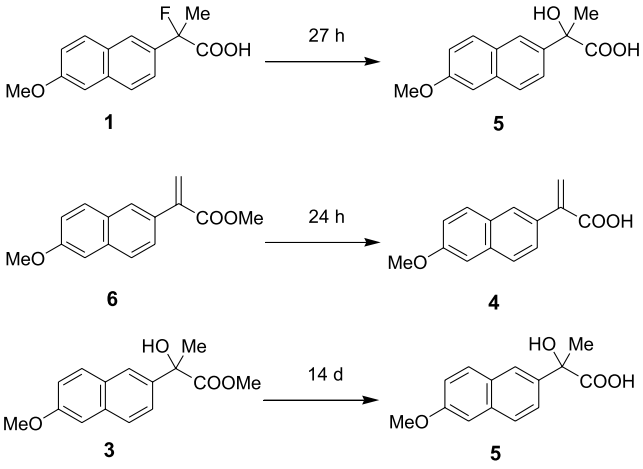
2. Results and discussion

Candida rugosa lipase (CRL) and lipase OF-360 have been used successfully for kinetic resolutions of various racemic 2-arylpropionic[12] and 2-aryloxypropionic acids.[13,14] Thus, we first tried to use these biomaterials for the kinetic resolution of 2-fluoro-2-(6-methoxynaphth-2-yl)propionic acid **1** by the lipase-catalyzed hydrolysis of the corresponding methyl ester **2**. The latter was prepared in two steps, in 51% overall yield, by adding 6-methoxy-2-naphthylmagnesium bromide to methyl pyruvate followed by fluorination of the resulting methyl 2-hydroxy-2-(6-methoxynaphth-2-yl)propionate **3** with diethylaminosulfur trifluoride (DAST).

The racemic α-fluoroester **2** was hydrolysed by crude lipase OF-360 (C-OF) and crude CRL (C-CRL) to give mostly, the racemic 2-hydroxy-2-(6-methoxy-2-naphtyl)propionic acid **5**, a small amount of the corresponding acid **1** of very low optical purity and traces of 2-(6-methoxy-2-naphth-2-yl)acrylic acid **4** (Scheme 1). The recovered ester **2** had low optical purity (entries 1 and 2 in Table 1).

Since arylacrylic acid **4** was clearly formed by HF elimination, at first glance, the α-hydroxypropionic acid **5** could have come from either the lipase-catalysed hydration of the intermediate acrylic ester or by enzyme catalysed nucleophilic substitution of fluorine by H_2O or, even better, OH^-. These results are somewhat intriguing if one considers that when lipases co-ordinate the substrate in their active pocket, the hydrolysis or esterification are the only reactions catalysed.[15] In our case, the presence of fluorine in the α-position of propionate should induce interactions that cause specific transformations.

In order to investigate the origin of the above products, the arylpropionic acid **1**, the methyl acrylate **6** and the α-hydroxyester **3**, initially considered probable intermediates in the whole process, were prepared by standard procedures and submitted to the action of C-CRL. Under the same conditions employed in the hydrolysis of ester **2**, acid



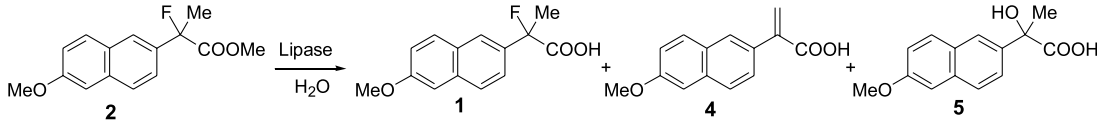
Scheme 2.

1 was exclusively converted into α-hydroxyacid **5** after 1 d (Scheme 2).

Methyl acrylate **6** was partially hydrolysed (47%) to give the corresponding acid **4** after 1 d. No traces of α-hydroxyacid were found in the reaction mixture, thus excluding a lipase-promoted hydration of the acrylic double bond. The hydrolysis of hydroxyester **3** turned out to be a very slow process giving 18% of acid **5** (ee 42%) after 14 d, while 66% of the starting material **3** (ee 9%) was recovered. No trace of elimination product was detected. As expected, replacing the fluorine with an OH group completely inhibited this process.

All of these observations are in line with the following mechanistic hypothesis. CRL catalyses the hydrolysis of racemic α-fluoroester **2** in a relatively fast process, through the action of the OH group of Ser_{209} (Scheme 3).[15]

Afterwards, a nucleophilic substitution reaction of fluoride takes place leading to the α-hydroxyacid **5** (Scheme 4). In this step, an acid group present in the active site, probably the imidazolium moiety of His_{449}, plays an important role in the removal of the fluoride ion and formation of carbocation **7** that is further stabilized by the α-carboxylate group. The intermediate **7** may undergo either deprotonation of the methyl group, catalysed by the imidazole moiety, to give the elimination product **4**; or a nucleophilic attack by H_2O, leading to α-hydroxyacid **5**. Of course, the medium polarity

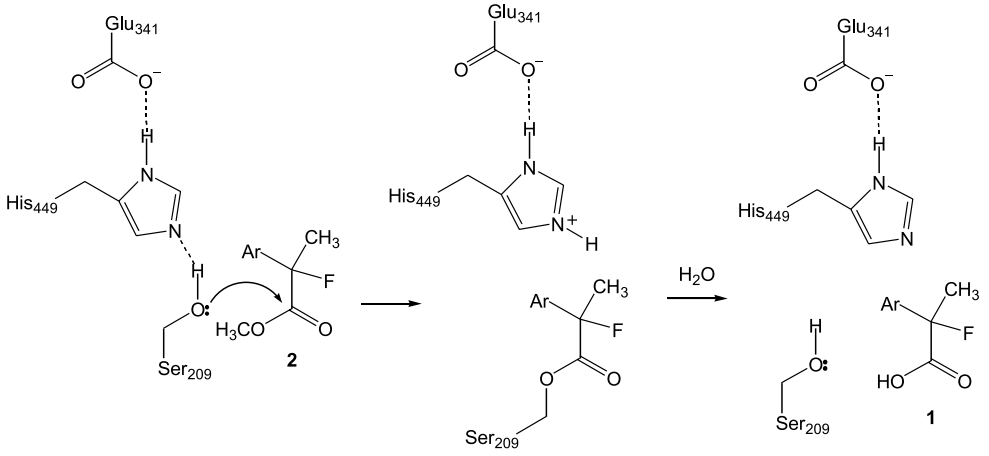


Scheme 1.

Table 1. CRL and OF-360 lipase-catalysed hydrolysis of racemic methyl 2-fluoro-2-arylpropionate **2** in water at pH 7.2 and 37 °C

Entry	Enzyme	C, %[a]	Time, d	Product, % (ee)[b]			
				α-Fluoro-ester **2**	α-Fluoro-acid **1**	Acrylic acid **4**	α-Hydroxy-acid **5**
1	C-OF	60	3	40 (49)	12 (23)	traces	48 (0)
2	C-CRL	60	6	40 (16)	18 (10)	—	42 (0)

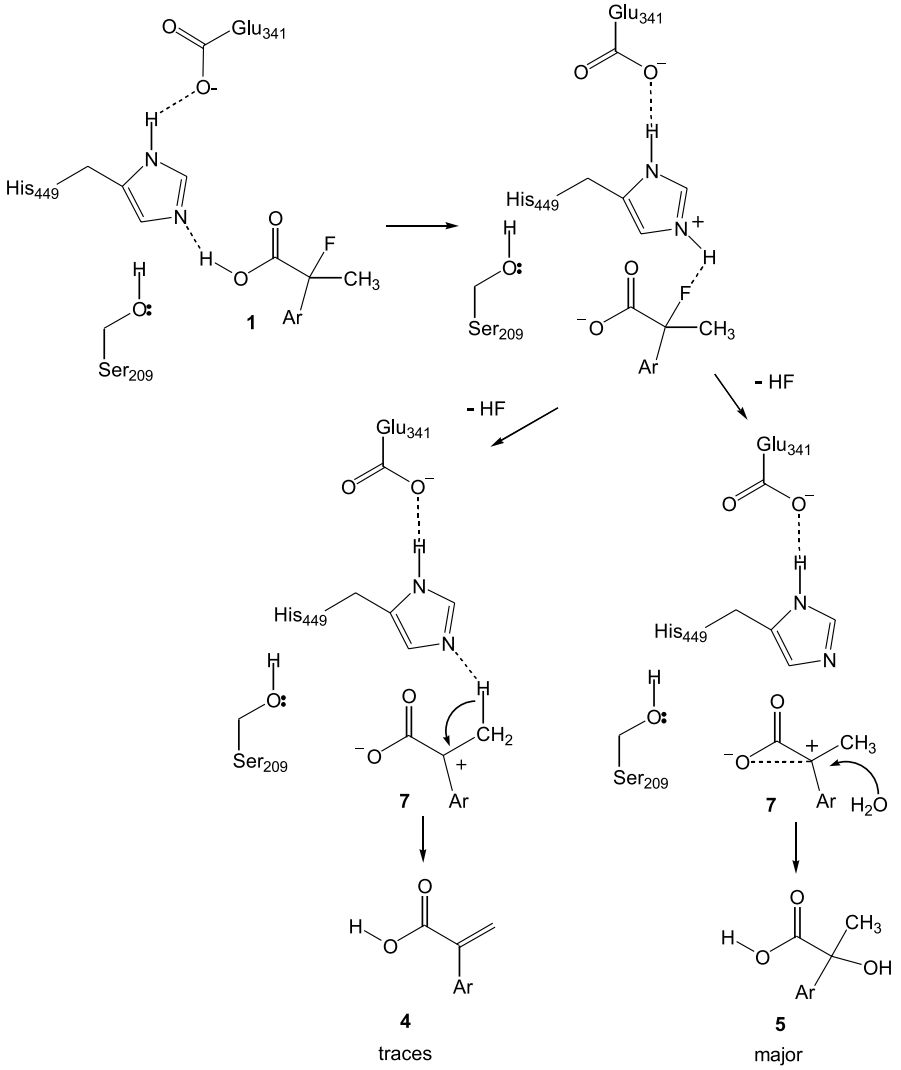
[a] Conversion, % (recovered α-fluoro-ester).
[b] Enantiomeric excess determined by ^1H NMR spectroscopy using $Eu(hfc)_3$ as a chiral shift reagent (substrate/$Eu(hfc)_3$ 1/6, $CDCl_3$).



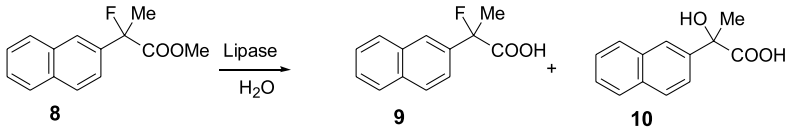
Scheme 3.

and mild temperature favour the latter process. In this particular case, the hypothesis of an E2 HF-elimination from the starting ester **2** should be discarded, because this high energy process would require the intervention of a much stronger base. On the other hand, the direct hydration of the acrylic double bond giving the α-hydroxyacid **5** is quite improbable, at least under these mild conditions, since the Markovnikov addition of water would be strongly disfavoured by the presence of the carboxylic group.[16]

The above mechanistic hypothesis would also explain why the lipase-catalyzed hydrolysis of methyl acrylate **6** stops at



Scheme 4.



Scheme 5.

acid **4** and, more importantly, why the lipase-catalyzed hydrolysis of **2** produces the racemic α-hydroxyacid **5**, while the corresponding α-fluoroacid **1** is recovered with some optical purity.

In order to assess the electronic effects of the substituents in the aromatic ring on the kinetics, as well as on the product distribution of the enzymatic resolution, methyl 2-fluoro-2-(naphth-2-yl)propionate **8** was prepared from the corresponding α-hydroxyester by an identical procedure and submitted to hydrolysis catalyzed by C-CRL. Products **9** (39%; ee 89%) and **10** (13%; ee 0) were obtained after 1 d analogous to what was observed with ester **2** (Scheme 5). The unreacted ester **8** exhibited high optical purity (ee 90%) (Table 2, entry 1).

The absence of a methoxy group in the naphthyl ring increases the rate of hydrolysis and the enantioselectivity of C-CRL toward **8**, leading to the same products, but in different molar ratios compared to the hydrolysis of **2**. According to the proposed mechanism, electronic effects substantially affect the product distribution, while leaving the pattern unchanged. In fact, in this case there was a substantial decrease of α-hydroxyacid **10** with respect to α-fluoroacid **9**, in contrast to what was observed with substrate **2**. As expected, the absence of an electron-releasing group makes the removal of the fluoride ion more difficult.

For comparison purposes, racemic methyl 2-fluoro-2-(4-isobutylphenyl)propionate (α-fluoroibuprofen methyl ester) **11** and racemic methyl 2-fluoro-2-(4-isopropoxyphenyl)propionate **14**, prepared from the corresponding α-hydroxy esters, were submitted to the action of C-CRL (Schemes 6 and 7). At 45% conversion, after 20 h, a modest kinetic resolution was noted for substrate **11** (48% ee for the remaining ester, and 59% ee for the α-fluoroacid **12**), and the racemic α-hydroxyacid **13** was obtained (14%) (entry 2 in Table 2).

The hydrolysis rate was practically unchanged, when the isobutyl group was replaced with an isosteric isopropoxy group in the α-fluoroibuprofen, while the enantioselectivity was substantially increased, with the remaining α-fluoroester **14** exhibiting high optical purity (ee, 95%, entry 3 in Table 2).

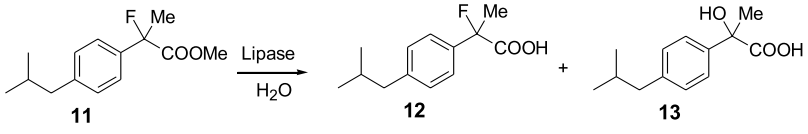
Moreover, the resulting α-fluoroacid was not detected because it was rapidly transformed into the corresponding racemic α-hydroxy-acid **15**. In this case, a small amount of the α-hydroxyester **16** (10%) was also detected. It was probably formed from α-fluoro-ester **14** in the same way as α-hydroxyacid **15**, according to the above mechanism. Clearly, the electron-releasing effect of the isopropoxy group is responsible for the stabilization of the benzylic carbocation by the carboxylate ion.

The substrate structure seems to play a decisive role in the lipase-promoted hydrolysis of condensed polycyclic 2-arylpropionates. High enantioselectivity or complete inhibition of the hydrolytic process may be observed. This was the case when we attempted to use the above OF-360 or

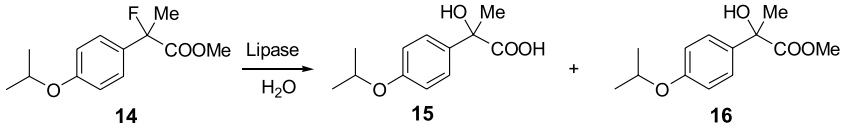
Table 2. CRL-catalysed hydrolysis of racemic methyl 2-fluoro-2-arylpropionate **8**, **11** and **14** in water at pH 7.2 and 37 °C

Entry	Enzyme	C, %[a]	Time, h	Product, % (ee)[b]				
				α-fluoro-ester	α-fluoro- acid	Acrylic acid	α-hydroxy- acid	α-hydroxy- ester
1	C-CRL	52	24	**8** 48 (90)	**9** 39 (89)	—	**10** 13 (0)	—
2	C-CRL	45	20	**11** 55 (48)	**12** 31 (59)	—	**13** 14 (0)	—
3	C-CRL	60	18	**14** 40 (95)	—	—	**15** 50 (0)	**16** 10 (0)

[a] Conversion, % (recovered α-fluoro-ester).
[b] Enantiomeric excess determined by ^{1}H NMR spectroscopy using Eu(hfc)$_3$ as a chiral shift reagent (substrate/Eu(hfc)$_3$ 1/6, $CDCl_3$).

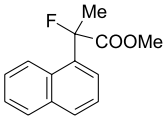


Scheme 6.



Scheme 7.

CRL lipases in the kinetic resolution of methyl 2-fluoro-2-(naphth-1-yl)propionate **17.**



17

The starting α-fluoro ester was recovered unchanged after 9 d. Clearly, the success of the enzymatic kinetic resolution of 2-fluoro-2-naphthylpropionic esters by lipases is strongly dependent on the position of the propionyl group in the naphthalene nucleus. This sheds light on the geometry of the active site of the enzyme, as well as on the structural requirements of the substrate for the correct binding to this.

3. Conclusion

Lipase from *C. rugosa* and lipase from *C. cylindracea* (OF-360) catalyze the hydrolysis of methyl 2-fluoro-2-arylpropionates **2**, **8**, **11** and **14**. A careful analysis of the reaction products revealed that 2-hydroxy-2-arylpropionic acid and traces of 2-arylacrylic acid are formed in addition to the expected 2-aryl-2-fluoropropionic acid. Powerful electron-releasing groups in the aromatic ring of the substrate increase the amount of 2-hydroxypropionic acid obtained. A mechanistic hypothesis has been formulated according to which the enzyme facilitates the elimination of the fluoride ion from the hydrolysed acid and the formation of a stable carbocation which produces 2-hydroxypropionic acid by nucleophilic attack of H_2O and 2-arylacrylic acids by eliminating a proton from the methyl group.

The success of the enzymatic kinetic resolution of 2-fluoro-2-naphthylpropionic esters by lipases is strongly dependent on the position of the propionyl group in the naphthalene nucleus. Whereas methyl 2-fluoro-2-(naphth-2-yl)propionate is easily hydrolysed by CRL and lipase OF-360, the corresponding regioisomer 2-fluoro-2-(naphth-1-yl)propionate is completely inert toward these kinds of lipases.

4. Experimental

4.1. General

If not specified otherwise, ^{1}H NMR and ^{1}H-decoupled ^{13}C NMR spectra were recorded at 400 and 200 MHz, respectively, in $CDCl_3$ solution using tetramethylsilane as an internal standard. ^{19}F NMR spectra were recorded at 376 MHz in $CDCl_3$ solution using $CFCl_3$ as a reference standard. IR spectra were registered in $CHCl_3$ solution in the 4000–625 cm^{-1} range. Gas-chromatographic analyses were performed using 30 m×0.32 mm×25 μm capillary columns loaded with two different stationary phases: DB-5 MS (5% phenyl-methylpolysiloxane) and DB-35 MS (5% phenyl-methylpolysiloxane) at 70–310 °C. Mass spectra were obtained at 70 eV. Melting points were corrected after calibration performed with authentic standards.

Commercial 1-bromonaphthalene, 2-bromonaphthalene, 2-bromo-6-methoxynaphthalene, methyl pyruvate and other inorganic reagents of the highest purity were used as purchased. Tetrahydrofuran and diethyl ether were distilled from potassium hydroxide in the presence of cuprous chloride and redistilled from sodium wire in the presence of benzophenone.

C. rugosa lipase (E.C.3.1.1.13 type VII, LOT. 033K0612) was purchased from Sigma Chemicals Co. and lipase from *C. cylindracea* (OF-360) was a gift from Amano Pharmaceutical Co.

4.2. General method to prepare methyl 2-fluoro-2-arylpropionates 2, 8, 11, 14 and 17

The suitable arylmagnesium bromide (10 mL, 1.0 M in THF, 10 mmol), prepared from the corresponding bromoarene and magnesium chips by standard procedure) was added drop-wise to freshly distilled methyl pyruvate (11 mmol) in THF (5 mL) at −78 °C. The temperature was allowed to rise at 25 °C, a sat. aq solution of NH_4Cl (30 mL) was added and the mixture was extracted with diethyl ether (3×25 mL). The collected organic phases were dried with Na_2SO_4. After the solvent was evaporated, chromatography of the crude product on silica gel (eluent, 7:3 petroleum ether/diethyl ether) gave the pure α-hydroxyester. The following α-hydroxy esters were prepared and characterized as follows:

4.2.1. Methyl 2-hydroxy-2-(6-methoxynaphth-2-yl)propionate (3). 76%: mp 86–88 °C; ^{1}H NMR δ 7.94 (d, J=1.6 Hz, 1H), 7.74 (d, J=8.5 Hz, 1H), 7.71 (d, J=8.2 Hz, 1H), 7.59 (dd, J=8.7, 1.9 Hz, 1H), 7.14 (dd, J=8.8, 2.5 Hz, 1H), 7.11 (d, J=2.4 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 1H), 3.76 (s, 3H), 1.87 (s, 3H); ^{13}C NMR δ 176.2, 157.9, 137.7, 133.9, 129.7, 128.4, 126.9, 123.8, 123.8, 119.0, 105.4, 75.8, 55.2, 53.2, 26.6; IR ($CHCl_3$) ν_{max} 3529, 3020, 2958, 2843, 1729, 1606, 1456, 1262, 1289, 1185 cm^{-1}; MS m/z (%) 260 (M^+, 35), 242 (1), 201 (100), 185 (12), 159 (18), 144 (14), 43 (52). Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.13; H, 6.11.

4.2.2. Methyl 2-hydroxy-2-(naphth-2-yl)propionate. 70%: mp 47–48 °C; ^{1}H NMR δ 8.03 (d, J=1.6 Hz, 1H), 7.88–7.81 (m, 3H), 7.65 (dd, J=8.7, 1.9 Hz, 1H), 7.51–7.45 (sym. m, 2H), 3.90 (s, 1H), 3.78 (s, 3H), 1.89 (s, 3H); ^{13}C NMR δ 176.1, 140.0, 133.0, 132.8, 128.3, 128.1, 127.5, 126.2, 126.2, 124.0, 123.4, 75.9, 52.2, 26.6; IR ν_{max} 3498, 3058, 2985–2847, 1732, 1508, 1261, 1246, 1140, 821, 753; MS m/z (%) 230 (M^+, 19), 171 (100), 155 (12), 127 (23), 43 (76). Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13. Found: C, 73.12; H, 6.02.

4.2.3. Methyl 2-hydroxy-2-(4-isobutylphenyl)propionate. 83%: colourless oil; ^{1}H NMR δ 7.43–7.10 (AA′BB′ system, 4H), 3.76 (s, 3H), 3.72 (br s, 1H), 2.44 (d, J=7.2 Hz, 2H), 1.84 (sept, J=6.7 Hz, 1H), 1.76 (s, 3H), 0.88 (d, J=6.7 Hz, 6H); ^{13}C NMR δ 176.3, 141.3, 140.0, 129.0, 124.9, 75.7, 53.1, 44.9, 30.1, 26.6, 22.3; IR ν_{max} 3511, 3027, 2955–2848, 1732, 1510, 1606, 1254, 1150 cm^{-1}; MS m/z (%) 236 (M^+, 1), 177 (100), 161 (5), 134 (5), 91 (8), 43 (41). Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 71.37; H, 8.39.

4.2.4. Methyl 2-hydroxy-2-(4-isopropoxyphenyl)propionate (16). 38%: viscous oil; ^{1}H NMR δ 7.43–6.81 (AA′BB′ system, 4H), 4.52 (sept, J=6.1 Hz, 1H), 3.74 (s, 3H), 3.73 (s, 1H), 1.74 (s, 3H), 1.31 (d, J=6.1 Hz, 6H); ^{13}C NMR δ 176.3, 157.4, 134.5, 126.4, 115.3, 75.3, 69.7, 53.1, 26.6, 22.0; IR ν_{max} 3506 (broad), 3041, 2978–2937, 1736, 1609, 1509, 1247, 1119, 955 cm^{-1}; MS, *m/z* (%) 238 (M^+, 4), 223 (1), 179 (76), 137 (100), 121 (15), 43 (68). Anal. Calcd for $C_{13}H_{14}O_4$: C, 65.53; H, 7.61. Found: C, 65.62; H, 7.55.

4.2.5. Methyl 2-hydroxy-2-(naphth-1-yl)propionate. 76%: mp 80–82°C; ^{1}H NMR δ 8.21–8.17 (m, 1H), 7.88–7.83 (m, 2H), 7.65 (dd, J=7.3, 1.1 Hz, 1H), 7.51–7.43 (m, 3H), 3.67 (s, 3H), 3.66 (s, 1H), 2.01 (s, 3H); ^{13}C NMR δ 177.7, 136.7, 134.3, 131.0, 129.5, 129.0, 126.3, 125.5, 124.7, 124.7, 124.1, 76.2, 53.2, 27.2; IR ν_{max} 3535, 3054, 3015, 2956, 1729, 1512, 1259, 1136, 805; MS, *m/z* (%) 230 (M+, 17), 171 (100), 155 (11), 127 (20), 43 (76). Anal. Calcd for $C_{14}H_{14}O_3$: C, 73,03; H, 6,13. Found: C, 73.19; H, 6.06.

The above esters were submitted to fluorination with diethylaminosulfur trifluoride (DAST). DAST (0.17 mL, 0.21 g, 1.28 mmol) was added to a solution of the hydroxyester (1.0 mmol) in dry dichloromethane (2 mL) at 0 °C under nitrogen atmosphere. The mixture was made to react for 30 min before water (10 mL) was carefully added. The organic phase was separated, washed with water (20 mL) and dried with sodium su1fate. After solvent evaporation, chromatography of the crude product on silica gel (100 mL, eluent 4:1 (v/v) petroleum ether/diethyl ether) allowed the pure α-fluoroesters **2**, **8**, **11**, **14** and **17** to be obtained.

4.2.6. Methyl 2-fluoro-2-(6-methoxynaphth-2-yl)propionate (2). 68%: mp 95–97 °C; ^{1}H NMR δ 7.89 (s, 1H), 7.75 (d, J=8.9 Hz, 1H), 7.74 (d, J=8.6 Hz, 1H), 7.55 (dd, J=8.6, 1.8 Hz, 1H), 7.17 (dd, J=8.9, 2.5 Hz, 1H), 7.12 (d, J=2.3 Hz, 1H), 3.90 (s, 3H), 3.76 (s, 3H), 2.02 (d, J=22.3 Hz, 3H); ^{13}C NMR δ 171.5 (d, J=27 Hz), 158.2, 134.4, 134.1 (d, J=22.6 Hz), 129.8, 128.2, 127.2, 123.6 (d, J=9.2 Hz), 122.8 (d, J=7.4 Hz), 119.4, 105.5, 94.8 (d, J=185 Hz), 55.3, 52.8, 24.6 (d, J=23.7); ^{19}F NMR δ −150.56 (q, J=22.3 Hz); IR ($CHCl_3$) ν_{max} 3028, 2956, 2844, 1747, 1608, 1485, 1270, 1128 cm^{-1}; MS *m/z* (%) 262 (M^+, 19), 242 (8), 203 (100), 159 (17). Anal. Calcd for $C_{15}H_{15}FO_3$: C, 68.69; H, 5.76. Found: C, 68.83; H, 5.69.

4.2.7. Methyl 2-fluoro-2-(naphth-2-yl)propionate (8). 73%. Mp 44–46 °C; ^{1}H NMR δ 7.99 (s, 1H), 7.89–7.81 (m, 3H), 7.60 (dd, J=8.6, 1.8 Hz, 1H), 7.53–7.48 (m, 2H), 3.77 (s, 3H), 2.04 (d, J=22.3 Hz, 3H); ^{13}C NMR δ 171.3 (d, J=26.6 Hz), 136.4 (d, J=22.2 Hz), 133.1, 132.8, 128.4, 128.3, 127.6, 126.6, 126.5, 123.7 (d, J=9.4 Hz), 122.2 (d, J=7.6 Hz), 94.7 (d, J=185.0 Hz), 52.9, 24.7 (d, J=23.7); ^{19}F NMR δ −151.56 (q, J=22.3 Hz); IR ($CHCl_3$) ν_{max} 3061, 2998–2874, 1715, 1608, 1266, 855 cm^{-1}; MS, *m/z* (%) 232 (M^+, 24), 173 (100), 153 (21). Anal. Calcd for $C_{14}H_{13}FO_2$: C, 72.40; H, 5.64. Found: C, 72.22; H, 5.69.

4.2.8. Methyl 2-fluoro-2-(4-isobutylphenyl)propionate (11). 71%. Oil; ^{1}H and ^{19}F NMR spectra were identical to those reported in the literature; [7] ^{13}C NMR δ 171.5 (d, J=27.0 Hz), 142.2, 136.4 (d, J=22.6 Hz), 129.2, 124.3 (d, J=8.1 Hz), 94.7 (d, J=184.0 Hz), 52.8, 45.0, 30.1, 24.7 (d, J=23.8 Hz), 22.3; IR ν_{max} 3035, 2957–2870, 1762, 1745, 1513, 1284, 1263, 1125 cm^{-1}; MS, *m/z* (%) 238 (M^+, 5), 179 (100), 137 (17), 115 (6). Anal. Calcd for C_{14} $H_{19}FO_2$: C, 70.56; H, 8.04. Found: C, 70.68; H, 8.17.

4.2.9. Methyl 2-fluoro-2-(4-isopropoxyphenyl)propionate (14). 95%. Oil; ^{1}H NMR d 7.39–6.85 (AA′BB′ system, 4H), 4.53 (sept, J=6.1 Hz, 1H), 3.74 (s, 3H), 1.90 (d, J=22.2 Hz, 3H), 1.31 (d, J=6.1 Hz, 6H); ^{13}C NMR δ 171.6 (d, J=27.4 Hz), 158.2, 130.7 (d, J=23.0 Hz), 126.0 (d, J=7.8 Hz), 115.4, 94.4 (d, J=184 Hz), 69.8, 52.7, 24.5 (d, J=23.7 Hz), 21.9; ^{19}F NMR δ −148.8 (q, J=22.2 Hz); IR ν_{max} 3028, 2980, 1759, 1744, 1611, 1511, 1247, 1121, 955, 836 cm^{-1}; MS, *m/z* (%) 240 (M^+, 6), 181 (14), 139 (100), 109 (5), 91 (5). Anal. Calcd for $C_{13}H_{17}FO_3$: C, 64.98; 7.13. Found: C, 65.19; H, 7.20.

4.2.10. Methyl 2-fluoro-2-(naphth-1-yl)propionate (17). 81%. Mp 75–77 °C; ^{1}H NMR δ 8.22 (dt, J=8.3, 1.3 Hz, 1H), 7.88 (m, 2H), 7.66 (dt, J=7.3, 1.3 Hz, 1H), 7.54–7.46 (m, 3H), 3.70 (s, 3H), 2.18 (d, J=22.6 Hz, 3H); ^{13}C NMR δ 172.3 (d, J=26.6 Hz), 134.1 (d, J=20.2 Hz), 134.0, 130.5, 130.3 (d, J=1.4 Hz), 128.9, 126.8 (d, J=1.2 Hz), 125.8, 124.7, 124.6, 124.2 (d, J=7.8 Hz), 95.0 (d, J=182 Hz), 52.9, 24.1 (d, J=24.6 Hz); ^{19}F NMR δ −140.9 (q, J=22.0 Hz); IR ν_{max} 3034, 2957–2848, 1752, 1602, 1514, 1270, 1124 cm^{-1}; MS *m/z* (%) 232 (M^+, 28), 173 (100) 153 (37). Anal. Calcd for $C_{14}H_{13}FO_2$: C, 72.40; H, 5.64. Found C, 72.19; H, 5.58.

4.2.11. Methyl 2-(6-methoxynaphth-2-yl)acrylate (6). The compound was prepared as an authentic specimen by refluxing methyl 2-hydroxy-2-(6-methoxynaphth-2-yl)-propionate **3** in toluene for 6 h in the presence of *p*-toluensulfonic acid as catalyst while removing water by a Dean–Stark apparatus. The solvent evaporation at reduced pressure, after neutralization of the acid catalyst, left an oily residue which was chromatographed on silica gel (eluent petroleum ether/diethyl ether 8:2) to give pure methyl acrylate **6** (30%, 61% of the starting hydroxyester recovered). Mp 69–71 °C; ^{1}H NMR δ 7.84 (d, J=1.5 Hz, 1H), 7.73 (m, 2H), 7.49 (dd, J=8.5, 1.8 Hz, 1H), 7.17–7.12 (m, 2H), 6.40 (d, J=1.2 Hz, 1H), 5.99 (d, J=1.2 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H); ^{13}C NMR δ 167.5, 158.0, 141.2, 134.2, 131.8, 129.7, 128.5, 127.2, 126.5, 126.4, 126.3, 119.0, 105.5, 55.2, 52.2; IR ν_{max} 3062, 3025, 1955–2844, 1719, 1633, 1606, 1264, 1171, 855 cm^{-1}; MS *m/z* (%) 242 (M^+, 100), 199 (9), 183 (61), 168 (13), 139 (27). Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.21; H, 5.76.

4.3. General method for enzymatic hydrolysis in presence of lipase

In a standard experiment (Table 1, entry 1), crude CRL (120 mg) was suspended in phosphate buffer (0.02 M, pH 7.2, 12 mL). Racemic ester **2** (0.131 g, 0.5 mmol) was added and the mixture was stirred at 37 °C. After 3 d, the reaction was terminated by addition of NaCl and then filtered through Celite. The solution was then acidified with HCl to pH 2 and extracted with ethyl acetate (3×20 mL).

The combined extracts were treated with an aqueous solution of NaOH 0.2 M (3×20 mL) and separated. The organic layer was dried with Na_2SO_4 and concentrated in vacuo giving ester **2**. The combined aqueous layers were acidified to pH 2, extracted with ethyl acetate (3×20 mL), combined and dried with Na_2SO_4 and then concentrated in vacuo to give a mixture of acids **1**, **4** and **5**. The enantiomeric excess of ester **2** obtained in the enzymatic hydrolysis was determined by recording ^{1}H NMR spectra in presence of Eu(hfc)$_3$ as a chiral shift reagent (substrate/Eu(hfc)$_3$ 1/6, $CDCl_3$).

The enantiomeric excess of acids **1** and **5** were determined on the corresponding methyl esters obtained by treatment with CH_2N_2 and separated by silica gel chromatography (petroleum ether/ethyl acetate 8/2).

4.4. General procedure for the preparation of the 2-aryl-2-fluoropropionic acids 1, 9, 12, 2-aryl-2-hydroxypropionic acids 5, 10, 13 and 15 and 2-(6-methoxynaphth-2-yl)acrylic acid 4

The reaction products were identified by their spectroscopic and analytical properties, by comparison with authentic specimens prepared as follows:

The suitable ester (1.0 mmol) was made to react for 2 h in 5% methanolic KOH (25 mL) at 20 °C. The solvent was evaporated, the resu1ting so1id was dissolved in water (30 mL) and the mixture was extracted with diethyl ether (2×20 mL). The clear solution was acidified with 5% aqueous HCl until pH 1, the resulting white suspension was extracted with ethyl acetate (3×20 mL) and the collected organic phases were dried with Na_2SO_4. After solvent evaporation, the remaining white solid was crystallized from 1:1 ethanol/hexane to give the pure acid. The acids were identified on the basis of their spectroscopic and analytical characteristics.

4.4.1. 2-Fluoro-2-(6-methoxynaphth-2-yl)propionic acid (1). 78%: mp 119 °C (dec); all spectroscopic and analytical characteristics were identical to those reported in the literature.[6]

4.4.2. 2-Fluoro-2-(naphth-2-yl)propionic acid (9). Mp 115 °C (dec.); ^{1}H NMR δ 8.07 (br s, 1H), 7.99–7.89 (m, 3H), 7.66 (dd, J=8.7, 1.9 Hz, 1H), 7.56–7.52 (symmetric m, 2H); ^{13}C NMR δ 171.0 (d, J=27.1 Hz), 137.1 (d, J=21.2 Hz), 133.2, 132.9, 128.3, 128.2, 127.5, 126.6, 126.5, 123.7 (d, J=9.0 Hz), 122.5 (d, J=7.3 Hz), 94.4 (d, J=183 Hz), 23.9 (d, J=23.9 Hz); ^{19}F NMR δ −149.7 (q, J=22.1 Hz); IR ($CHCl_3$) ν_{max} 3400–2300 (very broad), 2882, 1706, 1274, 1140, 827 cm^{-1}. Anal. Calcd for $C_{13}H_{11}FO_2$: C, 71.55; H, 5.08. Found: C, 71.80; H, 5.12.

4.4.3. 2-Fluoro-2-(4-isobutylphenyl)propionic acid (12). 86%: mp 69–71 °C; All spectroscopic and analytical characteristics were identical to those reported in the literature.[6]

4.4.4. 2-Hydroxy-2-(6-methoxynaphth-2-yl)propionic acid (5). 88%. Mp 170 °C (dec.); ^{1}H NMR δ 12.6 (very br s, 1H), 7.93 (br s, 1H), 7.81 (d, J=9.0 Hz, 1H), 7.75 (d, J=8.6 Hz, 1H), 7.59 (broad d, J=8.6 Hz, 1H), 7.26 (d, J=2.3 Hz, 1H), 7.13 (dd, J=8.9, 2.3 Hz, 1H), 5.98 (br s, 1H), 3.84 (s, 3H), 1.70 (s, 3H); ^{13}C NMR δ 176.6, 157.7, 139.9, 133.8, 130.0, 128.3, 126.7, 124.8, 123.9, 119.0, 106.0, 75.3, 55.5, 27.6; IR ($CHCl_3$) ν_{max} 3495, 3200–2000 (very broad), 3021, 2962–2895, 1718, 1607, 1270, 1132, 1027 cm^{-1}. Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.39; H, 5.67.

4.4.5. 2-Hydroxy-2-(naphth-2-yl)propionic acid (10). 84%. Mp 155 °C (dec.); ^{1}H NMR δ 12.70 (very br s, 1H), 8.02 (br s, 1H), 7.92–7.84 (m, 3H), 7.64 (dd, J=8.7, 1.9 Hz, 1H), 7.50–7.44 (symmetric m, 2H), 5.96 (br s, 1H), 1.72 (s, 3H); ^{13}C NMR δ 176.4, 142.3, 132.9, 132.5, 128.4, 127.8, 127.7, 126.5, 126.3, 124.4, 124.0, 75.4, 27.6; IR ($CHCl_3$) ν_{max} 3417 (broad), 3200–2000 (very broad), 3031, 2988, 1722, 1599, 1267, 1133, 883 cm^{-1}. Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.38; H, 5.71.

4.4.6. 2-Hydroxy-2-(4-isobutylphenyl)propionic acid (13). 91%. Mp 101–103 °C; ^{1}H NMR δ 7.52–7.11 (AA′BB′ system, 4H), 4.81 (br s, 1H), 2.44 (d, J=7.2 Hz, 2H), 1.83 (sept, J=6.7 Hz, 1H), 1.71 (s, 3H), 0.86 (d, J=6.7 Hz, 6H); ^{13}C NMR δ 175.8, 141.3, 140.6, 128.6, 125.0, 75.0, 44.6, 30.0, 26.6, 21.7; IR ($CHCl_3$) ν_{max} 3521, 3200–2300 (very broad), 3031, 2958–2870, 1718, 1510, 1262 cm^{-1}; Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.06; H, 8.18.

4.4.7. 2-Hydroxy-2-(4-isopropoxyphenyl)propionic acid (15). 87%. Mp 103–104 °C; ^{1}H NMR (acetone-d_6) δ 7.51–7.47 (sym m, 2H), 6.87–6.83 (sym m, 2H), 4.58 (sept, J=6.0 Hz, 1H), 1.70 (s, 3H), 1.26 (d, J=6.0 Hz, 6H), 13.0–11.0 (very broad 2H); ^{13}C NMR (acetone-d_6) d 176.0, 157.3, 135.6, 126.5, 115.0, 74.8, 69.2, 26.6, 21.4. IR ($CHCl_3$) ν_{max} 3512, 3400–2380 (very broad), 1721, 1500, 1247 cm^{-1}. Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.05; H, 7.22.

4.4.8. 2-(6-Methoxynaphth-2-yl)acrylic acid (4). Mp 172–174 °C; All spectroscopic and analytical characteristics were identical to those reported in the literature.[17]

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Cu-mediated Stille reactions of sterically congested fragments: towards the total synthesis of zoanthamine

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Abstract—A study on the Stille reaction of alkenyl iodides and stannanes with structural resemblance to retrosynthetic fragments of a projected total synthesis of the marine alkaloid zoanthamine was carried out. A range of reaction conditions was examined, and a protocol developed by Corey utilizing excess copper(I) chloride and lithium chloride was found to be most efficient. The methodology was successfully applied to join two major fragments of the zoanthamine skeleton.

1. Introduction

Since their discovery,[1] the zoanthamine alkaloids (Fig. 1) have been intensively investigated targets in marine natural product chemistry.[2] Yet, the mechanism of biological action and the biosynthetic origin of these fascinating molecular entities remain uncertain. Several related compounds have been isolated from *Zoanthus* sp.,[3] and a range of synthetic studies have been carried out, most notably with focus on the construction of the unusual double hemiaminal core of zoanthamine (**1**) and norzoanthamine (**2**).[4] Other studies have dealt with the synthesis of the AB ring system of norzoanthamine,[5] the ABC ring systems of zoanthamine[6] and zoanthenol,[7] and the incorporation of the quarternary C_9 and C_{22} methyl groups on the C ring.[8] However, more than 20 years after the initial discovery went by before Miyashita and co-workers recently reported the first total synthesis of norzoanthamine in 41 steps in an overall yield of 3.5%.[9]

It is not only the unique and challenging structural topology that has made the zoanthamine alkaloids attractive targets for synthetic organic chemists. The zoanthamine alkaloids have also been reported to exhibit a range of interesting biological properties. For example, zoanthamine (**1**) has displayed potent inhibitory activity towards phorbol myristate acetate induced inflammation of the mouse ear,[10] and norzoanthamine (**2**) has been reported to be a potent inhibitor of growth of murine leukemia cells.[11] Also

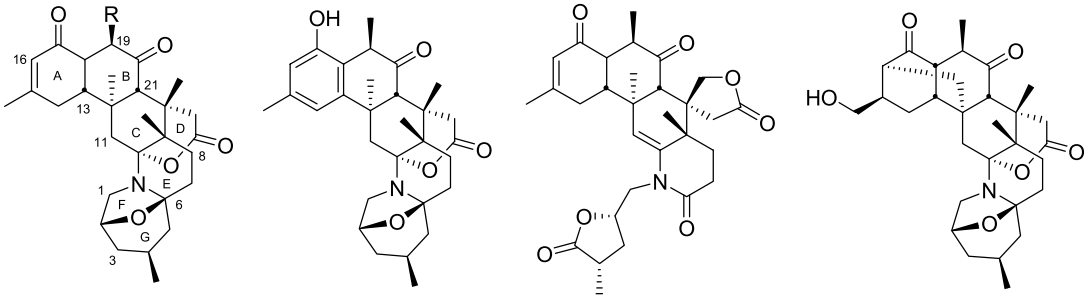

Figure 1. Representative zoanthamine alkaloids.

Keywords: Zoanthamine; Stille coupling.
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osteoporosis may be prevented, as indicated by the observation of significant suppression of bone weight decrease and weakening in ovariectomized mice treated with norzoanthamine (**2**).[12]

Palladium-catalyzed Stille reactions of organic electrophiles with organostannane reagents represent straightforward and highly effective methods for carbon–carbon bond formation in total syntheses of natural products.[13] Despite having lost some of its popularity to environmentally more benign protocols,[14] the Stille reaction remains a significant tool for the assembly of advanced and often highly complex fragments. Bearing in mind the unique functional group tolerance, mild reaction conditions and high reliability of this coupling process, in addition to a variety of well-established routes towards organo-stannanes,[15] we decided to investigate the coupling of two sterically congested major fragments B and C (Fig. 2) via the Stille reaction in a projected total synthesis of the marine alkaloid zoanthamine.[16]

A crucial disconnection in the proposed retrosynthesis of zoanthamine suggests the late-stage multi-condensation reaction of advanced intermediate **6**. Removal of the protecting groups of **6** in acid media is expected to facilitate a one-pot formation of the double hemiaminal core of **1**, as first suggested by Tanner et al. and elegantly demonstrated in model studies by Kobayashi and co-workers.[4a,b,d] Arrival at advanced intermediate **6** requires the proper assembly of fragments A, B and C. The Stille coupling between fragments B (C_6–C_{10}) and C (C_{11}–C_{22}) should set up the olefin geometries of **8** for a subsequent intramolecular Diels–Alder reaction. This is expected to give the desired stereochemistries at both C_{12} and C_{21} of zoanthamine, with the stereochemistry at C_{19} as a key controlling element. In principle, the halide or stannane functionality could be present in either fragments B and C of the Stille coupling. Furthermore, C_{20} of fragment C may be either at the ketone or alcohol stage, anticipating that each oxidation state displays fundamentally different steric and electronic properties, which should greatly affect the coupling process. Finding the optimal combination (stannane/halide, and alcohol/ketone) for the proposed Stille coupling represents the major research objective of the present study. Since stannanes may be easily converted to halides by halodestannylation reactions, it was decided to follow synthetic strategies directed towards each fragment in its stannylated form.

2. Results and discussion

The synthesis of fragment B commenced with a Pd-catalyzed hydrostannation of methyl propiolate (Scheme 1). In their original report,[17] Guibé et al. reported a 100% regioselectivity in favour of the α-stannylated product at rt. However, in our hands 10–15% of other regioisomers were also formed. Only by lowering the temperature to 0 °C, and adding tributyltin hydride dropwise, were we able to exclusively obtain the desired α-regioisomer **10**. Subsequent reduction to the alcohol **11** was conveniently effected with DIBAL-H (complete conversion and excellent purity according to crude NMR), although isolated yields of **11** varied. Initial attempts to isolate the stannylated acrylic aldehyde **12** from the subsequent TPAP-oxidation failed. Flash column chromatography on various commercial sources of silica gel or fluorisil, with miscellaneous eluent systems, including co-elution with triethylamine, was tried, but all combinations resulted in decomposition, and only traces of the pure compound could be isolated. We discovered that demetallated silica gel prepared according to the procedure described in Section 4 was a strict

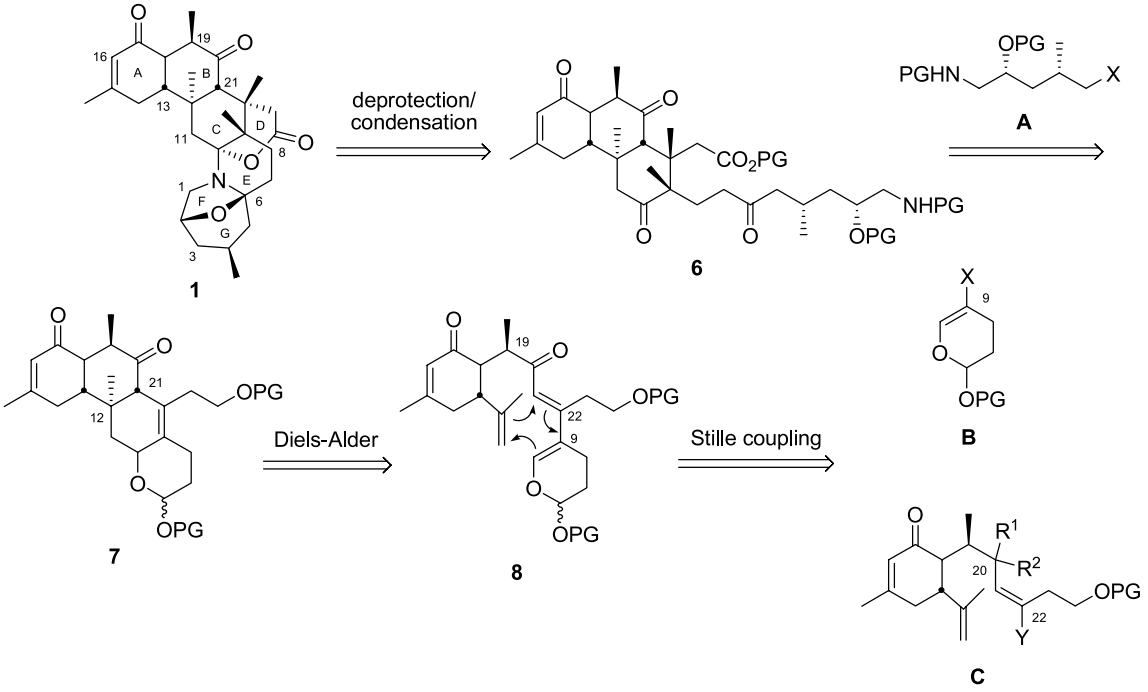
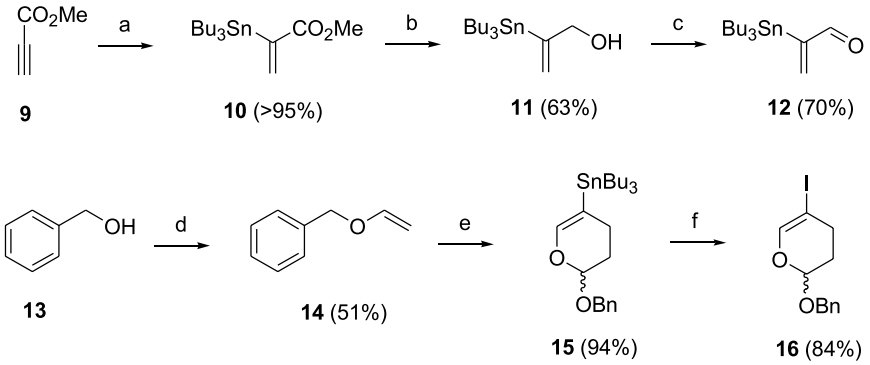


Figure 2. Major fragments A, B and C in the retrosynthesis of zoanthamine: X = halide or metal; Y = SnR_3 or I; PG = protecting group; R^1,R^2 = O, or R^1 = OH, R^2 = H.

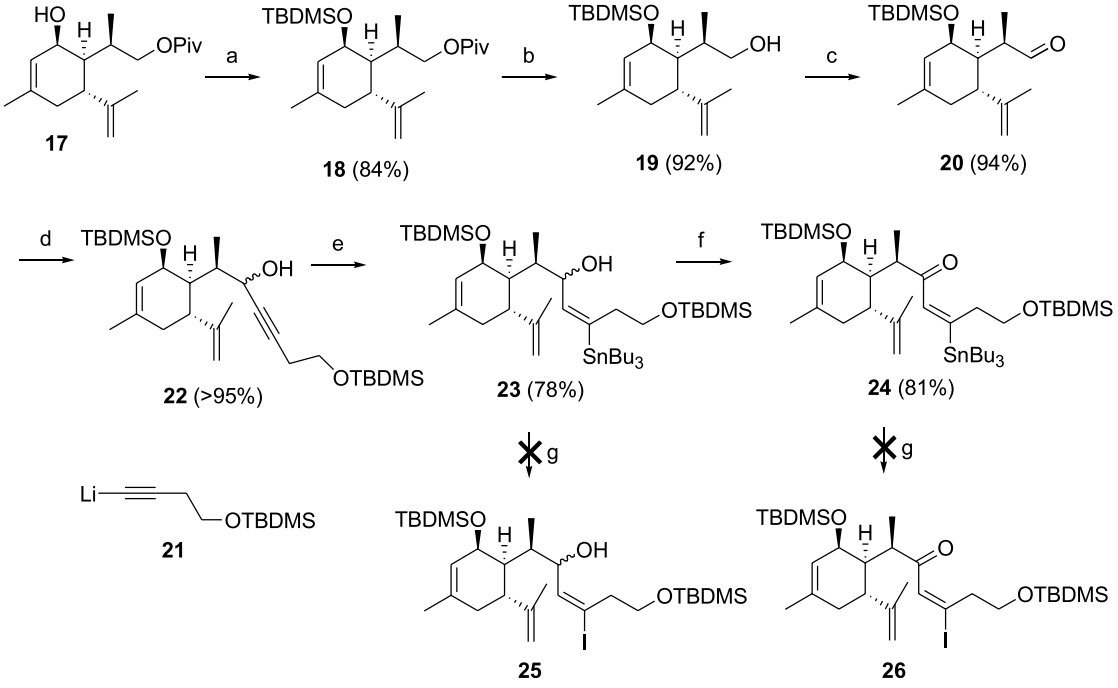


Scheme 1. Synthesis of fragment B (**15**–**16**): (a) Bu_3SnH, $Pd(PPh_3)_2Cl_2$ (0.5 mol%), THF, 0 °C; (b) DIBAL-H, THF, −78 °C; (c) TPAP (7 mol%), NMO, 4 Å MS, CH_2Cl_2, rt; (d) ethylvinyl ether, $Hg(OAc)_2$ (3 mol%); (e) **12**, $Yb(fod)_3$ (17 mol%), 4 Å MS, 30 °C; (f) I_2, CH_2Cl_2, rt, then *n*-$Bu_4NPh_2PO_2$, CH_2Cl_2, rt.

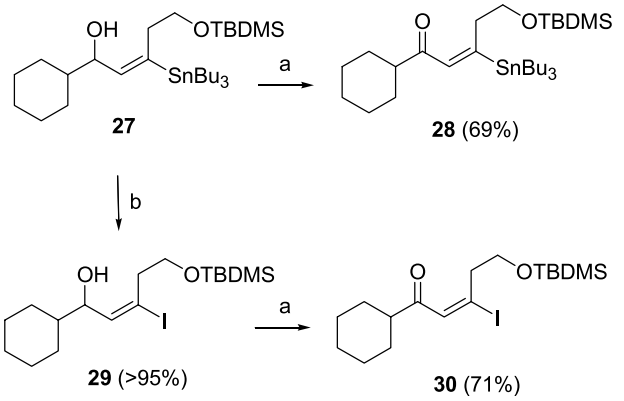
requirement in the purification of **12**. The key step in the synthesis of fragment B was the subsequent lanthanide-catalyzed inverse electron demand Diels–Alder (IEDDA) reaction, where benzyl vinyl ether was found to be an excellent dienophile, providing fragment B as the stable dihydropyrane **15**, in 94% yield with a readily removable protecting group (Bn). The addition of molecular sieves (4 Å) proved to be crucial in obtaining reproducible yields. Benzyl vinyl ether **14** was conveniently prepared by transetherification of ethyl vinyl ether with benzyl alcohol via Hg(II)-catalysis.[18] Iododestannylation with iodine gave the stable halide **16** in excellent yield, even on gram scale. For the purification of **15** and **16**, the use of demetallated silica gel was mandatory, and removal of tributylstannyl iodide by-products from crude **16** could only be accomplished by treatment with tetrabutylammonium diphenylphosphinate (*n*-$Bu_4NPh_2PO_2$) as a tin scavenger.[19]

Fragment C was synthesized from known[16,20b] compound **17**, starting with protection of the secondary hydroxyl group as the TBDMS ether **18**. Removal of the Piv protecting group with DIBAL-H afforded primary alcohol **19**, which was smoothly converted to the corresponding aldehyde **20** with the Dess–Martin periodinane. Addition of lithium acetylide **21** to the aldehyde gave propargylic alcohol **22** as a 2:1 diastereomeric mixture, which was hydrostannated according to the method of Greeves,[20] thus providing fragment C variant **23**. Ketone counterpart **24** could be obtained via the TPAP procedure (Scheme 2).

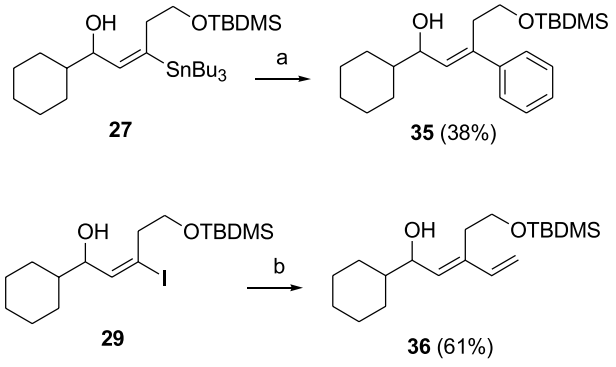
In a model study, alkenyl stannane **27** was easily iododestannylated with iodine to provide alkenyl iodide **29** or TPAP-oxidized to the α,β-unsaturated ketone **28** (Scheme 3). Surprisingly, stannylated derivatives **23** and **24** could not be converted into the corresponding alkenyl iodides **25** and **26**, the major products deriving instead from desilylation of the TBDMS-protected secondary alcohol



Scheme 2. Synthesis of fragment C (**23**–**24**): (a) TBDMSCl, imidazole, DMF; (b) DIBAL-H, CH_2Cl_2, −78 °C; (c) Dess–Martin periodinane, CH_2Cl_2, rt; (d) **21**, THF, −78 °C; (e) Bu_3SnH, $Pd(P(o\text{-}Tol)_3)_2Cl_2$ (1 mol%), THF, rt; (f) TPAP (16 mol%), NMO, 4 Å MS, CH_2Cl_2, rt; (g) I_2, CH_2Cl_2, rt.



Scheme 3. Iododestannylation of fragment C model compounds: (a) TPAP (7 mol%), NMO, 4 Å MS, CH_2Cl_2, rt; (b) I_2, CH_2Cl_2, rt.

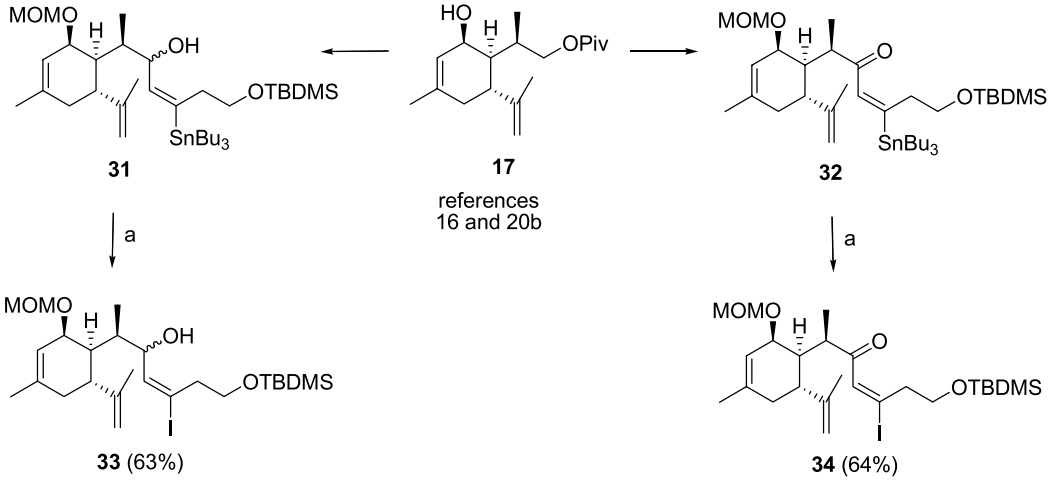


Scheme 5. Stille couplings of fragment C model compounds: (a) Ph-I (**37**), $Pd_2dba_3 \cdot CHCl_3$ (5 mol%), DMF, 65 °C; (b) $CH_2{=}CHSnBu_3$ (1.1 equiv), $Pd_2dba_3 \cdot CHCl_3$, TFP, THF, reflux.

moiety. Lowering the temperature to 0 °C or changing the solvent to THF at −78 °C, did not yield the desired products. This prompted us to prepare the analogous MOM-protected derivatives. Gratifyingly, stannanes **31** and **32** were converted into the desired alkenyl iodides **33** and **34** (Scheme 4),[21], which after rapid purification (filtration through a small plug of demetallated silica gel) were used immediately in the subsequent coupling reaction (Scheme 6).

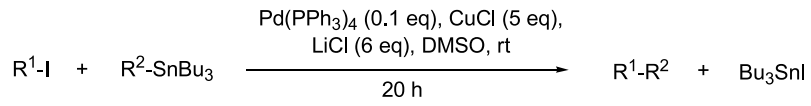
Having successfully prepared iodinated and stannylated fragments B (**15**–**16**) and C (**23**–**24**, **31**–**34**), our attention turned to the Stille coupling between these fragments. Attempts to couple alkenyl iodide **16** with stannanes **27** and **28** (readily available fragment C model compounds) failed completely under a variety of Stille reaction conditions, where many combinations of ligands (e.g., TFP, PPh_3, $AsPh_3$, Cy_2P(2-biphenyl), *t*-Bu_2P(2-biphenyl), P(*o*-Tol)$_3$), solvents (e.g., THF, DMF, NMP, toluene) Pd-sources (e.g., $Pd_2dba_3 \cdot CHCl_3$, $Pd(PPh_3)_4$, $Pd(CH_3CN)_2Cl_2$, Pd(P(*o*-Tol)$_3$)$_2$$Cl_2$), Cu-additives (e.g., CuI, CuBr, CuTC),[22] and reaction temperatures (e.g., rt, reflux) were tried. In parallel experiments, the coupling of iodobenzene with stannane **27** was examined. Curiously, this coupling proceeded under ligandless conditions (Scheme 5), albeit in low yield. Stannane **15** could not be coupled with alkenyl iodides **29** and **30** under traditional Stille reaction conditions. This problem appeared to be associated with sterical hindrance, as indicated by the smooth reaction of vinyltributylstannane with **29** (Scheme 5).

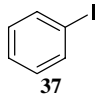
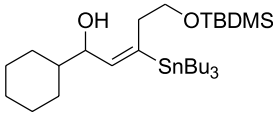
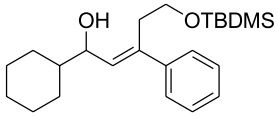
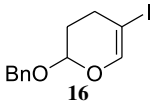
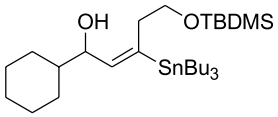
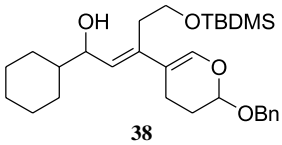
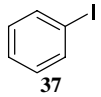
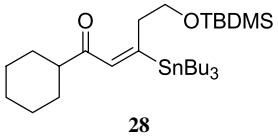
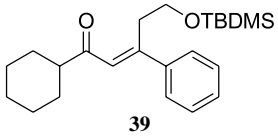
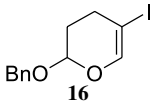
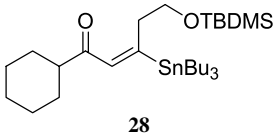
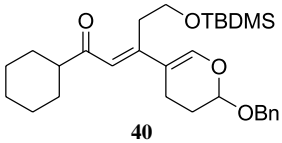
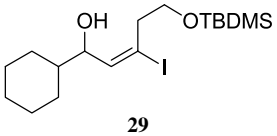
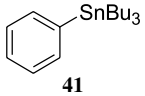
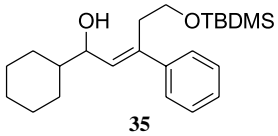
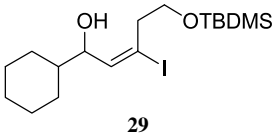
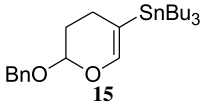
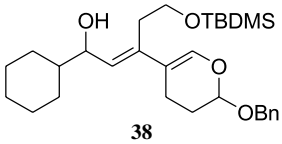
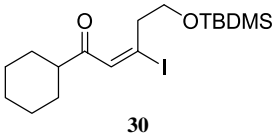
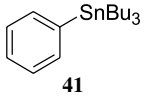
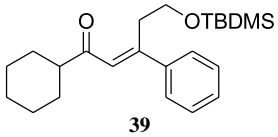
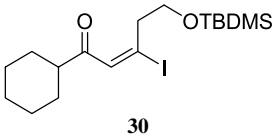
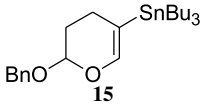
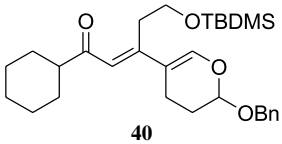
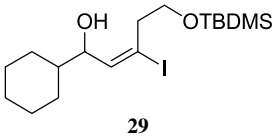
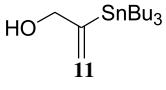
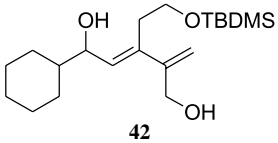
It was then decided to investigate a recent protocol reported by Corey for Stille couplings of sterically congested stannanes (Table 1).[23] It was found that use of an excess of both CuCl and LiCl under rigorously dry and anaerobic conditions in DMSO using $Pd(PPh_3)_4$ as the catalyst, was far superior to other Cu-mediated Stille couplings available. Pleasingly, a range of fragment B and C model compounds could be joined via this procedure (Table 1). Stannane **15** (fragment B) could be joined with both alkenyl iodides **29** and **30** in high yields (Table 1, entries 6 and 8), whereas the coupling of alkenyl iodide **16** (fragment B) with stannanes **27** and **28** remained problematic (Table 1, entries 2 and 4). Stille coupling product **38** could be oxidized to the unsaturated ketone **40** (yield: 67%, via the TPAP/NMO-procedure similar to that of Schemes 2–4). Further scope of the reaction conditions was demonstrated by the coupling of tributylstannyl benzene with alkenyl iodides **29** and **30**, providing the coupling products **35**



Scheme 4. Synthesis of iodinated and stannylated fragment C (**31**–**34**): (a) TPAP (7 mol%), NMO, 4 Å MS, CH_2Cl_2, rt; (b) I_2, CH_2Cl_2, 0 °C, then *n*-$Bu_4NPh_2PO_2$, CH_2Cl_2, rt.

Table 1. Stille couplings between fragment B and C model compounds via the Corey-modified Stille reaction

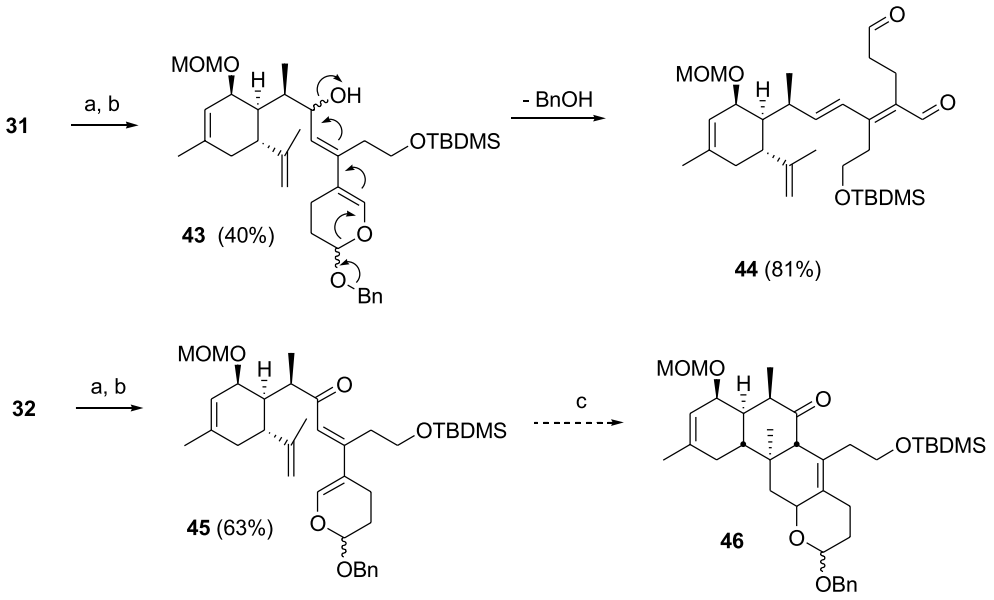


Entry	R^1-I	R^2-$SnBu_3$	Coupling product (R^1–R^2)	Yield (%)[a]
1	 **37**	 **27**	 **35**	51
2	 **16**	 **27**	 **38**	0
3	 **37**	 **28**	 **39**	37
4	 **16**	 **28**	 **40**	0
5	 **29**	 **41**	 **35**	82
6	 **29**	 **15**	 **38**	81
7	 **30**	 **41**	 **39**	41
8	 **30**	 **15**	 **40**	74
9	 **29**	 **11**	 **42**	84

[a] Isolated yield after flash column chromatography.

(yield: 82%) and **39** (yield: 41%), respectively, (Table 1, entries 5 and 7). Interestingly, stannane **11** was efficiently coupled to alkenyl iodide **29** to provide the primary alcohol **42** in excellent yield (84%, Table 1, entry 8).

The results of Table 1 pointed to the use of fragment B as the stannane (**15**) and fragment C as the alkenyl iodide (freshly prepared as either **33** from **31**, or **34** from **32**), for the Stille reaction towards a Diels–Alder precursor of type **8**. The strategy turned out most efficient for the synthesis of the Diels–Alder substrate **45**. Unfortunately, the coupling product **43** was rather unstable due to ring-opening of the dihydropyrane moiety, thus affording the dialdehyde **44**. However, **45** was formed as a stable coupling product in 63% yield (Scheme 6). So far we have not been able to promote the desired Diels–Alder reaction with **45** (e.g., no reaction in refluxing toluene, decomposition in refluxing xylene), but we are continuing our efforts and results will be reported in due course.



Scheme 6. Coupling of fragments B and C: (a) I_2, CH_2Cl_2, 0 °C, then *n*-$Bu_4NPh_2PO_2$, CH_2Cl_2, rt; (b) **15**, $Pd(PPh_3)_4$ (10 mol%), LiCl (6 equiv), CuCl (5 equiv), DMSO, rt, 3 h; (c) heat.

3. Conclusion

In summary, efficient synthetic routes towards two major fragments B and C in a projected total synthesis of the marine alkaloid zoanthamine have been established. The methodology allows the preparation of each fragment in its stannylated and iodinated forms. The relative positions of the stannyl and iodo moieties were found to be crucial for the Stille coupling between these fragments. Whereas fragment B in its iodinated form was completely unreactive under a range of Stille reaction conditions, the corresponding stannylated form reacted readily with alkenyl iodides using reaction conditions developed by Corey, utilizing excess of CuCl and LiCl in DMSO. Fragment B in its stannylated form reacted smoothly with two iodinated versions of fragment C (with C_{20} at either the ketone or alcohol stage). Although, the resulting allylic alcohol coupling product **43** was too unstable for further synthetic manipulations, the α,β-unsaturated coupling product **45** was found to be stable and constitutes therefore a promising intermediate for the projected total synthesis of zoanthamine.

4. Experimental

4.1. General

^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded using $CDCl_3$ as the solvent, and signal positions (δ-values) were measured relative to the signals for $CHCl_3$ (7.27) and $CDCl_3$ (77.0), respectively. Tin–hydrogen coupling constants, *J*(SnH), are given as the ^{117}Sn and ^{119}Sn values, or average values. IR spectra were obtained for thin films on AgCl plates, and only the strongest/structurally most important peaks (ν_{max}/cm^{-1}) are listed. Microanalyses were performed by the Microanalysis Laboratory, Department of Physical Chemistry, University of Vienna, Austria, and at the Department of Chemistry, University of Bath, England. HRMS was performed at the Department of Chemistry, University of Copenhagen, Denmark, and the Department of Chemistry, University of Bath, England. Molecular mass determinations (high-resolution mass spectrometry) for substances containing Bu_3Sn are based on ^{120}Sn and typically made on the $[M-Bu]^+$, unless otherwise stated. All compounds, on which HRMS were performed exhibited clean ^1H NMR spectra and showed one spot on TLC analysis, using UV light, and a solution of 5–10% phosphomolybdic acid in ethanol for visualization.

Column chromatography was performed using Amicron Matrex silica gel (35–70 μm). Demetallated silica gel was prepared by a modification of the procedure of Harris:[24] 1 kg of silica gel was mechanically stirred with 2 L of 10% hydrochloric acid until complete wetting was assured. Upon this treatment, the aqueous phase turned yellow, presumably due to the formation of chloro-complexes. The acidic aqueous phase was decanted off, and the silica subsequently washed 15–20 times with distilled water by decantation until pH≈4. Subsequently, 1 L of distilled water was added along with 1 mL of aqueous ammonia (25%). The aqueous phase was decanted and the silica oven-dried at 150 °C for a minimum of 24 h prior to use for chromatography. All solvents were distilled prior to use. THF was distilled under nitrogen from Na–benzophenone. CH_2Cl_2 and DMF were dried over calcium hydride and distilled under nitrogen. DMSO was dried over 4 Å MS, and degassed (4×) by the freeze-pump-thaw process (−78 °C-rt, argon). Copper(I) chloride was prepared according to Österlöf.[25] Copper(I) cyanide and lithium chloride were ovendried prior to use and used without further purification. Commercially available compounds were used as received unless otherwise indicated. All reactions were carried out under an atmosphere of dry argon using carefully flame-dried glassware. Argon gas was dried by passage through phosphorous pentoxide and silica gel.

4.1.1. 2-Tributylstannylacrylic acid methyl ester (10).[17] A 2 L three-necked roundbottomed flask equipped with a magnetic stirring bar, thermometer, and a septum was charged with freshly prepared bis(triphenylphosphine)-palladium(II)chloride (716 mg, 1.3 mmol) and methyl propiolate (17.18 g, 205 mmol). Degassed THF (820 mL) was added, and the solution was cooled to 0 °C. During 35 min tributyltin hydride (65.4 g, 225 mmol) was added dropwise, and the solution subsequently stirred at rt for 10 min during, which time the originally light-yellow solution turned orange-brown. The solvent was quickly removed by rotary evaporation. The obtained residue was dissolved in pentane (1.7 L), and washed with CH_3CN (2× 100 mL), water (2×200 mL) and brine (200 mL). The organic phase was isolated, dried over magnesium sulfate, filtered and rotary evaporated to afford the title compound **10** (83.2 g, quant.) as a red-brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (1H, d, J=3 Hz, $J(^{117}SnH)$=106 Hz, $J(^{119}SnH)$=112 Hz), 5.85 (1H, d, J=3 Hz, $J(^{117}SnH)$=51 Hz, $J(^{119}SnH)$=54 Hz), 3.66 (3H, s), 1.56–1.33 (6H, m), 1.33–1.12 (6H, m), 0.94–0.87 (6H, m), 0.86–0.77 (9H, t, J=7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 146.1, 139.8, 51.9, 29.0, 27.4, 13.8, 10.2.

4.1.2. 2-Tributylstannylprop-2-en-1-ol (11).[17] A three-necked roundbottomed flask equipped with a magnetic stirring bar and septa was charged with ester **10** (1.13 g, 3.0 mmol) and THF (18 mL). The solution was cooled to −78 °C, and dropwise added diisobutylaluminium hydride (1.0 M in hexanes, 6.2 mL, 6.2 mmol) over 2 h using a syringe pump, and stirred overnight at rt. Methanol (6 mL) was added, followed by benzene (15 mL), and water (6 mL). The mixture was immediately filtered, and the precipitated aluminum salts were thoroughly washed with methanol. The filtrate and washings were combined, and the volatiles removed by rotary evaporation. The residue was dissolved in pentane (100 mL) and washed with water (10 mL) and brine (10 mL). The organic phase was dried over magnesium sulfate, filtered and rotary evaporated to give a thick, yellow oil, which was subjected to flash column chromatography on demetallated silica gel (hexane/ethyl acetate/triethylamine, 100:10:1) to afford the title compound **11** (0.66 g, 63%) as a colourless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.88 (1H, m, J=4, 2 Hz, J(SnH)=129 Hz), 5.25 (1H, m, J=4, 2 Hz, J(SnH)=61 Hz), 4.27 (2H, m, J(SnH)=29 Hz), 1.61–1.43 (6H, m), 1.41–1.24 (6H, m), 0.97–0.85 (15H, m, J=7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.0, 123.1, 69.8, 29.4, 27.7, 13.9, 9.8.

4.1.3. 2-Tributylstannanylpropenal (12).[16] A 500 mL roundbottomed flask equipped with a magnetic stirring bar and a septum was charged with 60 g of freshly activated (flame dried under high vacuum) finely pulverized 4 Å molecular sieves. Dichloromethane (100 mL) was added, followed by *N*-methyl morpholine *N*-oxide (NMO, 1.50 g, 11.5 mmol), and alcohol **11** (2.50 g, 7.0 mmol). The solution was stirred for 10 min, then tetra-*n*-propylammonium perruthenate (TPAP, 115 mg, 0.5 mmol) was added in one portion, followed by the appearance of a green-brown colouration. The reaction mixture was stirred overnight, by which time the solution had become brown, and TLC showed a complete conversion of the alcohol into one product. The reaction mixture was applied directly to a column packed with demetallated silica gel, and the product was rapidly eluted (hexane/triethylamine, 100:1) affording the aldehyde **12** (1.7 g, 70%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.67 (1H, s, $J(^{117}SnH)$=52 Hz, $J(^{119}SnH)$=55 Hz), 6.85 (1H, d, J=2 Hz, $J(^{117}SnH)$=101 Hz, $J(^{119}SnH)$=106 Hz), 6.69 (1H, d, J=2 Hz, J(SnH)=48 Hz), 1.63–1.37 (6H, m), 1.37–1.20 (6H, m), 1.03–0.97 (6H, m), 0.91–0.84 (9H, m, J=7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.4, 156.7, 148.2, 29.2, 27.4, 13, 9.9.

4.1.4. Benzyl vinyl ether (14).[26] A 1000 mL flask equipped with a Claisen-adapter, thermometer and reflux condenser fitted with a calcium chloride-tube was charged with freshly distilled ethyl vinyl ether (400 mL, 301.2 g, 4.2 mol), benzylalcohol (38.2 mL, 40.0 g, 0.37 mol) and mercuric acetate (4.0 g, 12.6 mmol). The resulting solution was refluxed for 14 h, then treated with additional mercuric acetate (2.0 g, 6.3 mmol) and refluxed for another 4 h. It was then extracted twice with cold 10% potassium carbonate solution, dried with potassium carbonate and concentrated to a light oil. Efficient distillation was achieved using a 10 cm Vigreux column on top of a 10 cm column packed with small glass rings, to afford benzyl vinyl ether (25.5 g, 51%) as a colourless liquid: bp 128–132 °C/60 Torr (bp 88–90 °C /25 Torr);[27] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.27 (5H, m), δ 6.57 (1H, dd, J=7, 14 Hz), δ 4.77 (2H, s), δ 4.31 (1H, dd, J=2, 14 Hz), δ 4.08 (1H, dd, J=2, 7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.9, 137.2, 128.8, 128.2, 128.0, 87.7, 70.4.

4.1.5. 2-Benzyloxy-5-tributylstannyl-3,4-dihydro-2*H*-pyran (15). A Schlenk tube was charged with *tris*(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium ($Yb(fod)_3$, 210 mg, 0.198 mmol) under argon, and the flask was heated to 60 °C with stirring for 4 days under high vacuum. The aldehyde **12** (417 mg, 1.2 mmol) dissolved in freshly distilled benzyl vinyl ether **14** was subsequently added together with pulverized MS 4 Å (3 g). The solution was stirred overnight at 30 °C, followed by rapid filtration through a plug of demetallated silica gel (hexane/ethyl actetate/triethylamine, 40:1:1). The filtrate and washings were concentrated in vacuo. Benzylvinylether was removed under high vacuum (<0.5 mmHg) and collected using a freeze trap. The residue was subjected to flash column chromatography on demetallated silica gel (hexane/ethyl acetate/triethylamine, 40:1:1), affording dihydropyrane **15** (543 mg, 94%) as a colourless oil: IR ($CDCl_3$): 2925, 1605, 1455, 1040; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.23 (5H, m), 5.99 (1H, dd (app. t), J=2 Hz, J(SnH)=34 Hz), 5.07 (1H, dd (app. t), J=3 Hz), 4.86 (1H, d, J=12 Hz), 4.61 (1H, d, J=12 Hz), 2.37–2.22 (1H, m), 2.06–1.94 (1H, m), 1.93–1.84 (2H, m), 1.62–1.41 (6H, m), 1.38–1.25 (6H, m), 0.93–0.84 (15H, m, J=7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.3, 138.6, 128.5, 127.9, 127.7, 109.0, 96.1, 69.5, 29.4, 28.2, 27.6, 21.9, 14.0, 9.3. Anal. Calcd for $C_{24}H_{40}O_2Sn$: C, 60.13; H, 8.43. Found: C, 60.37; H, 8.32.

4.1.6. 2-Benzyloxy-5-iodo-3,4-dihydro-2*H*-pyran (16). A solution of the stannane **15** (114 mg, 0.24 mmol) in CH_2Cl_2 (16 mL) was cooled to 0 °C and slowly added a 0.04 M solution of iodine (60 mg, 0.24 mmol) in CH_2Cl_2 (7.5 mL). The addition of iodine was stopped when a permanent red

colouration of the reaction mixture appeared (this was taken as an indication of complete conversion of the stannane), and the reaction was stirred for further 30 min. The reaction mixture was diluted with ether (100 mL) and washed with satd sodium thiosulfate (2×10 mL), then dried over magnesium sulfate, filtered and rotary evaporated. The residue was immediately subjected to flash column chromatography on demetallated silica (hexane/ethyl acetate, 40:1) to give a colourless oil. Traces of tributylstannyl iodide were removed by dissolving the oil in ether (50 mL), followed by addition of tetra-*n*-butylammonium diphenylphosphinate. The precipitated salts were removed by filtration. Subsequent rotary evaporation of the volatiles gave the title compound **16** (61 mg, 84%) as a colourless oil: IR ($CDCl_3$): 2937, 1634, 1134, 1094, 1017; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.25 (5H, m), 6.48 (1H, dd, J=1, 2 Hz), 5.10 (1H, dd (app. t), J=3 Hz), 4.75 (1H, d, J=12 Hz), 4.52 (1H, d, J=12 Hz), 2.62–2.48 (1H, dddd, J=2, 8, 11, 17 Hz), 2.29–2.18 (1H, dddd, J=1, 5, 6, 17 Hz), 1.95–1.80 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.8, 137.8, 128.6, 128.0 (two signals), 94.7, 69.9, 69.5, 29.0, 28.9. Anal. Calcd for $C_{12}H_{13}O_2I$: C, 45.59; H, 4.14. Found: C 45.85; H 4.01; HRMS (FAB) calcd for $C_{12}H_{13}O_2I$ [M^+] 315.9960, found 315.9961.

4.1.7. (*R*)-2-[(1*R*,2*S*,6*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-methyl-6-(prop-1-en-2-yl)cyclohex-3-enyl]propyl pivalate (18). *tert*-Butyldimethylsilyl chloride (387 mg, 2.57 mmol) and imidazole (583 mg, 8.56 mmol) were dissolved in DMF (15 mL). Compound **17** (630 mg, 2.14 mmol) was dropwise added and the resultant solution stirred overnight. Hexane (60 mL) and a mixture of water/DMF (10:1, 55 mL) was added, and the mixture was transferred to a separation funnel. The organic phase was separated, washed with water (4×20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and evaporated to give a residue that was further purified by flash column chromatography (hexane/ethyl acetate, 20:1) to give the title compound **18** (734 mg, 84%) as a colourless oil: $[\alpha]_D^{25}$ −130.8 (*c* 4.2, CH_2Cl_2); IR ($CDCl_3$): 2958, 2931, 2858, 1728, 1463, 1284, 1254, 1165, 1035, 835, 774; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.49 (1H, m), 4.82 (2H, m), 4.33 (1H, m), 4.19–4.14 (1H, dd, J=4, 11 Hz), 4.11–4.05 (1H, dd, J=9, 11 Hz), 2.77–2.68 (2H, ddd, J=7, 11, 16 Hz), 2.05–1.92 (3H, m), 1.71 (3H, s), 1.67 (3H, s), 1.61–1.55 (1H, m), 1.19 (9H, s), 1.05 (3H, d, J=7 Hz), 0.91 (9H, s), 0.10 (3H, s), 0.09 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 146.7, 136.5, 124.1, 112.9, 68.3, 66.2, 44.6, 40.1, 38.7, 36.3, 33.3, 27.3, 26.1, 23.0, 18.9, 18.3, −3.6 (two signals); HRMS (EI) calcd for $C_{24}H_{44}O_3Si$ [M]$^+$408.3060, found 408.3076.

4.1.8. (*R*)-2-[(1*R*,2*S*,6*R*)-2-(*tert*-Butyldimethylsilyloxy)-6-isopropenyl-4-methylcyclohex-3-enyl]propan-1-ol (19). A solution of compound **18** (515 mg, 1.26 mmol) in CH_2Cl_2 (50 mL) was prepared and cooled to −78 °C. The solution was then dropwise added diisobutylaluminum hydride (1 M in hexanes, 3.15 mL, 3.15 mmol). After 45 min, TLC indicated complete reaction, and an aqueous solution of Rochelle salt (50 mL) was added. The resultant mixture was stirred at rt for 1 h. The separated aqueous phase was extracted with CH_2Cl_2 (3×100 mL) and the combined organic phases were washed with brine (100 mL), dried over magnesium sulfate and rotary evaporated to a residue that was purified by flash column chromatography (hexane/ethyl acetate, 5:1) to give the title compound **19** (376 mg, 92%) as a colourless oil: $[\alpha]_D^{25}$ −100.5 (*c* 2.0, CH_2Cl_2); IR ($CDCl_3$): 3420, 2956, 2857, 1644, 1472, 1378, 1252, 1031, 935, 890, 835, 805, 774; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.45 (1H, m), 4.87 (1H, s), 4.77 (1H, s), 4.40 (1H, br s), 3.60 (2H, m), 3.09 (1H, m, −OH), 2.71 (1H, ddd, J=7, 9, 16 Hz), 2.11–1.88 (2H, m), 1.81 (1H, m), 1.73 (3H, s), 1.68 (3H, s), 1.61–1.54 (1H, m), 1.05 (3H, d, J=7 Hz), 0.92 (9H, s), 0.11 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.3, 136.1, 124.2, 112.2, 67.3, 65.9, 45.5, 40.9, 36.0, 34.8, 33.4, 26.1, 34.8, 26.1, 22.9, 19.7, 18.4, 16.7, −3.9; HRMS (EI) calcd for $C_{19}H_{36}O_2Si$ [M]$^+$324.2485, found 324.2485.

4.1.9. (*R*)-2-[(1*R*,2*S*,6*R*)-2-(*tert*-Butyldimethylsilyloxy)-6-isopropenyl-4-methylcyclohex-3-enyl]propionaldehyde (20). A suspension of Dess–Martin periodinane (421 mg, 0.99 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C and stirred for 5 min. A solution of alcohol **19** (258 mg, 0.79 mmol) in CH_2Cl_2 (8 mL) was then dropwise added. After 30 min TLC indicated complete conversion of the alcohol. The reaction was quenched with a mixture of 10% sodium thiosulfate (aq) and sat. sodium bicarbonate (1:1, 10 mL) and stirred until two homogeneous layers were observed. The resultant mixture was extracted with CH_2Cl_2 (3×50 mL) and the combined organic phases were washed with water (50 mL), dried over magnesium sulfate and rotary evaporated to a residue that was further purified by flash column chromatography (hexane/ethyl acetate, 20:1) to give the title compound **20** (240 mg, 94%) as a colourless oil: $[\alpha]_D^{25}$ −168.9 (*c* 3.5, CH_2Cl_2); IR ($CDCl_3$): 2931, 2858, 1719, 1459, 1438, 1379, 1257, 1079, 1032, 935, 880, 837, 805, 778; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (1H, s), 5.48 (1H, m), 4.90 (1H, s), 4.87 (1H, s), 3.01 (1H, m), 2.41 (1H, m), 2.14–1.92 (2H, m), 1.85 (1H, m), 1.73 (3H, s), 1.71 (3H, s), 1.11 (3H, d, J=7 Hz), 0.86 (9H, s), 0.07 (3H, s), 0.02 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.6, 146.0, 136.0, 123.4, 113.9, 65.3, 45.7, 44.1, 40.4, 36.0, 26.0, 23.1, 18.8, 18.3, 12.4, −3.9 (two signals); HRMS (EI) calcd for $C_{19}H_{34}O_2Si$ [M]$^+$322.2328, found 322.2325.

4.1.10. (*R*)-7-(*tert*-Butyldimethylsilyloxy)-2-[(1*R*,2*S*,6*R*)-2-(*tert*-butyldimethylsilyloxy)-6-isopropenyl-4-methylcyclohex-3-enyl]hept-4-yn-3-ol (22). To a solution of freshly distilled but-3-yn-1-ol (92 mg, 0.5 mmol) in THF (3 mL) was added dropwise *n*-butyllithium (1.6 M in hexanes, 0.31 mL, 0.5 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 30 min, and then at −30 °C for another 30 min, before recooling to −78 °C and dropwise addition of aldehyde **20** (161 mg, 0.5 mmol). After stirring for 1 h at −78 °C, the reaction mixture was allowed to reach rt. A mixture of ether (10 mL) and sat. sodium bicarbonate (10 mL) was added and the organic layer separated and washed with brine (2×5 mL), then dried over magnesium sulfate, filtered and rotary evaporated. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 20:1) to give the title compound **22** (241 mg, quant.) as a colourless oil (2:1 diastereomeric mixture): $[\alpha]_D^{25}$ −46 (*c* 1.0, CH_2Cl_2); IR ($CDCl_3$): 3336, 2928, 2857, 1742, 1379, 1256, 1106, 1006, 890, 838, 778, 733, 668; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.39 (1H, br s), 4.87 (1H, m), 4.77–4.69 (1H, m), 4.68–4.54 (1H,

m), 4.48–4.31 (1H, m), 3.72 (2H, t, $J=7$ Hz), 2.94 (1H, m), 2.71–2.62 (1H, m), 2.47–2.40 (2H, m), 2.13–1.81 (4H, m), 1.76 (3H, s), 1.67 (3H, s), 1.14–1.06 (3H, m), 0.93–0.89 (18H, app. s), 0.14–0.06 (12H, app. s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.8, 147.1, 135.9, 135.6, 124.4, 124.0, 111.8, 82.1, 82.0, 81.1, 68.6, 68.3, 66.1, 64.3, 62.1, 43.6, 41.2, 41.0, 39.6, 39.1, 34.6, 33.6, 26.0, 25.9, 23.2, 22.7, 20.7, 20.5, 18.4, 18.3, 14.9, 14.4, −4.0, −4.2, −4.4; HRMS (EI) calcd for $C_{29}H_{54}O_3Si_2$ $[M]^+$506.3611, found 506.3608.

4.1.11. (*R*)-7-(*tert*-Butyldimethylsilyloxy)-2-[(1*R*,2*S*,6*R*)-2-(*tert*-butyldimethylsilyloxy)-6-isopropenyl-4-methyl-cyclohex-3-enyl]-5-tributylstannylhept-4-en-3-ol (23). To a solution of dichlorobis(tri-*o*-tolylphosphine)-palladium(II) (5 mg, 0.025 mmol) and propargylic alcohol **22** (253 mg, 0.5 mmol) in THF (6 mL) was added dropwise tributyltin hydride (218 mg, 0.75 mmol) during 0.5 h. The reaction mixture was stirred at rt for 1 h, and another amount of tributyltin hydride (218 mg, 0.75 mmol) was dropwise added during 0.5 h, followed by stirring overnight. The reaction mixture was added ether (15 mL), and filtered through a pad of Celite. The volatiles were removed in vacuo, and the residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 20:1) giving the title compound **23** (311 mg, 78%) as a colourless oil (2:1 diastereomeric mixture): $[\alpha]_D^{25}$ −25.9 (*c* 1.2, CH_2Cl_2); IR ($CDCl_3$): 2956, 2927, 2856, 1644, 1377, 1254, 1076, 939, 890, 837; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.74–5.59 (1H, m), 5.54–5.45 (1H, m), 4.92–4.74 (2H, m), 4.44–4.27 (2H, m), 4.13+3.14 (1H, m, −OH), 3.64–3.49 (2H, m), 2.79–2.62 (2H, m), 2.43–2.36 (1H, m), 2.19–1.71 (4H, m), 1.77 (3H, 2×s), 1.68 (3H, 2×s), 1.54–1.41 (6H, m), 1.40–1.25 (6H, m), 1.04 (3H, d, $J=7$ Hz), 0.94–0.85 (15H, m), 0.91 (18H, s), 0.122 (6H, app. s), 0.07 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.7, 146.6, 142.1, 139.9, 136.3, 136.1, 124.5, 124.4, 112.3, 70.0, 69.2, 68.3, 67.4, 63.2, 62.9, 45.4, 42.1, 40.9, 40.6, 40.4, 40.0, 36.8, 35.4, 29.2, 27.4, 26.2, 26.1, 23.0, 22.8, 20.5, 20.0, 18.5, 18.4, 14.8, 13.7, 12.6, 9.8, −3.8, −3.9, −4.1, −5.2; HRMS (FAB) calcd for $C_{37}H_{73}O_3Si_2Sn$ $[M-C_4H_9]^+$ 741, 4120, found 740.4121.

4.1.12. (*R*)-7-(*tert*-Butyl-dimethyl-silyloxy)-2-[(1*R*,2*S*,6*R*)-2-(*tert*-butyl-dimethyl-silanyloxy)-6-isopropenyl-4-methyl-cyclohex-3-enyl]-5-tributylstannyl-hept-4-en-3-one (24). To a slurry of freshly activated and finely pulverized 4 Å molecular sieves (2 g) in CH_2Cl_2 (10 mL) was added NMO (115 mg, 0.85 mmol) and a solution of the β-stannylated allylic alcohol **23** (399 mg, 0.5 mmol) in dichloromethane (2 mL). The reaction mixture was stirred for 10 min, before the addition of tetra-*n*-propylammonium perruthenate (26 mg, 0.08 mmol). The reaction mixture was stirred overnight and diluted with CH_2Cl_2 (20 mL) before filtering off the molecular sieves, which was washed with further amounts of CH_2Cl_2 (3×10 mL). The combined organic phases were evaporated, and the residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate; 20:1) to give the title compound **24** (322 mg, 81%) as a colourless oil: $[\alpha]_D^{25}$ −21.5 (*c* 1.3, CH_2Cl_2); IR ($CDCl_3$): 2956, 2928, 2856, 2362, 1686, 1464, 1377, 1253, 1091, 1006, 836; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.38 (1H, s, J(SnH)=68 Hz), 5.21 (1H, br s), 4.81 (1H, br s), 4.59 (1H, br s), 4.23 (1H, br s), 3.78–3.56 (2H, m), 3.08–2.98 (1H, m), 2.87–2.78 (1H, m), 2.72–2.61 (1H, m), 2.51–2.42 (2H, m), 2.14–1.85 (2H, m), 1.81 (3H, s), 1.71 (3H, s), 1.55–1.44 (6H, m), 1.40–1.27 (6H, m), 1.05–0.84 (36H, m), 0.07 (6H, s), 0.01 (3H, s), −0.05 (3H, s); ^{13}C NMR (75 MHz, C_6D_6) δ 201.6, 161.5, 147.8, 141.4, 134.4, 125.4, 110.4, 69.2, 63.6, 43.9, 42.7, 40.8, 40.1, 31.4, 29.6, 27.8, 26.6, 26.3, 22.9, 22.1, 19.0, 18.7, 15.4, 14.0, 10.4, −4.4, −4.7, −4.9; HRMS (FAB) calcd for $C_{37}H_{71}O_3Si_2Sn$ $[M-C_4H_9]^+$739.3964, found 739.3969.

4.1.13. 5-(*tert*-Butyldimethylsilyloxy)-1-cyclohexyl-3-iodopent-2-en-1-ol (28). A solution of the known β-stannylated allylic alcohol **27** (176 mg, 0.3 mmol) in CH_2Cl_2 (30 mL) was cooled to 0 °C, and a solution of iodine (76 mg, 0.3 mmol) in CH_2Cl_2 (15 mL) was added dropwise until a permanent red colouration was observed. The reaction mixture was stirred for further 30 min, before pouring the reaction mixture into sat. sodium thiosulfate (15 mL). The red-pink organic phase became immediately colourless when washed with sat. sodium thiosulfate (2× 10 mL) and brine (10 mL). The organic phase was dried over magnesium sulfate, and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 20:1) to give the title compound **28** (124 mg, 98%) as a slightly pink oil (the pink colouration disappeared when the compound was maintained under a slight vacuum): IR ($CDCl_3$): 3448, 2928, 1628, 1471, 1450, 1256, 1102, 1009, 927, 834, 777; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.43 (1H, d, $J=8$ Hz), 4.04–3.95 (1H, m, $J=8$ Hz), 3.83–3.67 (2H, m), 2.94 (1H, ddd, $J=4$, 5, 14 Hz), 2.74 (1H, d, $J=1$ Hz), 2.57 (1H, ddd, $J=4$, 5, 14 Hz), 1.97–1.86 (1H, m), 1.80–1.62 (4H, m), 1.50–0.80 (6H, m), 0.92 (9H, s), 0.12 (6H, 2×s); ^{13}C NMR (75 MHz, C_6D_6) δ 147.0, 103.8, 73.4, 61.3, 43.5, 42.9, 29.1, 26.5, 26.4, 26.2, 18.8,, −5.2 (two signals). Anal. Calcd for $C_{17}H_{33}IO_2Si$: C, 48.11; H, 7.84. Found: C, 48.37; H, 7.80.

4.1.14. 5-(*tert*-Butyldimethylsilyloxy)-1-cyclohexyl-3-iodopent-2-en-1-one (30). To a slurry of freshly activated and finely pulverized 4 Å molecular sieves (1.5 g) in CH_2Cl_2 (3 mL) was added NMO (41 mg, 0.3 mmol) and a solution of the β-iodinated allylic alcohol **28** (64 mg, 0.15 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred for 10 min, before the addition of TPAP (2×2.5 mg, 0.014 mmol). The reaction mixture was stirred overnight, and diluted with CH_2Cl_2 (50 mL) before filtering off the molecular sieves, which was washed with further amounts of CH_2Cl_2 (3×25 mL). The combined organic phases were evaporated, then added the smallest possible amount of CH_2Cl_2 to redissolve the thick, dark oil, which was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 25:1) to give the title compound **30** (49 mg, 77%) as a colourless oil: IR ($CDCl_3$): 2929, 1690, 1583, 1256, 1105, 836, 776; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (1H, s), 3.79 (2H, t, $J=6$ Hz), 3.27 (2H, dt, $J=1$, 6 Hz), 2.35–2.21 (1H, m), 1.91–1.61 (4H, m), 1.40–0.81 (6H, m), 0.89 (9H, s), 0.07 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.6, 139.5, 124.1, 62.5, 51.2, 44.4, 28.2, 25.9, 25.8, 25.6, 18.3, −5.3. Anal. Calcd for $C_{17}H_{31}IO_2Si$: C, 48.34; H, 7.40. Found: C, 48.35; H, 7.11.

4.1.15. 5-(*tert*-Butyldimethylsilyloxy)-1-cyclohexyl-3-phenylpent-2-en-1-ol (35). A solution of alkenyl stannane

27 (59 mg, 0.1 mmol), iodobenzene (20 mg, 0.1 mmol) *tris*(dibenzylideneacetone)dipalladium(0)chloroform complex (5.2 mg, 0.005 mmol) in DMF (2 mL) was heated to 65 °C overnight. Upon cooling, the DMF was removed by rotary evaporation, and the resulting residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 20:1) to give the title compound **35** (14 mg, 38%) as a yellow oil: IR ($CDCl_3$): 3446, 2929, 1711, 1492, 1471, 1450, 1257, 1090, 1006, 911; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.23 (5H, m), 5.90 (1H, d, J=8 Hz), 4.18 (1H, ddd, J=2, 8, 15 Hz), 3.70 (1H, m), 3.51 (1H, m), 2.97 (1H, m), 2.74 (1H, m), 2.61 (1H, d, J=2 Hz), 2.03 (1H, d, J=13 Hz), 1.86–0.91 (10H, m), 0.89 (9H, s), 0.01 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9, 140.1, 138.4, 128.3, 127.2, 126.7, 71.9, 61.3, 43.7, 33.4, 29.0, 26.7, 26.2, 26.0, 18.5, −5.5; HRMS (FAB) calcd for $C_{23}H_{37}OSi$ $[M-OH]^+$357.2601, found 357.2590.

4.1.16. (*E*)-3-[2-(*tert*-Butyldimethylsilyloxy)ethyl]-1-cyclohexylpenta-2,4-dien-1-ol (36). A solution of alkenyl iodide **28** (42 mg, 0.10 mmol), trifurylphosphine (4.6 mg, 0.02 mmol) and *tris*(dibenzylideneacetone)-dipalladium(0)-chloroform complex (5.2 mg, 0.005 mmol) in THF (2 ml) was stirred at rt for 10 min, before dropwise addition of vinyltributylstannane (35 mg, 0.11 mmol). The resulting solution was stirred overnight at reflux, then filtered through a pad of Celite, and concentrated by rotary evaporation. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 15:1) to give the title compound **36** (20 mg, 61%) as a colourless oil: IR ($CDCl_3$): 3446, 2926, 1472, 1451, 1257, 1096, 1004, 895, 836, 777; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.29 (1H, dd, J=11, 18 Hz), 5.70 (1H, d, J=8 Hz), 5.17 (1H, d, J=18 Hz), 5.04 (1H, d, J=11 Hz), 4.07 (1H, app. t, J=7 Hz), 3.80, 3.62 (2H, m), 2.73–2.61 (2H, m), 2.51 (1H, ddd, J=5, 5, 14 Hz), 1.97 (1H, m), 1.81–0.79 (10H, m), 0.89 (9H, s), 0.06 (6H, 2×s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.7, 137.4, 136.5, 112.4, 71.4, 61.6, 43.5, 29.7, 29.0, 28.8, 26.7, 26.1, 18.6, −5.3. Anal. Calcd for $C_{19}H_{36}O_2Si$: C, 70.31; H, 11.18. Found: C, 69.92; H, 10.80.

4.2. General procedure for Cu-mediated Stille reactions[23]

A 30 mL Schlenck-tube was charged with lithium chloride (31 mg, 0.74 mmol) and flame-dried under high vacuum. Upon cooling, tetrakis(triphenylphosphine)-palladium(0) (28 mg, 0.03 mmol) and copper(I) chloride (61 mg, 0.62 mmol) were added, and the mixture was degassed (4×) under high-vacuum with an argon purge. DMSO (2 mL) was introduced with concomitant stirring, followed by the addition of the iodide (0.12 mmol) and the stannane (0.15 mmol). The resulting mixture was rigorously degassed (4×) by the freeze-thaw process (−78 °C to rt, Ar). The reaction mixture was stirred at rt for 1 h, then heated to 60 °C and left under stirring overnight. The reaction mixture was then allowed to reach rt, and diluted with ether (15 mL) and washed with a mixture of brine and 5% aqueous ammonium hydroxide (5 mL, 1:1). The aqueous layer was further extracted with ether (2×10 mL) and the combined organic layers were washed with water (2×10 mL), then brine (10 mL), dried over sodium sulfate and rotary evaporated. The residue was then purified by flash column chromatography on silica gel (hexane/ethyl acetate) to give the coupling product.

4.3. Analytical data for Stille coupling products

4.3.1. 3-(6-Benzyloxy-5,6-dihydro-4*H*-pyran-3-yl)-5-(*tert*-butyldimethylsilyloxy)-1-cyclohexylpent-2-en-1-ol (38). Yellow oil (diastereomeric mixture): IR ($CDCl_3$): 3424, 2929, 2855, 1720, 1654, 1629, 1451, 1257, 1090, 908; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.26 (5H, m), 6.53 (1H, d, J=4 Hz), 5.55 (1H, t, J=8 Hz), 5.07 (1H, m), 4.83 (1H, d, J=12 Hz), 4.62 (1H, d, J=12 Hz), 4.07 (1H, m), 3.71 (2H, m), 2.71 (1H, m), 2.80–0.91 (17H, m), 0.89 (9H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.8, 138.6, 137.8, 137.7, 136.8, 136.5, 128.2, 127.7 (two signals), 127.6 (two signals), 127.4, 127.3, 114.1 (two signals), 95.7, 95.5, 71.4, 71.2, 69.5 (two signals), 62.2, 62.0, 43.8, 43.7, 32.7, 30.5, 30.3, 29.1 (two signals), 29.0, 26.7, 26.4, 26.2 (two signals), 26.1 (three signals), 26.0, 25.9, (three signals), 25.8, 25.3, 18.6, 18.0, 17.9, 11.4, −5.4 (four signals); HRMS (FAB) calcd for $C_{29}H_{45}O_3Si$ $[M-OH]^+$469.3138, found 469.3105.

4.3.2. 5-(*tert*-Butyldimethylsilyloxy)-1-cyclohexyl-3-phenylpent-2-en-1-one (39). Yellow oil: IR ($CDCl_3$): 2929, 1678, 1596, 1449, 1257, 1102, 909; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–7.29 (5H, m), 6.52 (1H, s), 3.77 (2H, t, J=6 Hz), 3.25 (2H, t, J=7 Hz), 2.45 (1H, m), 1.97–0.82 (10H, m), 0.83 (9H, s), 0.02 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.8, 156.1, 142.3, 128.7, 128.3, 127.1, 124.8, 62.8, 52.1, 35.2, 28.7, 26.0, 25.9, 25.8, 25.7, 18.2, −5.4. Anal. Calcd for $C_{23}H_{36}O_2Si$: C, 74.14; H, 9.74. Found: C, 74.42; H, 9.41.

4.3.3. 3-(6-Benzyloxy-5,6-dihydro-4*H*-pyran-3-yl)-5-(*tert*-butyldimethylsilyloxy)-1-cyclohexylpent-2-en-1-one (40). Yellow oil: IR ($CDCl_3$): 3450, 2930, 1723, 1668, 1614, 1564, 1162, 1092; ^{1}H NMR (300 MHz, $CDCl_3$) δ; 7.41–7.24 (5H, m), 7.13 (1H, s), 6.10 (1H, s), 5.16 (1H, t, J=3 Hz), 4.84 (1H, d, J=12 Hz), 4.64 (1H, d, J=12 Hz), 3.77 (2H, t, J=7 Hz), 3.00 (2H, t, J=6 Hz), 2.47–0.90 (17H, m), 0.89 (9H, s), 0.04 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.5, 154.2, 147.0, 128.4, 127.8, 127.7, 117.6, 115.3, 95.9, 69.8, 63.8, 52.3, 31.7, 31.6, 29.0, 26.0, 25.9, 17.7, −5.3; HRMS (FAB) calcd for $C_{29}H_{44}O_4Si$ $[M-C_6H_{11}]^+$401.2148, found 401.2133.

4.3.4. 2-(*tert*-Butyldimethylsilyloxy)ethyl]-1-cyclohexyl-4-methylenepent-2-ene-1,5-diol (42). Yellow oil: IR ($CDCl_3$): 3406, 2929, 1450, 1257, 1090, 910, 835, 734; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (1H, d, J=8 Hz, 5.25 (1H, s), 5.16 (1H, s), 4.30 (2H, s), 4.08 (1H, t, J=8 Hz), 3.69 (2H, m), 2.73 (2H, m), 2.52 (1H, m), 2.06–0.91 (10H, m), 0.89 (9H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.0, 136.9, 132.2, 112.4, 71.5, 64.4, 61.8, 43.6, 31.6, 28.9, 26.6, 26.2, 26.0, 18.6, −5.4. Anal. Calcd for $C_{20}H_{38}O_3Si$: C, 67.74; H, 10.80. Found: C, 67.52; H, 10.78.

4.3.5. (2*E*)-2-((*S*,4*E*)-1-(*tert*-Betyldimethylsilyloxy)-6-((1*S*,2*S*,6*R*)-2-(methoxymethoxy)-4-methyl-6-(prop-1-en-2-yl)cyclohex-3-enyl)hept-4-en-3-ylidene)pentanedial (44). Following Section 4.2 using alkenyl iodide **33** (62 mg, 0.11 mmol), freshly prepared from **31** according to a procedure similar to that of Section 4.1.13 and purified by

rapid filtration through a small plug of silica gel, the reaction with lithium chloride (25 mg, 0.59 mmol), copper chloride(I) (49 mg, 0.49 mmol), tetrakis(triphenylphosphine)palladium(0) (11 mg, 0.01 mmol) and stannane **13** (58 mg, 0.12 mmol) gave after flash column chromatography on silica gel (hexane/ethyl acetate; 20:1) the coupling product **43** (30 mg, 40%, two steps from **31**) as a slightly yellow oil (diastereomeric mixture). The coupling product rapidly decomposed, and the resulting mixture was purified by flash column chromatography on silica gel (hexane/ethyl acetate; 20:1) to afford the title compound **44** (20 mg, 81%) as a colourless oil: $[\alpha]_D^{25}$ −76.8 (*c* 0.5, CH_2Cl_2); IR ($CDCl_3$): 3155, 2930, 2254, 1794, 1719, 1654, 1618, 1560, 1466, 1381, 1258, 1096, 1039, 912, 742, 651; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.13 (1H, s), 9.76 (1H, t, $J=2$ Hz), 7.34 (1H, m), 6.79 (1H, q, $J=8$ Hz), 6.35 (1H, d, $J=16$ Hz), 5.68 (1H, br s), 4.85 (1H, s), 4.77 (1H, s), 4.69 (1H, d, $J=7$ Hz), 4.56 (1H, d, $J=7$ Hz), 4.21 (1H, br s), 3.73 (2H, m), 3.36 (3H, s), 3.07–2.91 (2H, m), 2.75–2.60 (3H, m), 2.47–2.40 (2H, m), 2.03 (2H, m), 1.71 (6H, s), 1.20 (3H, d, $J=6$ Hz), 0.96–0.86 (1H, m), 0.85 (9H, s), 0.00 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.8, 192.0, 151.2, 146.5, 145.0, 138.7, 138.4, 135.7, 125.4, 121.1, 113.4, 95.1, 69.9, 62.6, 55.8, 45.1, 43.4, 41.0, 37.7, 36.9, 30.0, 25.9, 23.2, 18.6, 18.2, 17.8, −5.4; HRMS (FAB) calcd for $C_{30}H_{50}O_5Si$ $[M]^+$518.3428, found 518.3424.

4.3.6. (*R*,4*E*)-5-(6-(Benzyloxy)-5,6-dihydro-4*H*-pyran-3-yl)-7-(*tert*-butyldimethyloxy)-2-((1*R*,2*S*,6*R*)-2-(methoxymethoxy)-4-methyl-6-(prop-1-en-2-yl)cyclohex-3-enyl) hept-4-en-3-one (45). Following Section 4.2 using alkenyl iodide **34** (75 mg, 0.13 mmol), freshly prepared from **32** according to a procedure similar to that of Section 4.1.13 and purified by rapid filtration through a small plug of silica gel, the reaction with lithium chloride (53 mg, 1.26 mmol), copper chloride(I) (104 mg, 1.05 mmol), tetrakis(triphenylphosphine)palladium(0) (22 mg, 0.02 mmol) and stannane **13** (121 mg, 0.25 mmol) gave after flash column chromatography on silica gel (hexane/ethyl acetate; 20:1) the coupling product **45** (83 mg, 63%, two steps from **32**) as a colourless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.26 (5H, m), 7.08 (1H, s), 6.05 (1H, s), 5.32 (1H, br s), 5.14–5.12 (1H, m), 4.85–4.80 (2H, m), 4.65 (1H, s), 4.62 (1H, d, $J=12$ Hz), 4.42 (1H, dd, $J=3$ Hz), 4.27 (1H, dd, $J=5$, 7 Hz), 4.04 (1H, br s), 3.77 (2H, app. t, $J=6$ Hz), 3.26 (3H, d, $J=2$ Hz), 3.02 (1H, dddd, $J=7$, 7, 7, 13 Hz), 2.85 (1H, dddd, $J=2$, 7, 7, 9 Hz), 2.72–2.66 (1H, m), 2.53–2.48 (2H, m), 2.38 (1H, dddd, $J=6$, 11, 17, 17 Hz), 2.18 (1H, dddd, $J=5$, 5, 17, 17 Hz), 2.08–1.92 (3H, m), 1.88–1.82 (1H, m), 1.79 (3H, s), 1.73 (3H, s), 1.05 (3H, d, $J=6$ Hz), 0.88 (9H, m), 0.03 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.1, 152.6, 146.5, 138.1, 135.1, 128.3, 127.3, 127.0, 122.4, 120.0, 115.5, 95.9, 95.5, 93.4, 72.4, 64.2, 54.9, 45.4, 40.7, 39.8, 31.9, 26.3, 23.2, 21.8, 18.3, 16.7, −5.2; HRMS (EI) calcd for $C_{37}H_{56}NaO_6Si$ $[M+Na]^+$647.3744, found 647.3481.

Acknowledgements

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Stereoselectivity ratios in a simple Diels–Alder reaction in aqueous salt solutions of alcohols

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Abstract—This is the first exhaustive report on the variation of stereoselectivity ratios for a simple Diels–Alder reaction between cyclopentadiene and methyl acrylate. The reaction was carried out in aqueous mixtures of methanol, ethanol, propan-1-ol and butan-1-ol in presence of $LiClO_4$, LiCl, NaCl, KCl, $CaCl_2$ and $MgCl_2$. The *endo* stereoisomer decreases with the increase in carbon chain length of the alcohol. However, $LiClO_4$, a salting-in agent in water becomes salting-out in aqueous mixtures of alcohols. The solvent properties, thus can be attuned by adjusting the amount of solvents and salts.

1. Introduction

Diels–Alder (DA) reactions involving isopolar activated complexes are considered, in general, insensitive to changes in solvents. However, the spectacular rate enhancement of DA reactions in water reported by Breslow and group paved the way to establish the role of water in promoting a variety of organic reactions.[1,2] The group of Breslow has also examined the effect of various salts in accelerating or inhibiting several DA reactions.[3] LiCl, NaCl, $CaCl_2$, etc. are known as salting-out agents, while another group of salts including guanidinium chloride and $LiClO_4$ are known to be salting-in agents. The changes in rates and stereoselectivities of a variety of DA reactions in organic solvents have been analyzed using semiempirical models.[4] Further, the cumbersome synthesis of cantharidin under external high pressure of 15 kbar was made facile in 5 M $LiClO_4$–diethyl ether (LPDE), thus offering higher yields in ambient conditions.[5] The effect of water and its salt solutions on the kinetics of DA reactions has been ascribed to polarity, hydrophobic packing, hydrophobic hydration, salting-phenomena etc.[6a,b] The dramatic obviation of high pressure during the cantharidin synthesis and many other DA reactions has been discussed in terms of Lewis acid properties imparted by Li^+.[7] A critical account of the several factors responsible for rate variation has been discussed in a recent review from this laboratory.[8] In our ongoing efforts to recognize the causes responsible for rate acceleration and improvement in yields and stereoselectivity ratios, we recently showed that a salt that is salting-in in water, can become a salting-out agent in diethyl-ether.[9] For example, $LiClO_4$ in water is a salting-in agent, while it is salting-out in diethyl ether.

Engberts and co-workers have shown that the addition of small amount of alcohol to an aqueous medium enhances the rates of DA reactions.[10] The enhancement in rate constants is discussed in terms of enforced pairwise hydrophobic interactions between diene and dienophile. The hydrophobic interactions and the phenomenon of hydrophobic hydration is discussed in greater detail by Franks.[11] Recently, Bentley et al. studied the solvolysis of *p*-methoxybenzoyl chloride in aqueous alcohol and suggested that preferential solvation by either alcohol or water at the reaction site is not a major factor influencing rate.[12] A large number of studies are available, which explain the effect of binary aqueous organic mixtures on rates of DA and other organic reactions.[13] However, until now no report has been published that describes the variation of *endo*/*exo* ratios in the presence of salt solutions of aqueous alcohol. In this work, we wish to present a detailed study of the effect of addition of alcohol cosolvent to the aqueous solutions of various salt on the stereoselectivities of Diels–Alder reaction. In aqueous $LiClO_4$, a reduction in *endo*/*exo* ratio was observed for the reaction of cyclopentadiene with methyl acrylate than in water alone. The work from this laboratory shows that $LiClO_4$ can be forced to change its role to rate accelerator on addition of cosolvent to aqueous medium.[14]

2. Results and discussion

In order to study the effect of mixtures of aqueous organic

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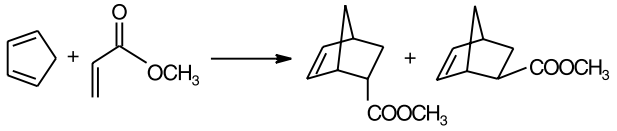
solutions containing salts, we examined the reaction of cyclopentadiene with methyl acrylate (Scheme 1) in pure alcohols such as methanol, ethanol, propan-1-ol and butan-1-ol. On going from lower homologous, methanol to higher homologous butan-1ol, the stereoselectivity ratio goes on decreasing. The reactions were then carried out in aqueous alcohol solutions of $LiClO_4$, a salting-in agent in water and in the LiCl, NaCl, KCl the salting-out agents in aqueous medium. 1 M $LiClO_4$ in aqueous mixture with 40% methanol offered about 89% *endo* isomer, which reduced to 83% in aqueous mixture of 40% butan-1-ol. Similarly in aqueous solution of 40% methanol containing 1 M NaCl *endo* product obtained was 85% whereas aqueous NaCl solution of 40% butan-1-ol offered 83% *endo* isomer. It seems that as the hydrophobic nature of alcohol goes on increasing with the carbon chain length, the stereoisomeric ratio decreases.



Scheme 1.

Attempts were made to analyze the experimental results by multi-parameter equations using a linear combination of existing empirical solvent parameters. A literature survey shows that several empirical parameters are employed to understand the correlation between kinetic parameters and solvent properties like Kamlet–Taft dipolarity–polarizability π^*, hydrogen bond donor α, hydrogen bond acceptor β as well as Kosower *Z*, Mayer–Gutmann AN, Dimroth–Reichardt $E_T(30)$ etc.[15a–f] We attempted to analyze the data to explain the rates and stereoselectivities taking into account the parameters dipolarity π^*, hydrogen bond donating (HBD) α and hydrogen bond accepting (HBA) β abilities of the solvents.[16] However, the unavailability of these parameters for the aqueous alcohol solutions restricted its further use. The influence of solvophobicity (represented by Sp) and solvent polarity (represented by E_T) on the *endo*/*exo* stereoselectivity was investigated.[13a,e,17,18] We analyzed the *endo*/*exo* ratios obtained in various salt solutions of aqueous alcohol using these two parameters Sp and E_T^N as given below.

$$\log(endo/exo) = a + b\mathrm{Sp} + cE_T^N \quad (1)$$

Multiple regression analysis of the data reveals that $LiClO_4$ in all aqueous alcohols shows significant regression coefficients (correlation coefficient, $r>0.9885$, standard deviation, s.d. <0.01, *F* statistics $=1462.53$). LiCl shows a good correlation coefficient in aqueous solutions of methanol, ethanol and propan-1-ol ($r>0.9000$, s.d. <0.0500, *F* statistics >5). For the other salts such as NaCl, $CaCl_2$, KCl and $MgCl_2$ significant regression coefficients are noted. In general, in aqueous butan-1-ol solution, poor correlation coefficients were noted as compared to other aqueous alcohol solutions. For $CaCl_2$ in butan-1-ol-water mixture a poor correlation coefficient was observed ($r=0.4913$, s.d. $=0.0295$, *F* statistics $=0.4831$). The regression coefficients were noted as compared to other aqueous alcohol solutions. For $CaCl_2$ in butan-1-ol-water mixture a poor correlation coefficient was observed ($r=0.4913$, s.d. $=0.0295$, *F* statistics $=0.4831$). The regression coefficients are listed in Table 1.

The experimental *endo*/*exo* ratios versus the *endo*/*exo* ratios calculated using the equation mentioned above are plotted as shown in Figure 1. For aqueous methanol solution containing $LiClO_4$ a good regression coefficient was

Table 1. The values of parameters *a*, *b* and *c* of Eq. 1 and regression coefficients such as correlation coefficient as *r*, standard deviation as s.d. and *F* statistics are listed

Aqueous mixtures of	*a*	*b*	*c*	*r*	s.d.	*F* statistics
$LiClO_4$						
Methanol	−15.8122	1.7385	19.1852	0.9997	0.0033	1462.53
Ethanol	3.7246	−0.9119	−3.3082	0.9979	0.0057	233.433
Propan-1-ol	−6.3663	1.9127	8.5101	0.9972	0.0062	175.810
Butan-1-ol	1.0598	−0.1454	−0.3566	0.9886	0.0051	43.4342
LiCl						
Methanol	1.4124	−0.5036	−0.3697	0.9887	0.0077	43.6511
Ethanol	−0.8434	−0.3001	2.5650	0.9099	0.0499	5.0522
Propan-1-ol	−2.6944	0.8196	4.1558	0.9961	0.0049	127.196
Butan-1-ol	0.1537	−0.3088	1.1798	0.9494	0.0062	9.3805
NaCl						
Methanol	−6.0636	0.7767	7.7475	0.9154	0.0181	5.4071
Ethanol	−0.2671	−0.1857	1.6266	0.9822	0.0134	27.6001
Butan-1-ol	1.5936	0.1544	−1.4766	0.9321	0.0134	6.8634
KCl						
Methanol	−5.8858	0.3744	7.9293	0.8846	0.046	3.8326
Ethanol	2.3156	−0.5439	−1.8119	0.9985	0.0029	339.210
Butan-1-ol	1.3307	−0.2574	−0.6071	0.9442	0.0181	25.3561
$CaCl_2$						
Methanol	−35.5819	5.3885	40.2253	0.9661	0.0211	14.233
Butan-1-ol	1.3950	0.2836	−1.3232	0.4913	0.0295	0.4831
$MgCl_2$						
Methanol	−14.6180	1.9073	17.3376	0.9306	0.0296	6.7043
Ethanol	4.1673	−1.0089	−3.9516	0.9767	0.0195	20.9306
Butan-1-ol	1.1099	0.1011	−0.7372	0.9133	0.0121	10.5272

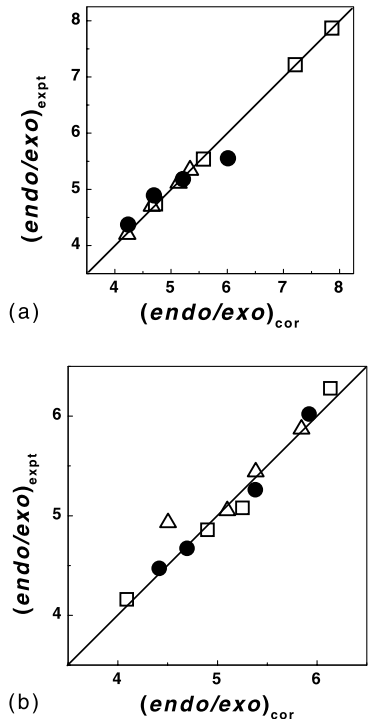


Figure 1. The plots of $(endo/exo)_{expt}$ versus $(endo/exo)_{cor.}$ by the model used for aqueous solutions of (a) 1 M $LiClO_4$ methanol (□), 1 M $CaCl_2$ in methanol (●), 1 M LiCl in propan-1-ol (△); (b) 1 M $MgCl_2$ in ethanol (●), 1 M NaCl in ethanol (□), 1 M KCl in butan-1-ol (△). Solid line shows the best fit.

obtained. Fairly good regression coefficients were obtained for the other systems and are given below. KCl in aqueous butan-1-ol shows the highest standard deviation.

$LiClO_4$ offered 73% *endo* products in 1 M aqueous medium with a reduction of 7% products as compared to that in water alone. On the other hand, *endo* products observed were 82% in aqueous LiCl. This change in *endo* product that is *endo*/*exo* ratio is related to the solubilities of diene and dienophile in aqueous medium. The studies suggested that the interactions of $LiClO_4$ with water are dramatically altered as compared to aqueous methanol. Thus the salt–solvent interactions are of paramount importance in governing the kinetic profile of DA reactions. Salting-in agents like guanidinium chloride and $LiClO_4$ increase the solubilities of the reactants in water, in turn decreasing the *endo*/*exo* ratio. In contrast, salting-out agents such as LiCl, NaCl, KCl etc. in aqueous medium increase *endo*/*exo* ratio by decreasing the solubility of the reactants. The alteration in *endo*/*exo* ratio is discussed in terms of salting parameters.

In order to understand how these salts in aqueous alcohols influence the stereoselectivity ratios, the reactions were conducted in aqueous $LiClO_4$, LiCl, NaCl, KCl, $CaCl_2$ and $MgCl_2$ using alcohol as a cosolvent with composition ranging from 10 to 40%. The effects of the cosolvents such as methanol, ethanol, propan-1-ol and butan-1-ol were studied for the above mentioned aqueous salt solutions.

The reaction of cyclopentadiene with methyl acrylate was carried out in 1 M aqueous $LiClO_4$ with 10–40% methanol as cosolvent. The *endo* isomer formed with 10% methanol was 86%, sharp rise of about 13% over the *endo* product observed in aqueous $LiClO_4$ alone (Fig. 2). On increasing the composition of methanol from 10 to 40%, the yield of *endo* product enhanced to 90%, a gain of 30% as compared to that in aqueous $LiClO_4$. When these results were compared with the *endo* isomer corresponding to 40% aqueous methanol, it was observed that the increase was about 1.6 fold. Furthermore, the increase in concentration of methanol to aqueous $LiClO_4$ decreases the *endo* product. Thus, the role of $LiClO_4$ as a salting-in agent in aqueous medium is forced to change to a salting-out agent just by addition of a small amount of alcohol as a cosolvent. As we have reported earlier, clathrate formation of $LiClO_4$ with methanol is the primary reason to enhance the *endo*/*exo* stereoselectivity.[14] It seems that a type of complex (clathrate) is formed involving $LiClO_4$, methanol and water that enhances the hydrogen bonded water structure, in other words the structuredness of water. The interaction of ClO_4^- with methanol–water plays an important role in the formation of the complex. This gives rise to a solvophobic effect. The increase in methanol composition increases the solvophobicity, which, in turn results in acceleration of *endo* product. This effect is maximum at 40% alcohol–water containing salt, in this case $LiClO_4$ in which the *endo*/*exo* ratio is also highest over the whole concentration range.

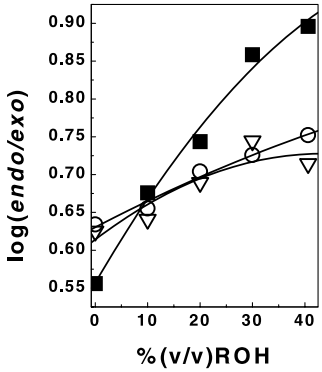


Figure 2. Variation of log(*endo*/*exo*) with increasing concentration of methanol containing salts $LiClO_4$ (■), LiCl (○), NaCl (▽).

Unlike salting-in agents, salting-out agents in aqueous medium retain their characteristic of increasing the *endo*/*exo* stereoselectivity ratio even on addition of cosolvent to aqueous salt solution. However, the magnitude of increase in *endo* product is lower in salts like LiCl, KCl, etc. than in $LiClO_4$ in aqueous mixture of alcohol. In the case of an aqueous solution of LiCl, the addition of cosolvent to the solution causes little difference in the *endo*/*exo* ratio. The *endo* product, which was about 82% in 1 M aqueous LiCl, increased to 86% on addition of 40% methanol. The increase in *endo* product, which was about 2.2 fold in 40% aqueous methanol containing $LiClO_4$ as compared to aqueous $LiClO_4$ now reduced to 1.3 times in the same composition of aqueous alcohol containing LiCl over that in its aqueous solution. The *endo* product obtained corresponding to 40% methanol is only 1.1 times greater than that obtained in aqueous methanol. These results highlights the central role played by the ClO_4^- ion in clathrate formation. In LiCl, no complex formation is possible due to absence of the counter ion effect.

Similar results were noted when a mixture of alcohol and water containing salts NaCl and KCl was employed as media. An 1 M aqueous NaCl solution with 40% methanol offered 84% *endo* product, an increase of about 1.2 fold over aqueous salt solution. KCl (1 M) in 40% aqueous methanol offered about 85% *endo* product, which was 1.4 times more than that in its aqueous solution. When $CaCl_2$ and $MgCl_2$ in 40% aqueous methanol were employed, the yielded *endo* product observed were 84%. From these results, KCl emerges as an effective salt for increasing the *endo*/*exo* ratio out of salting-out agents in aqueous medium. However, $LiClO_4$ shows the maximum increase in *endo*/*exo* ratio.

As the cosolvent changed from methanol to ethanol, the increase in *endo* product decreases for the DA reaction. In 1 M aqueous $LiClO_4$ using ethanol as a cosolvent, the *endo* product observed was 87%, while it was 90% corresponding to methanol as a cosolvent (Fig. 3). In other words, increasing the carbon chain length of the cosolvent reduces the *endo* products. It is reported that the increase in carbon chain length of the cosolvent weakens the structuredness of water, which was gained because of the addition of the methanol. This tendency becomes more prominent in case of propan-1-ol and butan-1-ol as a cosolvent. In the mixture of water and propan-1-ol containing $LiClO_4$ the *endo* product reduced to 85%, which was about 1.4 times reduction as compared to that in 40% methanol as a cosolvent. Employing higher homologous of alcohol as cosolvent, reduces the *endo* products more. Thus, on going from methanol to butan-1-ol, a decrease of 10% in *endo* product was observed. It is clear that the effect of cosolvent on stereoselectivity ratio reduces with increase in carbon chain length.

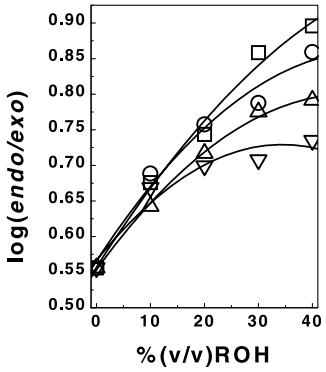


Figure 3. Log(*endo*/*exo*) as a function of various aqueous alcohols methanol (□), ethanol (○), propan-1-ol (△), butan-1-ol (▽), containing $LiClO_4$.

LiCl, as we have seen, increases the *endo* product in presence of cosolvent. When ethanol was used as a cosolvent instead of methanol, the *endo* product increased to 87% (Fig. 4). Further increase in carbon chain length of the cosolvent reduces the increase in *endo* isomer. In aqueous LiCl solution of 40% propan-1-ol as well as butan-1-ol, the *endo* product observed was 84%. In 1 M aqueous LiCl solution of butan-1-ol the increase in *endo*/*exo* ratio is not significant even on increasing the composition of butan-1-ol as compared to that in 40% ethanol. The rate increase from 10 to 40% methanol was 1.7 times, which reduces to

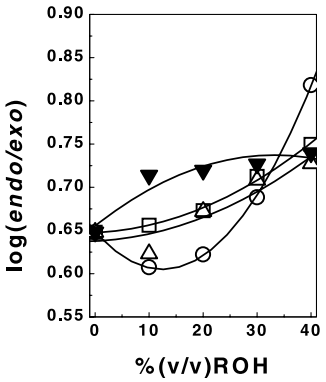


Figure 4. The plot of log(*endo*/*exo*) in aqueous methanol (□), ethanol (○), propan-1-ol (△) and butan-1-ol (▼) containing 1 M KCl.

only 1.1 times on going from 10 to 40% butan-1-ol for aqueous LiCl. The enhancement in rate vanishes with the increase in carbon chain length of the cosolvent. The results suggest that when aqueous LiCl is employed, ethanol is the most effective cosolvent in order to increase the *endo*/*exo* ratio. Similar results were noted for 1 M aqueous NaCl using cosolvent. The *endo* product obtained is about 84% in 40% methanol with an increase in *endo* product of about 1.2 fold as compared to *endo* isomer obtained only in 1 M aqueous NaCl. When 40% ethanol was added to 1 M aqueous NaCl instead of methanol, the *endo* product observed was 86%, an increase of about 1.4 fold over that in its aqueous solution only. Using higher homologues such as butan-1-ol, the *endo* isomer observed was only 83%. For NaCl, 40% ethanol offered maximum *endo*/*exo* ratio.

In the case of KCl, the alcohols such as methanol, ethanol and butan-1-ol offered about the same amount of *endo* isomer (84%) at 40% composition of alcohol (Fig. 5). In 40% methanol, the *endo* isomer increased by about 1.3 times as compared to its aqueous solution only. Further increase in carbon chain length does not alter the *endo* product significantly.

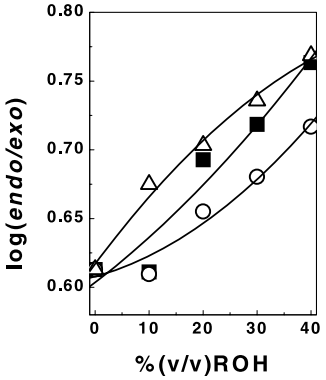


Figure 5. Log(*endo*/*exo*) as a function of aqueous alcohol composition for methanol (■), ethanol (○), and butanol (△) containing KCl.

For $CaCl_2$, methanol and butan-1-ol enhances the *endo*/*exo* ratio by about 1.2 times as compared its aqueous solution. The same is the case with $MgCl_2$. An increase in the carbon chain length does not alter the *endo* product significantly.

To support the experimental *endo*/*exo* data, we measured

the solubility of methyl acrylate in 40% aqueous solutions of methanol and butan-1-ol containing LiCl and $LiClO_4$. It was observed that the solubility of methyl acrylate in $LiClO_4$/methanol/water (0.046 M) is higher than that in LiCl/methanol/water (0.04 M). Also methyl acrylate is more soluble in $LiClO_4$/butanol/water than in aqueous butanol solution containing LiCl. These studies have shown that the solubility of methyl acrylate is maximum in 40% aqueous methanol solution containing $LiClO_4$. This system offered the highest *endo*/*exo* ratio.

3. Conclusion

The effects of various inorganic salts in aqueous mixtures of various alcohols on the *endo*/*exo* stereoselectivities of the reaction of methyl acrylate and cyclopentadiene were studied. It is noted that out of all the salts studied, $LiClO_4$ in aqueous solution of 40% methanol offered highest *endo*/*exo* ratio. Employing higher homologues of alcohol, decreases the *endo* product. It is shown that $LiClO_4$, a salting-in agent in aqueous medium acted as a salting-out agent on manipulation of the solvent. On the contrary, salting-out agents such as NaCl, LiCl, $CaCl_2$ etc. in water increase the *endo*/*exo* ratio even on addition of alcohol cosolvent. However, the increase in *endo* product is prominent in the presence of $LiClO_4$ (a salting-in agent in water) as compared to in LiCl, NaCl, $CaCl_2$, etc. (the salting-out agents in water).

4. Experimental

All commercially available (Merck) salts were recrystallized from alcohol or aqueous alcohol, then dried under vacuum for 6 h. These recrystallized salts were used throughout the experiments. AR grade alcohols (Merck) were used without further purification. The water content in alcohols as determined by gas chromatography was $<0.1\%$. Aqueous alcohol solutions (v/v) were prepared using de-ionized water. The salts were dissolved to these aqueous alcohol solutions to make the salt solutions.

Cyclopentadiene was freshly cracked from its dimer (Merck) just before use. Methyl acrylate obtained from Merck was used immediately after its distillation.

In a typical run, 0.6 ml (7.21 mmol) of freshly cracked cyclopentadiene was transferred into 5 ml of salt solution. Then, 0.6 ml (6.67 mmol) of methyl acrylate was dissolved in 5 ml of the salt solution. The solution containing cyclopentadiene was added to the solution having methyl acrylate. The reaction mixture was magnetically stirred at 30 °C for about 5 h.

The *endo*- and *exo*- products were characterized using NMR as described in the literature.[19] The *endo*/*exo* ratios were determined by gas chromatography. Nitrogen as carrier gas, injector temperature 250 °C detector temperature 280 °C, retention times: *exo* cycloadduct 8.559 min, *endo* cycloadduct 8.969 min. Each reaction was carried out three times and the *endo*/*exo* ratios were reproducible to within 5%. GC and NMR were used to check the dimerization of cyclopentadiene, which was found to be negligible.

Methyl acrylate was equilibrated with the respective salt solutions for 5 h with stirring. The aqueous layer was removed. The dissolved methyl acrylate was then extracted with chloroform. The absorbance was measured at 270 nm. The concentrations were determined by the calibration curve already plotted for the known concentrations. The solubilities as determined by triplicate measurements were precise to $\pm 2\%$.

Acknowledgements

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Regio- and stereoselective annelation of phenanthridines with α,β-acetylenic γ-hydroxyacid nitriles

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Abstract—Phenanthridines (3,4-, 7,8-benzoquinolines and methyloctahydrophenanthridine) are annelated regio- and stereoselectively with α,β-acetylenic γ-hydroxyacid nitriles to form new polyfunctional condensed systems, 4-cyanomethylene-1,3-oxazolidino-1,2-dihydrophenanthridines.

1. Introduction

Recently, we have discovered a facile annelation of α,β-acetylenic γ-hydroxyacid nitriles with pyridines,[1a] tris-[2-(4-pyridyl)ethyl]phosphine oxide,[1b] quinoline and quinoxaline,[1c] which proceeds under mild conditions providing a novel family of heterocyclic compounds, 1,3-oxazolidino-dihydroazines. This reaction can be of special interest for modification of phenanthridine skeleton and synthesis of novel functionalized highly condensed alkaloid-like systems.[2a–j] The *meso* moiety 'HC═N' and non-equality of the ring positions towards substitution make the phenanthridine chemistry more complicated compared to chemistry of other benzoquinolines.[3] Due to the sterically limited access to the nitrogen atom in the phenanthridine nucleus for such a bulky reagent as α,β-acetylenic γ-hydroxyacid nitriles, the result was difficult to predict.

Phenanthridine (3,4- and 7,8-benzoquinoline) structures are found in various natural alkaloids.[2a–j] The new methodology for modification and functionalization of phenanthridine skeleton can be useful for the targeted synthesis of biologically active compounds with desired properties.

2. Results and discussion

In order to develop this methodology for modification of natural compounds, we have studied the annelation of phenanthridines: 3,4-benzoquinoline **1**, 7,8-benzoquinoline **2**, and 6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine **3** with α,β-acetylenic γ-hydroxyacid nitriles **4a**,**b**. The results of this investigation are reported herein.

Reaction of 3,4-benzoquinoline **1** with cyanoacetylenes **4a**,**b** (equimolar amounts) proceeds under mild conditions (20–25 °C, 145 h, no catalyst, in CH_3CN) to afford regio- and stereoselectively, *Z*-(5,5-dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)phenanthridine **5a** (74% yield) and *Z*-(5-spirocyclohexyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)phenanthridine **5b** (60% yield), respectively, (Scheme 1).

A probable scheme of the formation of 1,3-oxazolidino-phenanthridines **5a**,**b** is likely to involve the zwitterionic mesomeric intermediates **A** and **B**.

A $CDCl_3$ solution of *Z*-**5b** at 20–25 °C results in rapid isomerization. After 10 h, in the ^1H NMR spectrum, along with the signal of olefinic proton at δ 4.81, related to *Z*-isomer of **5b**, a new signal appears at δ 3.91, attributable to *E*-**5b**. The concuration of the latter gradually increases and after 2 weeks the spectrum shows almost complete isomerization (5:95%, respectively).

Unlike 3,4-benzoquinoline **1**, 7,8-benzoquinoline **2** reacts with cyanoacetylene **4a** under more harsh conditions (110–120 °C, 60 h, no solvent), apparently, due to lower spatial availability of the nitrogen atom, to give stereoselectively *E*-(5,5-dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-

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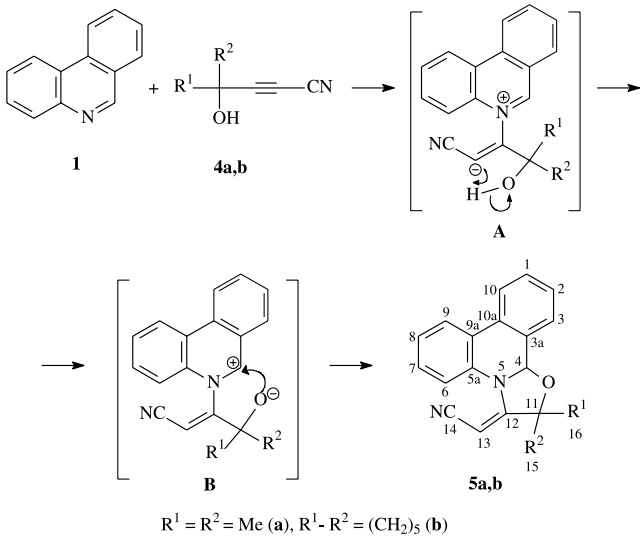
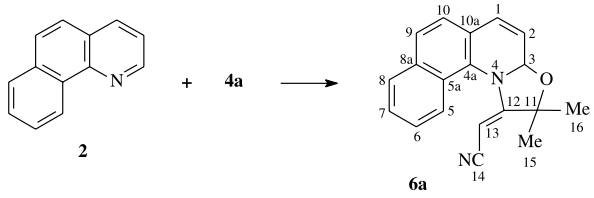


Scheme 1.

1,2-dihydro)phenanthridine **6a** (50%) (Scheme 2). Such a stereochemical outcome can be explained by the fact that *Z*-isomer does not exist in the form of the flat condensed system **6a**.

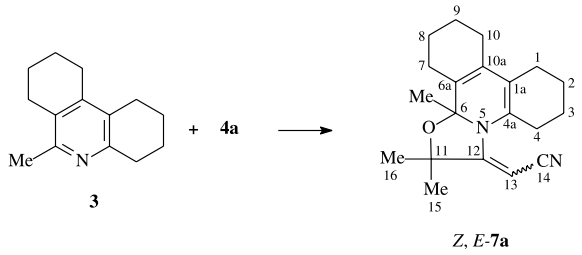


Scheme 2.

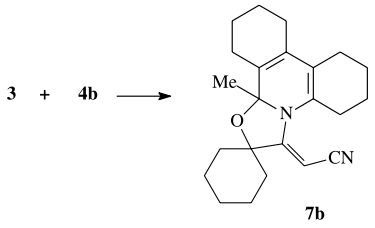
At lower temperature (70–80 °C, 30 h), in the presence and absence of CH_3CN, 7,8-benzoquinoline **2** fails to react with cyanoacetylene **4a**. Additionally, it does not react with cyanoacetylene **4b** at 110–120 °C and higher temperatures, presumably due to steric factors (in both cases, the starting compounds are recovered unchanged from the reaction mixture).

6-Methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine **3** reacts with an equimolar amount of cyanoacetylene **4a** in the absence of catalysts or solvents to give at 20–25 °C (50 h) mainly *Z*-(5,5-dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)-6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine **7a**, although the yield of the latter is rather low (20%). At higher temperature (60–70 °C, 8 h), another isomer, *E*-(5,5-dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)-6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine **7a**, is formed in 84% yield (Scheme 3).

In comparison to the reaction with cyanoacetylene **4a**, the annelation of methyloctahydrophenanthridine **3** with cyanoacetylene **4b** requires more harsh conditions (60–70 °C, 63 h), proving selectively *Z*-(5-spirocyclohexyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)-6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine **7b** in 40% yield (Scheme 4).



Scheme 3.



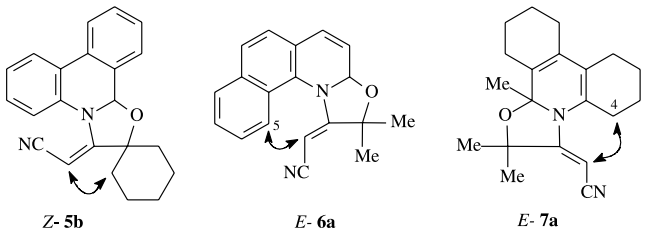
Scheme 4.

Increasing the reaction temperature to 90 °C did not improve the yield of *Z*-1,3-oxazolidinophenanthridine **7b** any further. In this experiment, no *E*-isomer was detected in the reaction mixture.

The 1,3-oxazolidinodihydrophenanthridines synthesized are crystalline solids **5a**,**b** or oils **6**, **7**, which are soluble in most organic solvents. Their structures have been confirmed using IR, ^1H and ^{13}C NMR and NOESY spectroscopies. The IR spectra of 1,3-oxazolidinodihydrophenanthridines **5**–**7** show absorption bands at 2180–2220 (=CH*CN*), 3050–3080 and 930–960 (C=CH), 1620–1640 (C=C), 2830–2960 (CH_2 and Me) cm^{-1}. No absorption bands the hydroxyl moieties are observed.

The ^1H NMR spectra of 1,3-oxazolidinodihydrophenanthridines **5**–**7** contain singlets for the olefinic protons (=C*H*CN) at δ 3.69–4.81. Such a wide dispersion of chemical shift values for *E*- and *Z*-isomers is caused by different effects of anisotropy of condensed aromatic fragments on the olefinic proton. Their structures were determined using 2D ^1H–^1H NOESY. Thus, in the spectrum of the compound **5b**, a cross-peak between the signal of the olefinic proton and that of *ortho*-protons in the cyclohexyl moiety is observed, indicating a *Z*-configuration. Similarly, a cross-peak between the olefinic proton and the proton in position-5 of the ring was observes in the spectrum of compound **6a**. A cross-peak was seen between the olefinic proton and protons in position-4 of phenanthridine ring in the spectrum of compound **7a**, indicating an *E*-configuration in these compounds (Fig. 1).

Therefore, annelation of phenanthridines **1**–**3** with cyanoacetylenes **4** results in the stereoselective assembly of novel condensed systems, 1,3-oxazolidinodihydrophenanthridines **5**–**7**, possessing an active *N*-2-cyanovinyl (acrylic) functional group of different configurations, which provides broad opportunities for further modification of the compounds synthesized.



Z- **5b** *E*- **6a** *E*- **7a**

Figure 1.

3. Experimental

3.1. General

IR spectra of compounds synthesized were recorded on a Specord IR-75 spectrometer (liquid film, KBr). ^{1}H and ^{13}C NMR spectra were taken on a Bruker instrument (250, 400 MHz) with HMDS as an internal standard. UV–vis spectra were measured on a Perkin-Elmer Lambda 35 spectrometer at rt (EtOH, $d=0.1$ cm). Phenanthridines **1**, **2** are chemically pure grade commercial products. Octahydrophenanthridine **3** and cyanoacetylenes **4a**,**b** were prepared according to published procedures.[4,5] Column and thin-layer chromatography was performed on neutral Al_2O_3 (chloroform/benzene/alcohol, 20:4:1 or ether/hexane, 1:1 as eluents).

3.1.1. *Z*-(5,5-Dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)phenanthridine (5a). To a solution of phenanthridine **1** (0.18 g, 1 mmol) in CH_3CN (1 mL), was added dropwise a solution of cyanoacetylene **4a** (0.11 g, 1 mmol) in CH_3CN (1 mL). The reaction mixture was stirred for 145 h at 20–25 °C. After removal of the solvent in vacuo, 1,3-oxazolidinophenanthridine **5a** (0.14 g, 74% taking into account phenanthridine conversion, white solid, mp 164–166 °C) and unreacted **1** (0.06 g, 67% conversion) were isolated from the residue through column chromatography (eluent: chloroform/benzene/ethanol, 20:4:1); [Found: C, 78.98; H, 5.60; N, 9.69. $C_{19}H_{16}N_2O$ requires C, 79.14; H, 5.59; N, 9.72%]; ν_{max} (KBr) 3060 (C=*CH*), 2960, 2920 (Me), 2180 (=CH*CN*), 1620 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.86–7.28 (8H, m, H-1-3, 6-10), 6.20 (1H, s, H-4), 4.64 (1H, s, =C*H*CN), 1.79 (3H, s, Me), 1.69 (3H, s, Me); δ_C (400 MHz, $CDCl_3$) 162.77 (C-12), 135.38–121.16 (C-1-3, 3a, 5a, 6-9, 9a, 10, 10a), 119.24 (C-14), 88.46 (C-4), 87.10 (C-11), 57.04 (C-13), 26.85, 25.88, (C-15, 16).

3.1.2. *Z*-(5-Spirocyclohexyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)phenanthridine (5b). Analogously, from phenanthridine **1** (0.18 g, 1 mmol) and cyanoacetylene **4b** (0.15 g, 1 mmol) in CH_3CN (2 mL), 1,3-oxazolidinophenanthridine **5b** (0.12 g, 60% taking into account phenanthridine conversion, white solid, mp 187–189 °C) and the phenanthridine **1** (0.07 g, 61% conversion) were obtained; [Found: C, 80.28; H, 5.95; N, 8.15. $C_{22}H_{20}N_2O$ requires C, 80.46; H, 6.14; N, 8.53%]; ν_{max} (KBr) 3060 (C=*CH*), 2930, 2850 [$(CH_2)_5$], 2190 (=CH*CN*), 1620 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.03–7.36 (8H, m, H-1-3, 6-10), 6.38 (1H, s, H-4), 4.81 (1H, s, =C*H*CN), 1.65–1.85 (10H, m, cyclohexyl); δ_C (400 MHz, $CDCl_3$) 161.77 (C-12), 135.46–121.83 (C-1-3, 3a, 5a, 6-9, 9a, 10, 10a), 119.06 (C-14), 88.33 (C-4), 87.74 (C-11), 56.92 (C-13), 33.86–21.47 (cyclohexyl); λ_{max} (log ε) 205 (4.56), 222 (4.36), 240 (4.18), 253 (4.21), 269 (4.29), 309 (4.12) nm.

3.1.3. *E*-(5-Spirocyclohexyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)phenanthridine (5b). Compound (**5b**) was prepared by allowing a $CDCl_3$ solution of *Z*-**5b** to stay in a spectrometer ampoule for 2 weeks; δ_H (400 MHz, $CDCl_3$) 7.91–7.30 (8H, m, H-1-3, 6-10), 6.21 (1H, s, H-4), 3.91 (1H, s, =C*H*CN), 1.80–1.62 (10H, m, cyclohexyl); δ_C (400 MHz, $CDCl_3$) 158.17 (C-12), 134.48–121.05 (C-1-3, 3a, 5a, 6-9, 9a, 10, 10a), 118.10 (C-14), 87.42 (C-4), 86.90 (C-11), 56.20 (C-13), 33.15–21.38 (cyclohexyl).

3.1.4. *E*-(5,5-Dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)phenanthridine (6a). To phenanthridine **2** (0.18 g, 1 mmol), cyanoacetylene **4a** (0.11 g, 1 mmol) was added dropwise. The reaction mixture was stirred for 60 h at 110–120 °C. After cooling to 20–25 °C, 1,3-oxazolidinophenanthridine **6a** (0.08 g, 50% taking into account phenanthridine conversion, yellow oil) and phenanthridine **2** (0.08 g, 55% conversion) were obtained using column chromatography (eluent: chloroform/benzene/ethanol, 20:4:1); [Found: C, 79.50; H, 5.35; N, 9.43. $C_{19}H_{16}N_2O$ requires C, 79.14; H, 5.59; N, 9.72.%]; ν_{max} (liquid film) 3050 (C=*CH*), 2990, 2940, 2870, 2850 (Me), 2190 (=CH*CN*), 1630 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.25–7.38 (6H, m, H-5-10), 6.43 (1H, d, $^{3}J_{1-2}=9.5$ Hz, H-1), 5.97 (1H, d, H-2), 5.94 (1H, s, H-3) (the vicinal coupling constant value between H-2 and H-3 protons, which conforms to the Carplus equation, is very low due to the fact that the corresponding dihedral angle is close to 90° and therefore, the H-2 proton signal appears as doublet, while that of H-3 shows up as a singlet), 3.75 (1H, s, =C*H*CN), 1.81 (3H, s, Me), 1.75, (3H, s, Me); δ_C (400 MHz, $CDCl_3$) 148.41 (C-12), 146.19–121.36 (C-1, 2, 4a, 5, 5a, 6-8, 8a, 9, 10, 10a), 118.62 (C-14), 89.24 (C-3), 89.23 (C-11), 61.53 (C-13), 26.76, 26.44 (C-15, 16).

3.1.5. *Z*-(5,5-Dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)-6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine (7a). Analogously, from phenanthridine **3** (0.20 g, 1 mmol) and cyanoacetylene **4a** (0.11 g, 1 mmol) (20–25 °C, 50 h, eluent: ether/hexane, 1:1), 1,3-oxazolidinooctahydrophenanthridine **7a** (0.03 g, 20% taking into account phenanthridine conversion, yellow oil) and phenanthridine **3** (0.10 g, 50% conversion) were obtained; [Found: C, 77.54; H, 8.23; N, 9.40. $C_{20}H_{26}N_2O$ requires C, 77.38; H, 8.44; N, 9.02%.]; ν_{max} (liquid film) 3050 (C=*CH*), 2920, 2840, 2830 (Me), 2190 (=CH*CN*), 1650 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.69 (1H, s, =C*H*CN), 2.85–1.28 (25H, 3 Me, m, H-1-4, 7-10); δ_C (400 MHz, $CDCl_3$) 164.80 (C-12), 135.86–127.52 (C-1a, 4a, 6a, 10a), 120.60 (C-14), 97.12 (C-6), 85.15 (C-11), 55.64 (C-13), 38.39–21.32 (C-1-4, 7-10, 15,16).

3.1.6. *E*-(5,5-Dimethyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)-6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine (7a). Analogously, from phenanthridine **3** (0.20 g, 1 mmol) and cyanoacetylene **4a** (0.11 g, 1 mmol) (60–70 °C, 8 h), 1,3-oxazolidinoocta-

hydrophenanthridine **7a** (0.16 g, 84% taking into account phenanthridine conversion, yellow oil) and phenanthridine **3** (0.08 g, 60% conversion) were obtained; [Found: C, 77.48; H, 8.11; N, 9.39. $C_{20}H_{26}N_2O$ requires C, 77.38; H, 8.44; N, 9.02.%]; ν_{max} (liquid film) 3080 (C=*CH*), 2930, 2850 (Me), 2220 (=CH*CN*), 1640 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.78 (1H, s, =C*H*CN), 2.83–1.56 (25H, 3 Me, m, H-1-4, 7-10); δ_C (400 MHz, $CDCl_3$) 171.03 (C-12), 152.85–127.36 (C-1a, 4a, 6a, 10a), 113.96 (C-14), 79.09 (C-6), 78.51 (C-11), 32.80 (C-13), 29.42–21.69 (C-1-4, 7-10, 15,16).

3.1.7. *Z*-(5-Spirocyclohexyl-4-cyanomethylene-1,3-oxazolidino[3,2-*a*]-1,2-dihydro)-6-methyl-1,2,3,4,7,8,9,10-octahydrophenanthridine (7b). Analogously, from phenanthridine **3** (0.40 g, 2 mmol) and cyanoacetylene **4b** (0.30 g, 2 mmol) (60–70 °C, 63 h), 1,3-oxazolidinooctahydrophenanthridine **7b** (0.14 g, 40% taking into account phenanthridine conversion, yellow oil) and phenanthridine **3** (0.20 g, 50% conversion) were obtained; [Found: C, 78.65; H, 8.61; N, 7.58. $C_{23}H_{30}N_2O$ requires C, 78.82; H, 8.63; N, 7.99.%]; ν_{max} (liquid film) 3060 (C=*CH*), 2930, 2850 [$(CH_2)_5$], 2190 (=CH*CN*), 1640 (C=C) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.69 (1H, s, =C*H*CN), 2.98–1.24 (29H, Me, m, H-1-4, 7-10, cyclohexyl); δ_C (400 MHz, $CDCl_3$) 164.82 (C-12), 135.86–127.54 (C-1a, 4a, 6a, 10a), 120.63 (C-14), 97.20 (C-6), 85.11 (C-11), 55.62 (C-13), 38.37–21.39 (C-1-4, 7-10, cyclohexyl).

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Catalytic effects of hydrogen-bond acceptor solvent on nucleophilic aromatic substitution reactions in non-polar aprotic solvent: reactions of phenyl 2,4,6-trinitrophenyl ether with amines in benzene–acetonitrile mixtures

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Abstract—The effect of addition of small amounts of hydrogen-bond acceptor solvent, acetonitrile, to the benzene medium of the reactions of phenyl 2,4,6-trinitrophenyl ether with aniline and cyclohexylamine, respectively have been investigated. The addition produced similar effects in the two reactions—continuous rate increase with increasing amounts of acetonitrile. The results are interpreted in terms of the effect of amine–solvent interaction on the nucleophilicity of the amines and are in accord with our expectations based on the effects observed for hydrogen-bond donor solvent, methanol on the same reactions. It is also established from the results that the role of hydrogen-bond acceptor co-solvent could be played by an added more basic non-nucleophilic amine.

1. Introduction

Some unusual findings in the kinetics of aromatic nucleophilic substitution reactions in non-polar aprotic solvents reported in the literature in recent times include the sometimes observed third-order dependence of the second-order rate constant, k_A, on amine concentrations.[1–7] As a result of this, a number of mechanisms have been proposed. Attempts have thus been made by some authors to provide support for their proposed mechanisms. Remarkable among these attempts is the study of the effects of added hydrogen-bond donor and hydrogen-bond acceptor co-solvents on such reactions in benzene[8–11] and toluene.[12,13] Having successfully studied and rationalised the sometimes conflicting effects of hydrogen-bond donor co-solvent, methanol, on S_NAr reactions in non-polar aprotic solvent, benzene,[8,9] and toluene,[12,13] we found it necessary to investigate also the effect of hydrogen-bond acceptor solvent on these reactions so as to have an overall view of the mechanisms of the reactions involving these two different types of solvents in non-polar aprotic medium. Examination of the literature reveals that only few cases of the effect of hydrogen-bond acceptor (hba) co-solvent on S_NAr reactions in non-polar aprotic solvents have been reported. Surh[14] studied the reaction of p-fluoronitrobenzene with piperidine in benzene–DMSO mixtures while Bernasconi and Zollinger[15] studied the reaction of 2,4-dinitrochloro- and 2,4,-dinitrifluorobenzene in benzene–DMSO mixtures. The latest report was by Nudelman and Palleros[16] on the reaction of 2,6-dinitroanisole with cyclohexylamine in toluene–DMSO mixtures. As in the case of hydrogen-bond donor co-solvent methanol, treated in our last paper,[8] we have decided to investigate in detail the effects of addition of small amounts of another hydrogen bond acceptor co-solvent, acetonitrile, on the reaction of 2,4,6-trinitrophenyl ether (PTPE) with aniline and cyclohexylamine, respectively, the two reactions that have been previously studied by us[4,9] in pure benzene.

2. Results and discussion

The reaction of aniline is third order in amine,[4] being catalysed by two aniline molecules while that of cyclohexylamine is first order in amine, as it is not base-catalysed.[8] Addition of small amounts of acetonitrile to the benzene medium of the respective reactions produced remarkable increases in the rates of the reactions (Tables 1 and 2). Similar observations in the literature include the reactions of 2,4-dinitrofluoro- and 2,4-dinitrochlorobenzenes with piperidine in benzene–dimethyl sulfoxide

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Table 1. Second-order rate constants, k_A for the reaction of phenyl-2,4,6-trinitrophenyl ether with aniline in benzene and benzene–acetonitrile mixtures at 25 °C

[Amine]/mol dm^3	% Acetonitrile (v/v)	$10^3\,k_A$ dm^3 mol^{-1} s^{-1}
0.15	0	5.20
	0.1	6.27
	0.2	7.00
	0.3	7.87
	0.4	8.73
	0.6	10.04
	0.8	12.15
0.20	0	8.40
	0.1	9.85
	0.2	10.95
	0.3	12.05
	0.4	13.40
	0.6	15.80
	0.8	18.15
0.25	0	13.10
	0.1	15.22
	0.2	16.72
	0.3	18.50
	0.4	20.04
	0.6	23.92
	0.8	26.70
0.30	0	19.00
	0.1	21.30
	0.2	23.50
	0.3	25.90
	0.4	28.53
	0.6	32.12
	0.8	36.50

[Substrate] $= 5.0\times10^{-4}$ mol dm^{-3}.

Table 2. Second-order rate constants, k_A for the reaction of phenyl-2,4,6-trinitrophenyl ether with cyclohexylamine in benzene–acetonitrile mixtures at 25 °C

[Amine]/mol dm^3	% Acetonitrile (v/v)	$10^3\,k_A$ dm^3 mol^{-1} s^{-1}
25×10^{-4}	0	12.84
	0.1	13.96
	0.2	14.92
	0.3	15.96
	0.4	16.88
	0.6	17.88
	0.8	18.80

[Substrate] $= 2.5\times10^{-5}$ mol dm^{-3}.

(DMSO) mixtures by Bernasconi and Zollinger.[15] The reaction of the fluoro-substrate is second order in amine while that of the chloro is first order. Nudelman and Palleros[16] also observed similar increases in the rate of the reaction of 2,6-dinitroanisole with cyclohexylamine in toluene–DMSO mixtures. The above observations show that added hydrogen-bond acceptor solvents produce an increase in rates of S_NAr reactions in non-polar aprotic medium irrespective of whether the reaction is base-catalysed or not. This is unlike the case of hydrogen-bond donor co-solvent where conflicting effects were observed for base-catalysed and non-base catalysed reactions.[8]

2.1. Cause of rate increase

An in-depth study of the interaction of amine with added hydrogen-bond acceptor solvent, DMSO, was carried out by Angella and Scott.[17] Using Bronsted-acid–base studies involving some amines with *p*-nitrophenol in benzene–DMSO solvent system, these researchers established that DMSO, when present as a solvent, does increase the effective basicity of primary and secondary amines and that this is achieved through the formation of hydrogen-bond between amine hydrogens and DMSO thus:

$$RNH_2 + SO(CH_3)_2 \overset{K}{\rightleftharpoons} RHNH\cdots OS(CH_3)_2 \quad (1)$$

Such aggregates should also be possible with other hydrogen-bond acceptor solvents, for example, acetonitrile used in the present study as show in Eq. 2,

$$RNH_2 + CH_3CN \overset{K}{\rightleftharpoons} CH_3CN\cdots HNHR \quad (2)$$

where K is the association constant for aggregate formation.

The amine in the aggregate formed in Eq. 2 will be a better nucleophile than the free amine because of the increased nucleophilicity of the amine-acetonitrile aggregate. It is therefore proposed that the amine-acetonitrile aggregate of enhanced nucleophilicity attacks the substrate in the first step of the S_NAr reaction to produce the observed rate increase.

2.2. Catalysis by acetonitrile

Addition of small amounts of acetonitrile (0.019–0.153 M) to the benzene medium of the reaction of PTPE with cyclohexylamine caused a gradual increase in the second order rate constant, k_A (Table 2) with initial value being $k_1 = 12.84$ mol^{-1} s^{-1}, the rate constant for the non-base catalysed reaction in pure benzene. These observed increases in rate are due to the increased nucleophilicity of the amine in the amine-acetonitrile aggregate which attacks the substrate in the first step of the two-step S_NAr reaction, as well as the catalytic effect of acetonitrile on the reaction involving the remaining free amine with the substrate.

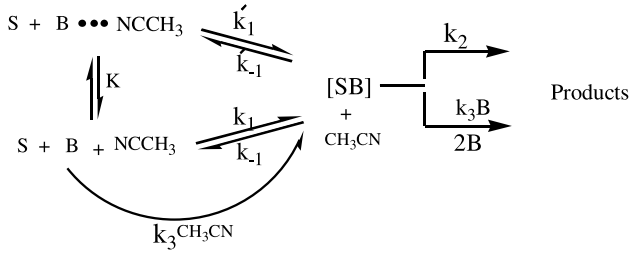
In earlier studies of S_NAr reactions carried out in benzene–DSMO mixtures by Bernasconi and Zollinger,[15] and by Suhr,[14] a considerable increase in reaction rate was observed in each case for small additions of hydrogen-bond acceptor solvent (hba) to the reaction medium. The rate increase was considered to exceed that expected based on the probable increase in the dielectric constant of the aprotic medium. Bernasconi ruled out base catalysis because the pK_a of DMSO in water is zero. Suhr, on the other hand, attributed the increase to a sort of base catalysis. We also rule out base catalysis for the following reason: the reaction of PTPE with cyclohexylamine which was found not to be base-catalysed in our last paper[8] has been found to be catalysed by the hba solvent, acetonitrile, in the present investigation. Since base catalysis occurs in the second step of S_NAr reactions, it means that the acetonitrile catalysis could only be taking place in the first step of the S_NAr reaction.

There is of course no doubt that the effect being observed is a form of catalysis because firstly, the interaction of the hba solvent with the amine results in increase in the rate of reaction and secondly, the increase in rate is proportional to the concentration of the added hba solvent. This is thus

catalysis resulting from the enhanced nucleophilicity of the amine in the formed amine-solvent aggregate which will be reflected in the enhanced value of the first step rate constant k_1' (compared to that of the free amine k_1) and the catalytic effect of the co-solvent, acetonitrile on the reaction of the substrate with the remaining free base, aniline, which will be reflected in the catalytic rate constant $k_3^{CH_3CN}$.

2.3. Mechanism of the base-catalysed reaction

As in the previously studied reaction involving methanol addition,[8] S_NAr reactions in non-polar aprotic solvents on addition of a small amount of acetonitrile can be assumed to involve the attack of the amine-acetonitrile aggregate, as well as the free amine, on the substrate to produce the zwitterionic intermediate. Since amine-acetonitrile aggregate formation via hydrogen bonding is likely to be a very rapid equilibrium process, two possible roots for conversion of the zwitterionic intermediate into products are proposed. The reaction of phenyl 2,4,6-trinitrophenyl ether with aniline in benzene–acetonitrile mixtures (at low acetonitrile concentrations) can be represented by Scheme 1, where S stands for the substrate, B for the base, $B\cdots NCCH_3$ for the amine-acetonitrile aggregate, [SB] for the zwitterionic intermediate and k_1', the enhanced first-step rate constant involving the attack of the amine-acetonitrile aggregate on the substrate, $k_3^{CH_3CN}$, the rate constant for the acetonitrile-catalysed reaction of the substrate with the remaining free amine and k_3^B, the amine catalytic rate constant for the conversion of the zwitterionic intermediate into products.



Scheme 1.

Since the amine may exist in free or hydrogen-bonded forms as given by Eq. 2, the stoichiometric measured concentration, B_{Stoich} will be related to the free base $[B]_{Free}$ by Eq. 3.

$$[B\cdots NCCH_3] + [B]_{Free} = [B]_{Stoich} \tag{3}$$

From Eqs. 2 and 3, the unmeasurable quantities $[B\cdots NCCH_3]$ and $[B]_{Free}$ are derived in terms of the measurable quantity $[B]_{Stoich}$.

Application of the steady-state hypothesis to Scheme 1 in terms of the stoichiometric base concentration leads to Eq. 4 for the observed overall rate-constant k_A,

$$k_A = \frac{\frac{k_1'K[CH_3CN]}{1+K[CH_3CN]} + \frac{k_1}{1+K[CH_3CN]} + \frac{k_{CH_3CN}[CH_3CN]}{1+K[CH_3CN]}\left(k_2 + \frac{k_3[B]^2}{(1+K[CH_3CN])^2}\right)}{k_{-1}'[CH_3CN] + k_{-1} + k_2 + \frac{k_3^B[B]^2}{1+K[CH_3CN]^2}} \tag{4}$$

where [B] is the total (stoichiometric) base concentration and K is the association constant for amine-acetonitrile aggregate formation. At low base concentration,

$$(1 + K[CH_3CN])^2 \approx (1 + K[CH_3CN])$$

in Eq. 4.

For the base-catalysed reaction, when the second step is rate-determining, Eq. 5 holds

$$k_{-1}'[CH_3CN] + k_{-1} \gg k_2 + \frac{k_3^B[B]^2}{1 + K[CH_3CN]^2} \tag{5}$$

and Eq. 4 becomes Eq. 6.

$$k_A = \frac{\frac{k_1'K[CH_3CN]}{1+K[CH_3CN]} + \frac{k_1}{1+K[CH_3CN]} + \frac{k_{CH_3CN}[CH_3CN]}{1+K[CH_3CN]}\left(k_2 + \frac{k_3[B]^2}{1+K[CH_3CN]}\right)}{k_{-1}'[CH_3CN] + k_{-1}} \tag{6}$$

If we assume that acetonitrile increases the nucleophilicity of the amine considerably, $k_1' \gg k_1$ and correspondingly, it can also be assumed that $k_{-1}' \gg k_{-1}$

$$\therefore k_A = \frac{\frac{k_1'K[CH_3CN]}{1+K[CH_3CN]} + \frac{k_1}{1+K[CH_3CN]} + \frac{k_{CH_3CN}[CH_3CN]}{1+K[CH_3CN]}\left(k_2 + \frac{k_3[B]^2}{1+K[CH_3CN]}\right)}{k_{-1}'[CH_3CN]} \tag{7}$$

On expanding and re-arranging, Eq. 7 becomes Eq. 8.

$$k_A = \frac{k_1'k_2K}{k_{-1}'} + \frac{k_1k_2K}{k_{-1}'} + \frac{k_2k_3^{CH_3CN}}{k_{-1}'} + \left(\frac{k_1'k_2K^2}{k_{-1}'} + \frac{k_2k_3^{CH_3CN}K}{k_{-1}'}\right)[CH_3CN] + \left(\frac{k_1'k_3^BK}{k_{-1}'} + \frac{k_3^Bk_3^{CH_3CN}}{k_{-1}'}\right)[B]^2 \tag{8}$$

At constant acetonitrile concentration, this equation reduces to Eq. 9.

$$k_A = k' + k''[B]^2 \tag{9}$$

where k' and k'' are defined by Eqs. 10 and 11, respectively.

$$k' = \frac{k_1'k_2K}{k_{-1}'} + \frac{k_1k_2K}{k_{-1}'} + \frac{k_2k_3^{CH_3CN}}{k_{-1}'} + \left(\frac{k_1'k_2K^2}{k_{-1}'} + k_2k_3^{CH_3CN}K\right)[CH_3CN] \tag{10}$$

$$k'' = \frac{k_1'k_3^BK}{k_{-1}'} + \frac{k_3^Bk_3^{CH_3CN}}{k_{-1}'} \tag{11}$$

The plots of k_A against $[aniline]^2$ gave straight lines giving credence to Eq. 8. The values of intercepts and slopes are listed in Table 3.

When the amine concentration is kept constant, while the acetonitrile concentration is varied, Eq. 12, becomes applicable.

$$k_A = k' + k''[CH_3CN] \tag{12}$$

Table 3. Values of intercepts and slopes of the plots of k_A against [aniline]^2 at constant acetonitrile concentrations for the reaction of phenyl 2,4,6-trinitrophenyl ether with aniline in benzene–acetonitrile mixtures

% Acetonitrile	$10^4\,k'$ /dm^3 mol^{-1} s^{-1}	$10^2\,k''$ dm^6/mol^{-2} s^{-1}
0.1	1.09	2.24
0.2	1.32	2.46
0.3	1.59	2.69
0.4	1.85	2.94
0.6	2.75	3.29

where k' and k'' are given by Eqs. 13 and 14, respectively.

$$k' = \frac{k'_1 k_2 K}{k'_{-1}} + \frac{k_1 k_2 K}{k'_{-1}} + \frac{k_2 k_3^{CH_3CN}}{k'_{-1}} + \left(\frac{k_1 k_3^{B} K}{k'_{-1}} + \frac{k_3^{B} k_3^{CH_3CN}}{k'_{-1}}\right)[B]^2 \quad (13)$$

$$k'' = \frac{k'_1 k_2 K^2}{k'_{-1}} + \frac{k_2 k_3^{CH_3CN} K}{k'_{-1}} \quad (14)$$

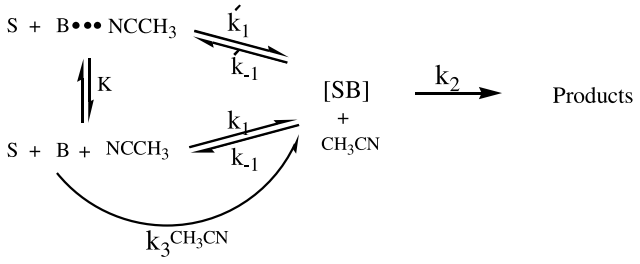
The plots of k_A against [acetonitrile] gave straight lines in accordance with Eq. 12. The values of the intercepts and slopes with the correlation coefficients are listed in Table 4.

Table 4. Values of intercepts and slopes of the plots of k_A against [acetonitrile] at constant aniline concentrations for the reaction of phenyl 2,4,6-trinitrophenyl ether with aniline in benzene–acetonitrile mixtures at 25 °C

[Aniline]/mol^{-1}	$10^2\,k'$/mol^{-1} s^{-1}	$10^2\,k''$ dm^6/mol^{-2} s^{-1}	r
0.15	0.538	4.30	0.999
0.20	0.8581	6.25	0.998
0.25	1.347	8.76	0.9998
0.30	1.933	11.25	0.9988

2.4. Mechanism of the reaction that is not base-catalysed

For the reaction of PTPE with cyclohexylamine which is not base-catalysed, but is catalysed by acetonitrile, Scheme 2 applies.



Scheme 2.

Application of steady-state hypothesis to Scheme 2, working in terms of the stoichiometric base concentration gives the observed overall second order rate constant k_A as

$$k_A = \frac{k_2\left(\frac{k'_1 K[CH_3CN]}{1+K[CH_3CN]}\right) + \frac{k_1}{1+K[CH_3CN]} + \frac{k_3^{CH_3CN}[CH_3CN]}{1+K[CH_3CN]}}{k'_{-1}[CH_3CN] + k_{-1} + k_2} \quad (15)$$

Since the reaction is not base-catalysed, the first step is rate-determining and the inequality in Eq. 16 holds.

$$k_2 \gg k'_{-1}[CH_3CN] + k_{-1} \quad (16)$$

$$\therefore k_A = \frac{k'_1 K[CH_3CN] + k_1 + k_3^{CH_3CN}[CH_3CN]}{1 + K[CH_3CN]}$$

At low acetonitrile concentration, $1 + K[CH_3CN] \approx 1$

$$\therefore k_A = k_1 + (k'_1 K + k_3^{CH_3CN})[CH_3CN] \quad (17)$$

The reaction of PTPE with cyclohexylamine in benzene is not base-catalysed but is catalysed by acetonitrile and so conforms with Scheme 2 and Eq. 17 derived from it.

A plot of the second-order rate constant k_A against [acetonitrile] thus gives a straight line with the intercept k_1 being 12.84×10^{-3} dm^3 mol^{-1} s^{-1} and slope given by $k'_1 K + k_3^{CH_3CN}$, showing that the reaction is catalysed by acetonitrile.

When no acetonitrile is present in the reaction medium Eq. 17 reduces to Eq. 18.

$$k_A = k_1 \quad (18)$$

The observed rate constant then becomes equal to the rate constant for the formation of the zwitterionic intermediate complex in the first step of the reaction in pure benzene, as is usually the case with non base-catalysed reactions.

2.5. Other hydrogen-bond acceptors

Other hydrogen-bond acceptors such as triethylamine or pyridine, being stronger bases with high pK_a values, 11.01 and 5.58, respectively, can easily act as hydrogen-bond acceptors by forming aggregates with weaker amines like aniline (pK_a, 4.61). These aggregates will be similar to that proposed for acetonitrile and aniline (Eq. 2). The formed amine–amine aggregate [$R_3N\cdots HNHR$] can further use the second proton on the aniline to engage in similar hydrogen-bond formation with a second tertiary amine molecule thus:

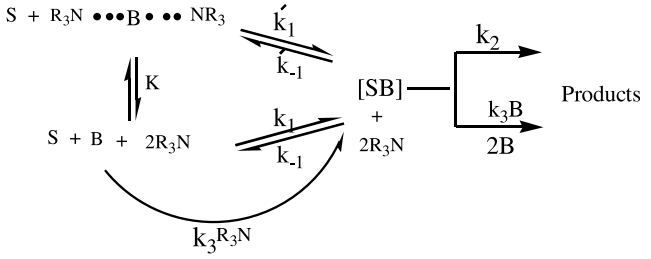
$$2R_3N + RNH_2 \overset{K}{\rightleftharpoons} R_3N\cdots HNRH\cdots NR_3 \quad (19)$$

where K is the association constant for the aggregate formation.

The aggregate formed in Eq. 19 will be a better nucleophile than that of the free amine molecule. It is this new aggregate with much enhanced nucleophilicity that now attacks the substrate in the first step of the S_NAr reaction to give the intermediate complex shown in Scheme 3, where S stands for the substrate, B for the base, and SB for the zwitterionic intermediate.

Scheme 3 is similar to Scheme 1 except that two molecules of hydrogen-bond acceptor (non-nucleophilic amine) are involved in aggregate formation with the nucleophilic amine.

Application of the steady-state hypothesis to Scheme 3 with the necessary assumptions gives the observable second-order rate constant k_A as shown in Eq. 20.



Scheme 3.

$$k_A = \frac{k'_1 k_2 K}{k'_{-1}} + \frac{k_1 k_2 K}{k'_{-1}} + \frac{k_2 k_3^{R_3N}}{k'_{-1}} + \left(\frac{k'_1 k_2 K^2}{k'_{-1}} + \frac{k_2 k_3^{R_3N} K}{k'_{-1}}\right)[R_3N]^2 + \left(\frac{k'_1 k_3^B K}{k'_{-1}} + \frac{k_3^B k_3^{R_3N}}{k'_{-1}}\right)[B]^2 \quad (20)$$

A plot of k_A against $[R_3N]^2$ should therefore give a straight line thus giving credence to the fact that two molecules of the non-nucleophilic amine as well as two molecules of the nucleophilic amine (aniline) are involved in the catalysis of the reaction.

This thus explains the kinetic behaviour observed by Nudelman and Montsserat in their reaction of 2,4-dinitrofluorobenzene with aniline in toluene when a non-nucleophilic amine such as pyridine was added as catalyst.[13] It is thus obvious that these authors' assertions[12] that such kinetic behaviour could not be explained by the mechanism of Banjoko et al. but by only the 'dimer nucelophile' mechanism is clearly erroneous as already established in our previous paper.[8]

3. Conclusion

Addition of hydrogen-bond acceptor solvent to S_NAr reactions involving a substrate and an amine in non-polar aprotic solvent results in the formation of amine-solvent aggregates of increased nucleophilicity thus causing an increase in the rate of reaction in addition to its catalytic effect. The role of the hydrogen-bond acceptor co-solvent could, however, be played by the addition of a more basic non-nucleophilic amine, that is, one having a higher pK_a than the nucleophilic amine. The resulting amine–amine aggregate will be a better nucleophile than the nucleophilic amine.

4. Experimental

Phenyl 2,4,6-trinitrophenyl ether (PTPE) was prepared by the reaction of potassium phenolate with picril chloride in aqueous ethanol. The product was precipitated with water and recrystallised from ethanol.[18] Aniline was dried over potassium hydroxide for 3 days and twice distilled over Zn powder (bp 182–183°C, lit.[19] 184°C). Cyclohexylamine was heated under reflux for 6 h and then distilled. The process was repeated twice and the middle fraction distilling at 132 °C was collected (lit. 132–133 °C).[19] Analar acetonitrile (500 cm^3) was poured over phosphorous pentoxide in a 1-dm^3 round bottomed flask, refluxed for 3 h and then distilled. The process was repeated twice and the fraction that distilled at 81 °C was collected and stored in a dessicator (lit.[19] bp 81 °C). Reaction products were prepared by the reaction of the substrate with twice its molar concentration of the appropriate amine in benzene. The volume of each reaction was reduced to about a third to allow the precipitation of the product.

N-(2,4,6-Trinitrophenyl)aniline was crystallised from glacial acetic acid and then toluene, mp 181 °C (lit.[20] 181–182 °C), λ_{max} (C_6H_6) 370 nm.

N-(2,4,6-Trinitrophenyl)cyclohexylamine was crystallised from toluene, mp 90–91 °C (lit.[20] 181–182 °C), λ_{max} (C_6H_6) 370 nm.

Kinetic procedure. The reactions were studied spectrophotometrically under conditions of excess nucleophile over substrate by measuring the increase in absorbance of the product of the reaction of each amine at the respective absorption maximum. The reaction of aniline with the substrate was carried out using pipette procedure. Solutions of PTPE (25 cm^3, 1.0×10^{-3} mol dm^{-3}) and aniline (50 cm^3, 1.5×10^{-1} to 3.0×10^{-1} mol dm^{-3}) were allowed separately to attain 29 °C in a thermostated bath. The aniline solution (25 cm^3) was quickly transferred into the substrate solution and thoroughly mixed. A 2 cm^3 aliquots of the reaction mixture was immediately pipetted and added to 20 cm^3 of quenching mixture (1 mol dm^{-3} H_2SO_4/methanol solution) in a small container. The instant of addition of the aliquot to the quenching mixture was noted as the initial time (zero time) for the reaction. Ten of such aliquots were afterwards pipetted at regular time intervals, t, and each added to 20 cm^3 of the quenching mixture. The absorbance of each quenched reaction mixture was determined. The reaction of cyclohexylamine with the substrate (which was much faster) was monitored directly in the spectrophotometer. For reactions in mixed solvents, the acetonitrile content (v/v) refers to its final volume in the reaction mixture. In all cases the absorption spectrum of the reaction mixture at 'infinity time' corresponded within 2% of the 'mock' infinity prepared by using the respective *N*-(2,4,6-trinitrophenyl)amine obtained as a product of the reaction. The observed pseudo-first-order rate constants were obtained by the least squares method as the slope of the correlation $\log(A_\infty - A_t)$ against t, where A_∞ is the optical density of the reaction solution measured at 'infinity' time (more than 10 half lives). In all cases, the reaction followed pseudo-first-order kinetics well to at least 70% reaction. The second-order rate constants k_A were obtained by dividing the pseudo-first-order rate constants by the amine concentrations. All rates were accurate to within $\pm2\%$.

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Synthesis of strapped zinc chlorophyll derivatives and their complexation with a single axial ligand

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Abstract—Zinc chlorins having a bridged moiety between 3- or 8- and 17-positions were designed as protected chlorophylls at one of π-faces, aiming at the investigation of asymmetric coordination ability towards the central metal. These strapped chlorins were synthesized by the cyclization of the dihydroxylated chlorins with dicarboxylic acid dichlorides under highly diluted conditions. The synthetic zinc chlorins complexed with pyridine as an axial ligand in benzene to form 5-coordinated species. The 1:1 binding constants determined by UV–vis titration method were smaller than those of the corresponding acyclic (unstrapped) compounds.

1. Introduction

Chlorophylls(Chls) are known as main pigment molecules that engage in light-harvesting, energy transfer, and charge transfer processes in natural photosynthetic systems.[1] Recent X-ray crystallographic analyses have successfully shown the detailed structure of pigment–protein complexes, where (bacterio)chlorophylls are fixed in proper locations by (in)direct coordination of the functional groups in a protein to the central magnesium.[2–5] In most cases, the nitrogen atom of histidine residue is used as the fifth ligand. Based on the structural data provided by Protein Data Bank and the molecular modelling, we and Balaban et al., reported that the back side of Chls (see Fig. 1) was the favored face for the fifth ligation.[6,7]

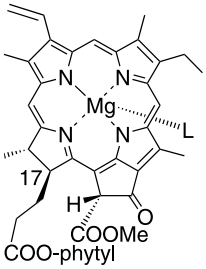

Figure 1. Molecular structure of chlorophyll-*a* having an axial ligand (L) on its back side. In this work, the front and back faces are defined as the same and opposite directions of the 17-propionate, respectively.

On the other hand, self-aggregates of chlorophylls without any support from proteins are available in a special light-harvesting antenna system of photosynthetic green bacteria.[8–10] Intermolecular interactions of composite Chls among the 3^1-hydroxy, central magnesium, and 13-carbonyl moieties together with π–π interaction of the chlorin macrocycles form a core part of the special antennas.

In both the above cases, coordination to central metal is a key factor to form the sophisticated supramolecular structures. However, there are only a few experimental reports of studying the relationship between the molecular structures of naturally occurring cyclic tetrapyrroles and their abilities to coordinate ligand molecules.[11,12] To investigate the stereoselective coordination chemistry of Chls, new model compounds that can control an axial-ligation towards central metal are required. Although, a large number of porphyrin derivatives possessing a bridged linkage over a macrocycle have been reported,[13–20] essentially no reports are available for this kind of synthetic chlorophylls to study the fifth ligation. A few related examples appeared as cyclic chlorophyll dyads, which were prepared as model structures of a special pair of photosynthetic reaction centers or the stacked supramolecular structures found in some innermembrane antennas.[21–23]

As model compounds that can restrict axial ligation to one side of the two macrocycle faces, we designed new zinc chlorins having a bridged structure. In this work, we report the synthetic route of strapped chlorins Zn-**1**/**2** (Fig. 2) and their binding ability towards pyridine.

Keywords: Chlorin; Coordination; Cyclization; Macrocycles.
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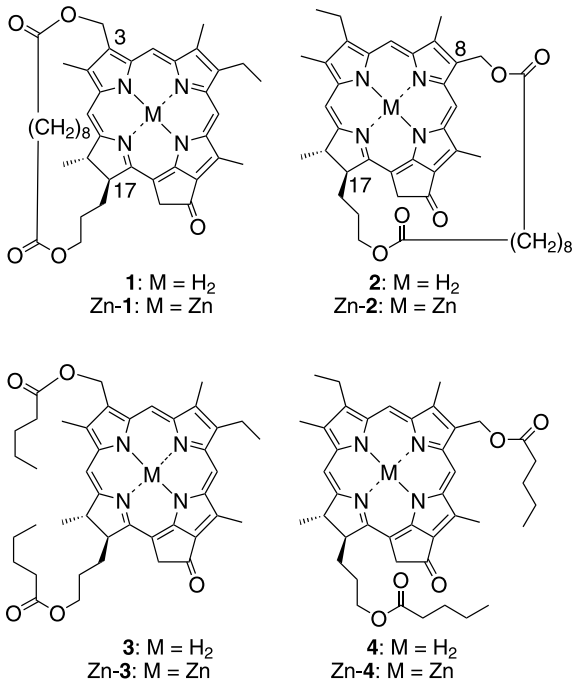


Figure 2. Molecular structures of strapped chlorins (Zn-)**1** and (Zn-)**2**, and their acyclic (unstrapped) reference compounds (Zn-)**3** and (Zn-)**4**.

2. Results and discussion

Two kinds of strapped zinc chlorins Zn-**1** and Zn-**2** were designed based on a molecular modeling study.[24–26] As illustrated in Figure 3, a bridge of octamethylene unit seems to be enough to act as a hood that would prevent an axial ligand from coordinating from the front side. The synthesis of these strapped chlorins (Zn-)**1/2** is outlined in Scheme 1. Methyl pyropheophorbide-*a* (**5**, Scheme 1),[27,28] which, is easily obtained from Chl-*a*, was used as a starting material. The 13-carbonyl group of **5** was protected as a ketal form,[29,30] and reduction of the 17^2-methoxycarbonyl group with $LiAlH_4$[31] followed by deprotection in an acidic condition gave chlorin **6** having 17^3-hydroxy group (>98% yield for the reduction). The 3-vinyl group of **6** was oxidatively cleaved by OsO_4 and $NaIO_4$[27,28,32] to give 3-formyl-chlorin in 92% yield in spite of the presence of the primary alcohol on the 17-position. Selective reduction of the 3-formyl group was done using *t*-$BuNH_2 \cdot BH_3$[27,28] to form diol compound **7** in 74% yield. In this mild reduction, the 13-keto carbonyl group was not altered. Cyclization of diol **7** with sebacoyl chloride ($ClCO(CH_2)_8COCl$) was performed under highly diluted conditions to give the desired chlorin **1** in 38% yield. Because **1** was obtained as a single conformer based on its HPLC analysis[†] and ^1H NMR spectrum,[‡] octamethylene bridge is assumed to be on the front side of the macrocycle. Double esterification of **7** with valeric acid gave the acyclic compound **3**. Zinc insertion was carried out by standard procedures[11,28] to form strapped zinc chlorin Zn-**1** and its reference compound Zn-**3**.

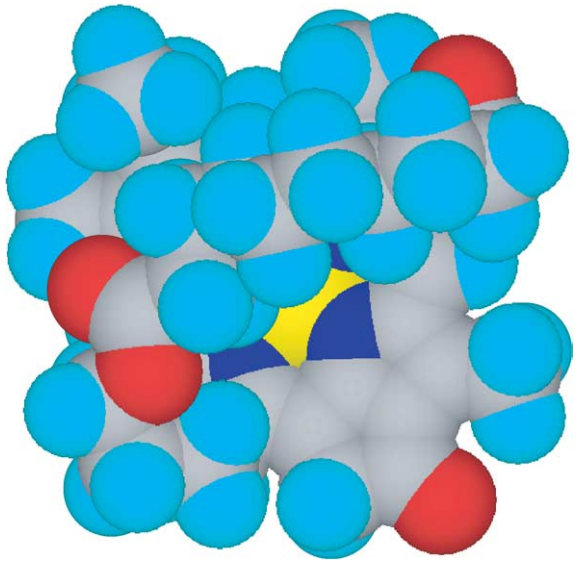


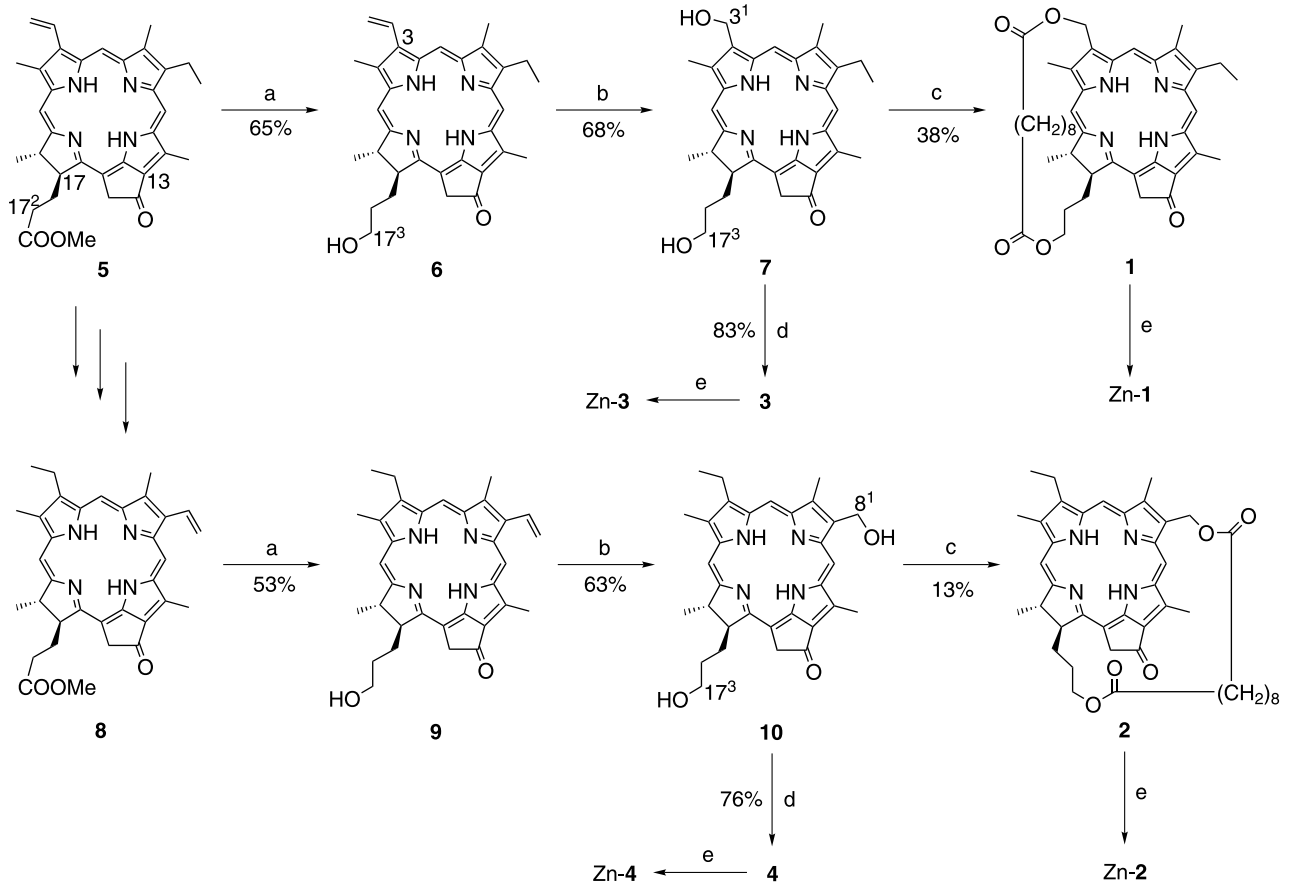
Figure 3. Space filling model of strapped chlorin Zn-**2** in an energy-minimized structure by MM+/PM3 calculation.[24–26] Hydrogen, carbon, nitrogen, oxygen, and zinc atoms are represented by light blue, gray/black, blue, red, and yellow, respectively.

For the preparation of the 8–17 strapped chlorin **2**, methyl 8-vinyl-mesopyropheophorbide-*a* (**8**)[33] was selected due to the presence of the reactive 8-vinyl group instead of the 3-vinyl group. The above synthetic route of **5**→ (Zn-)**1** was applied for **8**→ (Zn-)**2** as follows. The reduction of 17-propionate of **8** with $LiAlH_4$ was done via protection/deprotection of the 13-carbonyl group in 53% total yield. The oxidation of 8-vinyl to 8-formyl group and the following reduction to 8-hydroxymethyl group was performed in 87 and 72%, respectively. The cyclization of diol **10** with sebacoyl chloride gave the strapped chlorin **2** in 13% yield. The cyclization yield was about one-third of that in **7**→**1** (38%). The decrease is ascribable to the longer intramolecular distance between 8^1- and 17^3-hydroxy groups in **10** than that between 3^1- and 17^3-OHs in **7**. It should be noted that the reaction of diol **7** with suberoyl chloride ($ClCO(CH_2)_6COCl$) gave the corresponding 3–17 strapped chlorin **11** (Fig. 4) having a shorter methylene bridge in 23% yield, while no 1:1 cyclic compound was obtained in the case of diol **10** possessing more distant reactive hydroxy groups in a molecule. The ^1H NMR spectra of strapped chlorins (Zn-)**1**, (Zn-)**2**, and **11** showed characteristic upfield-shifted signals of the linked methylene protons up to 0 ppm (see Supplementary data), indicating that the bridge is located in a shielding region of the front side of a chlorin π-system.[‡]

Major absorption maxima of free-base and zinc chlorins (Zn-)**1/2** are summarized in Table 1. Because the absorption spectra of strapped chlorins (Zn-)**1**(or **2**) and acyclic references (Zn-)**3**(or **4**) are almost the same in less polar benzene or coordinatable THF, it is assumed that the bridging unit does not strongly affect the structure of the chlorin macrocycle but just covers the front face towards the fifth axial ligation. On the other hand, it is interesting to note that the Q_y peak shift between (Zn-)**1/3** and (Zn-)**2/4** caused by the different position of the substitutent is relatively large (ca. 10 nm), suggesting a possibility of

[†] HPLC analysis was carried out with a packed ODS column (Cosmosil 5C18-ARII, 10 mm∅×250 mm), t_R=32 min (MeOH, 2.0 ml/min).

[‡] The movement of strapped moiety from the front to back sides on chlorin macrocycle was excluded based on the molecular modeling study,[24–26] especially in the case of 8–17 strapped chlorins.



Scheme 1. Synthesis of strapped chlorins (Zn-)**1** and (Zn-)**2**, and their reference compounds (Zn-)**3** and (Zn-)**4**; (a) (i) $HOCH_2CH_2OH$, TMSCl, CH_2Cl_2; (ii) $LiAlH_4$, THF; (iii) 5% HCl–THF; (b) (i) OsO_4, $NaIO_4$, aq AcOH–THF; (ii) *t*-$BuNH_2 \cdot BH_3$, CH_2Cl_2; (c) sebacoyl chloride, Et_3N, CH_2Cl_2; (d) valeric acid, EDC·HCl, DMAP, CH_2Cl_2; (e) $Zn(OAc)_2 \cdot 2H_2O$, CH_2Cl_2–MeOH.

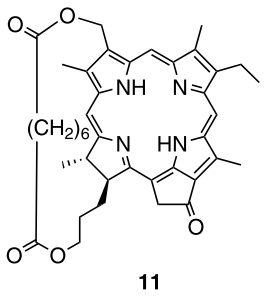


Figure 4. Molecular structure of strapped chlorin **11** having a hexamethylene bridge.

tuning an absorption maximum using a (carbonyloxy)-methyl group, $-CH_2OCO-$.

Figure 5a shows the change of the absorption spectra of Zn-**1** upon addition of pyridine in benzene. The spectra reveal isosbestic points at 425 and 655 nm, and the 1:1 binding constant (K) was calculated by curve fitting method[34] at several different wavelengths as shown in Figure 5b. The averaged K value was $2.3\times10^4\ M^{-1}$. The complex formation ability of Zn-**1** with other guest ligands was also examined. The K values of Zn-**1** with 3,5-lutidine and quinoline were 3.1×10^4 and $4.1\times10^2\ M^{-1}$, respectively. The slightly larger value in complexation of lutidine compared to pyridine is ascribed to an increase in basicity of

Table 1. Absorption maxima (λ_{max}/nm) of free-base and zinc chlorins[a]

Metal-free chlorins	Benzene		THF		Zinc chlorins	Benzene		THF	
	Soret	Q_y	Soret	Q_y		Soret	Q_y	Soret	Q_y
1	411	668	409	664	Zn-**1**	425	654	425	651
2	417	658	414	656	Zn-**2**	426	645	427	643
3	412	667	409	664	Zn-**3**	425	653	425	650
4	417	657	413	655	Zn-**4**	426	643	426	642

[a] Each sample was measured at ca. 10 μM.

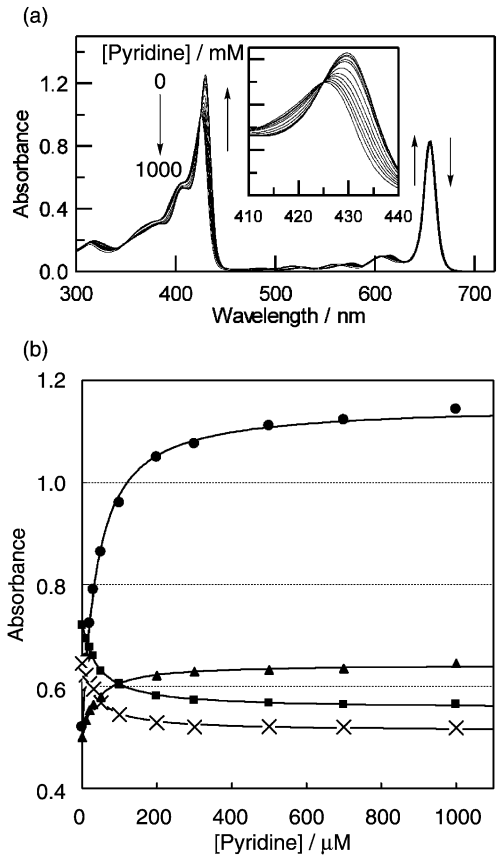


Figure 5. (a) UV–vis spectra of strapped chlorin Zn-**1** with addition of pyridine in benzene and (b) their absorption change at (■) 417, (●) 432, (×) 650, and (▲) 660 nm. [Zn-**1**] = 1.0×10^{-5} M.

the guest ligand, while the decrease by two orders of magnitude by pyridine → quinoline is due to its sterical hindrance and less basic nitrogen atom towards the coordination. It turned out that more sterically hindered and less basic acridine showed no complexation ability.

The averaged K values (K_{av}) of zinc chlorins (Zn-)**1**–**4** are summarized in Table 2. The K value of Zn-**3** is 1.4 times that of strapped Zn-**1**, and the K value of Zn-**4** is 1.6 times that of Zn-**2**. The observed difference would permit two interpretations. Because the binding constant is known to increase as the tetrapyrrole π-plane becomes more flexible in the order of bacteriochlorin > chlorin > porphyrin,[11] the observed smaller K values of the strapped chlorins can be explained by the reduced flexibility of the chlorin macrocycle. The bridged structure would disturb the formation of a pyramidal 5-coordinated complex with pyridine. Another possible explanation would be the preference of the front face for coordination under the experimental conditions. Strapped zinc chlorins Zn-**1/2** allow ligands to coordinate only from their back faces, and acyclic references Zn-**3/4** opening both the front and back faces might have the stronger binding ability of an axial ligand from the front face.

Table 2. Association constant K for 1:1 complexation of zinc chlorins Zn-**1**–**4** with pyridine[a]

Compounds	K/M^{-1} (λ/nm)	K_{av} (M^{-1})
Zn-**1**	26,200 (417)	2.9×10^4
	28,300 (432)	
	30,000 (650)	
	31,100 (660)	
Zn-**2**	23,800 (415)	2.3×10^4
	21,600 (431)	
	26,400 (640)	
	20,200 (650)	
Zn-**3**	44,600 (417)	4.2×10^4
	36,600 (431)	
	47,300 (650)	
	40,400 (660)	
Zn-**4**	34,300 (415)	3.7×10^4
	36,100 (430)	
	39,400 (640)	
	39,900 (650)	

[a] Association constants were determined in benzene by UV–vis titration method.

Zn-**1/3** possessing an oxymethyl group at the 3-position, in contrast, showed slightly larger K values compared to their corresponding 8-position analogues Zn-**2/4**. The difference is probably due to the stereoelectronic effect through a σ–π interaction of the C–O bond and chlorin π system.

In summary, we synthesized two kinds of strapped zinc chlorins and examined their binding ability towards the fifth ligand in benzene. The strapped chlorins showed smaller binding constants than acyclic reference compounds did. The concept of molecular design was shown to be useful for the development of model compounds that allow investigation of the (face-selective) coordination chemistry of photosynthetic systems.

3. Experimental

3.1. General

^{1}H NMR spectra were recorded on a Bruker AC-300 or a JEOL JNM-ECA600HR spectrometer. All chemical shifts are reported relative to the residual solvent peak: δ = 7.26 ppm ($CHCl_3$). UV–vis spectra were measured on a Hitachi U-3500 spectrophotometer. FAB-MS spectra were recorded on a JEOL GCmate II spectrometer. THF and CH_2Cl_2 were dried and distilled over CaH_2 before use. Other solvents and reagents were employed as purchased without further purification. All synthetic procedures were performed in the dark.

Methyl pyropheophorbide-*a* (**5**),[27,28] and 8-deethyl-8-vinyl-mesopyropheophorbide-*a* (**8**)[33] were prepared as previously reported. Zinc-metallation of free base chlorins was done according to the reported procedure.[11,28]

3.2. Preparation of pyropheophorbide derivatives

3.2.1. 17^2-Decarboxy-17^2-hydroxymethyl-pyropheophorbide-*a* (6). To a solution of **5** (1.10 g, 2.0 mmol) in CH_2Cl_2 (200 ml) was added ethylene glycol (12.4 g, 0.20 mol) and TMSCl (8.7 g, 0.080 mol), and stirred for 2 h at room temperature.[29,30] The reaction mixture was poured into 5% aqueous NH_3 and extracted with CH_2Cl_2. The extract was washed twice with water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by basic alumina column

chromatography deactivated with 5% water (eluent, CH_2Cl_2) followed by recrystallization from CH_2Cl_2–hexane to afford 778 mg (66%) of 13^1-ethylene ketal.

A suspension of $LiAlH_4$ (380 mg, 10 mmol) in 200 ml of THF was cooled in an ice-bath, and the above ketal (593 mg, 1.0 mmol) in 50 ml of THF was added dropwise. After an additional 10 min of stirring, 5% aqueous HCl was added dropwise. The mixture was warmed to room temperature, stirred for 10 min, diluted with water, and extracted with CH_2Cl_2. The extract was washed with 4% aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was recrystallized from CH_2Cl_2–hexane to give the titled compound **6** (510 mg, 98%) as a black solid: vis (CH_2Cl_2) λ_{max} 668 (rel. intensity, 44%), 611 (8), 539 (9), 509 (10), 414 nm (100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ=9.42, 9.34, 8.55 (each 1H, s, 5-, 10-, 20-H), 7.98 (1H, dd, J=12, 18 Hz, 3-CH), 6.27 (1H, dd, J=1, 18 Hz, 3^1-CH *trans* to 3-CH), 6.16 (1H, dd, J=1, 12 Hz, 3^1-CH *cis* to 3-CH), 5.23, 5.09 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.51 (1H, dq, J=2, 8 Hz, 18-H), 4.26 (1H, br d, J=8 Hz, 17-H), 3.64–3.83 (4H, m, 8-, 17^2-CH_2), 3.62, 3.40, 3.22 (each 3H, s, 2-, 7-, 12-CH_3), 2.40, 2.09, 1.78, 1.60 (each 1H, m, 17-CH_2CH_2), 1.82 (3H, d, J=8 Hz, 18-CH_3), 1.68 (3H, t, J=8 Hz, 8^1-CH_3), 0.46, −1.68 (each 1H, s, NH); MS (FAB) m/z 520 (M^+).

3.2.2. 17^2-Decarboxy-3-devinyl-3,17^2-bis(hydroxymethyl)-pyropheophorbide-*a* (7). According to the reported procedure,[27,28] the 3-vinyl group of **6** (469 mg, 0.90 mmol) was oxidatively cleaved by OsO_4 (ca. 30 mg), $NaIO_4$ (963 mg, 4.5 mmol), AcOH (1 ml) in water (10 ml) and THF (50 ml) to the 3-formyl group. The crude product was purified by silica gel chromatography (MeOH/CH_2Cl_2, 1:9) and recrystallization from CH_2Cl_2–hexane to give the 3-formyl compound (432 mg, 92%) as a black solid: vis (CH_2Cl_2) λ_{max} 695 (rel., 73%), 638 (10), 554 (18), 522 (17), 429 nm (100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ=11.5 (1H, s, 3-CHO), 9.42, 9.34, 8.55 (each 1H, s, 5-, 10-, 20-H), 5.31, 5.16 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.61 (1H, q, J=7 Hz, 18-H), 4.36 (1H, d, J=9 Hz, 17-H), 3.61–3.75 (4H, m, 8-, 17^2-CH_2), 3.75, 3.67, 3.27 (each 3H, s, 2-, 7-, 12-CH_3), 2.45, 2.14, 1.80, 1.64 (each 1H, m, 17-CH_2CH_2), 1.86 (3H, d, J=7 Hz, 18-CH_3), 1.69 (3H, t, J=8 Hz, 8^1-CH_3), −0.13, −1.68 (each 1H, s, NH); MS (FAB) m/z 522 (M^+).

According to the reported procedure,[27,28] the 3-formyl group of the above compound (366 mg, 0.70 mmol) was reduced by borane-*tert*-butylamine complex (87 mg, 1.0 mmol) in 50 ml of CH_2Cl_2 to the 3-hydroxymethyl group. The crude product was purified by silica gel chromatography (MeOH/CH_2Cl_2, 1:9) and recrystallization from CH_2Cl_2–hexane to give the titled compound **7** (271 mg, 74%) as a black solid: vis (CH_2Cl_2) λ_{max} 663 (rel., 47%), 607 (8), 536 (9), 505 (10), 410 nm (100); ^{1}H NMR (10% CD_3OD–$CDCl_3$, 300 MHz) δ=9.25, 9.23, 8.40 (each 1H, s, 5-, 10-, 20-H), 5.65 (2H, s, 3-CH_2), 5.08, 4.92 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.37 (1H, d, J=7 Hz, 18-H), 4.09 (1H, d, J=9 Hz, 17-H), 3.49–3.53 (4H, m, 8-, 17^2-CH_2), 3.45, 3.24, 3.08 (each 3H, s, 2-, 7-, 12-CH_3), 2.25, 1.88, 1.74, 1.46 (each 1H, m, 17-CH_2CH_2), 1.67 (3H, d, J=7 Hz, 18-CH_3), 1.53 (3H, t, J=8 Hz, 8^1-CH_3), NH peaks were too broad to be observed; MS (FAB) m/z 524 (M^+).

3.2.3. 3-17 $(CH_2)_8$-Strapped chlorin 1. To a stirred solution of Et_3N (61 mg, 0.60 mmol) in CH_2Cl_2 (20 ml) was added simultaneously a solution of diol **7** (105 mg, 0.20 mmol) in THF (2 ml) and CH_2Cl_2 (20 ml) and a solution of sebacoyl chloride (48 mg, 0.20 mmol) at the same rate over an hour. After additional 30-min of stirring, the mixture was poured into 3% aqueous HCl. The separated organic phase was washed with 4% aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (Et_2O/CH_2Cl_2, 1:9) to give **1** (52 mg, 38%) as a black solid: vis (CH_2Cl_2) λ_{max} 666 (rel., 54%), 607 (8), 537 (10), 507 (10), 411 nm (100); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.57 (1H, s, 10-H), 9.54 (1H, s, 5-H), 8.61 (1H, s, 20-H), 6.63, 6.29 (each 1H, d, J=13 Hz, 3-CH_2), 5.20, 5.17 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.47 (1H, q, J=8 Hz, 18-H), 4.24–4.30 (2H, m, 17-H and 17^2-CH), 3.79 (1H, m, 17^2-CH), 3.73 (2H, q, J=8 Hz, 8-CH_2), 3.68 (3H, s, 7-CH_3), 3.47 (3H, s, 2-CH_3), 3.31 (3H, s, 12-CH_3), 2.16, 1.80 (each 1H, m, 17-CH_2), 1.70 (3H, d, J=8 Hz, 18-CH_3), 1.80, 1.66 (each 1H, m, 17^1-CH_2), 1.60 (3H, t, J=8 Hz, 8^1-CH_3), 2.43, 2.27, 1.80, 1.60, 1.41, 1.27, 0.91, 0.81, 0.67, 0.53, 0.53, 0.45, 0.45, 0.45, 0.18, 0.04 (each 1H, m, $(CH_2)_8$), 0.28, −1.81 (each 1H, s, NH); HRMS (FAB) m/z 691.3885 (MH^+), calcd for $C_{42}H_{51}N_4O_5$ 691.3859.

3.2.4. 3-17 Strapped zinc chlorin Zn-1. Vis (benzene) λ_{max} 654 (ε, 84,900), 606 (10,100), 559 (4900), 517 (3700), 425 nm (102,000); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.27 (1H, s, 10-H), 9.22 (1H, s, 5-H), 8.44 (1H, s, 20-H), 6.47, 6.13 (each 1H, d, J=13 Hz, 3-CH_2), 4.89, 4.81 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.39 (1H, q, J=8 Hz, 18-H), 4.19 (1H, ddd, J=2, 2, 9 Hz, 17-H), 3.90, 3.35 (each 1H, m, 17^2-CH_2), 3.52–3.59 (2H, m, 8-CH_2), 3.48 (3H, s, 12-CH_3), 3.35 (3H, s, 2-CH_3), 3.15 (3H, s, 7-CH_3), 2.09, 1.79 (each 1H, m, 17-CH_2), 1.88 (3H, d, J=8 Hz, 18-CH_3), 1.66, 1.45 (each 1H, m, 17^1-CH_2), 1.60 (3H, t, J=8 Hz, 8^1-CH_3), 2.38, 2.28, 1.54, 1.43, 1.40, 1.34, 0.66, 0.61, 0.57, 0.48, 0.48, 0.39, 0.37, 0.33, 0.15, 0.09 (each 1H, m, $(CH_2)_8$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ=196.5, 173.7, 173.0, 168.2, 161.1, 157.1, 153.0, 150.1, 147.2, 146.8, 145.1, 143.7, 138.4, 135.8, 134.5, 133.6, 131.7, 106.1, 105.2, 98.9, 92.5, 63.3, 56.5, 50.7, 49.1, 47.7, 34.4, 34.0, 30.4, 28.4, 28.3, 28.2, 27.8, 25.4, 25.0, 24.6, 23.3, 19.3, 17.4, 12.6, 11.6, 10.9; HRMS (FAB) m/z 752.2934 (M^+), calcd for $C_{42}H_{48}N_4O_5{}^{64}Zn$ 752.2916.

3.2.5. 3-17 $(CH_2)_6$-Strapped chlorin 11. Reaction of diol **7** (105 mg, 0.20 mmol) with suberoyl chloride (42 mg, 0.20 mmol) was carried out similarly as described for the preparation of **1**. The product was purified by silica gel chromatography (Et_2O/CH_2Cl_2, 1:9) to give **11** (30 mg, 23%) as a black solid: vis (CH_2Cl_2) λ_{max} 666 (rel., 53%), 607 (8), 538 (10), 508 (10), 412 nm (100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ=9.54, 9.52, 8.59 (each 1H, s, 5, 10, 20-H), 6.56, 6.34 (each 1H, d, J=12 Hz, 3-CH_2), 5.24, 5.09 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.48 (1H, q, J=7 Hz, 18-H), 4.38 (1H, m, 17-H), 3.99, 3.29 (each 1H, m, 17^2-CH_2), 3.71 (2H, q, J=8 Hz, 8-CH_2), 3.68, 3.46, 3.31 (each 3H, s, 2, 7, 12-CH_3), 2.28, 2.23, 2.02, 1.94 (each 1H, m, 17-CH_2CH_2), 1.81 (3H, d, J=7 Hz, 18-CH_3), 1.71 (3H, t, J=8 Hz, 8^1-CH_3), 0.65–1.26, 0.31–0.38, 0.06–0.13 (6H, 3H, 3H, m,

$(CH_2)_6$), 0.38, −1.74 (each 1H, s, NH); HRMS (FAB) *m/z* 662.3474 (M^+), calcd for $C_{40}H_{46}N_4O_5$ 662.3468.

3.2.6. 3,17-Reference chlorin 3. To a solution of diol **7** (105 mg, 0.20 mmol) in CH_2Cl_2 (30 ml) was added valeric acid (102 mg, 1.0 mmol), EDC·HCl (192 mg, 1.0 mmol), and DMAP (244 mg, 2.0 mmol), and the mixture was stirred for 12 h at room temperature. The mixture was poured into 3% aqueous HCl and extracted with CH_2Cl_2. The extract was washed with 4% aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (Et_2O/CH_2Cl_2, 1:19) to give **3** (115 mg, 83%) as a black solid: vis (CH_2Cl_2) λ_{max} 664 (rel., 51%), 607 (8), 536 (9), 505 (10), 410 nm (100); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.50, 9.39, 8.61 (each 1H, s, 5-, 10-, 20-H), 6.36 (2H, s, 3-CH_2), 5.23, 5.13 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.53 (1H, dq, J=2, 8 Hz, 18-H), 4.31 (1H, br d, J=9 Hz, 17-H), 4.12 (2H, t, J=7 Hz, 17^2-CH_2), 3.69 (2H, q, J=8 Hz, 8-CH_2), 3.67, 3.45, 3.26 (each 3H, s, 2-, 7-, 12-CH_3), 2.45, 2.27 (each 2H, t, J=8 Hz, $CH_2COO\times2$), 2.41, 2.11, 1.89, 1.63 (each 1H, m, 17-CH_2CH_2), 1.84 (3H, d, J=8 Hz, 18-CH_3), 1.70 (3H, t, J=8 Hz, 8^1-CH_3), 1.69, 1.57 (each 2H, quintet, J=8 Hz, $CH_2CCOO\times2$), 1.34, 1.31 (each 2H, sextet, J=8 Hz, $CH_2C_2COO\times2$), 0.86, 0.87 (each 3H, t, J=8 Hz, $CH_3C_3COO\times2$), 0.30, −1.80 (each 1H, s, NH); HRMS (FAB) *m/z* 693.4033 (MH^+), calcd for $C_{42}H_{53}N_4O_5$ 693.4016.

3.2.7. 3,17-Reference zinc chlorin Zn-3. Vis (benzene) λ_{max} 653 (ε, 84,600), 605 (10,100), 555 (4800), 515 (4200), 425 nm (105,000); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.26, 9.09, 8.46 (each 1H, s, 5-, 10-, 20-H), 6.21 (2H, s, 3-CH_2), 4.80, 4.90 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.48 (1H, dq, J=2, 7 Hz, 18-H), 4.22 (1H, ddd, J=8, 2, 2 Hz, 17-H), 3.90–3.95 (2H, m, 17^2-CH_2), 3.55 (2H, q, J=8 Hz, 8-CH_2), 3.48, 3.34, 3.12 (each 3H, s, 2-, 7-, 12-CH_3), 2.40, 2.09 (each 2H, t, J=7 Hz, $CH_2COO\times2$), 2.31, 2.07, 1.82, 1.54 (each 1H, m, 17-CH_2CH_2), 1.66, 1.38 (each 2H, quintet, J=7 Hz, $CH_2CCOO\times2$), 1.88 (3H, d, J=7 Hz, 18-CH_3), 1.60 (3H, t, J=8 Hz, 3H, 8^1-CH_3), 1.21, 1.33 (each 2H, sextet, J=7 Hz, $CH_2C_2COO\times2$), 0.82, 0.84 (each 3H, t, J=7 Hz, $CH_3C_3COO\times2$); HRMS (FAB) *m/z* 754.3060 (M^+), calcd for $C_{42}H_{50}N_4O_5{}^{64}Zn$ 754.3073.

3.2.8. 17^2-Decarboxy-8-deethyl-17^2-hydroxymethyl-8-vinyl-mesopyropheophorbide-*a* (9). Protection of the 13-keto-carbonyl group of **8**, reduction of the 17^2-ester by $LiAlH_4$, and deprotection of the ketal with dilute aqueous HCl were performed as described for the transformation of **5** to **6** to give **9** (53% yield) as a black solid: vis (CH_2Cl_2) λ_{max} 658 (rel., 38%), 602 (7), 541 (5), 508 (8), 418 nm (100); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.54, 9.23, 8.48 (each 1H, s, 5-, 10-, 20-H), 7.88 (1H, dd, J=11, 18 Hz, 8-CH), 6.12 (1H, dd, J=2, 18 Hz, 8^1-CH *trans* to 8-CH), 5.96 (1H, dd, J=2, 11 Hz, 8^1-CH *cis* to 8-CH), 5.21, 5.07 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.50 (1H, dq, J=2, 7 Hz, 18-H), 4.25 (1H, ddd, J=9, 2, 2 Hz, 17-H), 3.83 (2H, q, J=8 Hz, 3-CH_2), 3.64–3.72 (2H, m, 17^2-CH_2), 3.57, 3.36, 3.29 (each 3H, s, 7-, 7-, 12-CH_2), 2.41, 2.08, 1.81, 1.61 (each 1H, m, 17-CH_2CH_2), 1.82 (3H, d, J=7 Hz, 18-CH_3), 1.73 (3H, t, J=8 Hz, 3^1-CH_3), 0.45, −1.67 (each 1H, s, NH); MS (FAB) *m/z* 520 (M^+).

3.2.9. 17^2-Decarboxy-8-deethyl-8,17-bis(hydroxymethyl)-mesopyropheophorbide-*a* (10). Oxidation[27,28] of the 8-vinyl of **9** to formyl group gave 8-formyl compound (87% yield) as a black solid: vis (CH_2Cl_2) λ_{max} 660 (rel., 26%), 607 (5), 572 (3), 521 (4), 413 (100); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=11.09 (1H, s, CHO), 10.25, 9.30, 8.57 (each 1H, s, 5-, 10-, 20-H), 5.22, 5.08 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.55 (1H, dq, J=2, 8 Hz, 18-H), 4.29 (1H, ddd, J=8, 2, 2 Hz, 17-H), 3.85 (2H, q, J=8 Hz, 3-CH_2), 3.68–3.75 (2H, m, 17^2-CH_2), 3.61, 3.55, 3.33 (each 3H, s, 7-, 7-, 12-CH_2), 2.45, 2.13, 1.80, 1.73 (each 1H, m, 17-CH_2CH_2), 1.85 (3H, d, J=8 Hz, 18-CH_3), 1.75 (3H, t, J=8 Hz, 3^1-CH_3), −0.08, −1.93 (each 1H, s, NH); MS (FAB) *m/z* 522 (M^+).

Reduction[27,28] of the resulting formyl group gave **10** (72% yield) as a black solid: vis (CH_2Cl_2) λ_{max} 656 (rel., 34%), 601 (7), 538 (7), 509 (9), 415 nm (100); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.60, 9.24, 8.50 (each 1H, s, 5-, 10-, 20-H), 5.71 (2H, s, 8-CH_2), 5.18, 5.02 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.50 (1H, dq, J=2, 8 Hz, 18-H), 4.21 (1H, br d, J=9 Hz, 17-H), 3.83 (2H, q, J=8 Hz, 3-CH_2), 3.63–3.71 (2H, m, 17^2-CH_2), 3.57, 3.35, 3.30 (each 3H, s, 2-, 7-, 12-CH_2), 2.38, 2.08, 1.78, 1.58 (each 1H, m, 17-CH_2CH_2), 1.80 (3H, d, J=8 Hz, 18-CH_3), 1.73 (3H, t, J=8 Hz, 3^1-CH_3), 0.34, −1.76 (each 1H, s, NH); MS (FAB) *m/z* 524 (M^+).

3.2.10. 8-17 Strapped chlorin 2. Cyclization of diol **10** (79 mg, 0.15 mmol) with sebacoyl chloride (42 mg, 0.20 mmol) was carried out as described for the preparation of **1**. The product was purified by silica gel chromatography (Et_2O/CH_2Cl_2, 1:9) to give **2** (13 mg, 13%) as a black solid: vis (THF) λ_{max} 656 (rel. intensity, 44%), 602 (6), 537 (7), 506 (8), 414 nm (100); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.72, 9.32, 8.55 (each 1H, s, 5-, 10-, 20-H), 6.59, 5.99 (each 1H, d, J=13 Hz, 8-CH_2), 5.32, 5.15 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.45–4.46 (2H, m, 17-, 18-H), 3.87 (2H, m, 3-CH_2), 3.68, 3.42, 3.32 (each 3H, s, 2-, 7-, 12-CH_3), 3.60, 3.55 (each 1H, m, 17^2-CH_2), 2.61, 2.38 (each 1H, m, 17-CH_2), 1.87 (3H, d, J=7 Hz, 18-CH_3), 1.73 (3H, t, J=8 Hz, 3^1-CH_3), 1.55, 0.11 (each 1H, m, 17^1-CH_2), 2.51, 2.25, 1.57, 1.57, 1.43, 1.32, 0.75, 0.69, 0.53, 0.30, 0.30, 0.30, 0.25, 0.13, −0.32, −0.45 (each 1H, m, $(CH_2)_8$), 0.24, −1.76 (each 1H, s, NH); HRMS (FAB) *m/z* 690.3785 (M^+), calcd for $C_{42}H_{50}N_4O_5$ 690.3781.

3.2.11. 8-17 Strapped zinc chlorin Zn-2. Vis (benzene) λ_{max} 645 (ε, 62,800), 599 (8900), 559 (4500), 513 (4700), 426 nm (98,200); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.29 (1H, s, 10-H), 9.06 (1H, s, 5-H), 8.41 (1H, s, 20-H), 6.28, 5.85 (each 1H, d, J=13 Hz, 8-CH_2), 5.01, 4.90 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.42 (1H, q, J=7 Hz, 18-H), 4.38 (1H, br s, 17-H), 3.74 (2H, q, J=7 Hz, 3-CH_2), 3.45 (3H, s, 12-CH_3), 3.29 (3H, s, 7-CH_3), 3.23 (3H, s, 2-CH_3), 3.43, 3.22 (each 1H, m, 17^2-CH_2), 2.48, 2.36 (each 1H, m, 17-CH_2), 1.91 (3H, d, J=8 Hz, 18-CH_3), 1.68 (3H, t, J=8 Hz, 3^1-CH_3), 1.55, 0.32 (1H, m, 17^1-CH_2), 2.44, 2.25, 1.41, 1.33, 1.33, 1.29, 0.82, 0.71, 0.55, 0.31, 0.08, −0.01, −0.07, −0.15, −0.27, −0.27 (each 1H, m, $(CH_2)_8$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ=196.4, 173.6, 173.0, 169.3, 161.3, 157.3, 154.7, 149.2, 147.6, 146.7, 144.9, 143.6, 136.5, 134.9, 134.2, 134.0, 131.4, 107.2, 105.0, 98.4, 92.2, 63.2, 57.0, 50.5, 47.8, 47.6, 35.1, 33.5, 29.3, 28.9, 28.8,

28.5, 28.1, 25.5, 24.2, 23.7, 23.2, 19.5, 17.3, 12.5, 11.2, 10.9;.HRMS (FAB) *m/z* 752.2932 (M^+), calcd for $C_{42}H_{48}N_4O_5^{64}Zn$ 752.2916.

3.2.12. 8,17-Reference chlorin 4. Reaction of diol **10** (21 mg, 0.040 mmol) with valeric acid (20 mg, 0.20 mmol) was carried out as described for the preparation of **3**. The product was purified by silica gel chromatography (Et_2O/CH_2Cl_2, 1:9) to give **4** (21 mg, 76%) as a black solid: vis (CH_2Cl_2) λ_{max} 655 (rel., 36%), 599 (6), 540 (6), 508 (7), 416 nm (100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ=9.60, 9.26, 8.53 (each 1H, s, 5-, 10-, 20-H), 6.20 (2H, s, 8-CH_2), 5.27, 5.09 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.52 (1H, dq, J=2, 7 Hz, 18-H), 4.28 (1H, ddd, J=9, 2, 2 Hz, 17-H), 4.11 (2H, t, J=6 Hz, 17^2-CH_2), 3.84 (2H, q, J=8 Hz, 3-CH_2), 3.66, 3.37, 3.31 (each 3H, s, 2-, 7-, 12-CH_3), 2.45, 2.26 (each 2H, t, J=8 Hz, $CH_2COO\times2$), 2.40, 2.08, 1.88, 1.61 (each 1H, m, 17-CH_2CH_2), 2.26 (2H, t, J=8 Hz), 1.83 (3H, d, J=7 Hz, 18-CH_3), 1.73 (3H, t, J=8 Hz, 3^1-CH_3), 1.57, 1.69 (each 2H, quintet, J=7 Hz, $CH_2CCOO\times2$), 1.30, 1.37 (each 2H, sextet, J=7 Hz, $CH_2C_2COO\times2$), 0.86, 0.84 (each 3H, t, J=7 Hz, $CH_3C_3COO\times2$), 0.35, −1.77 (each 1H, s, NH); HRMS (FAB) *m/z* 692.3907 (M^+), calcd for $C_{42}H_{52}N_4O_5$ 692.3938.

3.2.13. 8,17-Reference zinc chlorin Zn-4. Vis (benzene) λ_{max} 643 (ε, 61,200), 597 (7900), 559 (4500), 513 (4700), 426 nm (98,200); ^{1}H NMR ($CDCl_3$, 600 MHz) δ=9.33 (1H, s, 10-H), 9.03 (1H, s, 5-H), 8.41 (1H, s, 20-H), 6.04, 6.01 (each 1H, d, J=13 Hz, 8-CH_2), 5.01, 4.93 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.48 (1H, dq, J=2,8 Hz, 18-H), 4.22 (1H, ddd, J=9, 2, 2 Hz, 17-H), 3.96–4.02 (2H, m, 17^2-CH_2), 3.72 (2H, q, J=8 Hz, 3-CH_2), 3.52 (3H, s, 12-CH_3), 3.23 (6H, s, 7-, 7-CH_3), 2.38 (2H, t, J=8 Hz, 8^1-$OCOCH_2$), 2.33, 2.09 (each 1H, m, 17-CH_2), 2.14–2.18 (2H, m, 17^3-$OCOCH_2$), 1.88 (3H, d, J=8 Hz, 18-CH_3), 1.83, 1.59 (each 1H, m, 17^1-CH_2), 1.68 (3H, t, J=8 Hz, 3^1-CH_3), 1.64 (2H, quintet, J=8 Hz, CH_2CCOO), 1.41–1.46 (2H, m, CH_2CCOO), 1.30, 1.24 (each 2H, sextet, J=8 Hz, $CH_2C_2COO\times2$), 0.83, 0.82 (each 3H, t, J=8 Hz, $CH_3C_3COO\times2$); HRMS (FAB) *m/z* 754.3087 (M^+), calcd for $C_{42}H_{50}N_4O_5^{64}Zn$ 754.3073.

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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.2005.06.001

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Structural and stereochemical revision of isocyanide and isothiocyanate amphilectenes from the Caribbean marine sponge *Cribochalina* sp.

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Abstract—The absolute stereochemistry of amphilectene metabolites from *Cribochalina* sp. has been revised by a detailed NMR spectroscopic study of the Mosher ester derivatives of a related alcohol. The relative stereochemistry of the previously described amphilectenes has been reinvestigated and reassigned on the basis of the X-ray structural analysis carried out on one of them. The structure of a new amphilectene metabolite, which is an isothiocyanato analogue is also presented.

1. Introduction

Cytotoxic terpenes containing isocyano or isothiocyanato substituents are characteristic metabolites from marine sponges and associated molluscs.[1] Diterpene representatives of this bioactive suite of metabolites can be classified as oxygenated decalins of the kalihinol or kalihinene type,[2] usually associated with sponges of the genus *Acanthella*, or as non-oxygenated tricyclic or tetracyclic products with an amphilectene or cycloamphilectene ring system[3–12] that are characteristic of haplosclerid sponges such as *Amphimedon terpenensis*, *Ciocalypta* sp. or *Halichondria* sp. In both the ring systems, structure and stereochemical assignment by NMR spectroscopy is frequently complicated by poor signal resolution in the proton spectra, even when data are acquired at high field, thus X-ray analysis remains a basic structural tool for this group of metabolites. Amphilectene and cycloamphilectene structures, whose relative stereochemistry has been secured by X-ray, include (**1**)–(**3**);[3,5] Konig et al. have provided a comprehensive 2D NMR spectroscopic analysis of diterpenes from the tropical marine sponge *Cymbastela hooperi*.[8] Moreover, the relative stereochemistry of some of these metabolites has been secured by means of X-ray studies.[9] In 1999, we described a series of amphilectene metabolites, compounds **4**–**7**, and a related bifloradiene (**8**), from a marine sponge *Cribochalina* sp. collected off the Caribbean coast of Mexico.[10] We investigated the absolute stereochemistry of compound **4** by modified Mosher analysis of the alcohol derivative **9**. However, our study was hampered by the presence of many overlapping signals in the proton NMR spectra of these compounds in $CDCl_3$, even when data were acquired at 500 MHz. Now, we have examined these derivatives in d_5-pyridine, a solvent in which the proton NMR signals are better dispersed. Our results allow us to report a revised absolute stereochemistry for isocyanide (**4**) that matches that deduced for the cycloamphilectene 7,20-diisocyanoadociane (**1**) by total synthesis,[13] and by X-ray analysis on its *p*-bromobenzamide derivative.[14] Since our initial report, we have also isolated the minor compound **10**, the isothiocyanate derivative of **4**, as well as succeeded in obtaining single crystals of compound **7** suitable for an X-ray diffraction study, which fully clarified its relative stereochemistry and, in particular, firmly established the uncommon 8,13 *cis* ring-junction. This *cis* junction is then assured also for the biosynthetically-related metabolites (**5**)–(**8**).

Keywords: Diterpenes; NMR; Mosher analysis; X-ray; Stereochemistry; Sponges.
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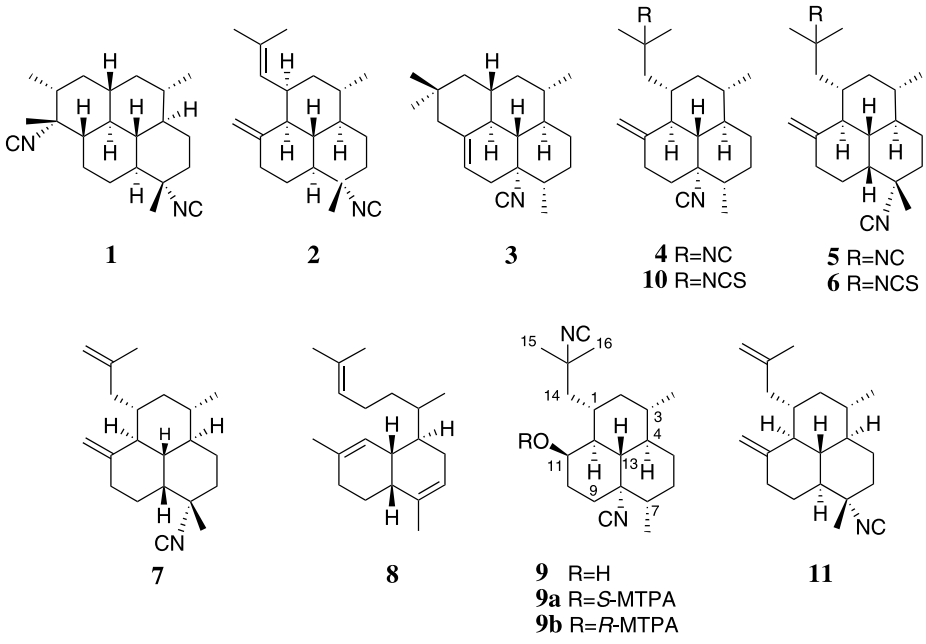
2. Results and discussion

An aliquot of the diethyl ether soluble portion (1 g) of the acetone extract of *Cribochalina* sp. was purified by silica-gel chromatography (light petroleum ether/diethyl ether gradient) according to our literature conditions.[10] Along with already reported compounds **4**–**8**, a novel minor related metabolite (**10**) was isolated from the less polar fraction.



First of all, we focused our attention on the main compound **4** to re-investigate its absolute stereochemistry proposed to be opposite to the related cycloamphilectenes (e.g., **1**) of known absolute configuration.[13,14] Alcohol **9**, re-obtained by reductive ozonolysis from compound **4**,[10] was dissolved in d_5-pyridine and submitted to a careful NMR spectroscopic analysis leading to a complete proton and carbon assignment (see Table 1 and Section 3), which confirmed the axial orientation of the hydroxy group at C11 (H-11, δ 4.16, $J_{W1/2}$ 7.5 Hz). According to the known procedure,[15] treatment of compound **9** with (*R*)- and (*S*)-MTPA chlorides in dry methylene chloride and DMAP afforded the corresponding (*S*)-MTPA (**9a**) and (*R*)- MTPA (**9b**) esters. The two Mosher derivatives were dissolved in d_5-pyridine and analysed by ^{1}H NMR spectroscopy. The NMR signals were significantly better dispersed than those recorded in $CDCl_3$,[10] thus allowing the full assignment, as reported in Table 1 and Section 3.

The chemical shift analysis of (*S*)-MTPA (**9a**) and (*R*)-MTPA (**9b**) esters clearly indicated an *R* absolute configuration at C11, opposite to that previously

Table 1. ^{1}H NMR assignments[a,b] in Pyr-d_5 of alcohol **9** and its Mosher'esters

Position	Compound **9**		**9a** (*S*-ester)		**9b** (*R*-ester)		$\Delta\delta$
	δ H	m (*J*, Hz)	δ H	m (*J*, Hz)	δ H	m (*J*, Hz)	
1	2.02	m	1.59	m	1.68	m	−0.09
2	2.14/0.83	m	2.24/0.83	m	2.26/0.85	m	−0.02/−0.02
3	1.03	m	0.93	m	0.98	m	−0.05
4	1.08	m	1.04	m	1.06	m	−0.02
5	1.85/0.77	m	1.83/0.69	m	1.84/0.70	m	−0.01/−0.01
6	1.86/1.43	m	1.88/1.40	m	1.85/1.36	m	+0.03/+0.04
7	1.32	m	1.20	m	1.15	m	+0.05
8	—		—		—	—	
9	—		1.39	m	1.39	m	0.00
10	—		2.12/1.96	m	2.05/1.92	m	+0.07/+0.04
11	4.16	br s	5.56	br s	5.57	br s	−0.01
12	1.33		1.54	t 10.9	1.55	t 1.05	−0.01
13	1.72	bt 10.5	1.28	m	1.33	m	−0.05
14	1.82/1.25	m	1.86/1.30	m	1.89/1.30		−0.03/0.00
15	—		—		—		
16	1.43	s	1.30	s	1.30	s	0.00
17	1.38	s	1.36	s	1.35	s	−0.01
18	0.80	d 6.2	0.79	d 6.2	0.80	d 6.5	−0.01
19	0.94	d 6.6	0.88	d 6.2	0.85	d 6.6	+0.03

[a] Bruker AMX 600 MHz, Pyr-d_5; δ values are reported referred to Pyr-d_5 (δ 8.71, 7.56, 7.19).
[b] Assignments determined by ^{1}H–^{1}H COSY, HSQC, HMBC experiments.

suggested,[10] so the absolute stereochemistry of compound **4** must be revised as indicated in the given structure.

Next, we also reconsidered the relative stereochemistry of amphilectenes **5**–**7**, previously suggested on the basis of NMR spectroscopic analysis. Compounds **5**–**7** are characterised by the same cyclic framework that exhibits the isocyanide function linked to carbon C7 bearing a methyl group. Even though, the spin systems of **5**–**7** were confidently assigned by NMR spectroscopic analysis, the relative stereochemistry was suggested in the first paper by analogy with the related main metabolite, compound **4**, as well as by a series of NOE experiments.[10] Indeed, re-analysis of these spectra led us to reconsider the previous stereochemical assignment and to further investigate this aspect by X-ray diffraction analysis.

We succeeded in growing good single crystals of compound **7**, which is the most abundant of these amphilectenes, from *n*-hexane. The X-ray structure has removed all the ambiguities regarding its relative stereochemistry. The structure was solved using SIR97[16] and refined by SHELXL package[17] to a conventional discrepancy *R* factor = 0.044 on 2466 observed reflections and 202 variables. A perspective view of the final X-ray model of **7** is shown in Figure 1.

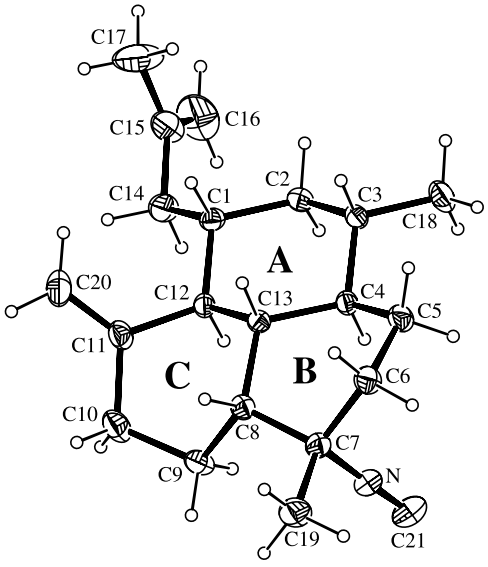


Figure 1. Perspective view of the final X-ray model of **7** with the atomic labelling for non-H atoms. Displacement ellipsoids are drawn at the 30% probability level.

In the absence of atoms with a strong anomalous scattering contribution, reliable evidence of the absolute stereochemistry could not be obtained. The enantiomer depicted is based on our reassigned absolute stereochemistry (NMR and Mosher analyses) here reported of compound **4**, and agrees with the literature data.[13,14] On this basis, the compound **7** is defined as (1*S*,3*S*,4*R*,7*S*,8*R*,12*S*, 13*S*)-7-isocyanoamphilecta-11(20),15-diene. It is the 8-epimer of 7-isocyanoamphilecta-11(20),15-diene (**11**), a metabolite from the tropical marine sponge *C. hooperi*, whose structure was assigned by 2D NMR spectroscopy[8] and confirmed by X-ray analysis.[9]

The crystal study revealed the *cis* ring-junction along the C8 and C13 bond (**B/C** *cis*-fusion), which is rather unusual in the amphilectene and cycloamphilectene classes. In particular, a *cis* fusion has been found in only two isomeric cycloamphilectenes from a Palauan sponge *Halichondria sp*.[6] and more recently[11] in *N*-formyl-7-amino-11-cycloamphilectene, a metabolite from Vanuatu sponge *Axinella* sp. This is the first case of *cis* ring junction in amphilectene diterpenoids. Indeed, the previously found *cis* junction in 7-isocyanoneoamphilecta-11,15-diene[7] regards a different kind of tricyclic neoamphilectene skeleton with a spirocyclic rearrangement.

Apart from the exocyclic methylene group at C11, the tricyclic system of **7** presents an isobutenyl substitution at C1 and a secondary methyl group at C3, both in equatorial orientations. Moreover, a methyl group and a isocyanide functionality (β and α oriented, respectively), are present at the C7 carbon. Intramolecular geometry agrees well with the generally accepted values for correlated molecules.[3,6,7,9,11,14,18] In particular, the carbon–carbon single bonds are in the range 1.498–1.567 Å and the longest bond distance C8–C13 = 1.567(3) Å corresponds to **B**, **C** ring *cis* fusion. The geometry of isocyanide group is: C7–N = 1.464(3) Å, N–C21 = 1.150(4) Å, C7–N–C21 = 177.7(5)°. The ring **A** is in a chair conformation, puckering parameters[19] are: $\theta = 4.0(2)°$ and $\phi = -4(3)°$, with C2 and C13 displaced 0.603(2) and 0.675(2) Å, respectively, and in the opposite direction with respect to the best plane through the remaining ring carbons. The other rings adopt twist-boat conformations, slightly distorted toward boat forms, with $\theta = 86.2(2)°$ and $\phi = 26.0(2)°$ for the **B** ring and $\theta = 86.5(1)°$ and $\phi = 20.6(1)°$ for the **C** ring. In the molecule, the shortest non-1,4 intramolecular distances are N–C4 = 3.052(3) and C14–C20 = 3.362(4) Å. The molecular packing is governed only by van der Waals interactions and the shortest contacts involve the nitrile carbon: C21···C19($\frac{1}{2}+x$, $\frac{1}{2}-y$, $1-z$) = 3.656(4) and C21···C18($1-x$, $y-\frac{1}{2}$, $\frac{1}{2}-z$) = 3.717(4) Å.

On the basis of these results, the structure of amphilectenes **5**–**7** must to be revised as now reported. The presence of the 8,13 *cis*-junction in these compounds is in agreement with the structure of co-occurring metabolite **8**,[20] which could be considered a possible putative precursor of amphilectenes **5**–**7**. The relative stereochemistry of **8** was erroneously reported in our previous paper.[10]

Finally, we have taken into consideration the novel compound **10**, which was obtained as a colourless oil (1 mg, R_f 0.75, light petroleum ether/diethyl ether 9:1). Its HRESIMS spectrum displayed a sodiated-molecular peak at *m/z* 379.2164 $(M+Na)^+$, consistent with the molecular formula $C_{22}H_{32}N_2S$. Both proton and carbon NMR spectroscopic data of **10** strongly resembled those of diterpene **4** suggesting a closely related structure. In particular, analysis of ^{13}C NMR spectrum clearly indicated that **10** differed from **4** only in the presence of an isothiocyanate function in the place of the isocyanate one at C-21 (δ 126.0 in **10**, δ 156.2 in **4**). All resonances of compound **10** were assigned as reported in Table 2 by NMR spectroscopic analysis and comparison with compound **4**.

In conclusion, we have presented here the stereochemical

Table 2. ^{1}H and ^{13}C NMR data[a,b] for compounds **10** and **4**[c]

Position	Compound **10**				Compound **4**			
	δ H	m (*J*, Hz)	δ C	m	δ H	m (*J*, Hz)	δC	m
1	1.92	m	33.0	CH	1.99	m	33.1	CH
2	2.18/0.90	m	41.0	CH_2	2.28/0.93	m	40.8	CH_2
3	1.10	m	35.5	CH	1.10	m	35.4	CH
4	1.13	m	42.6	CH	1.15	m	42.5	CH
5	2.00/0.86	m	29.7	CH_2	1.99/0.86	m	29.6	CH_2
6	1.53	m	29.9	CH_2	1.53/1.43	m	29.8	CH_2
7	1.40	m	40.9	CH	1.39	m	40.7	CH
8			67.0	C			66.9[e]	C
9	2.30/1.32	m	39.6	CH_2	2.30/1.32	m	39.5	CH_2
10	2.32	m	33.5	CH_2	2.32	m	33.4	CH_2
11			150.1	C			149.7	C
12	1.85	m	46.0	CH	1.88	bt 10.5	45.9	CH
13	1.05	m	55.5	CH	1.08	m	55.4	CH
14	2.05/1.28	m	46.7	CH_2	2.05/1.28	d 15.0/m	45.7	CH_2
15			60.6	C			56.5	C
16	1.43	br s	29.4	CH_3	1.43	br s	29.8	CH_3
17	1.45	br s	31.9	CH_3	1.45	br s	31.8	CH_3
18	0.93	d 6.7	19.8	CH_3	0.93	d 6.0	19.7	CH_3
19	0.99	d 6.0	15.7	CH_3	0.97	d 6.2	15.6	CH_3
20	4.86/4.61	s	106.8	CH_2	4.86/4.64	s	106.2	CH_2
21			156.3	C			156.2	C
22			126.0	C			154.3	C

[a] Bruker AMX 600 MHz, $CDCl_3$; δ values are reported referred to $CHCl_3$ (δ 7.26) and $CDCl_3$ (δ 77.0).
[b] Assignments determined by ^{1}H–^{1}H COSY, HSQC, HMBC experiments.
[c] NMR data of compound **4** are reported from Ref. 10.
[d] Erroneously reported in Ref. 10.

reassignment of isocyanide and isothiocyanate amphilectene diterpenes from Caribbean sponge *Cribochalina* sp. The structures of these compounds were incorrectly reported in our previous paper.[10] The revised absolute stereochemistry of the main isocyanide **4** matches that previously demonstrated for the related compound **1** whereas an uncommon 8,13-*cis* junction has been now defined for amphilectenes **5**–**7**. This stereochemical feature is unprecedented among amphilectenes with a regular tricyclic skeleton.

Isocyanide amphilectenes are typical metabolites of haplosclerid sponges of genera *Amphimedon*, *Ciocalypta* or *Halichondria*.[1] Interestingly, compound **4** has been recently isolated also from nudibranch *Phyllidiella pustulosa*, which most likely obtains this metabolite from an haplosclerid sponge prey.[21]

3. Experimental

3.1. General experimental procedures

Precoated TLC plates Merck (Darmstadt, Germany). Si gel 60 F254 were used for analytical TLC and Merck Kieselgel 60 powder was used for preparative column chromatography. Optical rotations were measured on a Jasco DIP 370 digital polarimeter; IR spectra were measured on a Biorad FTS 155 FTIR spectrophotometer; 1D and 2D NMR spectra were recorded on a Bruker AMX 400 (400.13 MHz), and on a Bruker AMX 600 equipped with a TXI CryoProbe; ^{13}C NMR were recorded on a Bruker AMX 300 (75.47 MHz); HRESIMS was carried out on a Micromass Q-TOF micro.

3.2. Biological material

See Ref. 10.

3.3. Extraction and isolation of diterpenes

The remaining part of the ether soluble extract of the sponge (1 g) was chromatographed on a Si-gel column (light petroleum ether and increasing amounts of diethyl ether) giving fractions containing all the known compounds **4**–**8** as reported in the previous paper.[6] One fraction was submitted to HPLC giving the new compound **10** (eluent *n*-hexane/ethyl acetate 99:1, flow 1 ml/min, column Kromasil, 5μ).

3.4. Single crystal X-ray analysis of compound 7

Single crystals were grown as colourless rectangular prisms by slow evaporation from *n*-hexane at room temperature. A sample of size $0.40\times0.20\times0.06$ mm^3 was selected for data collection on a Bruker KappaCCD diffractometer using graphite-monochromated Mo Kα radiation ($\lambda=0.71069$ Å).

Crystal data. $C_{21}H_{31}N$, $a=9.0224(8)$ Å, $b=10.2668(5)$ Å, $c=20.367(1)$ Å, $V=1886.6(2)$ Å^3, orthorhombic system, space group $P2_12_12_1$, $Z=4$, $M_w=297.47$, $D_c=1.047$ g cm^{-3}, $\mu=0.059$ mm^{-1}.

Accurate cell parameters were obtained by least-squares refinement of the setting angles of 374 reflections at medium θ ($4.3<\theta<16.6$). A total of 14380 reflections ($-12\le h\le 11$, $-13\le k\le 10$, $-22\le l\le 27$), 4652 of, which were independent ($R_{int}=0.0207$), were measured at room temperature. Data were collected up to $\theta=28.98°$ (96.5% of completeness) using COLLECT package.[22] The structure was solved by direct methods using SIR97[11] and refined by full-matrix least-squares calculations on F^2 using

SHELXL.[12] All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included at ideal positions, with isotropic thermal parameters set 1.2 times the U_{eq} of the parent atom, and not refined except for the torsion angles of methyl groups. At convergence, the final discrepancy index R was 0.0441 based on 2466 observed reflections [$I>2\sigma(I)$] and 202 variable parameters. The overall R_w value was 0.1173 with $w=1/[\sigma^2(F_o^2)+(0.0261P)^2+0.0153P]$ where $P=(F_o^2+2F_c^2)/3$; $S=1.626$; $(\Delta/\sigma)_{max}<0.001$. No residual electron density was outside the range -0.26 to 0.20 e $Å^{-3}$. The anomalous dispersion effect is small and no reliable evidence of the absolute configuration could be obtained, indeed the final Flack parameter $=-2(4)$, using 1902 Friedel opposite reflections, is not significant. All the crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC number 267942.

3.4.1. Compound 9. Compound **4** was submitted to a reductive ozonolysis[10] to obtain alcohol **9**. The structure of the alcohol was secured by comparison with chemical features of the previously reported compound **9**. Complete assignments of **9** was made in deuterated pyridine. ^{13}C NMR was recorded on a 300 MHz Bruker apparatus (75.47 MHz), resonances are given in ppm: 156.2 (C-21, C), 154.4 (C-22, C), 67.0 (C-8, C), 63.0 (C-11, CH), 57.0 (C-15, C), 45.5 (C-12 and C-13, CH), 45.3 (C-14, CH_2), 43.6 (C-2, CH_2), 42.5 (C-4, CH), 40.6 (C-7, CH), 36.6 (C-3, CH), 33.3 (C-1, CH), 31.1 (C-17, CH_3), 30.4, 30.3, 30.2, 29.9, (C-5, CH_2; C-6, CH_2; C-9, CH_2; C-10, CH_2, all these resonances could be interchangeable), 28.7 (C-16, CH_3), 20.1 (C-18, CH_3), 15.8 (C-19, CH_3).

3.4.2. Compound 9a(*S*-ester). ^{13}C NMR (75.47 MHz, C_5D_5N): 166.0*, 130.5*, 129.3*, 128.2* (* all resonances belonging to MTPA residue), 71.7 (C-11, CH), 66.3 (C-8, C), 55.6 (C-15, C), 46.5 (C-13, CH), 44.5 (C-14, CH_2), 44.1 (C-12, CH), 42.3 (C-4, CH), 40.7 (C-2, CH), 40.6 (C-7, CH), 36.1 (C-3, CH), 33.6 (C-1, CH), 32.3 (C-17, CH_3), 30.3 (C-9, CH_2), 30.1 (C-5, CH_2), 29.9 (C-6, CH_2), 28.1 (C-16, CH_3), 26.0 (C-10, CH_3), 19.8 (C-18, CH_3), 15.6 (C-19, CH_3).

3.4.3. Compound 9b (*R*-ester). ^{13}C NMR (75.47 MHz, C_5D_5N): 164.5*, 130.5*, 129.3*, 128.1* (* all resonances belonging to MTPA residue), 71.9 (C-11, CH), 66.3 (C-8, C), 56.0 (C-15, C), 46.6 (C-13, CH), 44.6 (C-14, CH_2), 43.8 (C-12, CH), 42.2 (C-4, CH), 41.0 (C-2, CH_2), 40.5 (C-7, CH), 36.3 (C-3, CH), 33.9 (C-1, CH), 32.1 (C-17, CH_3), 30.1 (C-9 and C-5, CH_2), 29.9 (C-6, CH_2), 28.6 (C-16, CH_3), 25.8 (C-10, CH_2), 19.8 (C-18, CH_3), 15.6 (C-19, CH_3).

3.4.4. Compound 10. 1 mg: pale yellow oil; $[\alpha]_D$ -52 (*c* 0.1, $CHCl_3$); IR (liquid film) ν_{max}: 2955, 2923, 2867, 2124, 2090, 1736, 1646, 1456, 895 cm^{-1}; HRESIMS: found 379.2164 (379.2184 calculated for $C_{22}H_{32}N_2NaS$).

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Shimalactone A, a novel polyketide, from marine-derived fungus *Emericella variecolor* GF10

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Abstract—Shimalactone A (**1**), a novel polyketide having bicyclo[4.2.0]octadiene and oxabicyclo[2.2.1]heptane units, was isolated from a cultured marine fungus of *Emericella variecolor* GF10. The stereo structure of **1** was deduced from 2D NMR and X-ray crystallographic analyses of **1** and its derivatives. The absolute structure of **1** was determined by application of the CD allylic benzoate rule to the benzoate derivative of **1**. Shimalactone A (**1**) induced neuritogenesis at 10 μg/mL against neuroblastoma Neuro 2A cells.

1. Introduction

In recent years, marine microorganisms have been paid much attention as a significant source in search for new drug leads.[1–3] In our continuing search for new biological active substances from marine microorganisms, we previously reported ophiobolins-type cytotoxic sesterterpenes as cytotoxic constituents of a marine-derived fungus, *Emericella variecolor* GF10.[4] Further study on this strain led us to isolate a novel polyketide named shimalactone A (**1**), which possesses a unique carbon skeleton such as bicyclo-[4.2.0]octadiene and oxabicyclo[2.2.1]heptane units. This paper presents the isolation and structure elucidation of this compound.

2. Results and discussion

The fungus strain of *E. variecolor* GF10 was separated from the marine sediment collected from a depth of 70 m off Gokasyo Gulf, Mie Prefecture, Japan. The GF10 strain was cultured at 30 °C for 2 weeks in a solid-state medium based on rice prepared from artificial seawater. The cultured medium was extracted with an organic solvent, and the resulting extract was partitioned into a 2-butanone–water mixture. The 2-butanone-soluble portion was further partitioned into an *n*-hexane-90% aq MeOH mixture to furnish an MeOH extract. Then the MeOH extract was fractionated by silica gel column chromatography and HPLC to obtain a new polyketide named shimalactone A (**1**).

Shimalactone A (**1**) was obtained as a colorless oil. The FAB-MS of **1** showed a quasi-molecular ion peak at *m/z* 475 $(M+Na)^+$, and the molecular formula was determined as $C_{29}H_{40}O_4$ by HRFAB-MS in conjunction with NMR analysis. The ^{1}H NMR spectrum of **1** indicated the presence of eight singlet-methyl protons (δ_H 1.71, 1.65, 1.65, 1.55, 1.34, 1.15, 1.10, 1.01), two doublet-methyl protons (δ_H 1.60, 1.19), and nine methine protons (δ_H 5.49, 5.46, 5.27, 5.10, 4.83, 3.84, 2.68, 2.60, 2.39). The ^{13}C NMR and HMQC (Heteronuclear Multiple Quantum Coherence) spectra of **1** further defined the presence of a keto-carbonyl carbon (δ_C 207.6), an ester carbonyl carbon (δ_C 171.8), four olefinic quaternary carbons (δ_C 138.2, 136.5, 132.5, 129.4), and four quaternary carbons (δ_C 70.1, 58.3, 49.6, 41.2). The four protons (δ_H 5.49, 5.46, 5.27, 4.83) among the nine methine protons were assigned for olefinic protons. The analysis of the COSY spectrum of **1** revealed four small partial structures figured by a thick line. The detailed analysis of the HMBC (Heteronuclear Multiple Bond Correlation) spectrum of **1** clarified the presence of bicyclo[4.2.0]octadiene unit (based on the correlations: between H-9 and C-10, C-16; H-11 and C-10, C-15; H-15 and C-10, C-11, C-16, C-17; H_3-25 and C-9, C-10, C-11; H_3-28 and C-16, C-17) and oxabicyclo[2.2.1]heptane unit (based on the correlations: between H-4 and C-3; H-5 and C-1, C-3, C-6; H_3-21 and C-1, C-2, C-3, C-6; H_3-22 and C-3; H_3-23 and C-5, C-6), respectively (Fig. 1). Connectivity of these

Keywords: Shimalactone A; Polyketide; Marine derived fungus; X-ray analysis; *Emericella variecolor*.
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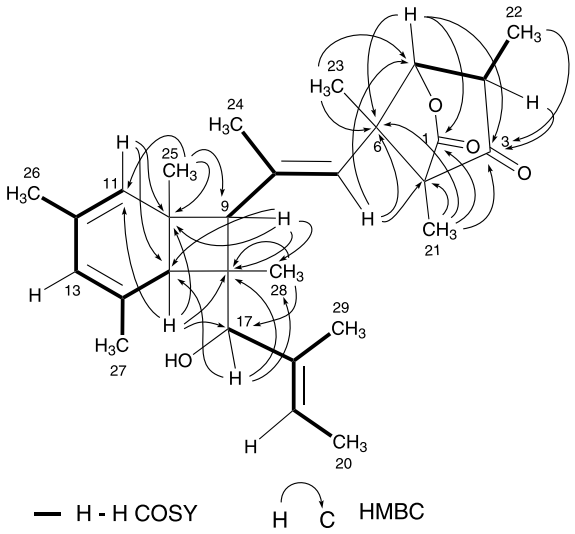


Figure 1. COSY and Key HMBC correlations of shimalactone A (**1**).

partial structures was also defined on the basis of the HMBC correlations shown in Figure 1 and Table 1, and the planar structure of **1** has been elucidated.

The geometry of the two olefins and the relative stereo structure of **1** were clarified by analysis of the NOESY spectrum of **1**. Thus, from the presence of the NOE correlation between H_3-20 (δ_H 1.60, d, J=6.6 Hz) and H_3-29 (δ_H 1.55, s) and the absence of the NOE between H-7 (δ_H 5.27, s) and H_3-24 (δ_H 1.71, s) the *E*-configuration for both trisubstituted olefins was deduced. The strong NOE

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for shimalactone A (**1**) in $CDCl_3$

No.	^{13}C δ_C	^{1}H δ_H (m, J (Hz))	HMBC correlations[a]
1	171.8		
2	58.3		
3	207.6		
4	44.0	2.60 (1H, qd, J=7.2, 2.2)	3, 22
5	85.2	5.10 (1H, d, J=2.2)	1, 3, 6
6	70.1		
7	123.8	5.27 (1H, s)	2, 5, 6, 9, 24
8	138.2		
9	60.7	2.68 (1H, s)	7, 8, 10, 16, 17, 24, 25, 28
10	41.2		
11	123.9	4.83 (1H, s)	10, 13, 15, 26
12	129.4		
13	123.5	5.46 (1H, s)	11, 15, 26, 27
14	132.5		
15	51.7	2.39 (1H, s)	10, 11, 14, 16, 17, 25, 27, 28
16	49.6		
17	86.3	3.84 (1H, s)	9, 15, 16, 18, 19, 28, 29
18	136.5		
19	122.5	5.49 (1H, q, J=6.6)	17, 20, 29
20	13.1	1.60 (3H, d, J=6.6)	18, 19
21	4.7	1.15 (3H, s)	1, 2, 3, 6
22	11.6	1.19 (3H, d, J=7.2)	3, 4, 5
23	16.8	1.34 (3H, s)	2, 5, 6, 7
24	20.6	1.71 (3H, s)	7, 8, 9
25	31.9	1.10 (3H, s)	9, 10, 11, 15
26	22.1	1.65 (3H, s)	12, 13
27	23.3	1.65 (3H, s)	13, 14, 15
28	13.2	1.01 (3H, s)	9, 15, 16, 17
29	14.4	1.55 (3H, s)	17, 18, 19

[a] C coupled with H.

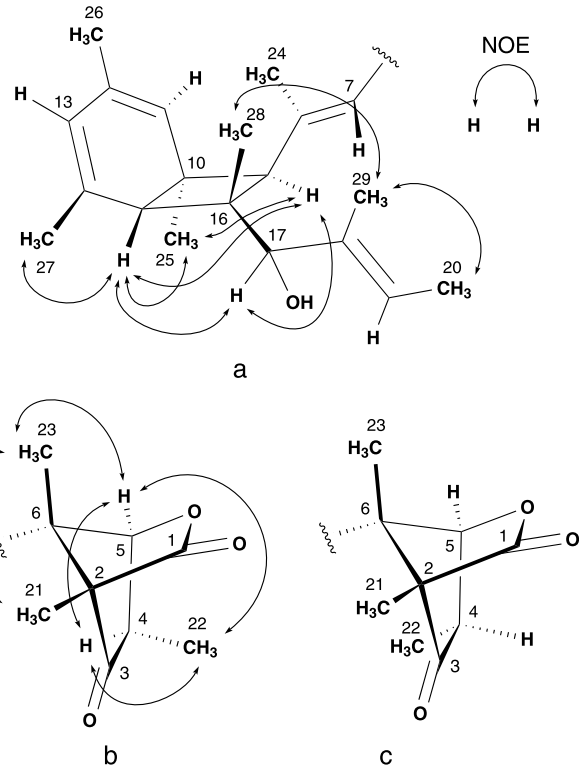


Figure 2. Key NOE correlations of shimalactone A (**1**).

correlations, which were observed among H-9 (δ_H 2.68, s), H-15 (δ_H 2.39, s), and H_3-25 (δ_H 1.10, s), clarified the relative configurations of the bicyclo[4.2.0]octadiene unit as shown in Figure 2, while the relative configurations of the oxabicyclo[2.2.1]heptane unit and the steric relationship between each partial structure were still unknown (Fig. 2b and c). $NaBH_4$ reduction of **1** in Et_2O gave two reduced products (**2** and **3**). Detailed analysis of the ^{1}H NMR spectra of **2** and **3** revealed that the H-3 signal of **2** having 3,4-*anti* configuration appeared at higher field (δ_H 3.23, d, J= 3.0 Hz) in comparison with that of **3** (δ_H 4.04, d, J=9.1 Hz)

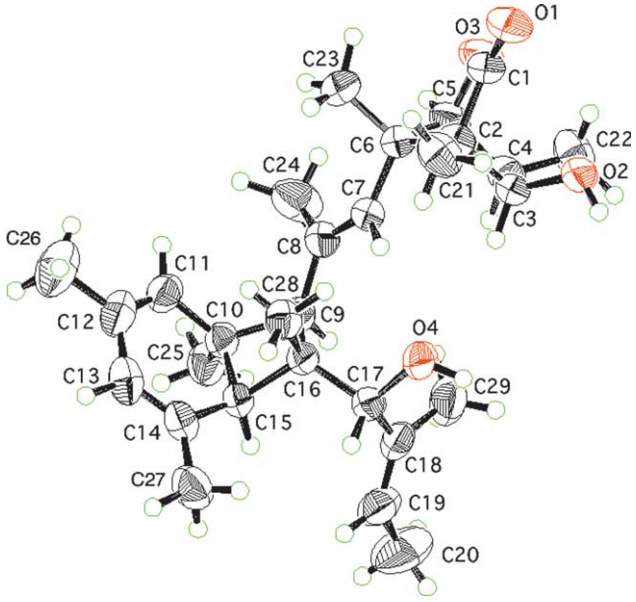


Figure 3. X-ray analysis of **3**.

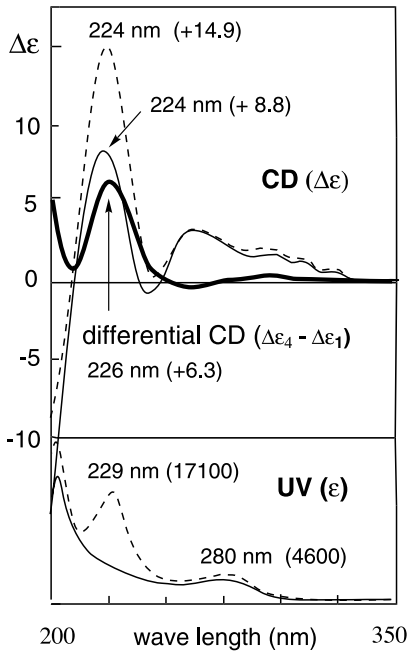


Figure 4. CD and UV spectra of shimalactone A (**1**, solid line) and **4** (dotted line) and differential CD spectrum of **4** and **1** (bold line) in methanol.

having 3,4-*syn* configuration by the anisotropic effect of the C-1 carbonyl group. This result suggested the oxabicyclo-[2.2.1]heptane unit has the **b**-type stereo structure shown in Figure 2. However, we could not elucidate the steric relationship of each partial structure from the NOESY analysis of these compounds. Fortunately, compound **3** could be crystallized from an *n*-hexane–Et_2O solution to give a colorless plate crystal, and the relative stereo structure of shimalactone A (**1**) was unambiguously defined by X-ray crystallographic analysis (Fig. 3).

Next, the absolute structure of **1** was elucidated by application of the CD allylic benzoate rule.[5] The pyridine solution of **1** was treated with benzoyl chloride and DMAP at 80 °C to give a benzoate **4**. Although, both compounds exhibited complex CD spectra, the differential CD spectrum of **4** and **1** showed a positive Cotton effect ($\Delta\varepsilon = +6.3$) at 226 nm, which reflects a plus chirality between the benzoate and the Δ^{18} olefin chromophores in **4** (Fig. 4). Based on this evidence, the 17*R* configuration and the absolute structure of shimalactone A (**1**) was elucidated as shown in Chart 1.

Shimalactone A (**1**) is a unique polyketide having two bicyclic ring systems (bicyclo[4.2.0]octadiene unit and oxabicyclo[2.2.1]heptane unit). Compound **1** was presumed to be biosynthesized from an acetate and nine propionates. Recently, two similar polyketides, SNF4435C and SNF4435D, having the bicyclo[4.2.0]octadiene unit have been found as immunosuppressive agents from the cultured broth of *Streptomyces spectabilis*.[6] The biomimetic synthesis of the bicyclo[4.2.0]octadiene unit has been also achieved from an all-*trans* tetraene precursor through a tandem double electrocyclization.[7]

Shimalactone A (**1**) induced neuritogenesis in neuroblastoma Neuro 2A cells at 10 μg/mL at 24 h after treatment. At higher concentration of 20 μg/mL, it showed cytotoxicity against the same cell line.

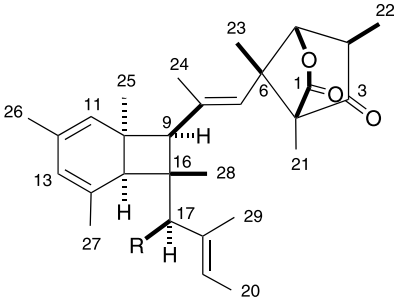
3. Experimental

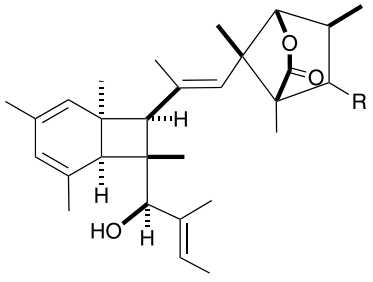
3.1. General experimental procedures

NMR spectra were recorded on a Varian Unity Inova 600 (600 MHz for ^1H) spectrometer. Spots on TLC were detected by spraying 1%$Ce(SO_4)_2$/10%H_2SO_4 with subsequent heating. Artificial seawater was prepared by Aquamarine (Yashima Pure Chemical Co. Ltd, Japan). Melting point was measured on a Yanagimoto micro melting point apparatus. CD spectra were recorded on a JASCO J-720 spectrometer ($L = 1$ mm). X-ray crystallographic analysis was made on a Rigaku RAXIS RAPID diffractometer equipped with imaging plate area detector with graphite monochromated Cu Kα radiation. Other instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[8]

3.2. Fungus material, culture conditions, and extraction

The *E. variecolor* GF10 strain was separated from the marine sediment collected from a depth of 70 m off Gokasyo Gulf, Mie Prefecture, Japan, in 2002 and deposited





shimalactone A (**1**): R = OH
4: R = $OCOC_6H_5$

2: R = α-OH
3: R = β-OH

Chart 1.

in our laboratory. The GF10 strain was classified as *E. variecolor* from its cultural characteristics and 16SrDNA sequence.[4] The GF10 strain was cultured in the MG medium (malt extract: 20 g, glucose: 20 g, bact peptone: 1 g, artificial seawater: 1000 mL) at 30 °C for 5 days. Then, the broth of the strain was inoculated into rice solid medium (rice: 25 g, artificial seawater: 50 mL, in a 500 mL flask) and cultured under static conditions at 30 °C for 2 weeks. The culture was extracted with acetone and a mixed solvent (EtOAc–MeOH–acetone, 1:2:4), and then the organic solvent was combined and evaporated under reduced pressure to give an extract. The extract was partitioned into a 2-butanone–H_2O mixture, and the 2-butanone-soluble portion was further partitioned into an *n*-hexane–90% aq MeOH mixture to furnish a MeOH extract (5.0 g).

3.3. Isolation of shimalactone A (1)

The MeOH extract (5.0 g) of the solid culture (75 g×40) was fractionated by SiO_2 column chromatography (*n*-hexane–EtOAc) to give seven fractions (A–G). Fraction B (460 mg) was then subjected to SiO_2 column chromatography ($CHCl_3$–MeOH) to afford four fractions (B-1 to B-4). Fraction B-3 (183 mg) was separated by reversed phase HPLC (Cosmosil 5C18-AR, MeOH–H_2O, 80:20) to obtain three fractions B-3.1, B-3.2 and B-3.3. Fraction B-3.3 (112 mg) was further purified by HPLC (Cosmosil 5SL, *n*-hexane–EtOAc, 88:12) to furnish shimalactone A (**1**, 77 mg).

3.3.1. Shimalactone A (1). Colorless oil. $[\alpha]_D^{23}$ +12 (*c* 0.57, MeOH). IR ν_{max} (KBr) cm^{-1}: 3528, 2973, 1795, 1751. UV λ_{max} (MeOH) nm (ε): 280 (3700). CD (0.6 mM, MeOH, 25 °C) λ_{max} nm ($\Delta\varepsilon$): 210 (0), 224 (+8.8), 240 (0), 243 (−0.4), 247 (0), 263 (+3.6). (^{1}H and ^{13}C NMR data: shown in Table 1. FAB-MS: *m/z* 475 $(M+Na)^+$. HRFAB-MS: found *m/z* 475.2833 $(M+Na)^+$. Calcd for $C_{29}H_{40}O_4Na$: 475.2824.

3.4. Reduction of shimalactone A (1)

An Et_2O (10 mL) solution of **1** (21.0 mg) was treated with $NaBH_4$ (5.0 mg) and the mixture was stirred at room temperature for 4 days.[9] The reaction mixture was filtered with Millipore (Millex^R-FH, 0.45 μM). The filtrate was evaporated under reduced pressure, and the resulting residue was purified by HPLC (Cosmosil 5SL, *n*-hexane–EtOAc, 4:1) to obtain **2** (9.1 mg) and **3** (7.2 mg) with the recovered **1** (4.4 mg).

3.4.1. Compound 2. Colorless amorphous. IR ν_{max} (KBr) cm^{-1}: 3485, 2970, 1778. ^{1}H NMR (600 MHz, $CDCl_3$, δ_H): 5.66 (1H, s, H-7), 5.51 (1H, q, *J*=6.7 Hz, H-19), 5.47 (1H, s, H-13), 4.97 (1H, s, H-11), 4.67 (1H, d, *J*=1.8 Hz, H-5), 3.79 (1H, s, H-17), 3.23 (1H, d, *J*=3.0 Hz, H-3), 2.83 (1H, s, H-9), 2.32 (1H, s, H-15), 2.18 (1H, m, H-4), 1.65 (6H, s, H_3-26, 29), 1.64 (3H, d, *J*=6.7 Hz, H_3-20), 1.63 (3H, s, H_3-24), 1.60 (3H, s, H_3-27), 1.22 (3H, s, H_3-28), 1.16 (3H, s, H_3-21), 1.16 (3H, d, *J*=7.2 Hz, H_3-22), 1.12 (3H, s, H_3-23), 1.09 (3H, s, H_3-25). ^{13}C NMR (150 MHz, $CDCl_3$, δ_C): 178.5 (C-1), 136.6 (C-8), 136.5 (C-18), 131.8 (C-14), 130.0 (C-12), 124.8 (C-7), 124.0 (C-19), 123.5 (C-11), 123.3 (C-13), 88.2 (C-5), 85.1 (C-17), 80.2 (C-3), 59.8 (C-6), 58.8 (C-9), 56.0 (C-2), 49.3 (C-15), 49.3 (C-16), 45.1 (C-4), 40.7 (C-10), 31.5 (C-25), 23.5 (C-27), 22.1 (C-26), 19.1 (C-24), 17.0 (C-23), 15.7 (C-28), 15.0 (C-29), 14.5 (C-22), 13.2 (C-20), 6.6 (C-21). FAB-MS *m/z*: 477 $(M+Na)^+$. HRFAB-MS: found *m/z* 477.2953 $(M+Na)^+$. Calcd for $C_{29}H_{42}O_4Na$: 477.2981.

3.4.2. Compound 3. Colorless plates. mp 158–159 °C. IR ν_{max} (KBr) cm^{-1}: 3499, 2974, 1775. UV λ_{max} (MeOH) nm (ε): 202 (15,000), 281 (3800). CD (0.6 mM, MeOH, 25 °C) λ_{max} nm ($\Delta\varepsilon$): 202 (−13.0), 213 (0), 227 (+14.2), 281 (+3.2). ^{1}H NMR (600 MHz, $CDCl_3$, δ_H): 5.51 (1H, q, *J*=6.7 Hz, H-19), 5.46 (1H, s, H-13), 5.40 (1H, s, H-7), 4.88 (1H, s, H-11), 4.61 (1H, s, H-5), 4.04 (1H, d, *J*=9.1 Hz, H-3), 3.87 (1H, s, H-17), 2.67 (1H, s, H-9), 2.43 (1H, dq, *J*=9.1, 7.2 Hz, H-4), 2.41 (1H, s, H-15), 1.66 (6H, s, H_3-26, 27), 1.61 (3H, s, H_3-24), 1.59 (3H, d, *J*=6.7 Hz, H_3-20), 1.57 (3H, s, H_3-29), 1.16 (3H, s, H_3-21), 1.11 (6H, s, H_3-23, 25), 1.08 (3H, s, H_3-28), 1.01 (3H, d, *J*=7.2 Hz, H_3-22). ^{13}C NMR (150 MHz, $CDCl_3$, δ_C): 177.0 (C-1), 136.6 (C-18), 135.8 (C-8), 132.8 (C-14), 129.0 (C-12), 125.4 (C-7), 124.5 (C-11), 123.5 (C-13), 122.4 (C-19), 86.7 (C-5), 86.4 (C-17), 74.7 (C-3), 61.2 (C-9), 60.8 (C-2), 54.5 (C-6), 51.8 (C-15), 49.6 (C-16), 41.2 (C-10), 36.3 (C-4), 32.0 (C-25), 23.3 (C-27), 22.1 (C-26), 20.1 (C-24), 16.5 (C-23), 14.5 (C-29), 13.3 (C-28), 13.1 (C-20), 9.1 (C-22), 7.8 (C-21). FAB-MS *m/z*: 477 $(M+Na)^+$. HRFAB-MS: found *m/z* 477.2977 $(M+Na)^+$. Calcd for $C_{29}H_{42}O_4Na$: 477.2981.

3.5. X-ray crystallographic analysis of 3

Single crystals suitable for X-ray analysis were obtained by recrystallization from *n*-hexane–Et_2O (10:1). A colorless platelet crystal having approximate dimensions of 0.60×0.40×0.10 mm was mounted on a glass fiber. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu Kα radiation (λ=1.5419 Å) at 296 K. Crystal data: $C_{29}H_{42}O_4$, (M_r 454.65), monoclinic, space group $P2_1$ (#4), a=7.463(2) Å, b=10.387(2) Å, c=17.898(4) Å, β=97.50(1)°, V=1375.6(5) Å^3, Z=2, and D_{calcd}=1.10 g/cm^3. The structure was solved by direct methods (SIR92) and expanded using Fourier techniques (DIRDIF99). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on F^2 was based on 4566 observed reflections and 342 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of R=0.055, R_w=0.150. All calculations were performed using the CrystalStructure[10] crystallographic software package.

Crystallographic data (excluding structure factors) for the structure analysis have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC267342. 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].

3.6. Benzoylation of shimalactone A (1)

A solution of **1** (6.0 mg) in pyridine (1.0 mL) was treated with benzoyl chloride (0.1 mL) and 4-dimethylaminopyridine (DMAP) (2.0 mg) and the mixture was stirred at 80 °C for 2 h. The reaction was quenched by saturated aq NH_4Cl (10 mL) and extracted with EtOAc (15 mL). After being washed with saturated aq NH_4Cl (10 mL), saturated aq $NaHCO_3$ (10 mL), and brine (10 mL) in sequence, the EtOAc layer was dried over $MgSO_4$ and evaporated in vacuo to afford a crude product. The crude product was purified by reversed phase HPLC (Cosmosil 5C18-AR, MeOH–H_2O, 80:20) to obtain **4** (6.0 mg).

3.6.1. Compound 4. IR ν_{max} (KBr) cm^{-1}: 2974, 1798, 1753, 1718. UV λ_{max} (MeOH) nm (ε): 202 (28,200), 229 (17,100), 280 (4600). CD (0.6 mM, MeOH, 25 °C) λ_{max} nm ($\Delta\varepsilon$): 209 (0), 224 (+14.9), 245 (+0.3), 264 (+3.3). ^1H NMR (600 MHz, CD_3OD, δ_H): 8.02 (2H, d, J=7.7 Hz, H-2′, 6′), 7.61 (1H, t, J=7.7 Hz, H-4′), 7.49 (2H, t, J=7.7 Hz, H-3′, 5′), 5.63 (1H, q, J=6.9 Hz, H-19), 5.47 (1H, s, H-13), 5.35 (1H, d, J=2.0 Hz, H-5), 5.32 (1H, s, H-7), 5.30 (1H, s, H-17), 4.93 (1H, s, H-11), 2.84 (1H, s, H-9), 2.61 (1H, dq, J=2.0, 7.4 Hz, H-4), 2.45 (1H, s, H-15), 1.79 (3H, s, H_3-24), 1.64 (3H, s, H_3-26), 1.63 (3H, s, H_3-29), 1.61 (3H, d, J=6.9 Hz, H_3-20), 1.44 (3H, s, H_3-27), 1.35 (3H, s, H_3-23), 1.22 (3H, s, H_3-28), 1.14 (3H, s, H_3-25), 1.11 (3H, d, J=7.4 Hz, H_3-22), 1.10 (3H, s, H_3-21). ^1H NMR (600 MHz, $CDCl_3$, δ_H): 8.02 (2H, dd, J=8.0, 1.0 Hz, H-2′, 6′), 7.55 (1H, td, J=8.0, 1.0 Hz, H-4′), 7.43 (2H, t, J=8.0 Hz, H-3′, 5′), 5.58 (1H, q, J=6.6 Hz, H-19), 5.43 (1H, s, H-13), 5.30 (1H, s, H-7), 5.26 (1H, s, H-17), 5.10 (1H, s, H-5), 4.82 (1H, s, H-11), 2.75 (1H, s, H-9), 2.56 (1H, dq, J=2.2, 7.4 Hz, H-4), 2.46 (1H, s, H-15), 1.72 (3H, s, H_3-24), 1.64 (3H, s, H_3-26), 1.60 (3H, s, H_3-29), 1.58 (3H, d, J=6.9 Hz, H_3-20), 1.44 (3H, s, H_3-27), 1.34 (3H, s, H_3-23), 1.18 (3H, s, H_3-28), 1.16 (3H, d, J=7.4 Hz, H_3-22), 1.15 (3H, s, H_3-21), 1.13 (3H, s, H_3-25). ^{13}C NMR (150 MHz, $CDCl_3$, δ_C): 207.5 (C-3), 171.6 (C-1), 165.6 (C-7′), 137.4 (C-8), 133.1 (C-4′), 132.3 (C-18), 131.9 (C-14), 130.4 (C-12), 129.5 (C-1′), 129.5 (C-2′, 6′), 128.5 (C-3′, 5′), 124.5 (C-19), 124.3 (C-7), 123.8 (C-11), 123.7 (C-13), 87.3 (C-17), 85.0 (C-5), 70.0 (C-6), 60.7 (C-9), 58.3 (C-2), 51.7 (C-15), 48.8 (C-16), 44.0 (C-4), 41.5 (C-10), 31.9 (C-25), 23.3 (C-27), 22.0 (C-26), 20.9 (C-24), 16.8 (C-23), 14.6 (C-29), 14.0 (C-28), 13.2 (C-20), 11.6 (C-22), 4.7 (C-21). FAB-MS m/z: 579 $(M+Na)^+$. HRFAB-MS: found m/z 579.3098 $(M+Na)^+$. Calcd for $C_{36}H_{44}O_5Na$: 579.3087.

3.7. Assay for neuritogenic activity in Neuro 2A cells

Neuro 2A cells were grown in Dulbecco's modified essential medium (DMEM) with 10% fetal bovine serum (FBS). The cells were kept in incubator at 37 °C with 5% CO_2. The cells were plated on 24-well plates at a density of 2×10^4 per well with 1 mL of culture medium. After 24 h cultivation, the medium was exchanged with fresh medium, and the testing sample as 10 μL of EtOH solution was added to each well. After 24 h incubation, morphological changes in the cells were observed under microscope.

Acknowledgements

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9. When EtOH was used as solvent, shimalactone A (**1**) gave **2** and **3** only in poor yield.
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Solid-state and solution photocycloadditions of 4-acyloxy-2-pyrones with maleimide

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Abstract—Solid-state photosensitized reactions of 4-acyloxy-2-pyrones (**1b**,**c**) with maleimide (**2**) afforded *endo–endo* double-[4+2] cycloadducts (**3b**,**c**) with high stereoselectivity. Sensitized photoreactions of **1a–d** with **2** in solution gave *exo–endo* double-[4+2] cycloadducts (**4a–d**). 2-Pyrones **1a–d** were photolyzed to give carboxylic acids (**5a–d**) via their valence isomerization in the solid state and in solution. Such kinds of photoreaction of the 4-acyloxy-2-pyrones were dramatically different from regio- and stereoselective [2+2] cycloadditions of 4-alkyloxy-2-pyrones. The photoreaction mechanisms of **1** with **2** and **1** itself were analyzed by powder X-ray diffraction analysis and MO calculations.

1. Introduction

Studies on solid-state photoreactions occurring in two different organic molecules or in solid mixtures are a current topic of interest because of the unusual and interesting results obtained.[1–4] In our recent papers,[5–7] we have reported a highly selective [2+2] cycloaddition reaction by irradiation to 1:1 complex crystals between 4-alkyloxy-2-pyrones and maleimide even by irradiation to the ground mixtures, together with the reaction between 2-pyrones and benzophenones. In contrast with the solid-state photoreaction, the reaction of 2-pyrones with maleimide in solution affording double-[4+2] cycloadducts (type **4**)[5] as shown in Scheme 1 was different from that of 1:1 complex crystals. We planned to extend the reaction to 4-acyloxy-2-pyrones to investigate the generality of these solid-state and solution-state photoreactions.

2. Results and discussion

A mixture of **1b** (0.2 mmol), **2** (0.4 mmol) and xanthone (0.05 mmol) as a sensitizer was crystallized from acetone to give a mixed crystal (mp 51–100 °C), which was ground for 10 min in a mortar with a pestle. The powder was sandwiched between two Pyrex glass plates and irradiated for 24 h with a 400 W high-pressure mercury lamp under nitrogen atmosphere at room temperature. The reaction mixture was recrystallized from acetonitrile to afford *endo–endo* double-[4+2]cycloadduct **3b** and benzoic acid **5b** in 66 and 18% yields, respectively, by ^{1}H NMR spectral analysis (Scheme 1). On the other hand, the same solid-state photoreaction without xanthone gave **5b** in 96% yield. Heating a mixture of **1b**, **2** and xanthone at 50 °C for 24 h without a solvent gave quantitative recovery of each starting material, but the reaction of **1b** with **2** in refluxing toluene for 15 h afforded **3b** in 95% yield (isolated yield). A solution of **1b** (0.66 mmol), **2** (1.3 mmol) and benzophenone (0.14 mmol) as a sensitizer in acetonitrile (15 ml) was irradiated for 5 h. After removal of the solvent, the oily residue was chromatographed by silica gel using ethyl acetate/hexane (1:1v/v) as eluent to give *exo–endo* double-[4+2]cycloadduct **4b** in 28% yield, together with **5b** (37% yield) by ^{1}H NMR spectral analysis. The same photoreaction in solution without benzophenone gave **5b** (84% yield). The results of similar photoreactions of **1** with **2** are summarized in Table 1. The solid-state photoreaction of **1a** with **2** in the presence of xanthone was not examined because a ground mixture of **1a**, **2**, and xanthone gave a viscous oil. The *exo–endo* configuration of **4** was assumed by inspection of the ^{1}H NMR spectral data in comparison with that of the *endo–endo* double-[4+2]adduct **3**, which was prepared from the thermal reaction. They have different 2- and 6-H chemical shifts due to the shielding effect of the C=C double bonds (**3b**: δ 2.81 (2-H), 3.23 (6-H), **4b**: δ 2.69 (2-H), 2.97 (6-H)).[8] It was found that the optimal molar ratio

Keywords: Solid-state photosensitized reaction; 2-Pyrones; Maleimides; [4+2] Cycloadducts.
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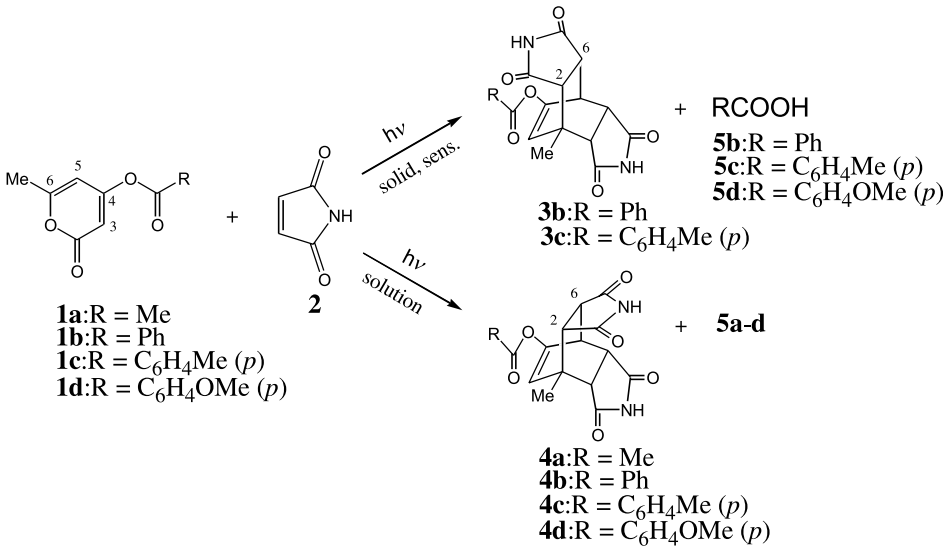


Scheme 1.

Table 1. Photoreaction of 2-pyrones (**1**) with maleimide (**2**) in the solid state[a] and in solution[b]

Pyrone	Yield (%) in the solid state					Yield (%) in solution					
	With sensitizer[c]			Without sensitizer[d]		With sensitizer[e]			Without sensitizer[d]		
	Conv.	Adduct **3**	Acid **5**	Conv.	Acid **5**	Conv.	Adduct **4**	Acid **5**	Conv.	Adduct **4**	Acid **5**
1a	—[f]	—[f]	—[f]	41	88	96	43	33	94	12	74
1b	45	66	18	45	96	97	28	37	96	0	84
1c	75	10	41	26	98	97	27	46	100	0	83
1d	49	0	55	33	98	100	22	47	100	0	92

[a] Mixture of **1** and **2** (1:2 molar ratio), and in the presence or in the absence of xanthone (0.25 molar ratio) was irradiated for 24 h.
[b] Solution of **1** and **2** (1:2 molar ratio), and in the presence or in the absence of benzophenone (0.2 molar ratio) was irradiated for 12 h.
[c] Reaction conversions and yields were determined by ^{1}H NMR spectroscopy with xanthone as an internal standard material.
[d] Reaction conversions and yields were determined by ^{1}H NMR spectroscopy with pyrazine as an internal standard material.
[e] Reaction conversions and yields were determined by ^{1}H NMR spectroscopy with benzophenone as an internal standard material.
[f] Mixed crystal was not obtained but gave viscous oil.

of **1b**:**2**:xanthone to give **3b** was 1.0:2.0:0.25 in the solid state because of the following results; **1b**:**2**:xanthone = 1.0:2.0:0.13 (**3b**, 0%), **1b**:**2**:xanthone = 1.0:2.0:0.25 (**3b**, 66%), **1b**:**2**:xanthone = 1.0:2.0:0.5 (**3b**, 13%), **1b**:**2**:xanthone = 1.0:2.0:1.0 (**3b**, 6%). In addition, the yield of **3b** was decreased by changing the molar ratio from 1.0:2.0:0.25 to 1.0:1.0:0.25.

Since it was difficult to obtain a single crystal of the 1:1 complex crystal between **1** and **2**, or the 1:1:1 complex crystal among **1**, **2**, and xanthone, the structure–reactivity correlation studies which have been shown in the 1:1 complex crystal of several 2-pyrones and maleimide[5] could not be carried out in this system. However, since the powder X-ray diffraction pattern for the ground mixed crystals **1b**·**2**, and **1b**·**2**·xanthone showed new peaks (Fig. 1), which phenomena have been shown in the 1:1 complex crystal of 2-pyrones and maleimide,[6] it was suggested that the crystalline complex also appeared in this system. Solid-state photosensitized reaction of **1d** (R = C_6H_4OMe (*p*)) with **2** did not afford double-[4 + 2]adduct **3**. Since, the crystal packing depends on a number of polar interactions, it is suggested that the change in the crystal structure caused by the polar nature and/or bulkiness of the *p*-methoxy group at the benzene ring prevents the favorable orientation in the ground state between **1d** and **2**. The

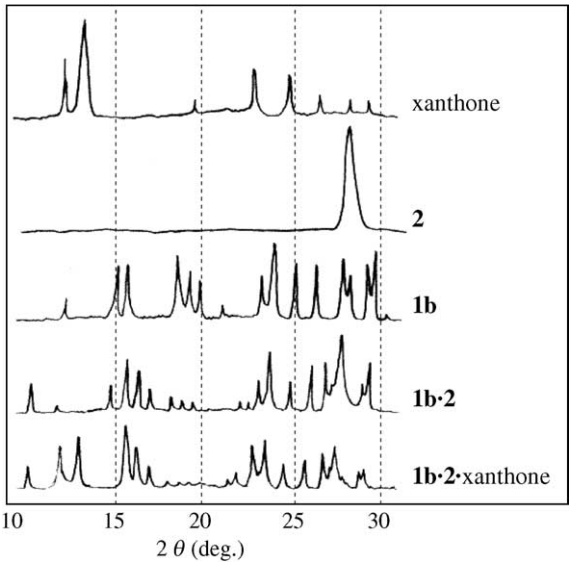
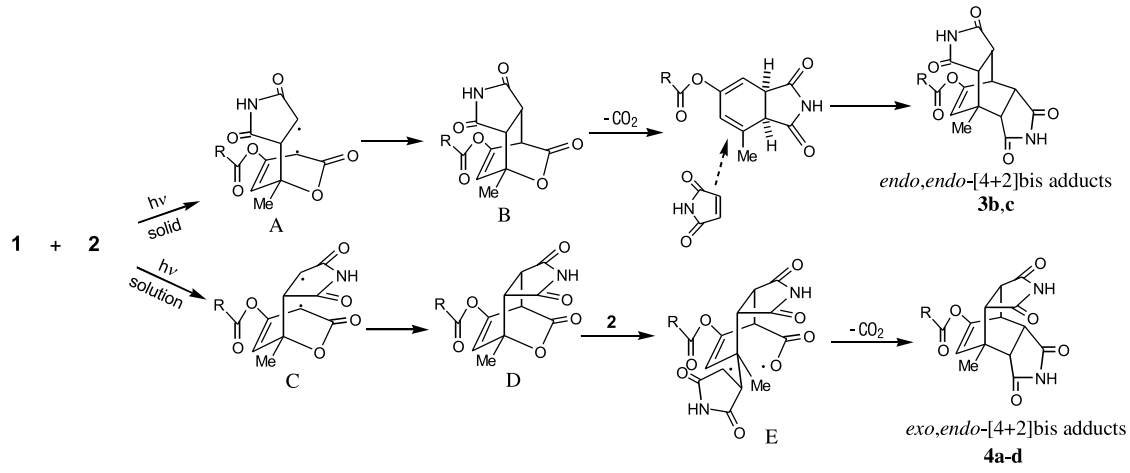


Figure 1. Powder X-ray diffraction patterns for **1b**, **2**, xanthone, **1b**·**2**, and **1b**·**2**·xanthone.



Scheme 2.

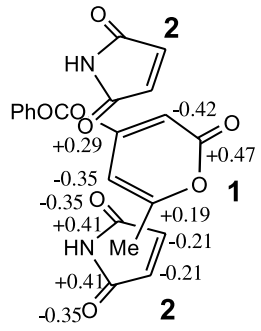


Figure 2. Estimated intermolecular packing of **1b** with **2** in the ground state from their electron densities and HOMO–LUMO interaction (for *endo* [4+2] addition) by the MO calculation.

selective formation of two types of double-[4+2]adduct in the solid-state and in solution photoreactions between **1** and **2** is estimated as follows (Scheme 2). In the solid-state sensitized photoreaction, **1** and **2** are regularly stacked in 1:2 ratio owing to their ground-state electrostatic interaction and the HOMO–LUMO interaction (for *endo* [4+2] addition) by the MO calculation using Win MOPAC (PM5) (Fig. 2) to give kinetically favorable *endo*-[4+2]adducts. In the reaction of **1b** with **2**, the mechanism is reasonably suggested from the FMO analysis (Win MOPAC (PM5)) via the triplet excited state of **1b** by considering the interaction between HSOMO (**1b**) and LUMO (**2**) (Fig. 3). That is, *endo*-[4+2]adduct **B** is formed via biradical **A** caused by the combination between the 6-position of **1b** and the olefinic carbon of **2** because of smaller energy gap and larger coefficients of the frontier molecular orbitals.[9] Double-[4+2]adduct **3b** is formed through decarboxylation of the [4+2] adduct **B** followed by addition of another molecule of **2** from the bottom-side attack. On the other hand, in the sensitized photoreaction in solution thermodynamically favorable *exo*-[4+2]adduct **D** is produced via biradical **C** due to the steric hindrance of benzoyloxy and methyl groups in the excited state.[9,10] Double-[4+2]adduct **4b** is formed via a biradical **E** from the *endo*-attack of **2** followed by decarboxylation. Namely, it is reasonable to consider that double-[4+2]adducts **3** and **4** were produced via a history of the initially formed *endo* or *exo* [4+2]adducts.

Photostability of **1a** was investigated because carboxylic acids **5** were obtained in all the photoreactions of **1a–d** with **2** (Table 1). A solution of **1a** (2.4 mmol) and ethanol (2.4 mmol) in chloroform (20 ml) was irradiated for 60 h. After removal of the solvent, the resulting oil was purified through preparative TLC to give **6** in 56% yield, whose structure was confirmed by X-ray crystallographic analysis, together with acetic acid **5a** (53% yield). It was estimated that compound **6** was produced via valence isomerization of **1a** first, followed by an addition of ethanol to a ketene intermediate formed from cyclobutene carboxylic acid (Scheme 3). The cleavage of the hydrogen bonded acetoxy group at the valence isomer was suggested from the PM5

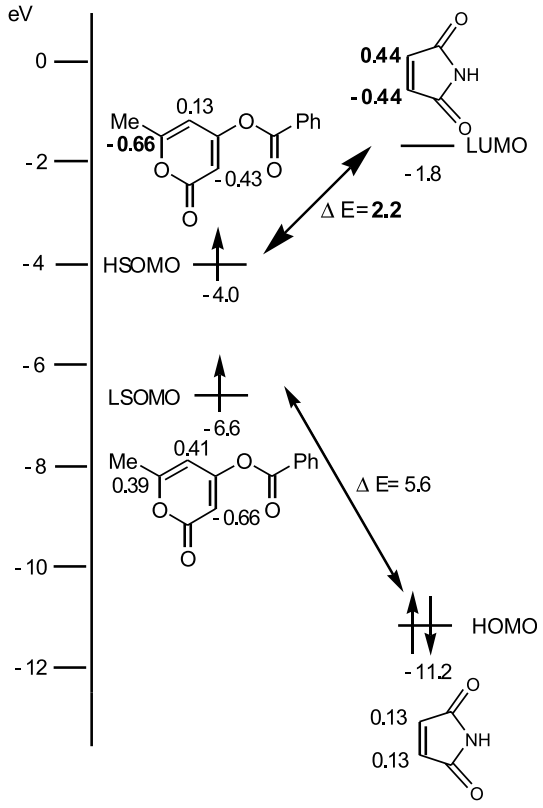
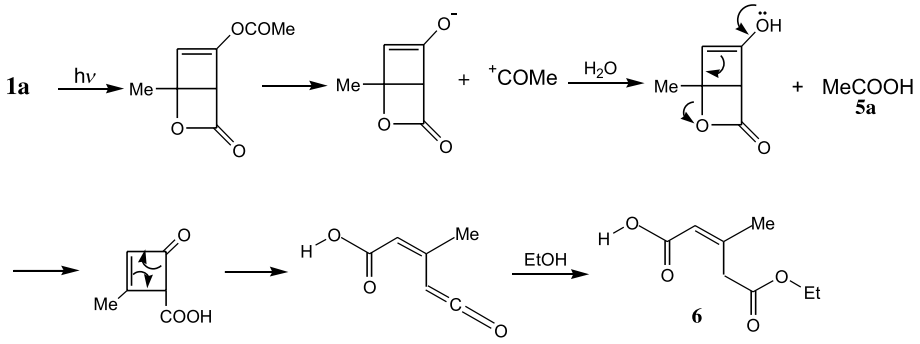


Figure 3. Estimated energies and coefficients of triplet 2-pyrone **1b** and ground-state maleimide by means of PM5 calculation.



Scheme 3.

calculation for the singlet excited state process of **1a** as shown in Figure 4. The intermolecular hydrogen bond between 3-H at 4-methoxy-2-pyrone and the carbonyl group of **2** is recognized to contribute to the selectivity of the [2+2] photocycloaddition of 2-pyrone with **2**.[6] In the case of 4-acetoxy-2-pyrone it was estimated that the intramolecular hydrogen bond, which was suggested from the MO calculation between 3-H at the 2-pyrone and carbonyl group of 4-acetoxy group, contributes to the elimination of the acetyl group. Namely, formation of the valence isomer by approach between the C_3 and C_6 on the singlet excited state of **1a** in the calculation showed a cleavage of the acyl part.

3. Conclusion

Sensitized photoreactions of ground mixtures of three components 4-acyloxy-6-methyl-2-pyrones **1b**,**c**, maleimide **2**, and xanthone (1:1:0.25 molar ratio), gave *endo–endo* double-[4+2]cycloadducts stereoselectivety, together with carboxylic acids by cleavage of the acyl groups. The mixed crystals of **1b**,**2**, and xanthone were predictable from the powder X-ray diffraction. The formation of the mixed crystals depends upon the feature of the *p*-substituent (polar nature and bulkiness) at the benzene ring of the 2-pyrone. It was concluded that the reaction mechanism for the [4+2] cycloaddition proceeded via the excited state of **1** and the ground state of **2** on the basis of frontier MO analysis. On the other hand, sensitized photoreactions of **1** with **2** in solution were found to give *exo–endo* double-[4+2]cycloadducts. Compounds **1** were found to be photolyzed easily to give carboxylic acids via the singlet excited state of **1** in the solid state and in solution. Compared with the solid-state photoreactions of 4-alkyloxy-2-pyrones with **2** giving [2+2] cycloadducts with high selectivity, the 4-acyloxy-2-pyrones showed a dramatic change in the photoreactions of 2-pyrone system in that they afforded [4+2] cycloadducts.

4. Experimental

4.1. General

All melting points are uncorrected. NMR spectra were measured at 400 MHz on the JNM GSX-400 (TMS as an internal standard). IR spectra were recorded with a JASCO IR Report-100 spectrometer as KBr disks. Mass spectra were recorded with a JEOL JMS-HX110A (FAB MS) using *m*-nitrobenzyl alcohol as matrix. Elemental analyses were made using a Yanaco MT-5. Photoirradiations were carried out in a Pyrex glass tube by using Riko 400 W high-pressure mercury lamp.

4-Acetoxy-6-methyll-2-pyrone (**1a**), 4-benzoyloxy-6-methyl-2-pyrone (**1b**), 4-(4-methylbenzoyloxy)-6-methyl-2-pyrone (**1c**), 4-(4-methoxybenzoyloxy)-6-methyl-2-pyrone (**1d**) were prepared according to the method described in the literature.[11]

Single crystal X-ray diffraction analysis of **6** was performed on a Rigaku RAXIS-RAPID imaging plate diffractometer with graphite-monochromated Mo Kα radiation. Lorentz and polarization corrections were applied to the intensity data. The structure was solved by direct methods using SIR92[12] and refined by a full-matrix least-squares method. The non-hydrogen atoms were refined anisotropically. All calculations were performed using the teXsan[13] crystallographic software package. Powder X-ray diffraction (PXD) patterns were obtained with Rigaku Corp. Model No. 2013 diffractometer equipped with Cu Kα radiation (1.64178 Å).

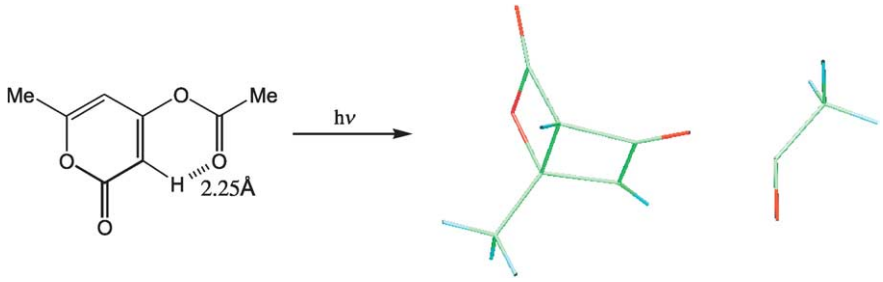


Figure 4. Estimation of the intramolecular hydrogen bonding at **1a** and cleavage of the acetoxy part by MO calculation.

Data were collected between 10 and 30° in 2θ at a scan rate of 1°/min.

4.1.1. 14-Benzoyloxy-1-methyl-4,10-diazatetracyclo-[5.5.2.0^{2,6}.0^{8,12}]tetradec-13-en-3,5,9,11-tetraone (3b) (*endo–endo* adduct). Compound **3b** was prepared from the photolysis of the mixed crystals **1b**·**2**·xanthone. The mixed crystals of **1b** (46 mg, 0.2 mmol), **2** (39 mg, 0.4 mmol), and xanthone (10 mg, 0.05 mmol) prepared by crystallization were ground for 10 min and sandwiched between two Pyrex glass plates and irradiated for 24 h under nitrogen atmosphere at room temperature. The reaction solid was washed with $CHCl_3$ (5 ml) to remove the starting materials and the resulting solid was filtered to give **3b** (40 mg, 52% yield), which was recrystallized from MeCN. The calculated yields of **3b** and benzoic acid (**5b**) were 66 and 18% (conversion 45%), respectively, by ^{1}H NMR spectroscopy with xanthone as an internal standard material. **3b**: mp 293–295 °C; IR (KBr) 1770, 1740, 1720 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.75 (3H, s), 2.81 (2H, d, J=8.0 Hz), 3.23 (3H, m), 5.75 (1H, s), 7.57–7.90 (5H, m), 11.26 (2H, s); LRMS m/z 381 (M+1). Anal. Calcd for $C_{20}H_{16}N_2O_6$:C, 62.99; H, 4.49; N, 7.35. Found: C, 62.86; H, 4.24; N, 7.39.

4.1.2. 14-(4-Methylbenzoyloxy)-1-methyl-4,10-diaza-tetracyclo[5.5.2.0^{2,6}.0^{8,12}]tetradec-13-en-3,5,9,11-tetra-one (3c) (*endo–endo* adduct). Compound **3c** was prepared from the irradiation of the mixed crystals **1c**·**2**·xanthone. The mixed crystals of **1c** (49 mg, 0.2 mmol), **2** (39 mg, 0.4 mmol), and xanthone (10 mg, 0.05 mmol) prepared by crystallization were ground for 10 min and sandwiched between two Pyrex glass plates and irradiated for 24 h. The same work-up gave **3c** (6 mg, 8% yield). The calculated yields of **3c** and 4-methylbenzoic acid (**5c**) were 10 and 41% (conversion 75%), respectively, by ^{1}H NMR spectroscopy with xanthone as an internal standard material. **3c**: mp 297–300 °C; IR (KBr) 1760, 1720, 1710 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.75, 2.40 (each 3H, s), 2.80 (2H, d, J=8.0 Hz), 3.22 (2H, dd, J=8.0 Hz), 3.30 (1H, s), 5.72 (1H, s), 7.37–7.77 (4H, m), 11.26 (2H, s); LRMS m/z 395 (M+1). HRMS (M+1) calcd for $C_{21}H_{19}N_2O_6$ 395.1243. Found 395.1242.

4.2. Photoreaction of 1 with 2 in solution

4.2.1. 14-Acetoxy-1-methyl-4,10-diazatetracyclo-[5.5.2.0^{2,6}.0^{8,12}]tetradec-13-en-3,5,9,11-tetraone (4a) (*exo–endo* adduct). A solution of **1a** (270 mg, 1.6 mmol), **2** (316 mg, 3.2 mmol), and benzophenone (59 mg, 0.32 mmol) in MeCN (33 ml) was irradiated for 24 h under nitrogen atmosphere at room temperature. After evaporating the solvent, the resulting residue was purified by preparative TLC (silica gel, ethyl acetate/hexane 1:1) to give **4a** (98 mg, 19% yield). The calculated yields of **4a** and acetic acid (**5a**) were 43 and 33% (conversion 96%), respectively, by ^{1}H NMR spectroscopy with benzophenone as an internal standard material. Compound **4a**: mp 213–216 °C; IR (KBr) 1770, 1720, 1710 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.65, 2.13 (each 3H, s), 2.64 (1H, d, J=8.0 Hz), 2.69 (1H, d, J=10.0 Hz), 2.88 (1H, dd, J=8.0, 3.2 Hz), 3.11 (1H, s), 3.28 (1H, dd, J=10.0, 4.8 Hz), 5.53 (1H, s), 11.29, 11.39 (each 1H, s); LRMS m/z 319 (M+1). HRMS (M+1) calcd for $C_{15}H_{14}N_2O_6$ 319.0930. Found 319.0928.

4.2.2. 14-Benzoyloxy-1-methyl-4,10-diazatetracyclo-[5.5.2.0^{2,6}.0^{8,12}]tetradec-13-en-3,5,9,11-tetraone (4b) (*exo–endo* adduct). Similar photoirradiation of a solution of **1b** (150 mg, 0.66 mmol), **2** (130 mg, 1.3 mmol), and benzophenone (24 mg, 0.14 mmol) in MeCN (15 ml), and the same work-up afforded **4b** (68 mg, 27% yield). The calculated yields of **4b** and benzoic acid (**5b**) were 28 and 37% yields (conversion 97%), respectively, (^{1}H NMR analysis). Compound **4b**: mp 250–253 °C; IR (KBr) 1775, 1725, 1720 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.71 (3H, s), 2.69 (1H, d, J=8.0 Hz), 2.79 (1H, d, J=10.0 Hz), 2.97 (1H, dd, J=8.0, 2.8 Hz), 3.26 (1H, dd, J=4.0, 2.8 Hz), 3.46 (1H, dd, J=10.0, 4.0 Hz), 5.75 (1H, s), 7.90–7.98 (5H, m), 11.34, 11.43 (each 1H, s); LRMS m/z 381 (M+1). HRMS (M+1) calcd for $C_{20}H_{17}N_2O_6$ 381.1087. Found 381.1128.

4.2.3. 14-(4-Methylbenzoyloxy)-1-methyl-4,10-diaza-tetracyclo[5.5.2.0^{2,6}.0^{8,12}]-tetradec-13-en-3,5,9,11-tetra-one (4c) (*exo–endo* adduct). Similar photoirradiation of a solution of **1c** (500 mg, 2.05 mmol), **2** (400 mg, 4.10 mmol), and benzophenone (73 mg, 0.40 mmol) in MeCN (70 ml), and the same work-up gave **4c** (162 mg, 20% yield). The calculated yields of **4c** and 4-methyl-benzoic acid (**5c**) were 27 and 46% yields (conversion 97%), respectively, (^{1}H NMR analysis). Compound **4c**: mp>300 °C; IR (KBr) 1760, 1700, 1690 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.69 (3H, s), 2.37 (3H, s), 2.68 (1H, d, J=8.0 Hz), 2.78 (1H, d, J=9.2 Hz), 2.96 (1H, dd, J=8.0, 3.2 Hz), 3.24 (1H, s), 3.48 (1H, dd, J=9.2, 3.2 Hz), 5.73 (1H, s), 7.38–7.85 (4H, m), 11.37, 11.43 (each 1H, s); LRMS m/z 395 (M+1). HRMS (M+1) calcd for $C_{21}H_{19}N_2O_6$ 395.1243. Found 395.1287.

4.2.4. 14-(4-Methoxybenzoyloxy)-1-methyl-4,10-diaza-tetracyclo[5.5.2.0^{2,6}.0^{8,12}]-tetradec-13-en-3,5,9,11-tetra-one (4d) (*exo–endo* adduct). Similar photoirradiation of a solution of **1d** (1.0 g, 3.9 mmol), **2** (750 mg, 7.7 mmol), and benzophenone (140 mg, 0.77 mmol) in MeCN (140 ml), and the same work-up gave **4d** (316 mg, 20% yield). The calculated yields of **4d** and 4-methoxybenzoic acid (**5d**) were 22 and 47% yields (conversion 100%), respectively, (^{1}H NMR analysis). Compound **4d**: mp>300 °C; IR (KBr) 1765, 1710, 1700 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.69 (3H, s),2.68 (1H, d, J=8.0 Hz), 2.78 (1H, d, J=9.6 Hz), 2.95 (1H, dd, J=8.0, 3.2 Hz), 3.23 (1H, s), 3.47 (1H, dd, J=9.6, 3.2 Hz), 3.86 (3H, s), 5.71 (1H, s), 7.09–7.91 (4H, m), 11.36, 11.43 (each 1H, s); LRMS m/z 411 (M+1). Anal. Calcd for $C_{21}H_{18}N_2O_7$:C, 61.31; H, 4.66; N, 6.81. Found: C, 61.61; H, 4.39; N, 7.07. HRMS (M+1) calcd for $C_{21}H_{19}N_2O_7$ 411.1192. Found 411.1228.

4.2.5. 4-Ethoxycarbonyl-3-methyl-2-butenoic acid (6). A solution of **1a** (400 mg, 2.4 mmol) and ethanol (110 mg, 2.4 mmol) in $CHCl_3$ (20 ml) was irradiated for 60 h. After evaporating the solvent, the resulting residue was purified by preparative TLC (silica gel, ethyl acetate/hexane 1:1) to give **6** (76 mg, 53% yield), which was recrystallized from hexane. Compound **6**: mp 56–59 °C; IR (KBr) 1730, 1695 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J=7.2 Hz), 1.89 (3H, s), 3.70 (2H, s), 4.06 (2H, J=7.2 Hz),

5.79 (1H, s), 12.15 (1H, s); ^{13}C NMR (DMSO-d_6) δ 14.1, 26.0, 38.7, 60.9, 118.6, 153.9, 170.0; LRMS *m*/*z* 173 (M+1). HRMS (M+1) calcd for $C_8H_{13}O_4$ 173.0814. Found 173.0813.

4.3. Powder X-ray diffraction data of 2-pyrone 1b, maleimide 2, xanthone, grinding mixtures of 1b·2, and 1b·2·xanthone

Compound **1b**: 2θ=12.5, 15.1, 16.0, 18.5, 19.2, 19.8, 21.3, 23.4, 23.9, 25.1, 26.4, 27.6, 28.3, 29.0, 29.5. Compound **2**: 2θ=28.2; xanthone: 2θ=13.2, 14.3, 20.0, 23.4, 24.7, 26.2, 28.3, 29.3; grinding mixture of **1b** and **2** for 10 min: 2θ= 10.9, 12.3, 14.8, 16.0, 16.7, 17.2, 18.3, 19.0, 19.5, 22.2, 22.6, 23.4, 23.8, 24.8, 26.2, 26.9, 27.9, 28.8, 29.3; grinding mixture of **1b**, **2**, and xanthone for 10 min: 2θ=10.7, 13.2, 14.3, 16.0, 16.7, 17.2, 21.5, 21.9, 22.6, 23.6, 24.6, 26.1, 26.8, 27.9, 28.7, 29.2.

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