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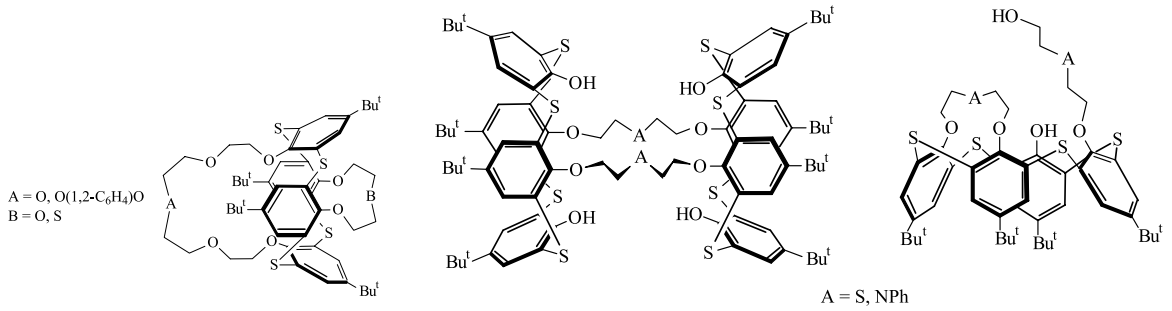
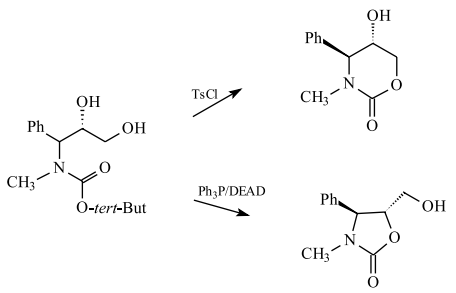
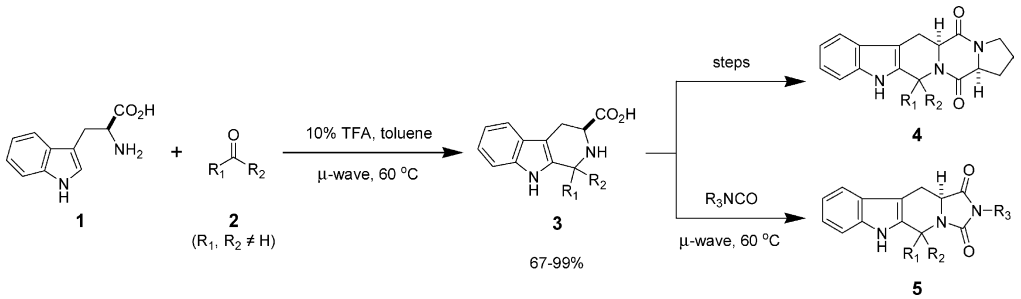
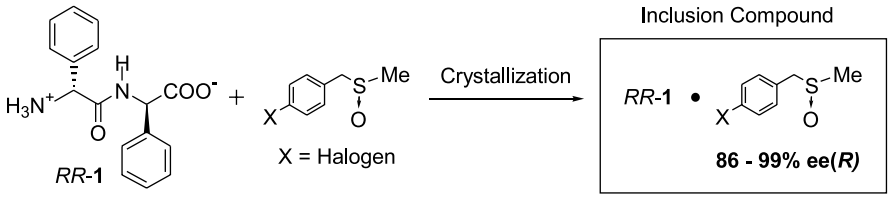
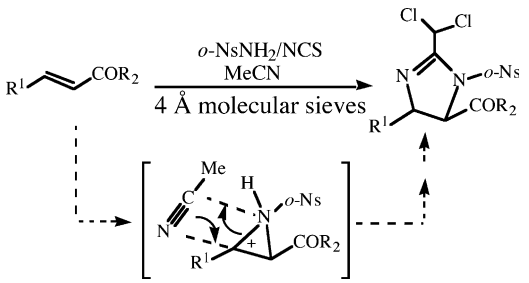


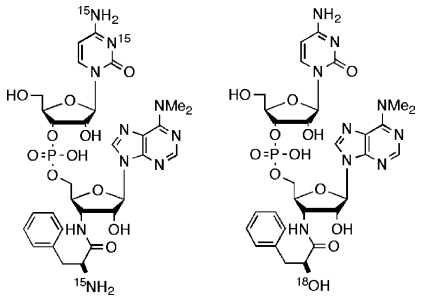


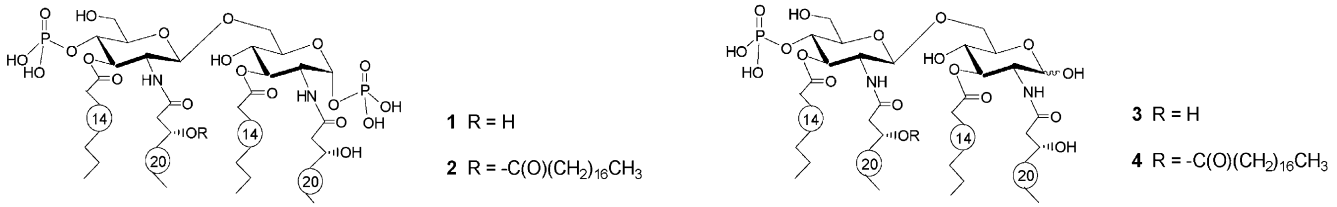


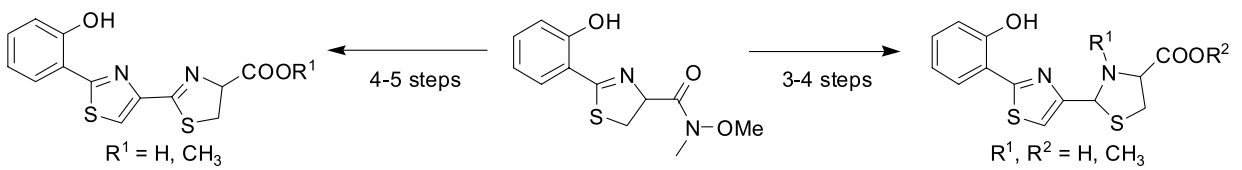


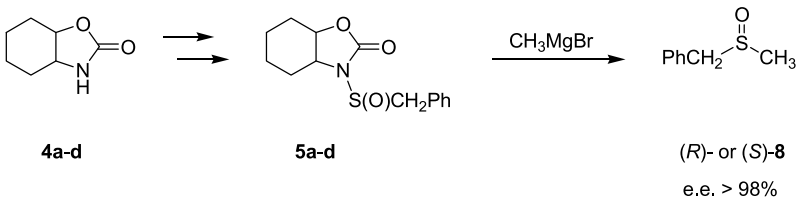
The regio-, stereo- and chemoselective imidazolination of enones has been established by using the new combination of 2-$NsNH_2$/NCS as the electrophilic nitrogen source and MeCN as the nucleophile. The reaction is very convenient to carry out simply by mixing olefin, 2-$NsNH_2$, NCS and 4 Å molecular sieves in freshly distilled acetonitrile at room temperature. The aziridinium ion formed from the reaction of 2-NsNCl with olefins and the corresponding [2+3] cycloaddition are proposed during the reaction process to control regio- and stereoselectivity.

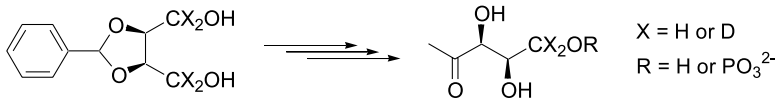


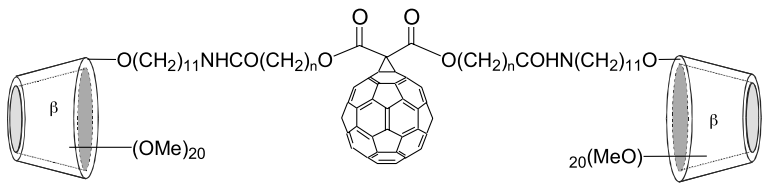


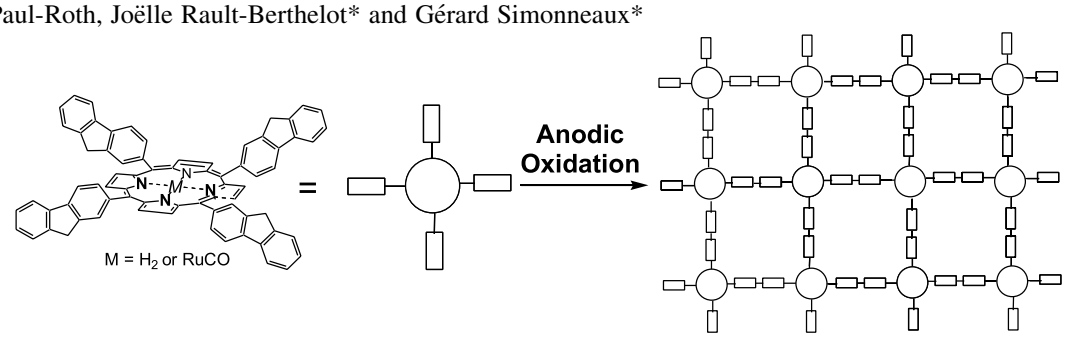


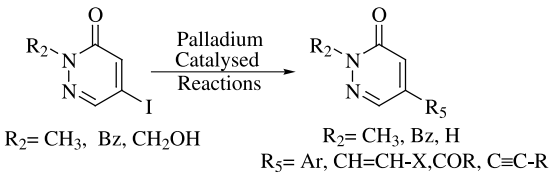


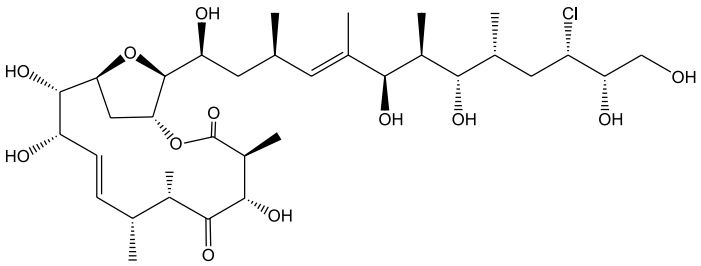


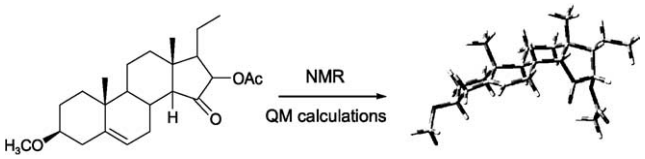


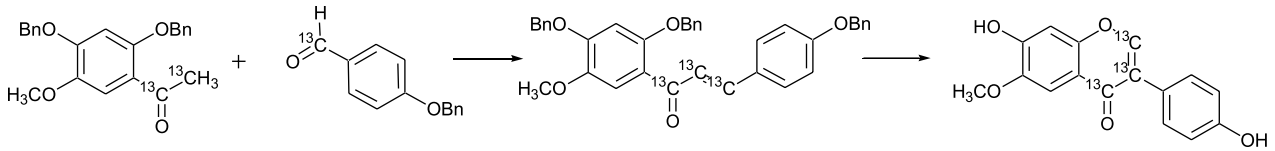


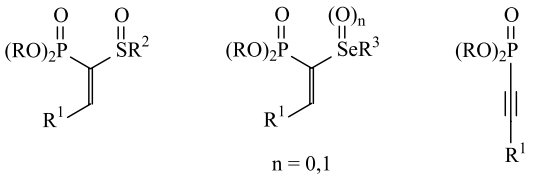


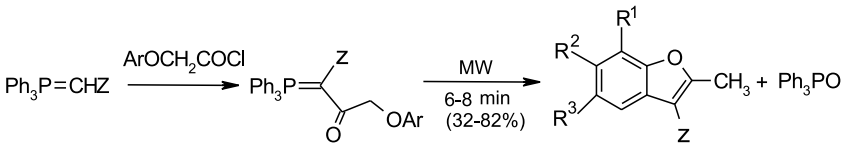


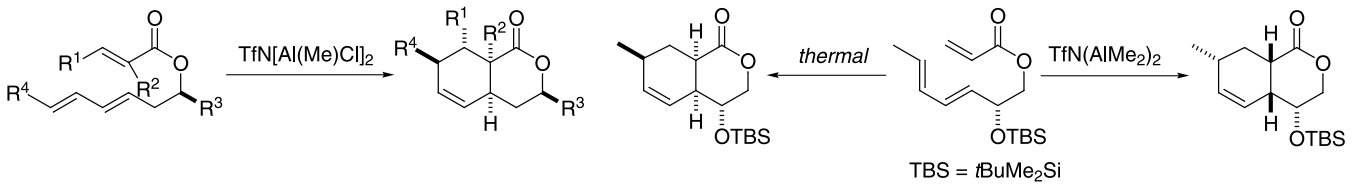


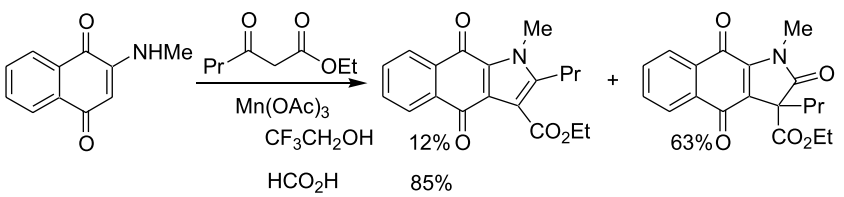


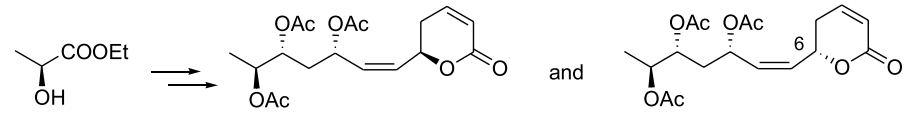












Ethyl L-lactate Hyptolide 6-*Epi*-hyptolide

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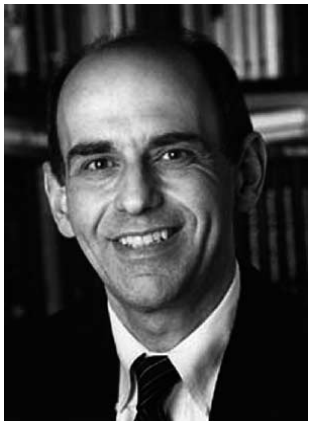
Publisher's Announcement— New Chairman of the Executive Board of Editors for Tetrahedron publications

The publisher of Tetrahedron Publications wishes to announce that commencing 1st January 2005, Professor Bruce Ganem will be appointed as the Chairman of the executive board of editors for Tetrahedron publications. Professor Ganem will succeed Professor Leon Ghosez, who stands down after four years of dedicated service to the journals. Professor Ghosez has been an exemplary ambassador for the journals, fostering effective communication within the family of five titles, and with the chemistry community at large. The publisher wishes to extend sincere thanks to Professor Ghosez for his term as Chairman, and wishes Professor Ganem every success during his appointment.



Professor Bruce Ganem
Chairman of the executive board of editors for Tetrahedron publications

Professor Ganem is Franz and Elisabeth Roessler Professor of Chemistry and Chemical Biology and J. Thomas Clark Professor of Entrepreneurship and Personal Enterprise at Cornell University, Department of Chemistry and Chemical Biology. His research is focused on modern applications of synthetic organic chemistry to a wide range of problems of biological interest, such as the improvement and enhancement of modern diversity-oriented synthesis.

Professor Ganem obtained his BA from Harvard University in 1969, and his PhD from Columbia University in 1972. He has won numerous awards both for his research and for his teaching, including the ACS Arthur C. Cope Scholar Award; the Catalyst Award; the American Cyanamid Award for the Advancement of the Art and Science of Chemical Synthesis; the Camille and Henry Dreyfus Teacher-Scholar Award; and the Clark Distinguished Teaching Award.

Professor Ganem has been US Editor for *Tetrahedron Letters* since 1998, and will continue to act in this capacity for the duration of his appointment as Chairman, dealing with papers in the field of general synthetic and combinatorial methods, bioorganic chemistry, heterocycles, natural products, carbohydrates and photochemistry.

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Recent advances in the total syntheses of oxazole-containing natural products

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Contents

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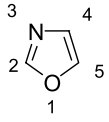
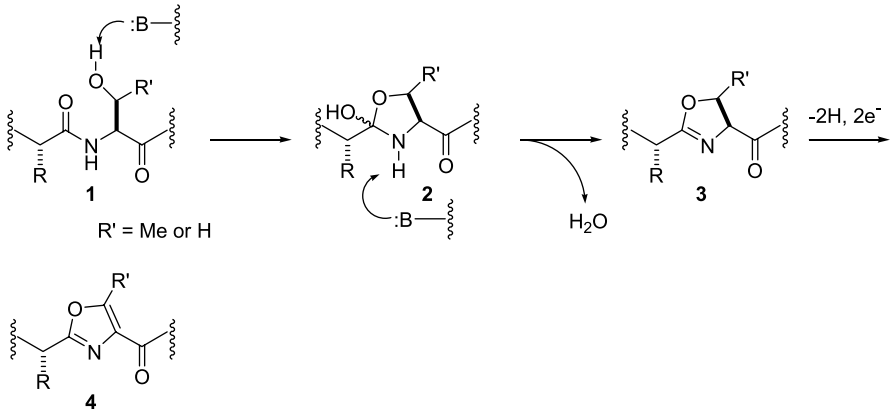
1. Introduction

The oxazole is a five-membered aromatic heterocycle that contains both oxygen and nitrogen. Oxazoles are numbered around the ring starting at the oxygen atom as shown below. The parent heterocycle is a liquid at room temperature, and has a boiling point of 69 °C.



Naturally occurring oxazoles were considered rare until the late 1980s when a number of unprecedented natural products such as mono-oxazole calyculins, bisoxazole hennoxazoles, and trisoxazole ulapualides were isolated from marine organisms.[1] These oxazoles are derived from enzymatic post-translational modifications of peptide based precursors.[2] The oxygen functionality on the side chain of *N*-acylated serines (R′=H) and threonines (R′=Me) is capable of undergoing heterocyclization onto the preceding carbonyl group to create five membered saturated heterocycles **2** (Scheme 1). These compounds, after dehydration and two electron oxidation, result in hetero-aromatic oxazole **4**.

Due to their fascinating structures and interest in their biological activities, oxazole-containing natural products have attracted the attention of many research groups who pursue their total syntheses. One of the key synthetic challenges is a mild and selective oxazole formation in the presence of other sensitive functional groups. Many new oxazole formation methodologies have been developed to meet these challenges,[3] which culminated in numerous total syntheses of these fascinating molecules. This review will focus on recently completed total syntheses of natural products that contain oxazole moieties as part of their structures covering literature up to December 2003. Rather than a comprehensive examination, this review will highlight syntheses that feature novel and unique oxazole construction methodologies. Moreover, key synthetic strategies and assemblage of fragments will also be discussed for each of the selected examples.

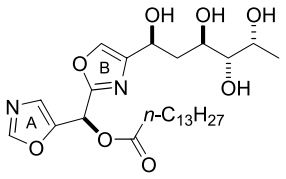


Scheme 1.

2. Total syntheses of oxazole containing natural products

2.1. Bengazole A

Bengazole A (**5**) (Fig. 1) and related homologues are bisoxazole containing natural products isolated from marine sponges of the genus *Jaspis*.[4] Bengazole A (**5**) exhibits potent in vitro antifungal activity against *Candida albicans*[5] and fluconazole-resistant *Candida* strains,[6] a property similar to that of amphotericin B. It is unknown if **5** has the same mode of action as amphotericin B, namely, the formation of ion-permeable pores in yeast cell-membranes, or whether it has a novel mode of action. Molinski and coworkers determined the structure of **5** by NMR and chiroptical methods.[4b] Bisoxazole **5** displays a biogenically rare 5-monosubstituted oxazole (ring A) in addition to the more common 2,4-disubstituted oxazole (ring B). In addition to determining the absolute stereochemistry of **5**, Molinski and co-workers were the first to report its total synthesis.[7] Recently, a second total synthesis of deacyl-bengazole was also reported by Shioiri and co-workers.[8]

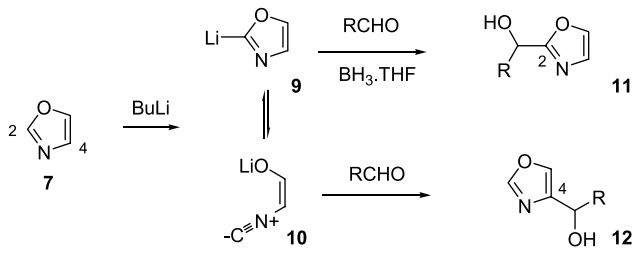


bengazole A (**5**)

Figure 1.

2.1.1. Molinski's total synthesis of bengazole A (5).[7] In Molinski and co-worker's total synthesis of bengazole A (**5**), the authors exploited two consecutive regiocontrolled metallation–addition reactions of the parent oxazole heterocycle (**7**) at C4 and C2 for the construction of the central oxazole unit (ring B), which served as a lynchpin for connecting the monosubstituted oxazole (ring A) to the polyol portion of the molecule (Scheme 2). This strategy is a conceptual departure from the common method of building 2,4-disubstituted oxazoles involving cyclodehydration of an *N*-acylserine amide to the corresponding oxazoline followed by oxidation to form the final oxazole.

C2-lithiated oxazoles can react with electrophiles at either C2 or C4 depending on the nature of the eletrophile and reaction conditions. Hodges and others have shown that 2-lithiooxazole **9** adds to aldehyde electrophiles exclusively at C4 through the isomeric ring-opened enolate-isonitrile **10** followed by ring closure (Scheme 3).[9] Complementary regioselectivity is observed in borane-mediated 2-lithio-



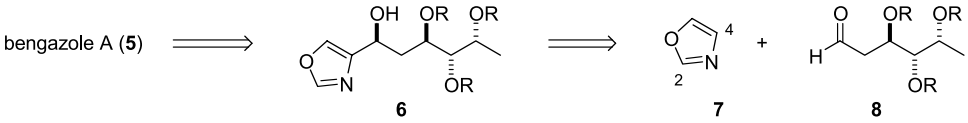
Scheme 3.

oxazole reaction with aldehydes that occur at C2, as demonstrated by Vedejs.[10]

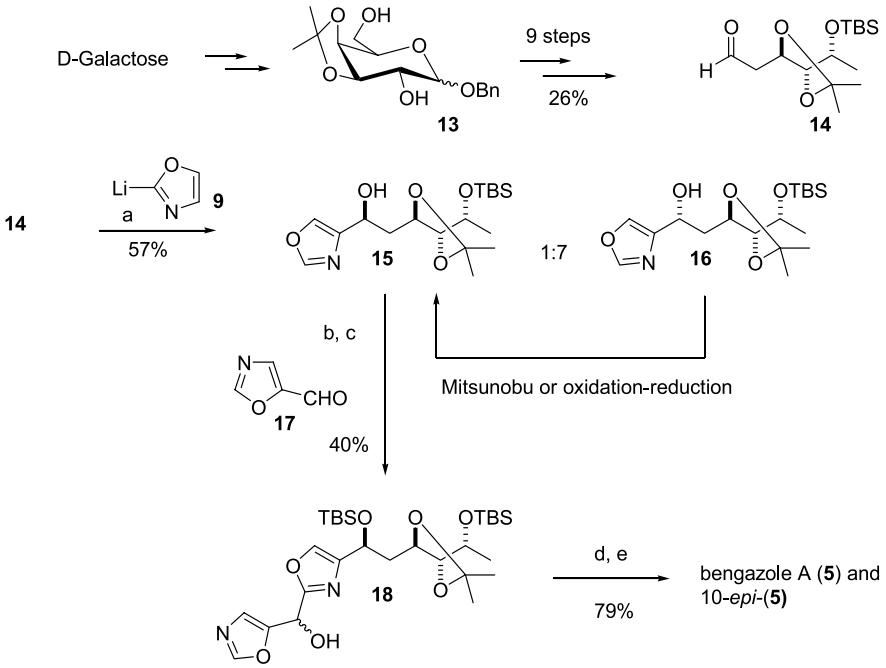
The polyol side chain segment **14** was prepared from D-galactose derivative **13** in nine steps (26% overall yield) (Scheme 4).[7b,c] The addition of 2-lithiooxazole **9** to **14** was diastereoselective (1:7) for the undesired epimer (i.e. **16**, 57% yield) for bengazole A synthesis. The diastereoselectivity was highly influenced by the solvent with a 1:20 v/v hex/THF giving the highest selectivity. The newly generated C6 stereogenic center could be inverted by either a Mitsunobu reaction or by an oxidation and reduction sequence (**16** to **15**). The second oxazole unit in bengazole A was assembled by treating the fully TBS protected **15** with $BH_3 \cdot THF$, deprotonating with *n*-BuLi, lithiating a second time with *t*-BuLi, and finally adding aldehyde **17**. The addition was not diastereoselective and gave **18** as 1:1 mixture of epimers at C10 in 40% yield. In contrast to the first lithiooxazole addition, TBS-**15** reacted with aldehyde **17** exclusively at C2 of the oxazole ring. Final acylation with myristol chloride gave bengazole A (**5**) and 10-*epi*-bengazole A as an inseparable mixture in 79% yield.

2.2. Calyculins

The calyculins are a family of structurally unique natural products isolated from the marine sponge *Discodermia calyx*.[11] The structure of calyculin A (**19**) (Fig. 2) was first reported by Fusetani and co-workers in 1986, and the relative stereochemistry was determined by X-ray analysis.[11] The absolute configuration was first tentatively assigned based on circular dichroism of the C33–C37 amino acid fragment obtained from degradation studies.[12] This assignment was confirmed by an asymmetric synthesis of the antipode of this degradation product by Shioiri.[13] In subsequent studies, seven additional congeners (Calyculins B–H) were isolated from the same source.[14] These structurally related analogues differ by the presence of an additional methyl group at C32, and/or geometric differences at the C2 and C6 olefin. Six other related compounds were reported recently, including calyculin J, caliculinamide A, B, F, des-*N*-methylcalyculin A, and desphosphono calyculin A.[15]

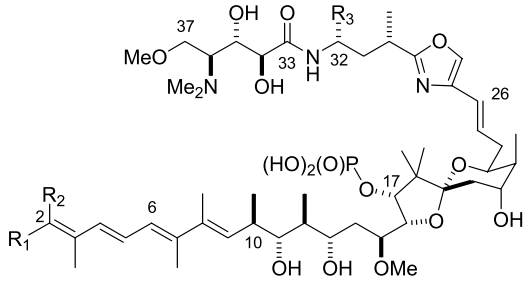


Scheme 2.



Scheme 4. (a) THF/hex, −78 °C. (b) TBSOTf, 2,6-lutidine. (c) $BH_3 \cdot THF$; *t*-BuLi; **17**, THF. (d) *n*-$C_{13}H_{27}COCl$, DMAP, Et_3N. (e) HF aq. CH_3CN.

The calyculins possess a number of interesting biological activities. It has been shown that most of the calyculins, including desphosphonocalyculin A, are potent inhibitors of protein phosphatase 1 and 2A with IC_{50} values on the order of 1 nM.[16] Calyculins A–D also exhibit potent cytotoxicity against L1210 leukemia cells.[14] In particular, calyculin A displays in vivo antitumor activity against Ehrlich and P388 leukemia in mice.[11] Calyculin A has also found application in the study of intracellular signal transduction due to its remarkable cell membrane permeability.[17]



calyculin	R_1	R_2	R_3	C_6 - geometry	
A	CN	H	H	*E*	(**19**)
B	H	CN	H	*E*	(**20**)
C	CN	H	CH_3	*E*	(**21**)
D	H	CN	CH_3	*E*	
E	CN	H	H	*Z*	
F	H	CN	H	*Z*	
G	CN	H	CH_3	*Z*	
H	H	CN	CH_3	*Z*	

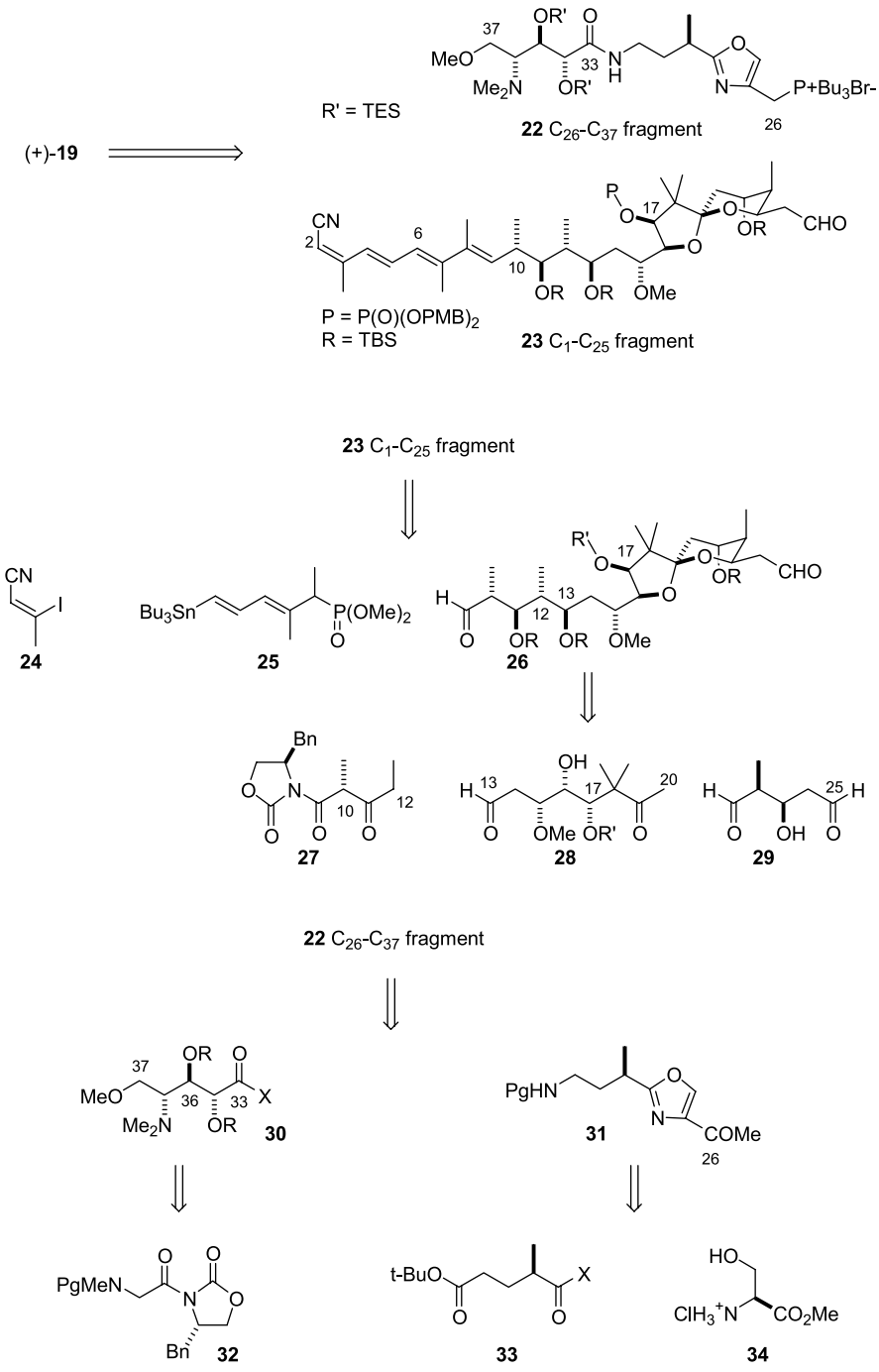
Figure 2.

The structure of calyculins features a central oxazole unit that bridges the C33–C37 amino acid fragment and the C1–C25 polypropionate portion. The C30 stereogenic center, adjacent to the oxazole, is prone to epimerization. Therefore, an efficient synthesis of the oxazole fragment must address this stability issue. Calyculins have attracted a great deal of attention and many research groups have pursued their syntheses.[18] Evans et al. were the first to report a total synthesis of (+)-**19**, the antipode of the natural product.[19] Following Evans' report, several other groups have also completed calyculin total syntheses: (−)-**19** by Masamune,[20] calyculin C (**21**) by Armstrong,[21] (+)-**19** and (−)-calyculin B (**20**) by Smith.[22] Furthermore, formal syntheses of advanced intermediates have also been disclosed by Shioiri[23a] and Barrett's group.[23b]

2.2.1. Evans total synthesis of (+)-calyculin A (19).[19] The key bond disconnection made by Evans' synthesis was at the C25–C26 olefin which divided the molecule into two major fragments of approximately equal complexity (Scheme 5).

It is interesting to note that the same bond disconnections are common to all subsequent calyculin syntheses. A phosphorus based olefination procedure was chosen to construct the C25–C26 double bond due to the mild conditions needed to unite the two complex fragments **22** and **23**. In their synthesis, Evans and co-workers demonstrated the utility of the phenylalanine-derived oxazolidinone chiral auxiliary[24] in the construction of several key stereogenic centers. This included the C17 and the C12–C13 of the spiral ketal fragment **26**, and the C34–C36 of γ-amino acid unit **30** (Scheme 5).

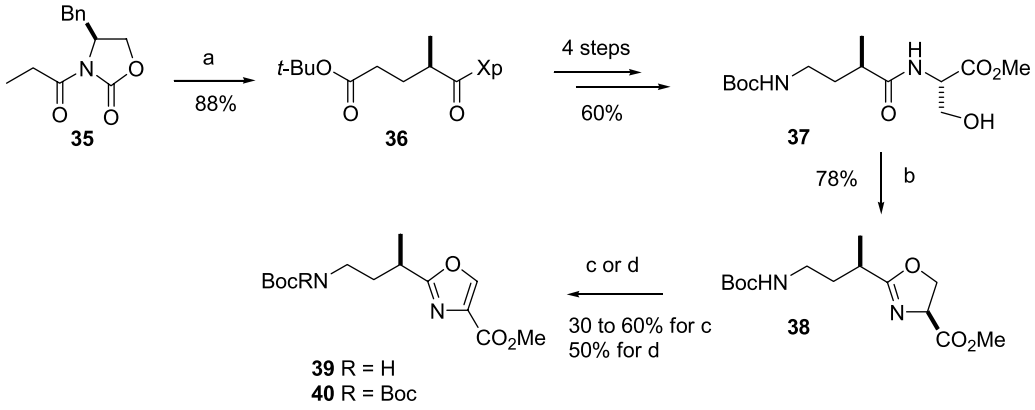
The synthesis of the C26–C32 oxazole fragment (**40**) began with a highly diastereoselective (>95:5) Michael addition



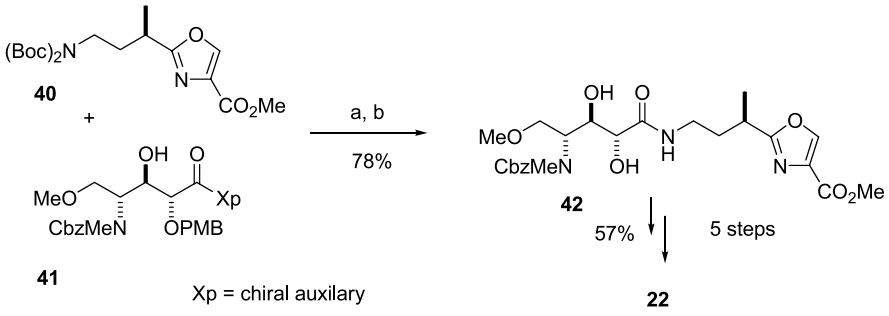
Scheme 5.

of *N*-propionyloxazolidinone **35** to *tert*-butyl acrylate to give adduct **36** in 88% yield (Scheme 6).[24f] Adduct **36** was then transformed into the serine amide **37** in four steps (60% yield). Cyclodehydration was achieved by treatment with $SOCl_2$ in pyridine to give oxazoline **38**. At this stage two different methods were examined to convert oxazoline **38** into oxazole **39** or **40**. Using a traditional method of dehydrogenation with nickel peroxide, an uncharacterized black powder produced by treatment of nickel(II) sulfate with sodium hypochlorite,[25] gave variable yields (30–60%). Therefore, a more laborious, but reliable, procedure which first involves full Boc-protection, then α-selenation followed by oxidative elimination to obtain **40** in large quantities (50% over three steps).

Oxazole **40** was effectively united with γ-amino acid derivative **41** using $AlMe_3$ as a promoter (Scheme 7). In the acylation step the chiral auxiliary was released from **41** and the C34 PMB ether was unexpectedly cleaved (78% over two steps). The coupled product was then elaborated in five steps (57% yield) to the desired tributylphosphonium salt **22** in preparation for the Wittig olefination with the C1–C25



Scheme 6. (a) Ti(O-*i*-Pr)Cl_3, *i*-Pr_2NEt, CH_2Cl_2; *tert*-butyl acrylate. (b) $SOCl_2$, pyridine. (c) Nickel peroxide. (d) (i) $(Boc)_2O$, DMAP, MeCN. (ii) KHMDS, PhSeCl. (iii) H_2O_2.

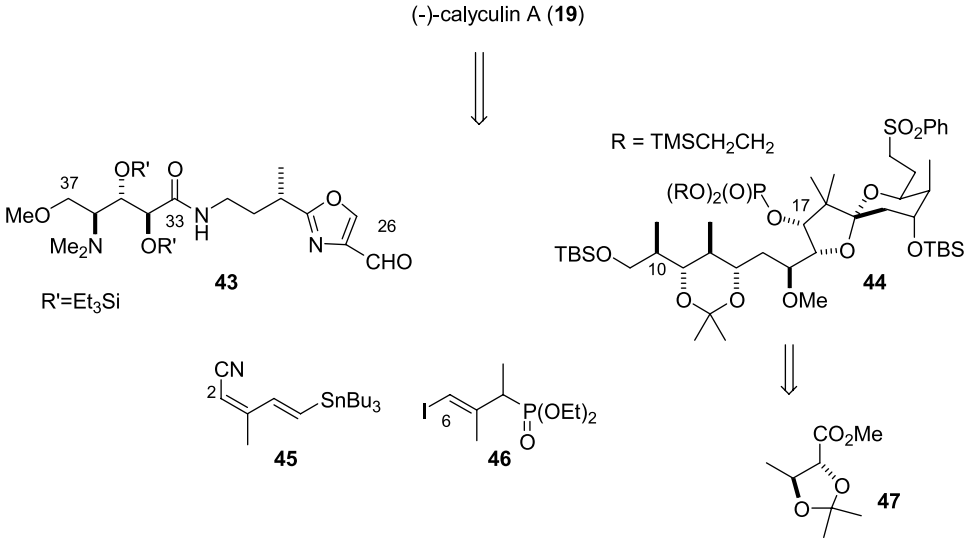


Scheme 7. (a) HCl(g), EtOAc. (b) $AlMe_3$, CH_2Cl_2.

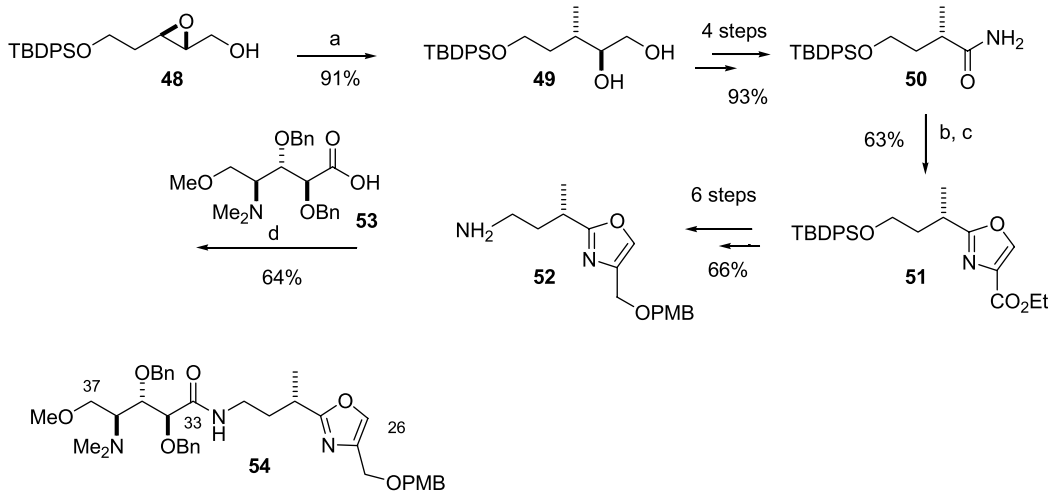
fragment **23**. The union of the two elaborate fragments (**22** and **23**) occurred in 65% yield and final deprotection using HF in wet CH_3CN gave synthetic (+)-**19**.

2.2.2. Masamune's total synthesis of (−)-calyculin A (19).[20] Scheme 8 summarizes the strategy utilized by Masamune et al. in their synthesis of (−)-**19**. In contrast to Evans' synthesis, the sensitive cyanotetraene unit was installed after the union of the C9–C25 spirolketal **44** and the C26–C37 oxazole **43** fragments. The C25–C26 olefin was formed by Julia olefination procedures and the construction of **44** featured various interesting aldol reactions starting from protected chiral diol **47**.

The preparation of the oxazole fragment was achieved by regioselective nucleophilic opening of chiral epoxide **48** by



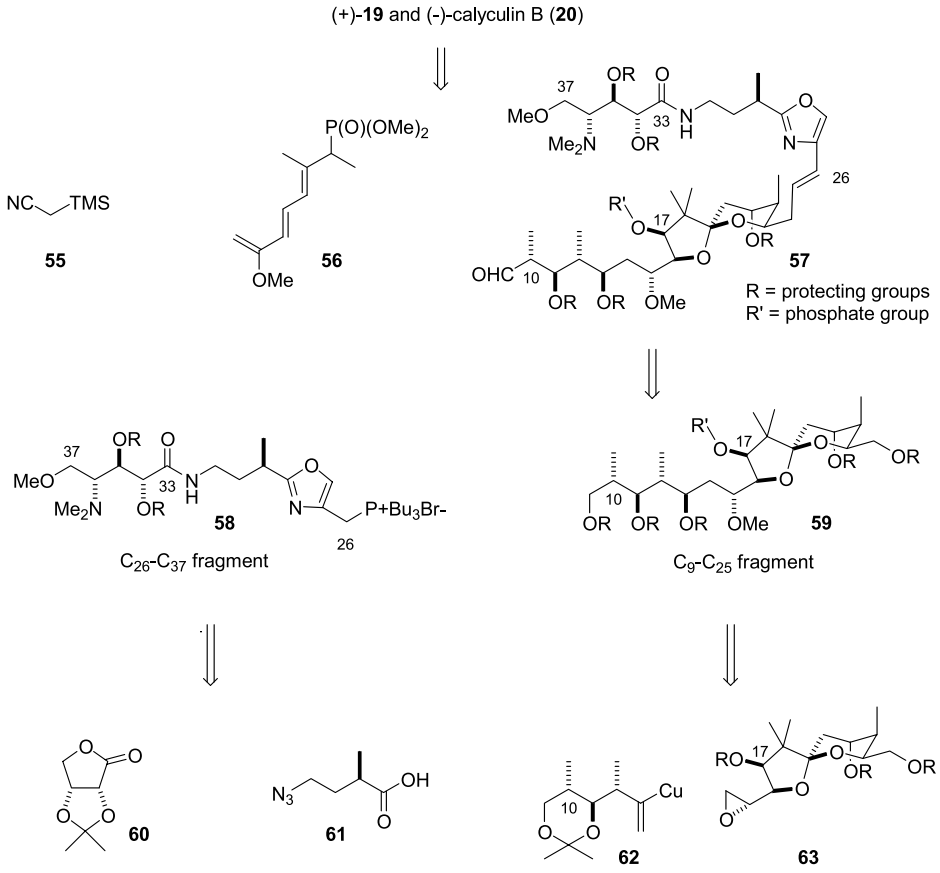
Scheme 8.



Scheme 9. (a) $AlMe_3$, pentane. (b) $BrCH_2C(O)CO_2Et$, 3,4-epoxycyclopentene, THF. (c) TFAA, pyr. (d) DCC, DMAP.

$AlMe_3$ which installed the C30 methyl group (Scheme 9).[18o] The resulting diol **49** was converted to amide **50** via a four step sequence (93% yield). Using the classical Hantzsch oxazole synthesis with amide **50** and an α-haloketone,[26] the authors found extensive epimerization at the α-stereogenic center at C30. However, they discovered that the epimerization could be completely suppressed when the reaction was conducted in the presence of excess 3,4-epoxycyclopentene. Hence, treatment of amide **50** with ethyl bromopyruvate and 3,4-epoxycyclopentene furnished the corresponding 4-hydroxyoxazoline which, upon dehydration, gave the desired oxazole **51** in 63% yield (two steps) with no epimerization. In six steps (66% yield), **51** was transformed into primary amine **52** which allowed the coupling with the amino acid **53** to give fragment **54** en route to the target molecule.

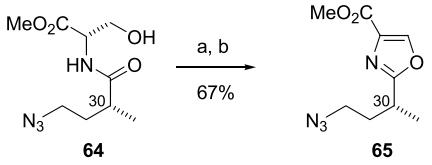
2.2.3. Smith's total syntheses of (+)-calyculin A (19) and (−)-calyculin B (20).[22] Smith and co-workers have recently published total syntheses of both (+)-calyculin A



Scheme 10.

(**19**) and (−)-calyculin B (**20**) (both are antipodes of the natural products). The authors utilized an advanced intermediate (ketone derived from **56** and **57**) to reach either compound through Peterson olefination at C2 (Scheme 10).[22] The remainder of the synthesis featured a diverse array of chemistry which included nucleophilic opening of epoxide **63** with a structurally complex vinyl cuprate **62**, attachment of the triene side chain **56** via Horner–Emmons chemistry, and synthesis of γ-amino acid from isopropylidene-D-erythronolactone **61**.

The authors examined three different oxazole constructions in the synthesis of the C26–C32 fragments including acid catalyzed Davidson cyclization and cyclodehydration of a serine amide using $SOCl_2$. However, both of those methods led to either unsatisfactory yields or extensive epimerization of the α-stereogenic center (C30). This difficulty was resolved by utilizing the Burgess reagent[29] for the cyclodehyration step introduced by Wipf et al. (Scheme 11).[30] The oxazoline to oxazole oxidation was achieved by the Barrish–Singh procedure using $CuBr_2$ as the oxidant in the presence of HMTA and DBU.[31] This procedure prevented any epimerization at C30 and afforded oxazole 40 in 67% yield over two steps.



Scheme 11. (a) $MeO_2CN^-SO_2N^+Et_3$, THF. (b) $CuBr_2$, HMTA, DBU, CH_2Cl_2.

A notable application of the selenium-77 NMR technique[32] for the determination of enantiomeric purity of the oxazole fragment was featured in Smith's synthetic studies. Compound **66** (Fig. 3) had clear baseline separation of ^{77}Se NMR signals between the two diastereomers.

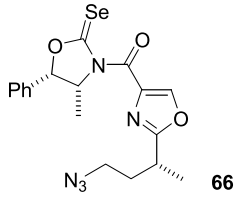
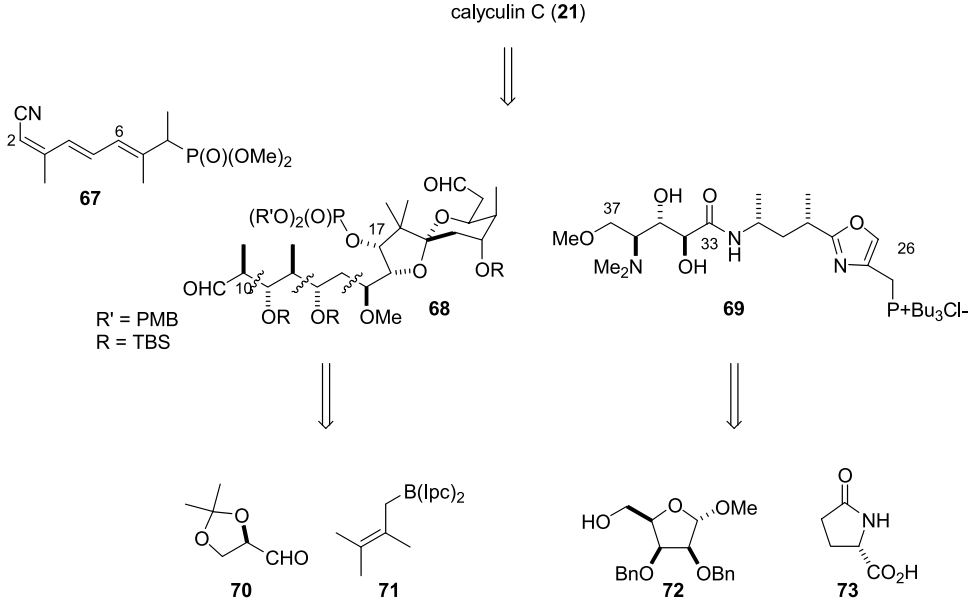


Figure 3.

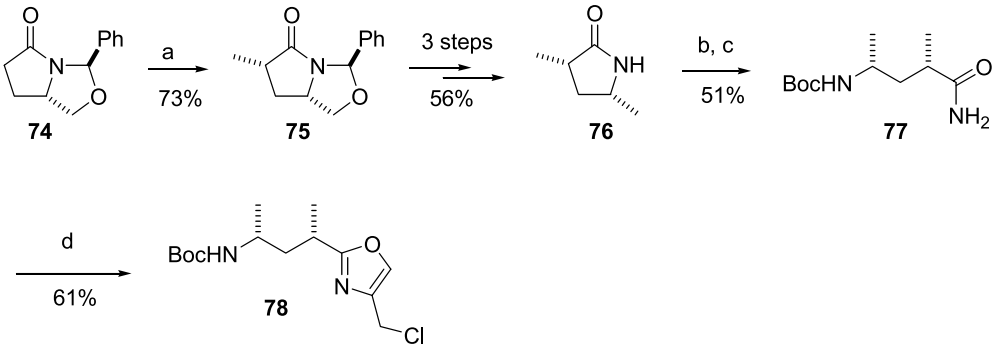
2.2.4. Armstrong's total synthesis of calyculin C (21).[21]

The first total synthesis of calyculin C (**21**) was achieved by Ogawa and Armstrong.[21] The initial disconnection at the C25–C26 olefin paralleled other synthetic efforts (Scheme 12). Brown's allylborane reagents were extensively utilized in the construction of the spiralketal unit **68**,[27] notably in the novel use of a tetrasubstituted allylborane **71** in the synthesis of the *gem*-dimethyl portion of this subunit. Calyculin C (**21**) has an additional methyl group at C32 which posed further synthetic challenge not present in the synthesis of either calyculin A or B.

The authors judiciously chose (*S*)-pyroglutamic acid (**73**) as a versatile chiral template for the construction of the C26–C32 chain, allowing them to access the oxazole unit of either calyculin A or calyculin C. Diastereoselective alkylation of pyroglutamic acid derived *N*,*O*-acetal **74** gave **75** as the major diastereomer (60% de, 73% yield) (Scheme 13).[28] In a three step sequence (56% yield) the acetal was hydrolysed and the hydroxyl was reductively removed to give **76**. After *N*-Boc-protection and $AlMe_3$-mediated ring opening, amide **77** was treated with 1,3-dichloroacetone in refluxing $CHCl_3$ and K_2CO_3 as base



Scheme 12.



Scheme 13. (a) LDA, CH_3I. (b) $(Boc)_2O$, Et_3N. (c) $AlMe_3$, NH_3. (d) 1,3-Dichloroacetone, K_2CO_3, $CHCl_3$, 100 °C.

buffer to form oxazole **78** in good yield (61%). The authors found that epimerization occurred if the oxazole forming reaction was run at a higher temperature.

2.3. Diazonamide

Diazonamide A (**82**) and its congener diazonamide B are structurally novel secondary metabolites isolated from the marine colonial ascidian *Diazona chinensis* by Fenical, Clardy, and co-workers in 1991.[33] The original assignments of structures **79** and **80** from the isolation paper are depicted in Figure 4. Diazonamide A (**82**) exhibits potent in vitro activity against HCT-116 human colon carcinoma and B-16 murine melanoma cancer cell-lines with IC_{50} values less than 15 ng/mL. An X-ray structural analysis was performed on the *p*-bromobenzamide of the major isolate diazonamide B (**81**), and it was proposed that the dehydration of the C11 hemiacetal occured during acylation. The structure of the minor isolate, diazonamide A, was then assigned by analogy and with the incorporation of a valine residue at C2 amine. The proposed structure (**79**) reconciled with observed HRMS data. Due to the scarcity of diazonamide A from the natural source and the unprecedented molecular architecture, this natural product has become an ideal target for synthetic studies. The daunting challenge posed by the **79** core structure stems from the densely functionalized macrocyclic framework which contains a biaryl linkage at C16–C18 of a single atropisomer, quaternary center at C10 and a rigid bisoxazole. Many innovative studies towards the core of the proposed structure of diazonamides have been reported.[34] A number of advanced intermediates have been disclosed by Magnus,[35] Moody,[36] Vedejs,[37] Wipf,[38] and Wood.[39] After, Harran and co-workers completed the total synthesis of the proposed structure of diazonamide A (**79**), they revealed significant discrepancies in the physical and spectroscopic data between the synthesized **79** and those of natural diazonamide A (**82**) from the isolation.[40] Through thorough detective work they concluded that the initially proposed structure was incorrect and put forth a revised structure of diazonamide A as **82**. Nicolaou and co-workers

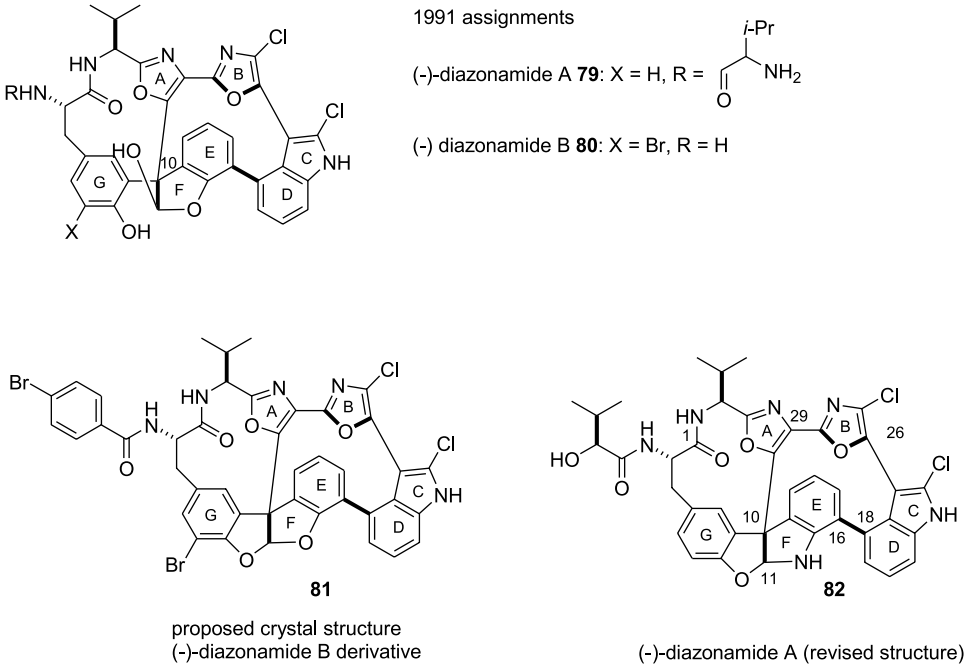
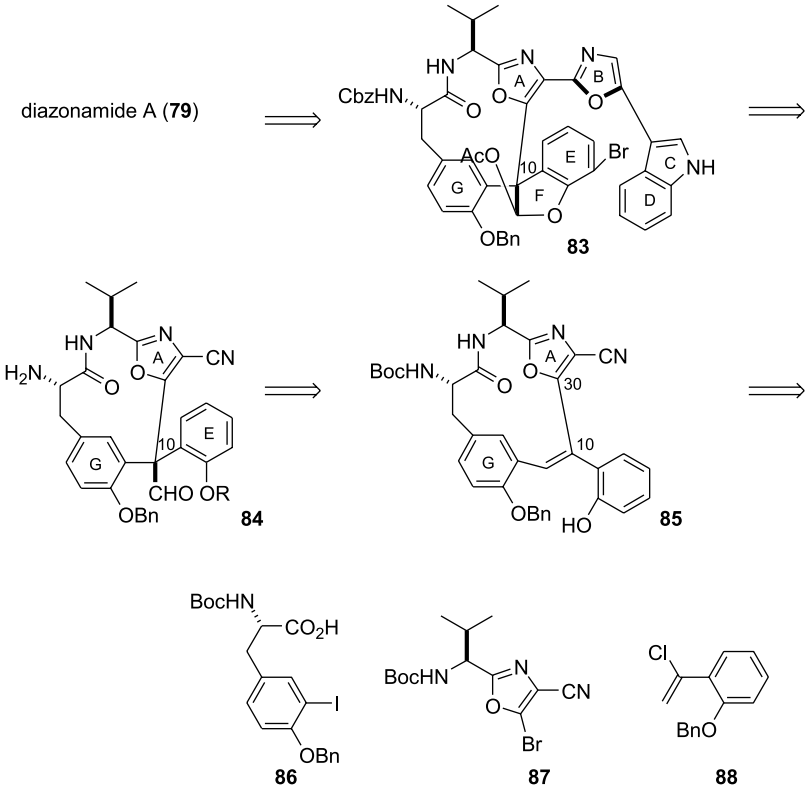


Figure 4.



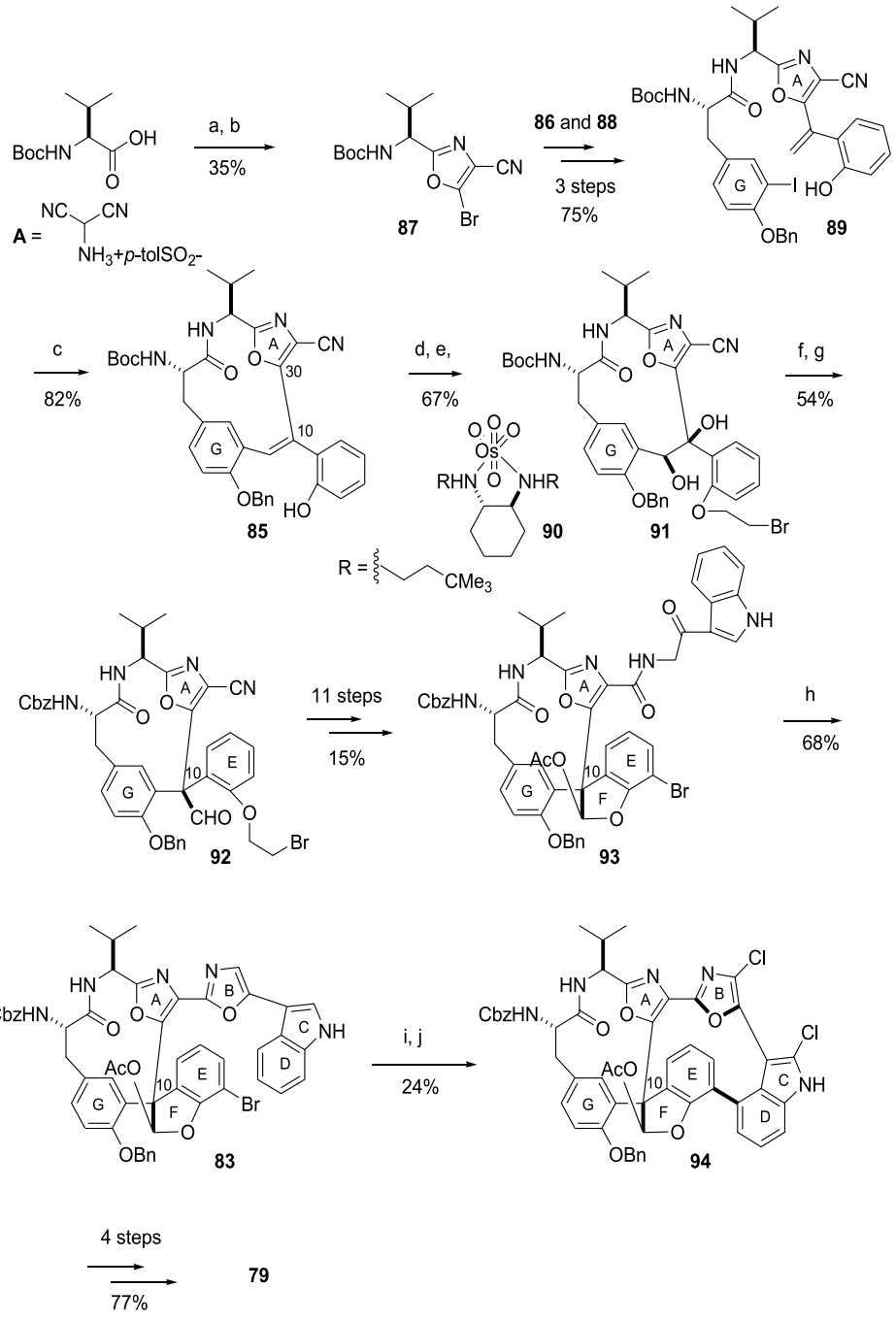
Scheme 14.

as well as Harran's group have since completed the total synthesis of **82** which confirmed the structure of diazonamide A.[41,42]

2.3.1. Harran's total synthesis of diazonamide A (79) (original proposed structure).[40] One of the key elements in Harran and co-workers' synthesis was the formation of the C16–C18 biaryl linkage late in the synthesis. The diastereoselective intramolecular biaryl formation was guided by the stereochemical information embedded in the correctly configured A–G macrolactam **83** (Scheme 14). This allowed them to avoid issues of forming the correct atropisomer early in the synthetic scheme. The A–G macrolactam **84** was derived from a ring-contracting pinacol rearrangement of a vicinal diol which originated from the face selective dihydroxylation of macrocyclic olefin **85**. The three building blocks **86**, **87**, and **88** for the macrolactam **85** were readily available from facile transformation of commercial materials.

The A ring oxazole building block **87** was prepared from acylation of *N*-Boc-L-Val-OH with aminomalononitrile providing an intermediate aminooxazole. Subsequent bromination of the aminooxazole gave bromide **87** (35% yield, two steps) (Scheme 15).[43] The oxazole forming step was based on a modified version of an efficient Freeman's oxazole synthesis.[44] Bromide **87** was then elaborated in three steps (75% yield) into amide **89**, which was cyclized via an intramolecular Heck reaction to give A–G macrolactam **85** in excellent yield (82%). It was found during the studies of cyclization reaction that employing 2-(di-*tert*-butylphophanyl)biphenyl as the Pd ligand greatly enhanced the stability of the catalyst allowing the reaction to be executed with low loading. In addition, a free phenol was necessary for the cyclization to achieve good yields. Hence, the authors suggested that pre-organization of a Pd(II) phenoxide facilitates the cyclization process.[40e] After protection of the phenol, the olefin was diastereoselectively oxidized using stoichiometric chiral osmium reagent **90**[45] which was able to override the inherent bias of the molecule to give diol **91** in excellent selectivity (93:7 dr) and good yield (67%). Acid mediated ring-contracting pinacol rearrangement gave **92** which established the desired configuration at the C10 quaternary center. Aldehyde **92** was efficiently converted to amide **93** in eleven steps (15% yield) which served as precursor for the formation of the second oxazole ring. Dehydration of **93** under Wipf's conditions gave bisoxazole **83** in 68% yield.[38b] A remarkable intramolecular biaryl macrocyclization using the photochemical method developed by Witkop et al.[46] followed by chlorination with NCS, gave **94** as a single atropdiastereomer which completed the scaffold of diazonamide (**79**). The total synthesis was then completed in four additional steps (77% yield). To the surprise of the authors, synthetic **79** was remarkably unstable and it was noticeably different in spectral and physical properties from natural diazonamide A. Through a series of reasoning and observation, the authors concluded that the structure of diazonamide A should be **82**.[40b] Harran et al. have also proposed a plausible biosynthesis of diazonamide A from four natural amino acids (Scheme 16).[40b]

2.3.2. Harran's total synthesis of (−)-diazonamide A (82).[41] Similar to his earlier synthesis of **79**, Harran's new

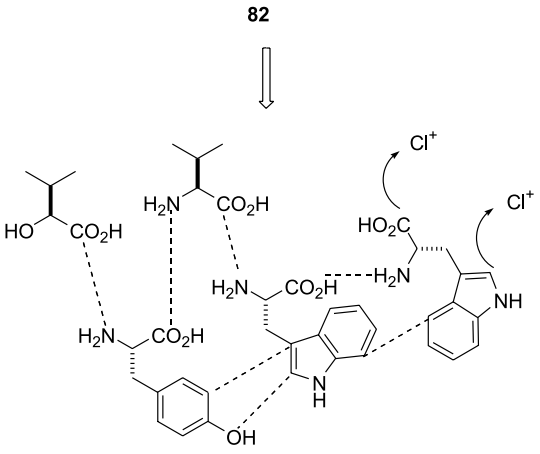


Scheme 15. (a) **A**, EDCI, pyr. (b) *t*-BuONO, $CuBr_2$, CH_3CN. (c) 3 mol% $Pd_2(dba)_3$, 6 mol% 2-(di-*tert*-butylphosphanyl)biphenyl, Ag_3PO_4, THF. (d) *t*-BuOK, 2-bromoethyltriflate, THF. (e) **90**, tol. (f) *p*-TsOH, tol. (g) *N*-(Benzyloxycabonyloxy)-succinamide. (h) $(Cl_3C)_2$, Ph_3P, Et_3N, THF. (i) *hν* (300 nm), LiOAc, epichlorohydrin, CH_3CN/H_2O. (j) NCS.

approach to diazonamide A (**82**) utilized the same late stage biaryl formation to construct the ring D–E biaryl bond (Scheme 17). The aminal **96** was formed by phenol oxidation followed by intramolecular capture of phenoxenium ion by the indole unit to generate the central dihydrobenzofuro[2,3*b*]indole.

Oxazole **99** was formed through the oxidation/dehydration of dipeptide **98** (two steps, 85% yield). Acylation of *N*-protected tyrosine **100** gave **101** which was oxidized with $PhI(OAc)_2$ to give the desired product **96** (25%) along with diastereomer **102** (8%) and **103** (15%) (Scheme 18). This efficient synthetic sequence provided the aminal core in only five steps from 7-bromotryptophan methyl ester and provided some evidence for Harran's conjecture on the biosynthesis of **82**.[40b]

Ester **96** was advanced in five steps (68% yield) to amide **104**. A two step (47% yield) benzylic oxidation-cyclodehydration sequence[38] afforded bisoxazole **95**. Light induced biradical generation resulted in the diastereoselective formation of the biaryl bond in 72% yield. The yield of



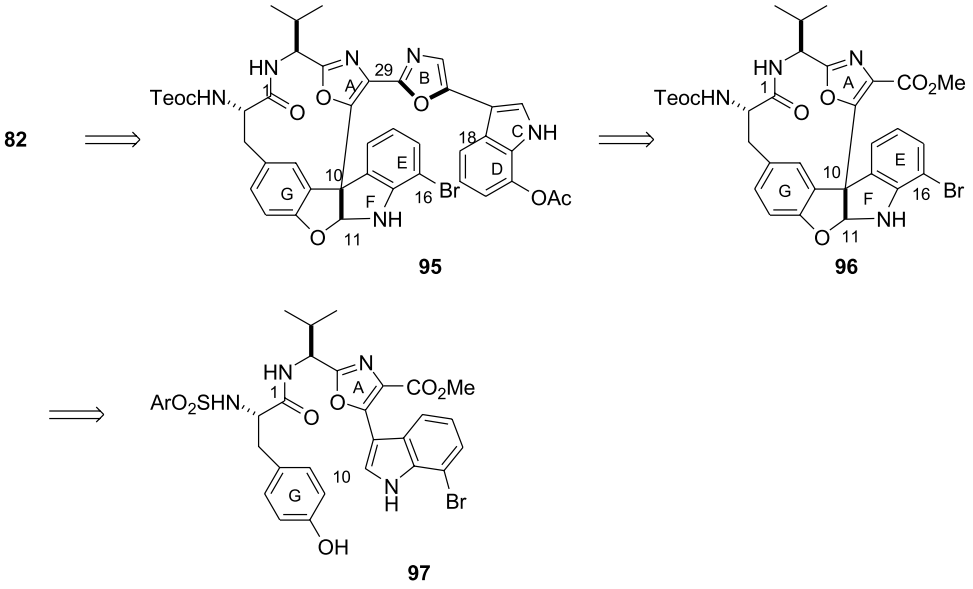
Scheme 16.

this step was significantly improved from the earlier synthesis (72% vs 24%) by placing a phenol functionality on ring D to increase the electron density. The remainder of the synthesis was accomplished in six additional steps (28% yield). The new synthesis of diazonamide A (**82**) involved only 19 operations and thus may be used to provide sufficient material for further biological studies (Scheme 19).

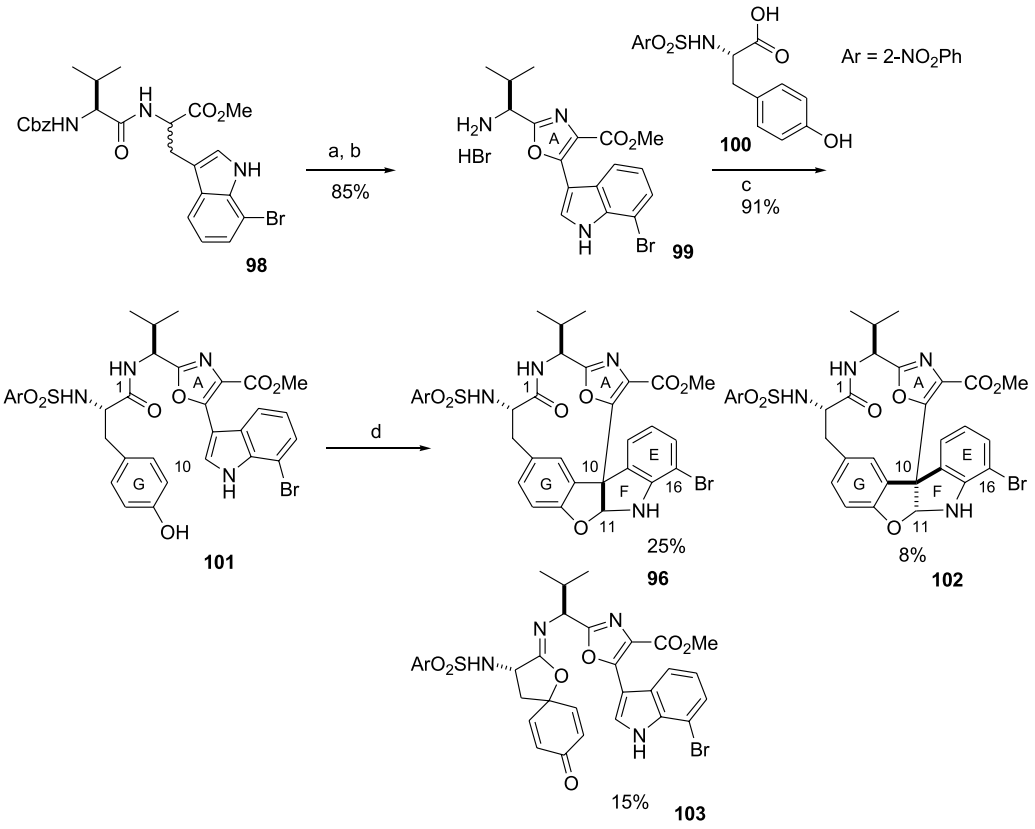
2.3.3. Nicolaou's total synthesis of diazonamide A (82).[42b] (first approach) Scheme 20 summarizes Nicolaou and co-workers' first approach to the total synthesis of the revised structure of diazonamide A (**82**). The isovaleric acid side chain and the aminal functionality in ring H were built late in the synthesis. The core was assembled by: (1) formation of the C10 quaternary center; (2) cyclization of the 12-membered macrolactam; (3) attachment of the indole-oxazole domain; and (4) formation of the 12-membered polycyclic framework. Akin to Harran's synthesis, the 12-membered polycyclic framework was assembled through intramolecular biaryl formation onto the macrocyclic lactam. The core was broken down into five building blocks **106** to **110** which were readily accessed from commercial materials.

The starting oxazole building block **107** was prepared following Meyer's procedure (discussed in more detail in Section 2.13).[169] Addition of the dianion of **107** to oxindole derivative **108** gave tertiary alcohol **111** in 73% yield (Scheme 21). The crucial quaternary center was formed unselectively by treating **111** with TsOH in the presence of excess **106** to give **112** as a mixture of diastereomers in 33% yield. After separation, both diastereomers were carried forward separately until the A–G macrolactam formation step. The correct diastereomer cyclized smoothly while the other did not undergo cyclization. After ten steps (0.05% yield), oxindole **112** was converted into indole **113** ready for oxazole formation. A Gabriel–Robinson cyclodehyration of keto amide **113** using $POCl_3$ in pyridine gave the corresponding oxazole. According to the authors, this reagent combination is superior to more conventional dehydraton methods such as neat $POCl_3$.[47] After the formation of the oxazole, the macrocycle was cyclized using a Witkop-type reaction, as seen previously in Harran's synthesis of diazonamide A.[40a,46] Although the cyclization yield was modest (30%), Ph_3SnH-mediated radical cyclization gave only 10% of the desired product **114**. With the establishment of both macrocycles, the culmination of the total synthesis was achieved in five additional steps (24% yield). This synthesis confirmed the revised structure of diazonamide A (**82**).

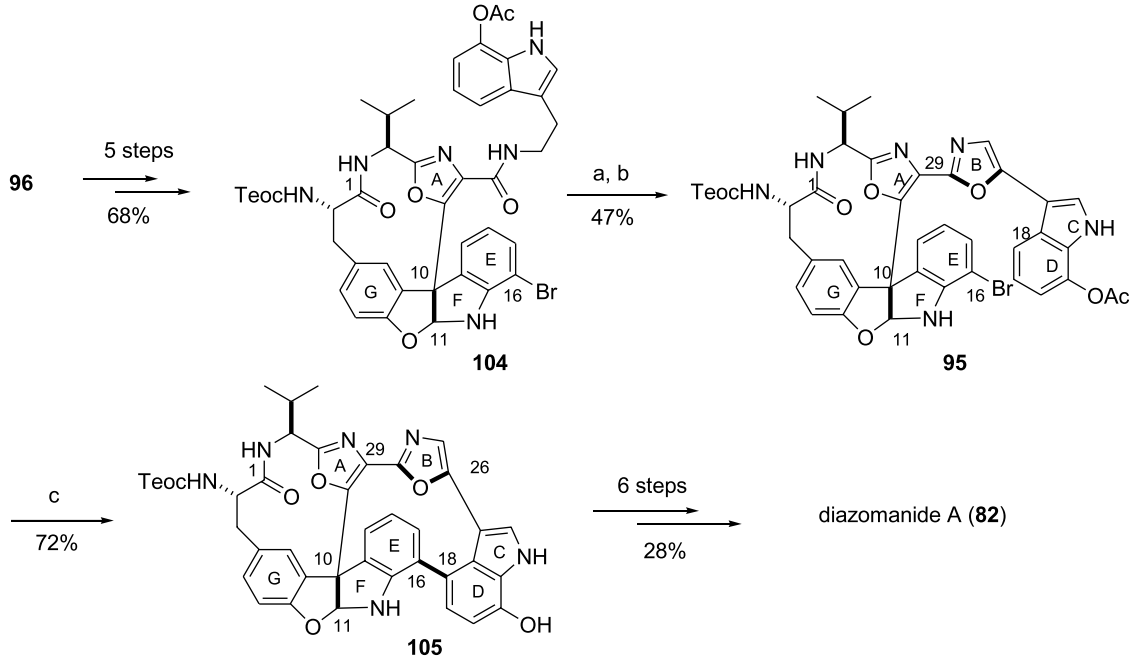
2.3.4. Nicolaou's second total synthesis of diazonamide A (82).[42a] (second approach) Nicolaou and co-workers recently completed a second total synthesis of diazonamide A (**82**). The key difference from their previous strategy is



Scheme 17.



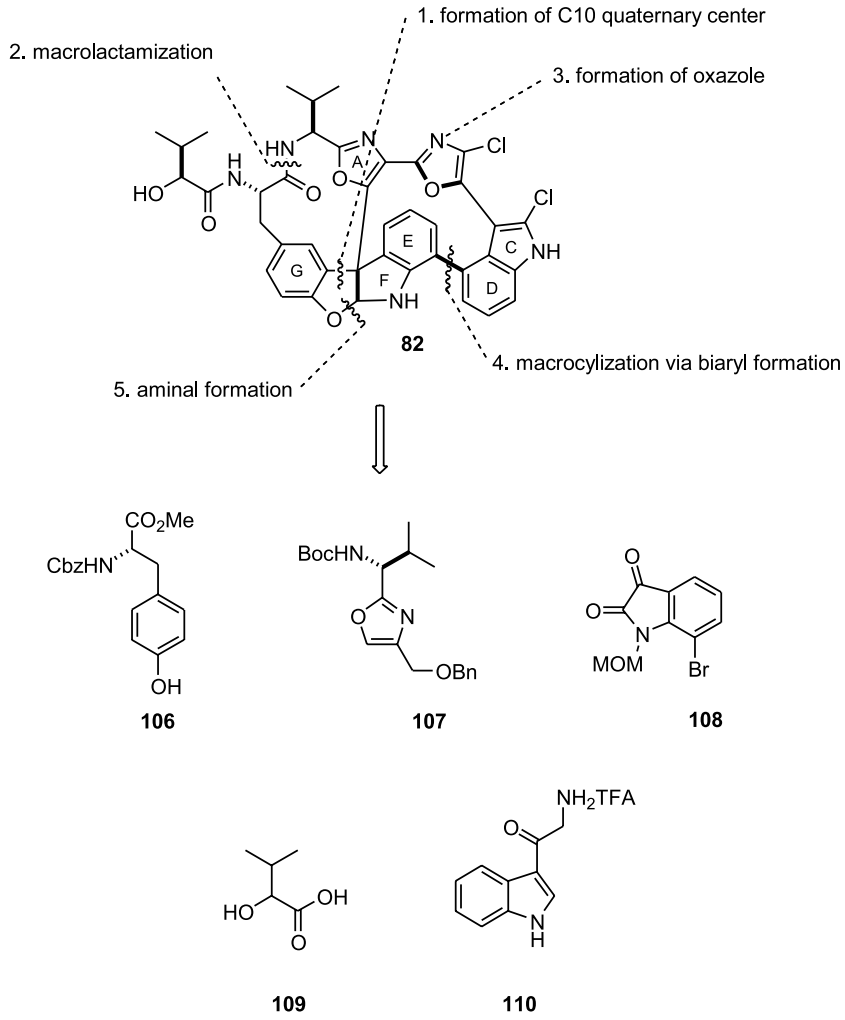
Scheme 18. (a) DDQ, THF, 70 °C. (b) HBr in AcOH. (c) TBTU, *i*-Pr_2NEt, DMF. (d) $PhI(OAc)_2$, LiOAc, 2,2,2-trifluoroethanol, −20 °C.



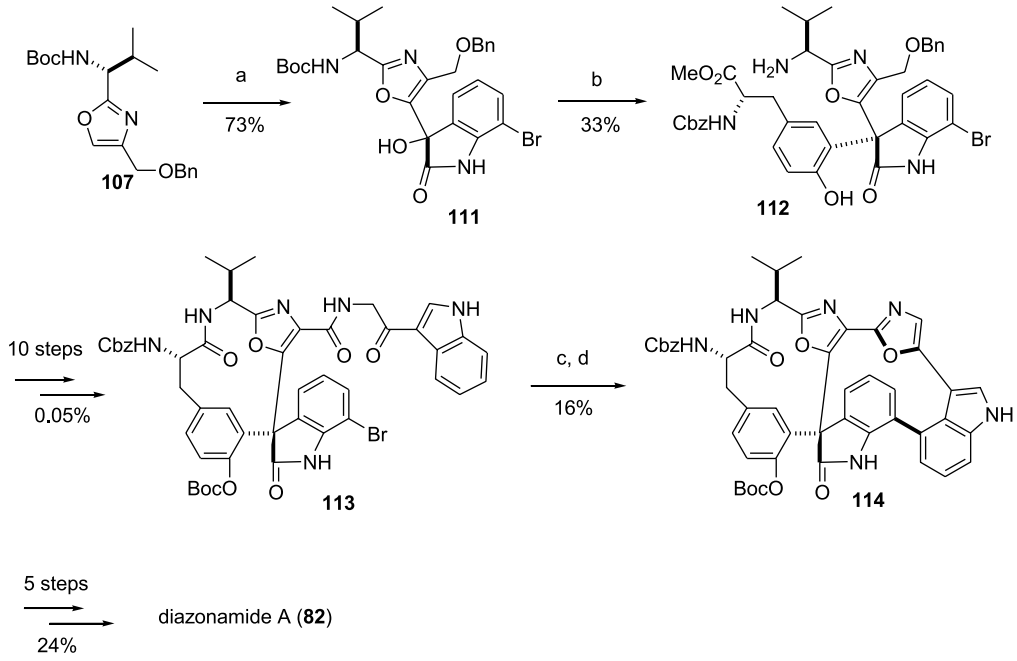
Scheme 19. (a) DDQ, THF/H_2O. (b) PPh_3, $(CCl_3)_2$, Et_3N, CH_2Cl_2. (c) *hν* (300 nm), CH_3CN/H_2O, LiOH.

reversed order in the construction of the two macrocycles. In their second approach, the A–G macrolactam was formed after the construction of A–F polycylic framework. The A–F framework was built via a novel heteropinacol coupling/oxime-cleavage macrocyclization cascade sequence (Scheme 22).[42a,c] The two main building blocks **106** and **107** were cross coupled to form the biaryl bond by a Suzuki reaction.

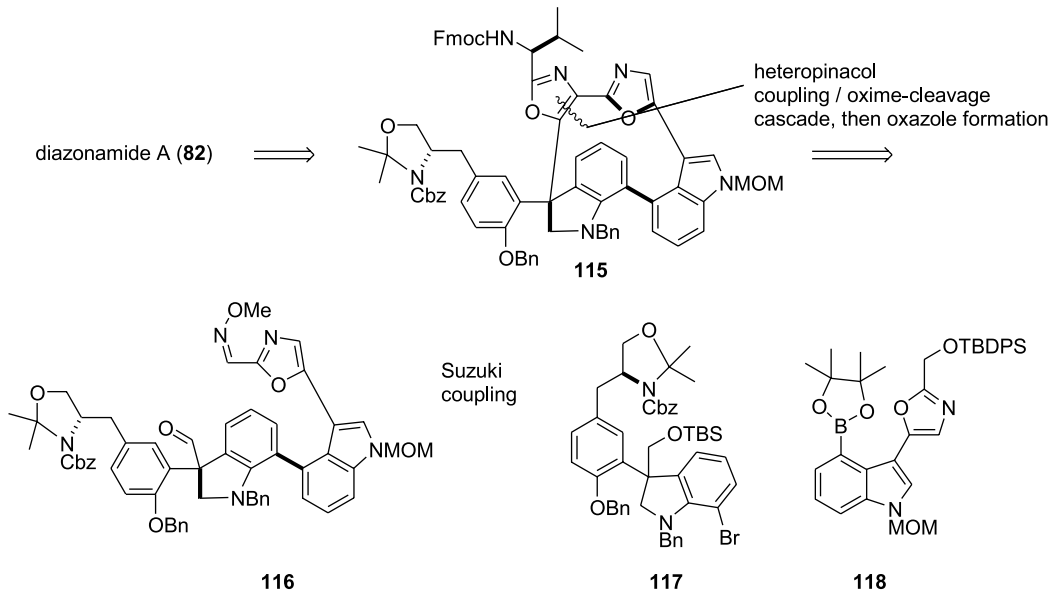
The indole amide **119** was synthesized from 4-bromoindole



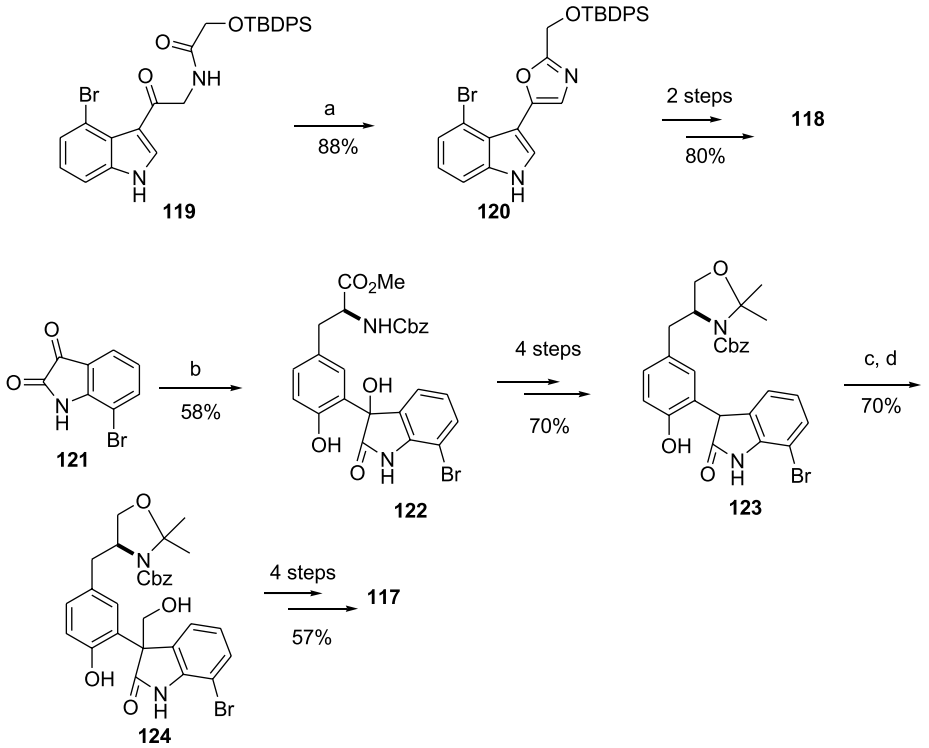
Scheme 20.



Scheme 21. (a) *n*-BuLi, THF, **108**. (b) **106** (4 equiv), *p*-TsOH, $ClCH_2CH_2Cl$. (c) $POCl_3$, pyr. (d) *hν* (200 nm), epichlorohydrin, LiOAc, MeCN/H_2O (30% yield) or Ph_3SnH, AIBN, C_6H_6 (10% yield).



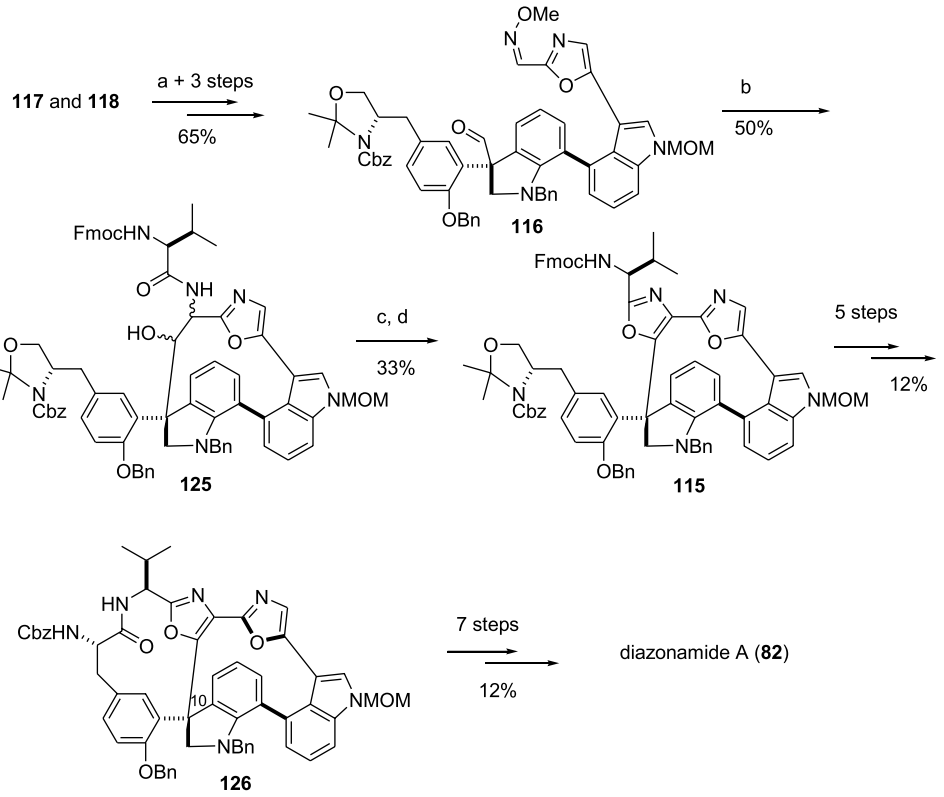
Scheme 22.



Scheme 23. (a) Cl_3CCCl_3, Ph_3P, Et_3N. (b) **106** (1.1 equiv), $TiCl_4$, CH_2Cl_2. (c) TMSCl, Et_3N, CH_2Cl_2. (d) HCHO, $Yb(OTf)_3$, THF.

in five steps (62% yield).[42d] The Gabriel–Robinson cyclodehydration, using Wipf's modified procedure,[48] gave the oxazole **120** in 88% yield (Scheme 23). In two additional steps (80% yield) **120** was converted into building block **118**. Protected tyrosine **106** reacted with 7-bromoisatin **121** in the presence of $TiCl_4$ to give hydroxyindole **122** (58%).

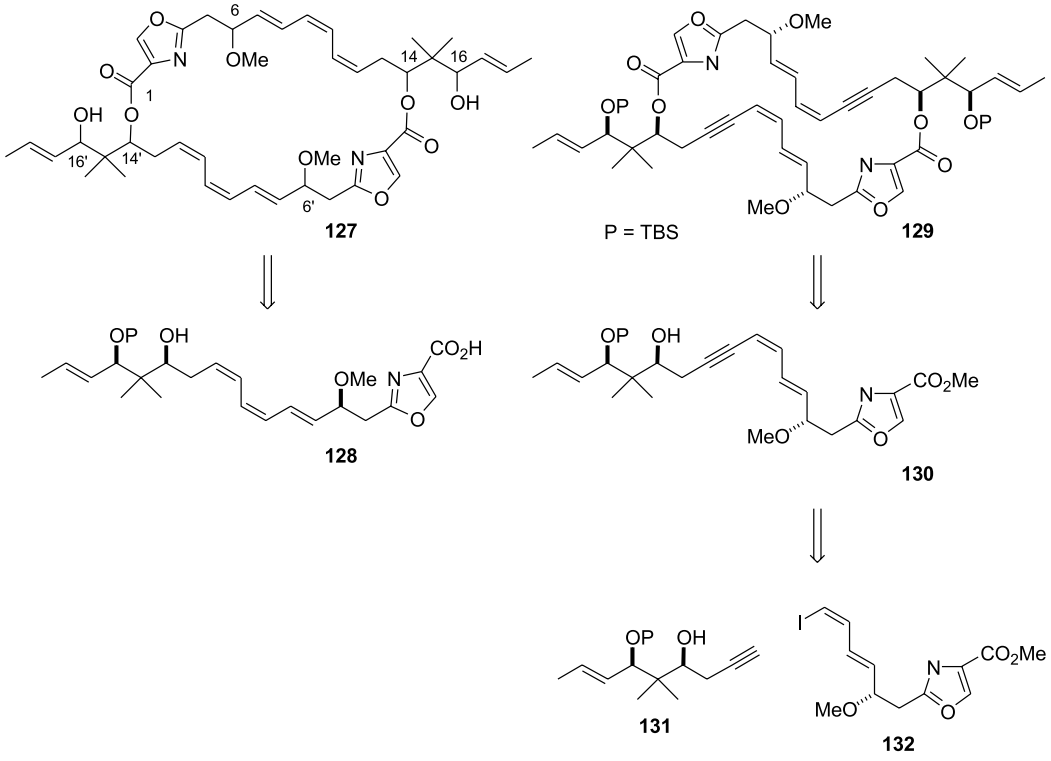
In four steps (70% yield), the newly generated tertiary alcohol was removed and the tyrosine ester group was reduced and protected as an acetonide (**122** to **123**). The required hydroxymethyl group at C10 (**124**) was installed by a two step procedure developed by Padwa which involves a $Yb(OTf)_3$-promoted aldol reaction between formaldehyde and the silyl enol ether of **123**.[49] This reaction generated an inseparable 1:1 mixture of diastereomers at C10 which was carried forward as a mixture until the macrolactamization step (**115** to **126**). Pd promoted Suzuki coupling united the two key fragments **117** and **118** in good yield (78%), and three additional steps of functional group manipulations



Scheme 24. (a) $[Pd(dppf)Cl_2]\cdot CH_2Cl_2$ (0.2 equiv), K_2CO_3, DME. (b) SmI_2 (9 equiv), DMA (36 equiv), THF, FmocValOH, EDCI, HOBt, DMF. (c) TPAP, NMO, CH_2Cl_2. (d) $POCl_3$/pyr (1:2).

gave the requisite aldehyde-oxime cyclization precursor **116** (Scheme 24).

The crucial heteropinacol reaction was promoted by an excess of SmI_2 and *N*,*N*-dimethylacetamide. The cascade reaction sequence involved heteropinacol macrocyclization, followed by a N–O bond cleavage to give an intermediate amino alcohol, which was directly acylated with



Scheme 25.

Fmoc-protected L-valine to give **125** in an 50% overall yield. After oxidation of alcohol **125**, the second oxazole was formed by Robinson–Gabriel cyclodehydration using $POCl_3$/pyridine mixture, as seen earlier in the first synthesis of diazonamide A (**82**) by Nicolaou's group,[42b] to give bisoxazole **115** (33%, two steps). Cleavage of the acetonide protecting group followed by functional group adjustment set the stage for the macrolactamization which occurred in a modest yield of 15% (**115** to **126**). The second synthesis of diazonamide A (**82**) was achieved in seven additional steps from **126** (12% yield).

2.4. Disorazole C_1

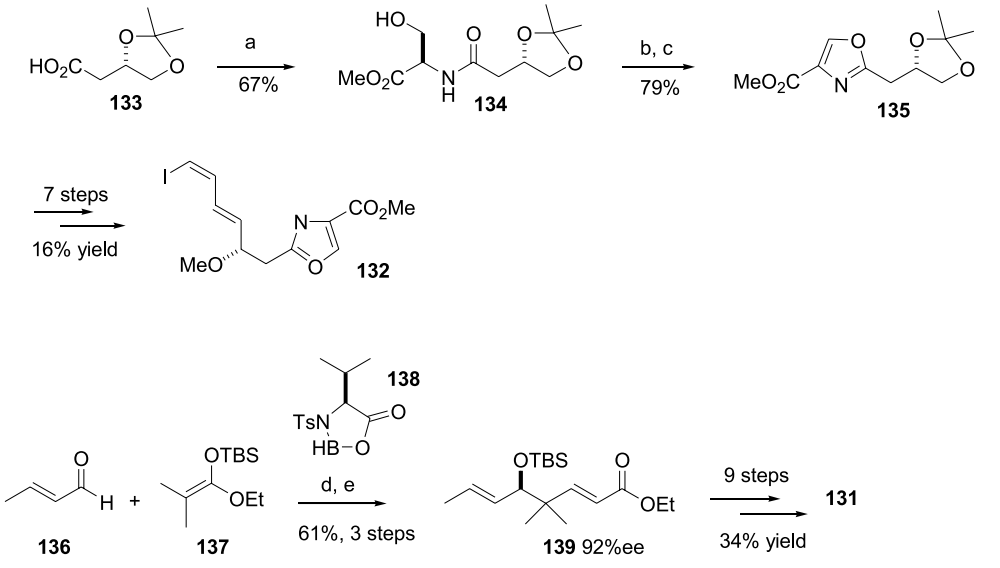
Disorazole C_1 (**127**) is a member of a family of 29 macrocylic polyketides which were isolated by Höfle and co-workers in 1994 from the fermentation broth of the gliding bacteria *Sorangium cellulosum*.[50] Disorazoles were found to be highly cytotoxic and possess moderately antifungal activity.[50] Structurally, disorazole C_1 is comprised of a highly functionalized 30-membered dimeric lactone which is a homodimer of trieneoxazole hydroxyl acid **128** (Scheme 25). The relative and absolute stereochemistry at C6–C6′, C14–C14′ and C16–C16′ has not been determined. Although no total synthesis has been completed for disorazoles, Meyers and co-workers have reported a synthesis of an advanced intermediate **129**.[51] Hoffman and co-workers have also disclosed a synthesis of the southern fragment of disorazole A_1 and C_1 which utilized a similar oxazole synthesis strategies as featured in Meyers' synthesis of **118**.[52]

2.4.1. Meyers' synthesis of disorazole C_1 (127).[51] In an earlier study, Meyers and co-workers attempted a highly convergent dilactonization of triene **128** to reach disorazole C_1 (Scheme 25). However, the effort was halted due to the unstable nature of the **128** triene system, which prohibited the construction of the macrocycle.[51b] In the new strategy reported by the authors,[51a] olefin C11–C12 was temporarily masked as an alkyne and the macrocycle was built from dienyne monomer **130**. Sonogashira coupling served to unite protected diol **131** and oxazole **132**. The stereogenic centers in **131** and **132** were chosen arbitrarily.

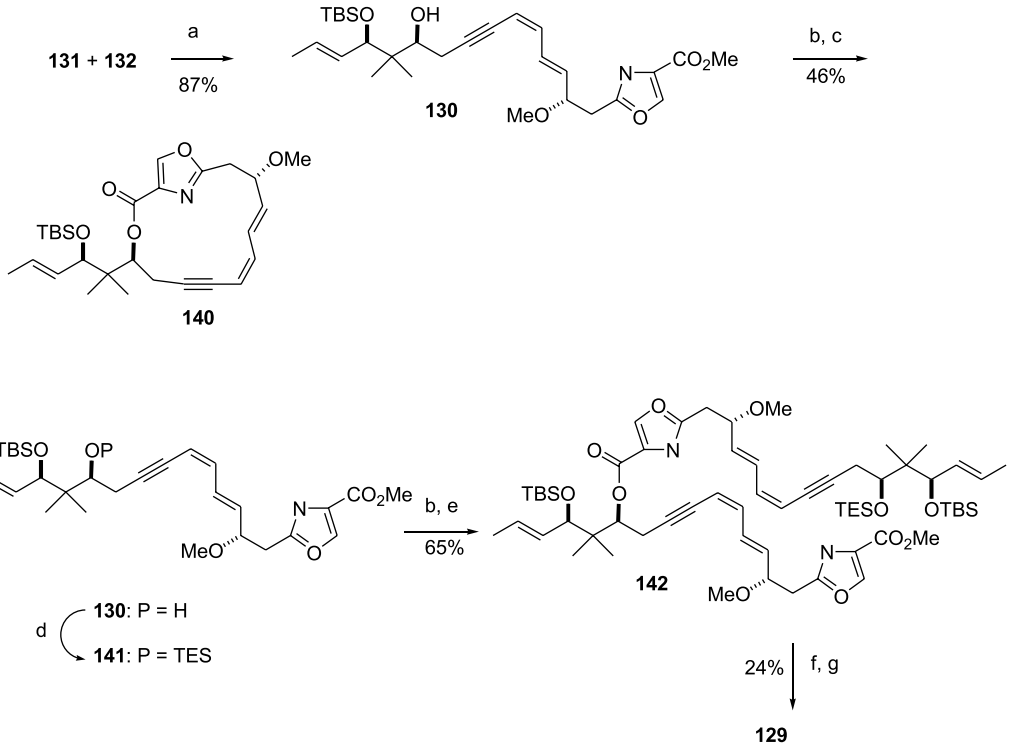
The synthesis of the oxazole fragment began with acylation of L-malic acid derivative **133** and L-serine methyl ester to give amide **134** in 67% yield (Scheme 26). The oxazole ring was then formed by a two step cyclodehydration/oxidation procedure routinely used in the synthesis of 2,4-disubstituted oxazoles. Treatment of **134** with diethylaminosulfur triflouride (DAST)[53] gave an intermediate oxazoline which was oxidized, under the mild condition reported by Williams[54] using $BrCCl_3$ and DBU, to give oxazole **135** in good yield (79%). Compound **135** was further elaborated into **132** in seven additional steps (16% overall yield).

The first step towards **131** was an enantioselective Mukaiyama aldol reaction between *E*-crotonaldehyde **136** and **137** promoted by L-valine derivative **138** as reported by Kiyooka[55] to give an aldol product which was directly homologated to **139**. Compound **139** was converted to **131** in nine steps (34% overall yield).

Fragments **131** and **132** were coupled via the Sonogashira reaction to give **130** in good yield (87%). After hydrolysis of the methyl ester, one step macrodilactonization was attempted by treating the corresponding hydroxy acid with dipyridyl thionocarbonate (DPTC)[56] but only the monomer **140** was obtained (Scheme 27). An alternate stepwise dilactonization was then achieved with the Yamaguchi protocol[57] to give protected dilactone **129** (Scheme 27). Unfortunately, further manipulation such as removal of silyl protecting groups or reduction of the alkyne caused decomposition of **129**. Although further work is needed to complete disorazole C1 (**127**), the authors have constructed the molecular framework for the total synthesis of the natural product.



Scheme 26. (a) HCl·L-serineOMe, EDCI, THF. (b) DAST, CH_2Cl_2. (c) DBU, $BrCCl_3$, CH_2Cl_2. (d) **138**, CH_2Cl_2. (e) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF.



Scheme 27. (a) $PdCl_2(PPh_3)_2$, CuI, Et_3N, CH_3CN. (b) LiOH. (c) DPTC, DMAP, tol. (d) TESOTf, 2,6-lutidine, CH_2Cl_2. (e) **130**, DPTC, DMAP. (f) TFA; LiOH. (g) 2,4,6-Trichlorobenzoyl chloride, DMAP, tol.

2.5. Hennoxazole A

Hennoxazole A (**143**) (Fig. 5) and congeners are bisoxazole natural products isolated by Scheuer, Higa, and co-workers from the marine sponge *Polyfibrospongia* sp. in 1991.[58] Hennoxazole A (**143**) displays potent activity against herpes simplex virus type 1 ($IC_{50}=0.6\ \mu g\ mL^{-1}$) and peripheral analgesic activity comparable to that of indomethacin. This natural product features a highly functionalized tetrahydropyran connected to a 2,4-disubstituted bisoxazole core which is connected to a nonconjugated triene sidechain. Compound **143** has attracted the attention of multiple synthetic research groups.[59] The first total synthesis of the antipode of **143** was achieved by Wipf and Lim which elucidated the absolute and relative configuration of C8 and C22.[60] Total syntheses of natural **143** have also been completed by Williams et al.[61] and Shioiri et al.[62] Shioiri's synthesis shares a similar approach in the construction of oxazoles to Wipf's synthesis, and therefore, only one of these syntheses is discussed in detail below.

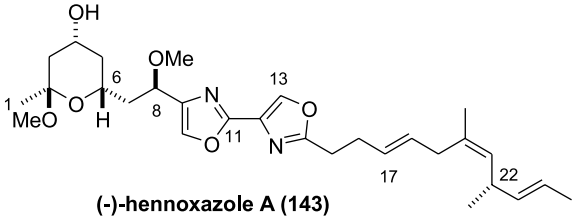
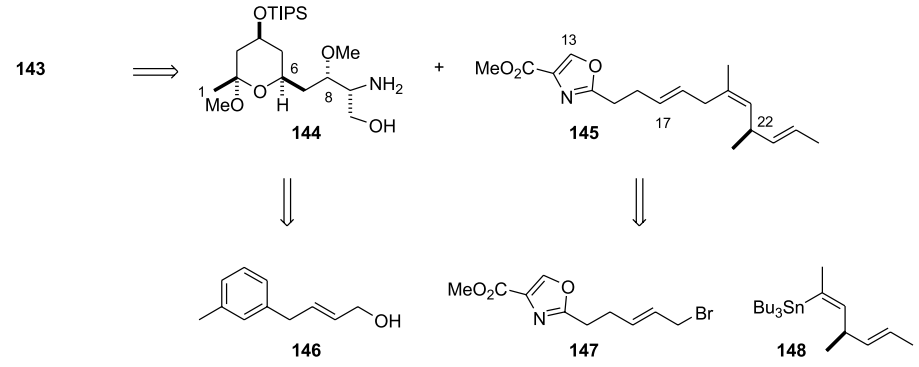


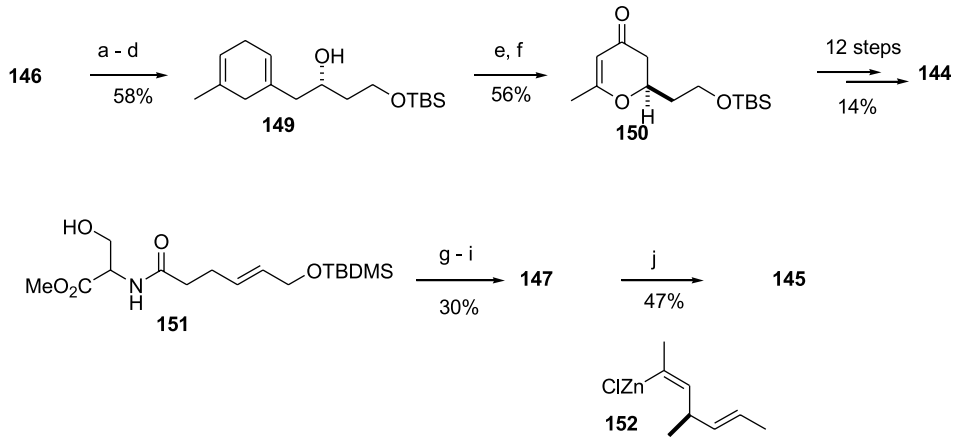
Figure 5.

2.5.1. Wipf's total synthesis of (+)-hennoxazole A.[60] (143)

The key disconnection in Wipf's synthesis of (+)-**143** was the cleavage of the oxazole ring at C11 which gave tetrahydropyran fragment **144** and triene oxazole **145** (Scheme 28). These two fragments were convergently coupled via a novel bisoxazole synthesis developed by



Scheme 28.



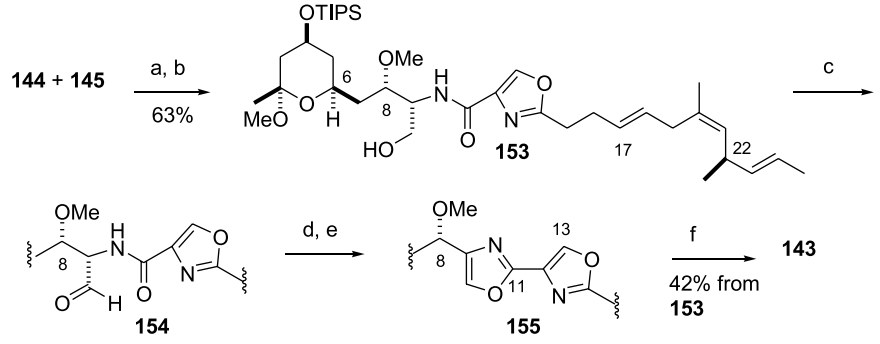
Scheme 29. (a) Sharpless epoxidation. (b) Red-Al, THF. (c) TBSCl, Im, CH_2Cl_2. (d) Li, NH_3, *tert*-amyl alcohol, THF. (e) O_3, EtOAc, H_2, $Pd(OH)_2$. (f) TsOH, THF. (g) $MeO_2CN^-SO_2N^+Et_3$, THF. (h) $CuBr_2$, DBU, HMPA, CH_2Cl_2. (i) TBAF; NBS, Ph_3P, CH_2Cl_2. (j) **152**, $Pd_2(dba)_3 \cdot CHCl_3$, $AsPh_3$, THF.

Wipf's group. Other features of the total synthesis included the use of *m*-xylene derivative **146** as a pyran synthon, and a vinyl cuprate S_N2 formation of **148** from an allylic ester.

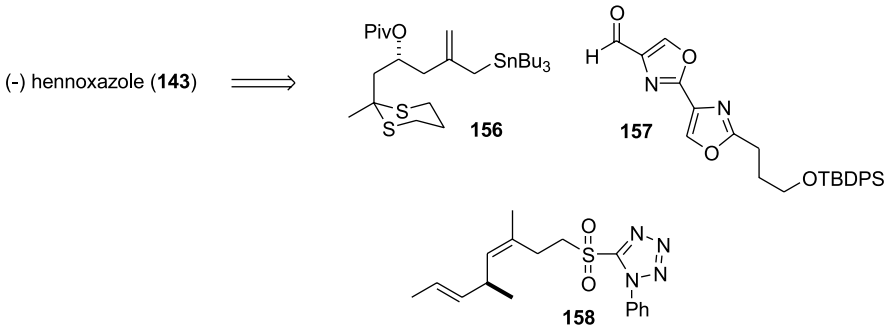
In the synthesis of the tetrahydropyran fragment, Sharpless epoxidation of allylic alcohol **146** served to install the C5 stereogenic center (Scheme 29). This was followed by reductive opening of the epoxide, protection of the primary alcohol, and dissolving metal reduction of the phenyl ring to give **149**. Ozonolysis and treatment with acid furnished pyranone **150**. Compound **150** was converted in twelve steps (14% yield) to give the key fragment **144** using a sequence which involved a Wittig olefination, a second Sharpless epoxidation to establish the C8 stereogenic center, and an isocyanate mediated installation of the C9 nitrogen.[63]

The first oxazole ring in the right hand fragment was synthesized via a two step cyclodehydration–oxidation sequence from serine amide **151**. Thus treating **151** with the Burgess reagent[30] gave an intermediate oxazoline which was oxidized by Barrish's procedure using $CuBr_2$ and DBU in HMPA.[31] The resulting oxazole **147** was coupled with the vinylzinc intermediate **152**, derived from stannane **148** using Negishi's procedure[64] to give right hand fragment in 47% yield.

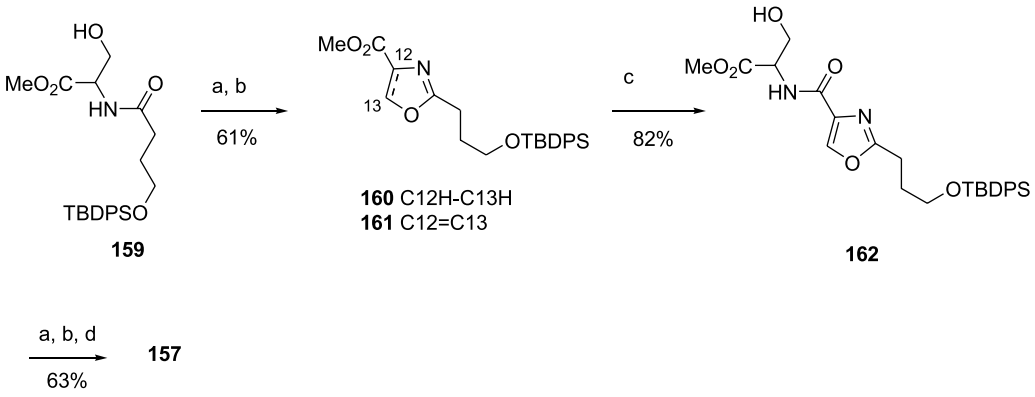
Fragments **144** and **145** were joined by PyBrop mediated acylation (Scheme 27).[65] The final oxazole was built via an oxidation–cyclodehydration sequence developed by Wipf's group.[48] Oxidation of the primary alcohol **153** gave an aldehyde intermediate **154** which was cyclodehydrated with



Scheme 30. (a) **145**, NaOH, MeOH/H_2O. (b) PyBrop, EtN(*i*-Pr)$_2$, CH_2Cl_2. (c) Dess–Martin CH_2Cl_2. (d) $BrCl_2CCCl_2Br$, Ph_3P, 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2. (e) DBU, CH_3CN. (f) TBAF, THF.



Scheme 31.



Scheme 32. (a) DAST; K_2CO_3, CH_2Cl_2. (b) $BrCCl_3$, DBU. (c) LiOH; *t*-BuOCOCl, Et_3N, HCl·serineOMe, CH_2Cl_2. (d) DIBAL, CH_2Cl_2.

$BrCl_2CCCl_2Br$ and Ph_3P in the presence of the hindered base 2,6-di-*tert*-butyl-4-methylpyridine. The resulting cyclodehydration intermediate 10-bromooxazoline was then dehydrohalogenated by DBU to give the oxazole moiety. Removal of the silyl protecting group gave synthetic (+)-hennoxazole **131** (42% from **153**, four steps). The above total synthesis showed that the oxazole formation methodology preserved the sensitive functionalities found in the natural product, which demonstrates that major retrosynthetic disconnections can be made at an oxazole to couple major fragments (Scheme 30).

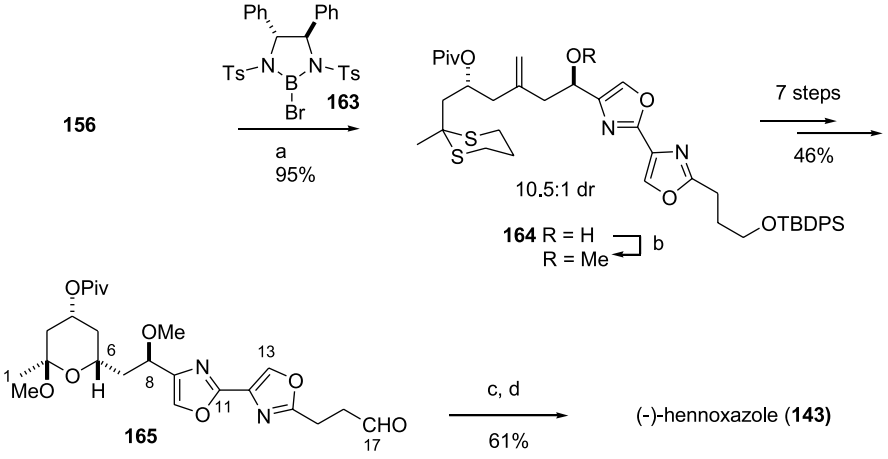
2.5.2. Williams' total synthesis of (−)-hennoxazole A (143).[61] In Williams et al.'s synthesis of (−)-hennoxazole A (**143**), the fragment **156** was convergently attached to the bisoxazole fragment by the formation of the C7–C8 bond with concurrent creation of the C8 stereogenic center. The skipped triene side chain was then appended in the final stages of the synthesis using sulfone **158** (Scheme 31).

The 2,4-disubstituted bisoxazole **157** was synthesized using an iterative cyclodehydration–oxidation strategy starting from serine amide **159**. Treatment of **159** with diethylaminosulfur trifluoride (DAST)[53] formed an intermediate oxazoline **160** (Scheme 32). The authors observed no evidence for the formation of an intermediate fluoride or fluoride-induced desilylation and the cyclization could be performed under mild conditions (1 equiv of DAST at −78 °C). Subsequent mild oxidation of **160** with $BrCCl_3$ and DBU cleanly effected dehydrogenation to give oxazole **161**.[54] After **161** was converted into serine amide **162**, the oxazole synthesis process was repeated for the formation of the second oxazole to reach **157** in good overall yield (51% from **161**, five steps) which illustrates the generality and effectiveness of this protocol for the synthesis of oxazole heterocycles.[53c]

The stannane **156** was united with bis-oxazole **157** via a mild asymmetric allylation strategy based on Corey's (*R*,*R*)-bromoborane **163**[66] to give a highly functionalized homoallylic alcohol **164** in excellent yield and diastereoselectivity (95%, 10.5:1 dr).[67] Compound **164** was elaborated in eight steps (46% yield) into aldehyde **165** that contained the C1–C17 portion of the natural product. The diene side chain **158** was attached using the Kocienski modification[68] of the Julia–Lythgoe olefination to give the C17–C18 alkene in 85% yield and excellent *E*-selectivity (*E*/*Z*, 91:9). Hydrolysis of the C4 pivaloate protecting group gave synthetic (−)-hennoxazole (**143**) (Scheme 33).

2.6. Leucascandrolide A

Leucascandrolide A (**166**) (Fig. 6) is a macrolide isolated from the marine sponge *Leucascandra caveolata* by Pietra and co-workers in 1996.[69] The natural product displayed potent cytotoxicity against KB and P388 tumor cell lines,



Scheme 33. (a) Add **156** to **163** then **157** CH_2Cl_2 (b) NaH, MeI, DMF. (c) **158**, KHMDS, DME. (d) LiOH.

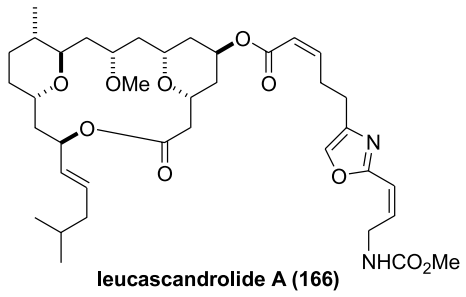
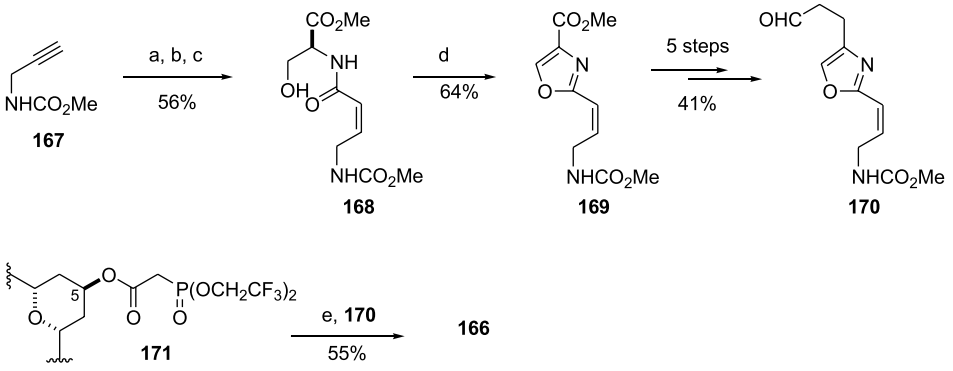


Figure 6.

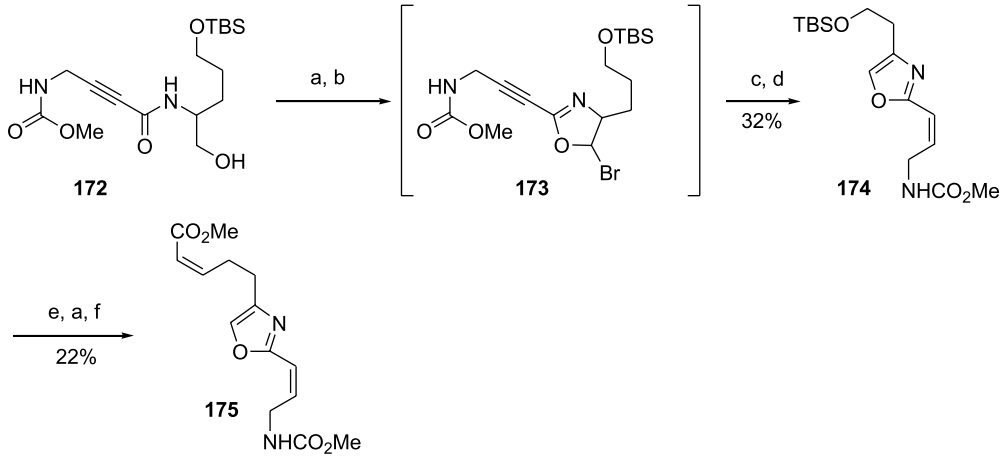
and strong antifungal activity against *Candida albicans*. Structurally, **166** possess several interesting features, including a doubly O-bridged 18-membered macrolactone, and an unsaturated oxazole containing side chain. It was later demonstrated by Pietra that the core macrocycle was responsible for the cytotoxic properties while the side chain contributed to the antifungal activity.[69b] Leucascandrolide A (**166**) has attracted considerable synthetic attention,[70] with the first total synthesis reported by Leighton and co-workers.[71] Total syntheses and formal syntheses of the natural product have also been completed by Rychnovsky et al.[72] Wipf et al.[73] Carreira et al.[74] Kozmin et al.[75] and Paterson et al.[76] Although each of the syntheses of the macrocyclic core feature fascinating chemistry, due to the scope of this review, the following discussion will be limited to selected examples of the C1′–C11′ oxazole side chain synthesis.

2.6.1. Leighton's total synthesis of leucascandrolide A (75).[71] In the first total synthesis of leucascandrolide A, reported by Leighton and co-workers,[71] the oxazole moiety was built from an efficient one-pot two-step cyclodehydration–oxidation of serine amide **168** using the protocol disclosed by Wipf and Williams (DAST; $BrCCl_3$, DBU) to give oxazole **169** in 64% yield (Scheme 30).[53c] Compound **169** was converted into aldehyde **170** in five steps (41% yield), which was appended onto the natural product core by Still's modification[77] of the Horner–Emmons reaction with phosphonoacetate **171**, resulting in synthetic leucascandrolide A (**166**) (Scheme 34).

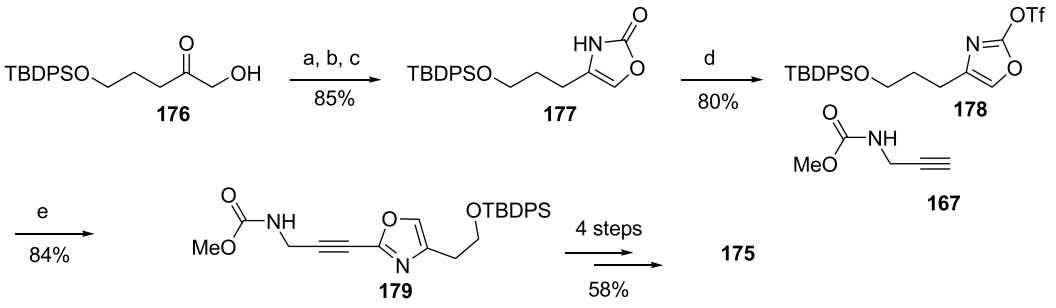
2.6.2. Wipf's synthesis of the C1′–C11′ segment of leucascandrolide A.[73b] Amide **172** was synthesized by acylation of the corresponding alkynoate and aminoalcohol (Scheme 35). It was subjected to a modified Robinson–Gabriel oxazole synthesis developed by Wipf and co-workers.[48] Oxidation of the primary alcohol **172** gave an intermediate aldehyde which was cyclodehyrated by treatment with Ph_3P and $BrCl_2CCCl_2Br$ in the presence of 2,6-di-*tert*-butyl-4-methylpyridine to provide bromooxazoline **173**. Elimination of HBr by DBU gave oxazole **174** in



Scheme 34. (a) *n*-BuLi, CO_2, THF. (b) Lindlar's cat., quinoline, H_2, EtOAc. (c) *i*-BuOCOCl, *N*-Me-morpholine, Ser-OMe·HCl, THF. (d) DAST, CH_2Cl_2; $BrCCl_3$, DBU. (e) KHMDS, 18-crown-6, THF.



Scheme 35. (a) Dess–Martin period. (b) Ph_3P, $(CBrCl_2)_2$, 2-6-di-*t*-butyl-4-methyl pyridine. (c) DBU, CH_2Cl_2. (d) Lindlar's cat., quinoline, H_2, EtOAc. (c) TBAF, THF. (f) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KHMDS, 18-C-6, THF.



Scheme 36. Cl_2CO, *N*,*N*-dimethylaniline. (b) NH_4OH. (c) H_2SO_4. (d) Tf_2O, 2,6-lutidine. (e) $Pd(PPh_3)_4$, CuI, dioxane.

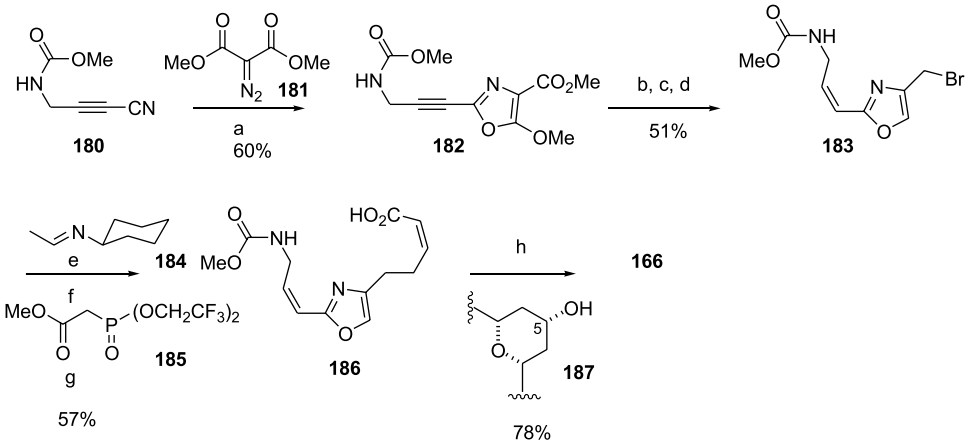
32% overall yield from **172**, after partial hydrogenation of the alkyne under Lindlar conditions. The (*Z*)-alkene **174** was converted in three steps (22% yield) into the desired methyl ester **175**.

2.6.3. Panek's synthesis of the C1′–C11′ segment of leucascandrolide A (75).[70d] In Panek and co-workers' synthesis of the C1′–C11′ oxazole side chain, the authors demonstrated the utility of Pd(0)-mediated Sonogashira coupling between a trifloyloxazole and an alkyne to form sp–sp^2 bonds.[78] Hydroxyketone **176** was treated with phosgene followed by NH_4OH, and acidification with H_2SO_4 gave oxazolone **177** in 85% yield (Scheme 36). Compound **177** was reacted with trifluoromethanesulfonic anhydride (Tf_2O) to afford trifloyloxazole **178** (80%). Sonogashira coupling with acetylene **167** was achieved at room temperature using catalytic $Pd(Ph_3P)_4$, and CuI with 2,6-lutidine as a base in dioxane in good yield (84%). The synthesis of the C1′–C11′ fragment was completed in 4 additional straightforward steps in 58% overall yield from **179**. The same strategy was employed by Paterson and co-workers for the construction of the oxazole side chain in their total synthesis of (+)-leucascandrolide A.[76]

2.6.4. Kozmin's total synthesis of leucascandrolide A (166).[75] The total synthesis of **166** achieved by Kozmin and co-workers featured an efficient $Rh_2(OAc)_4$ catalyzed cycloaddtion between alkynyl nitrile **180** and diazomalonate **181** in the construction of oxazole **182** (Scheme 33). This methodology was originally reported by Helquist[79] and **182** was obtained in 60% yield using 5 mol% Rh catalyst. After hydrogenation of the alkyne, the methoxy group was reductively removed by Super-Hydride, which also reduced the ester to the corresponding alcohol. This was followed by conversion to bromide **183**. Alkylation with lithiated imine **184**, *Z*-selective olefination, and hydrolysis completed the synthesis of oxazole side chain **186** in eight steps from **180**. Acid **186** was attached to the core of leucascandrolide **187** via Mitsunobu esterification to give the completed natural product in 78% yield (Scheme 37).

2.7. Madumycin, virginiamycin, and griseoviridin (group A streptogramin antibiotics)

The streptogramin antibiotics are a family of natural products that have been isolated from various strains of Streptomyces.[80] Streptogramin antibiotics can be divided into two groups: Group A consists of 23-membered macrolactones such as madumycin II (**188**),[81] virginiamycin M_2 (**189**),[82] and griseoviridin (**190**) (Fig. 7);[83] group B, represented by etamycin,[84] contains cyclic peptides. When group A and group B streptogramin antibiotics are used together, they exhibit potent synergistic effect against Gram-positive bacteria. Recently the FDA has approved a combination of group A and group B, marketed as Synercid (Aventis), for the treatment of vancomycin resistant infections.[85] Structurally, madumycin II (**188**), virginiamycin M_2 (**189**) and griseoviridine (**190**) all feature a 2,4-disubstituted oxazole, an (*E*,*E*)-dienylamine, and a 1,3-oxygenated backbone embedded in a macrolactone.



Scheme 37. (a) $Rh_2(OAc)_4$, 5 mol%, HF. (b) H_2, $Pd/CaCO_3$. (c) Et_3BHLi. (d) CBr_4, Ph_3P. (e) Et_2NLi, HMPA. (f) KHMDS. (g) LiOH. (h) DIAD, Ph_3P.

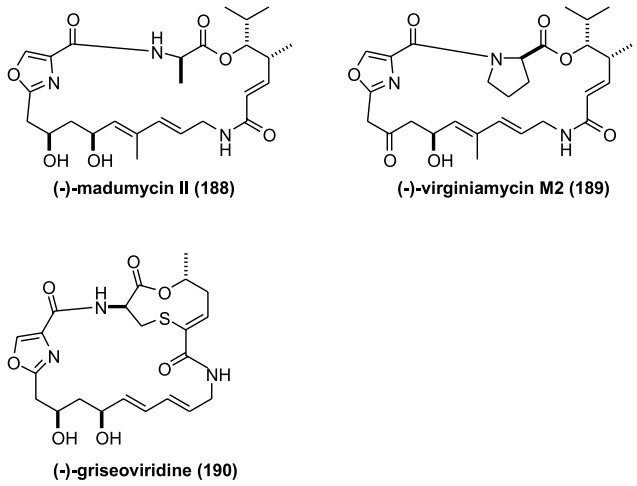
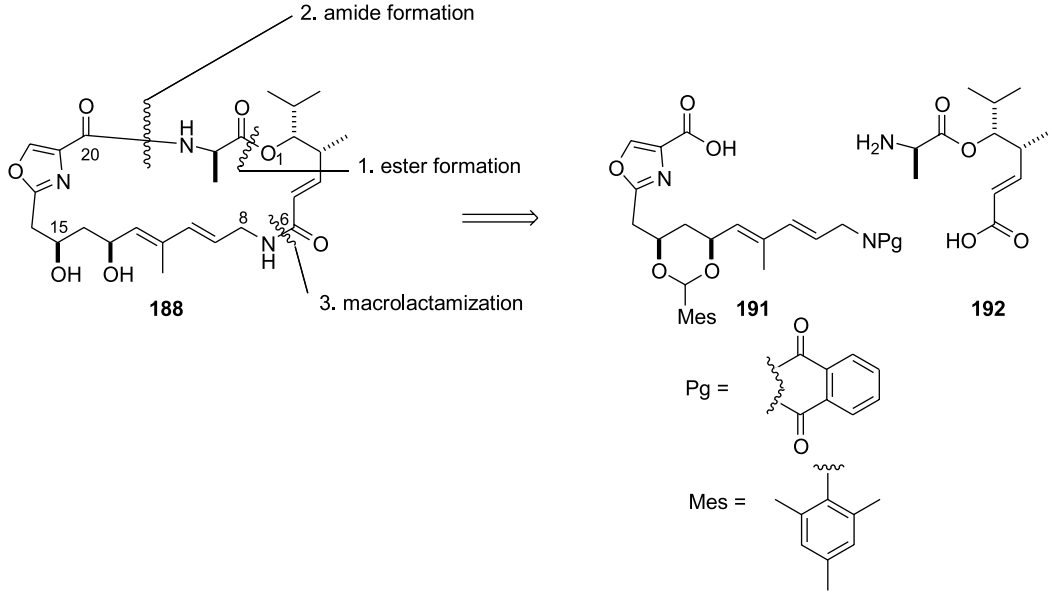


Figure 7.

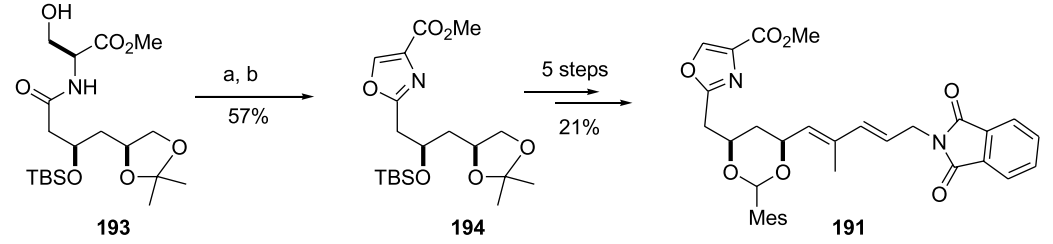
Griseoviridin has an additional 9-membered thiolactone within its structure. A number of total syntheses have been completed for the members of group A streptogramin antibiotics. Meyers et al. completed the synthesis of (−)-madumycin II (**188**) in 1996.[86] Simultaneously, Schlessinger et al. reported the synthesis of (−)-virginiamycin M_2 (**189**).[87,88] Subsequently, Pattenden et al. reported the synthesis of 14,15-anhydropristinamycin IIB in which the authors demonstrated an elegant application of an intramolecular Stille macrocyclization.[89] In addition, Ghosh, et al. have also reported a total synthesis of (−)-**188**.[90] Recently, (−)-griseoviridine (**190**) has yielded to a total synthesis by Meyers et al.[91,92]

2.7.1. Meyers' (−)-madumycin II (188) synthesis.[86] In the synthesis of (−)-madumycin II (**188**) by Meyers et al. the macrocycle was assembled in three key operations (Scheme 38). First, the ester linkage was formed between O1 and D-alanine to form the right hand fragment (**192**), which was then united with the C8–C20 oxazole fragment (**191**) through *N*-acylation at the alanine nitrogen. Finally, macrolactamization at C6 carbonyl and C8 amine completed the synthesis.

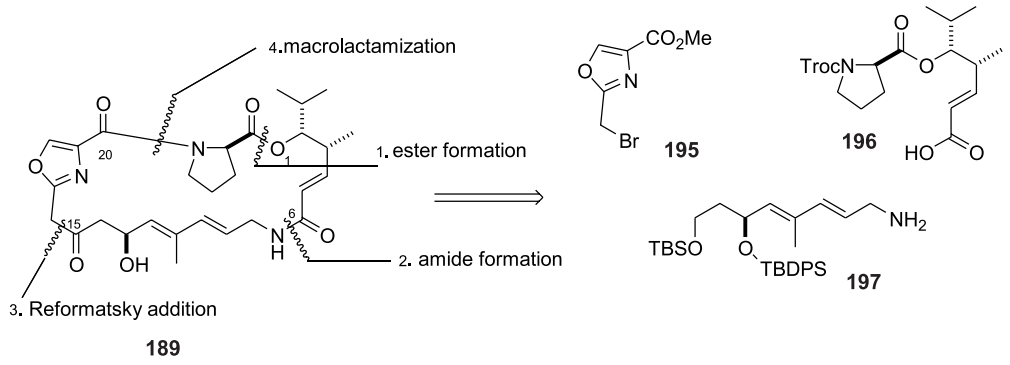
The synthesis of the oxazole ring was achieved via a two step procedure from the serine amide **193** which was prepared from (*S*)-malic acid in ten steps. Cyclodehydration of **193** using the Burgess reagent gave an intermediate oxazoline which was oxidized employing a Cu(I)–Cu(II) peroxide reagent (developed by the authors over the course of this natural product synthesis) to give oxazole **194** in 81% yield from the oxazoline (57% from **193**).[93] Oxazole **194** was elaborated into phthalimide **191** in five steps (21% overall yield) which was subsequently incorporated into the natural product as described above (Scheme 39).



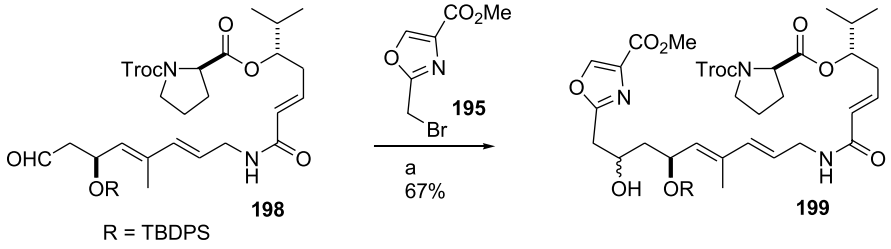
Scheme 38.



Scheme 39. (a) $MeO_2CN^-SO_2N^+Et_3$, THF. (b) *t*-butylperbenzoate, CuBr, $Cu(OAc)_2$, C_6H_6.

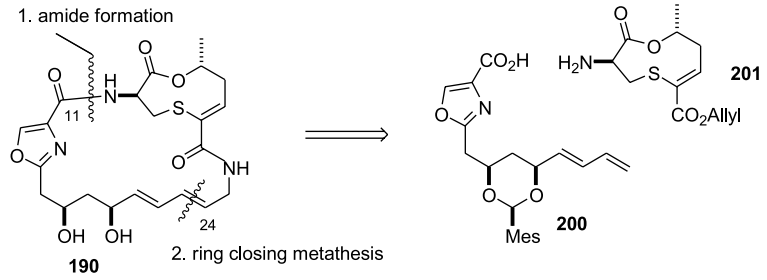


Scheme 40.

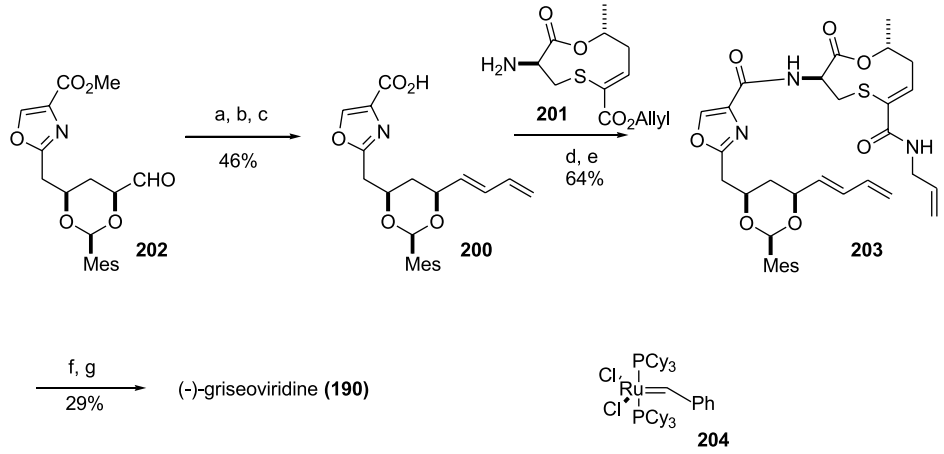


Scheme 41. (a) Zn dust, Et_2AlCl.

2.7.2. Schlessinger's (−)-virginiamycin M_2 (189) synthesis.[87] In Schlessinger et al.'s approach to (−)-virginiamycin M_2, the ester bond was formed between O1 and *N*-Troc-proline to give the right hand fragment **196** (Scheme 40). The fragment was then attached to the C8–C15 allylic amine fragment by amide formation at C6. The oxazole moiety was grafted onto the main carbon framework via a Reformatsky reaction between oxazole bromide **195** and aldehyde **198** (Scheme 41).[94] The process was efficient (67% yield) and convergent as no additional steps were required to form the oxazole ring. The end game of the synthesis included formation the macrocycle via lactam



Scheme 42.



Scheme 43. (a) Allyltriphenylphosphonium bromide, KHMDS, THF. (b) I_2, *hv*. (c) LiOH. (d) **201**, HOBt, EDCI, DMF. (e) $Pd(PPh_3)_4$, pyrrolidine; allyl amine, HOBt, EDCI, DMF. (f) **204**, tol. (g) PPTS, acetone/H_2O.

formation at the proline nitrogen and the C20 carbonyl, followed by oxidation at C15 and protecting group removal.

2.7.3. Meyers' synthesis of (−)-griseoviridine (190).[91] The first total synthesis of (−)-griseoviridin (**190**) accomplished by Meyers et al. featured an elegant application of ring closing metathesis for the formation of the macrocycle (Scheme 42).[91]

The key disconnections were made at the C11 amide bond and the C23–C24 olefin. The oxazole-containing aldehyde **202** was prepared from (*S*)-malic acid as previously described in the synthesis of (−)-madumycin II (Scheme 43).[86] Wittig olefination followed by iodine catalyzed photoisomerization gave the required *E*-diene which after hydrolysis afforded acid **200**. Amide formation between fragments **200** and **201** was achieved in good yield (78%). The allyl ester was exchanged to allyl amide to give the cyclization precursor **203**. Ring closing metathesis was promoted by 30% Grubbs' catalyst **204**[95] under high dilution (0.001 M) to give the cyclized product in 37–42% yield; subsequent deprotection afforded (−)-**190**.

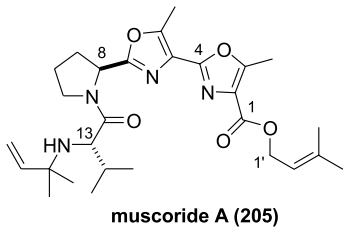


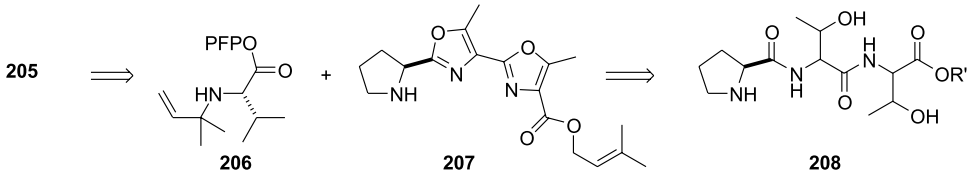
Figure 8.

2.8. Muscoride A

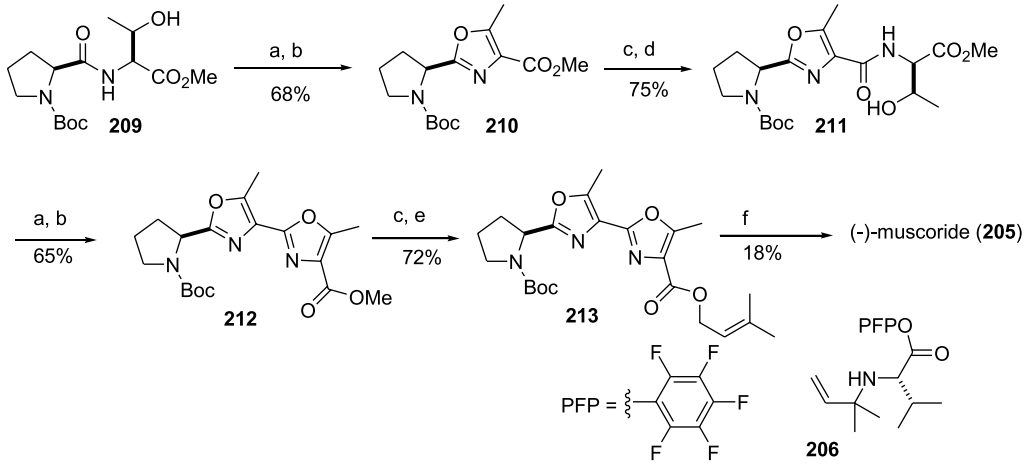
Muscoride A (**205**) (Fig. 8) is a bisoxazole natural product isolated from the freshwater cyanobacterium *Nostoc muscorum* by Sakakibara and co-workers.[96] Muscoride A (**205**) has only weak antibiotic activity, but its bis threonine-derived bisoxazole core attracted attention as a useful platform to test methods of oxazole construction. Wipf and coworkers have reported the first synthesis of **205**.[97] Subsequently, Pattenden et al.[98] and Ciufolini et al.[99] have also completed the total synthesis of this natural product. Pattenden's synthesis utilized oxazole construction methodologies discussed earlier in this review, and therefore, it has not been included in the following section.

2.8.1. Wipf's synthesis of (−)-muscoride (205).[97] In Wipf and Venkatraman's synthesis of (−)-muscoride (**205**), the disconnection was made between *N*-reverse-prenylated valine **206** and proline bisoxazole core **207**. The bisoxazole core, the cyclodehydrated equivalent of tripeptide **208**, was prepared by stepwise formation of the oxazole rings from a proline derivative using a modified variant of the Robinson–Gabriel oxazole synthesis developed in Wipf's group (Scheme 44).[48]

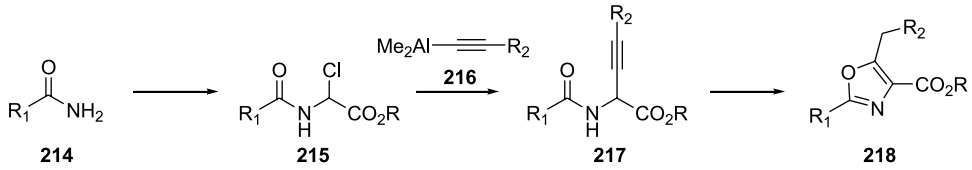
Oxidation of threonine amide **209** using Dess–Martin Periodinane[100] gave an intermediate ketone which was cyclodehydrated by exposure to electrophilic phosphorous reagent to give the first oxazole **210** (Scheme 45). The same procedure was applied for the formation of the second oxazole with good efficiency (**211** to **212**, 65% overall yield). The authors found that the iterative procedure was superior than the tandem oxidative cyclodehydration of tripeptide **208** (Scheme 44) due to the difficulty of handling this polar tripeptide in common organic solvents. After the



Scheme 44.



Scheme 45. (a) Dess–Martin. (b) Ph_3P, I_2, Et_3N, CH_2Cl_2. (c) LiOH (d) L-Thr-OMe, IBCF, NMM. (e) BOP, prenyl alcohol, CH_2Cl_2. (f) TFA; $ClCH_2CH_2Cl$, DMAP.



Scheme 46.

construction of the bisoxazole core **212**, the natural product was completed by converting methyl ester **212** to the 3,3-dimethylallyl ester **213** followed by attachment of *N*-reverse-prenylated valine derivative **206**.

2.8.2. Ciufolini's synthesis of (−)-muscoride (205).[99]

A novel methodology for oxazole synthesis was developed by Ciufolini et al. and applied to the total synthesis of (−)-muscoride (**205**).[99] The methodology was based on the ability of alkynylglycinates **217** to cyclize to give oxazole products **218** (Scheme 46).[101] The required alkynylglycine substrate was derived from the conversion of primary amide **214** to an α-chloroglycinate **215** followed by the addition of an alkynyl aluminum reagent **216**. The starting point for the total synthesis was the condensation of prolinamide **219** and ethyl glyoxylate to give α-hydroxyglycinate **220** (Scheme 47). Chlorination in neat $SOCl_2$ furnished an intermediate chloride **221** which was reacted with the dimethylaluminum derivative of TMS acetylene. The acetylene product **222** was not isolated, as basic workup using LiOH gave the cyclized oxazole directly with concurrent removal of the TMS group, as well as hydrolysis of the ester (**222** to **223**). This efficient oxazole synthesis protocol was repeated again on **223** to obtain the bisoxazole core of the natural product (**223** to **225**, 45% overall yield). The end game was completed in three additional steps (12% yield), similar to those used in the synthesis described by Wipf.[97]

2.9. Mycalolide A and ulapualide A

The isolation and the planar structure of (−)-Mycalolide A (**226**) (Fig. 9), a unique secondary metabolite from Mycale, a marine sponge, was reported by Fusetani and co-workers in 1989.[102] This macrolide belongs to a family of trisoxazole-containing natural products which includes ulapualides (**227**) (Fig. 9),[103] kabiramides,[104]

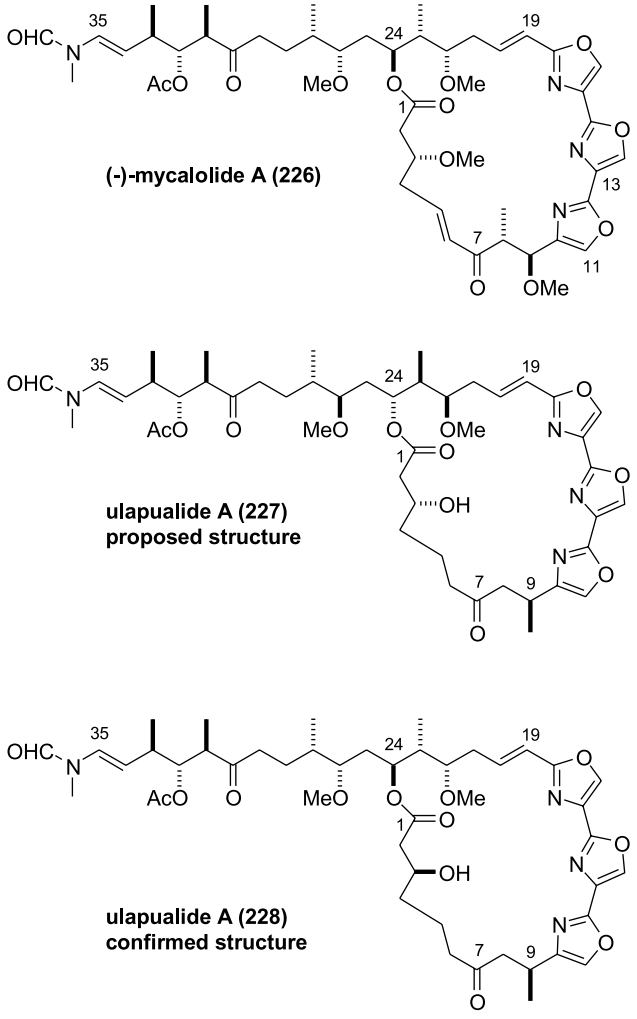
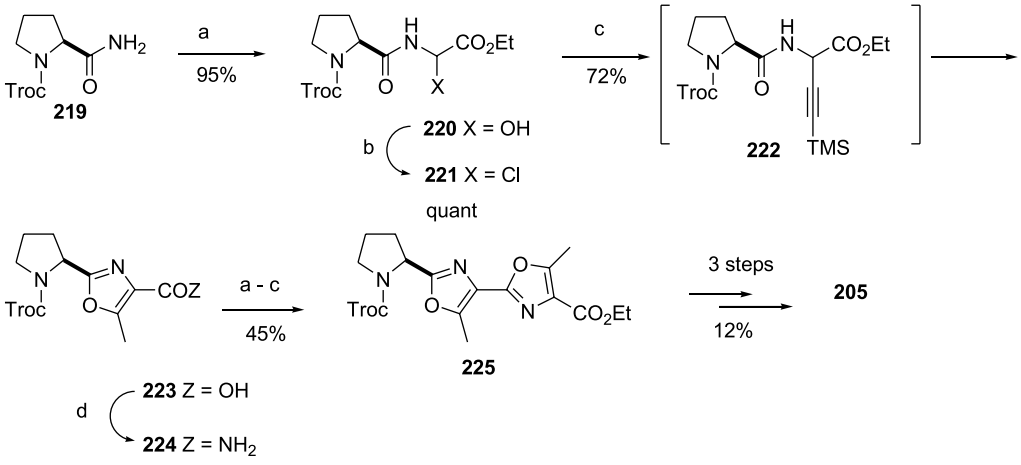
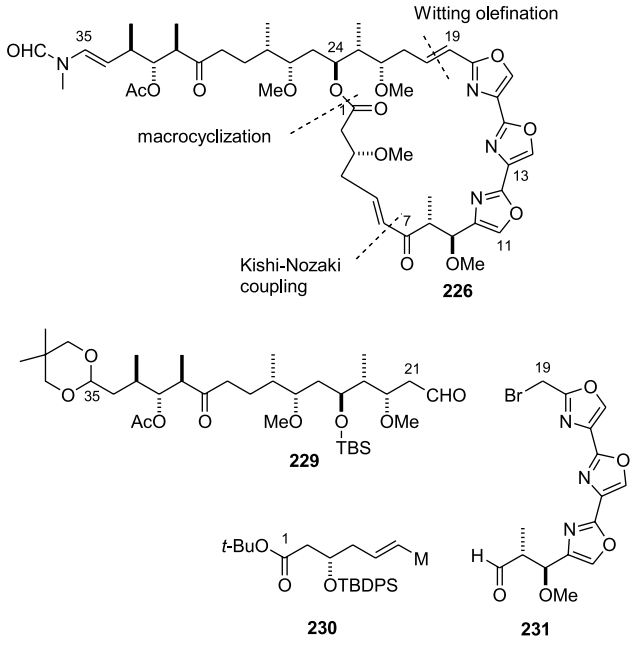


Figure 9.



Scheme 47. (a) $CHOCO_2Et$, THF. (b) $SOCl_2$. (c) $Me_2AlCCTMS$, Et_2O/THF; LiOH, THF/H_2O. (d) EtO_2CCl, Et_3N, then NH_3.

halichondramides,[105] and jaspiramides.[106] Biological profiles of these natural products include antileukemic, antifungal, and ichthyotoxic properties. Mycalolide A (**226**) displays potent antifungal and cytotoxic activities.[102] It was shown that **226** has the ability to selectively inhibit the actomyosin Mg^{2+}-ATPase,[107] which suggested that **226** acts as an actin-depolymerizing agent, a property that may find application in the studies of essential cell functions.[108] The relative and absolute stereochemistry of mycalolides has been determined by chemical degradation and extensive ^{1}H and ^{13}C NMR spectroscopy studies.[109] Due to their fascinating structure and biological activities, these trisoxazole natural products have attracted significant attention from a number of research groups.[110] Panek and Liu have reported the completion of the first synthesis of (−)-mycalolide A (**226**).[111] Furthermore, Pattenden and co-workers have also disclosed a total synthesis of a diastereomer of ulapualide A (**227**).[112] Recently, the absolute stereochemistry of ulapualide A was established by X-ray crystallography of the ulapaulide A–G-actin complex and it is shown as structure **228** (Fig. 9).[113]

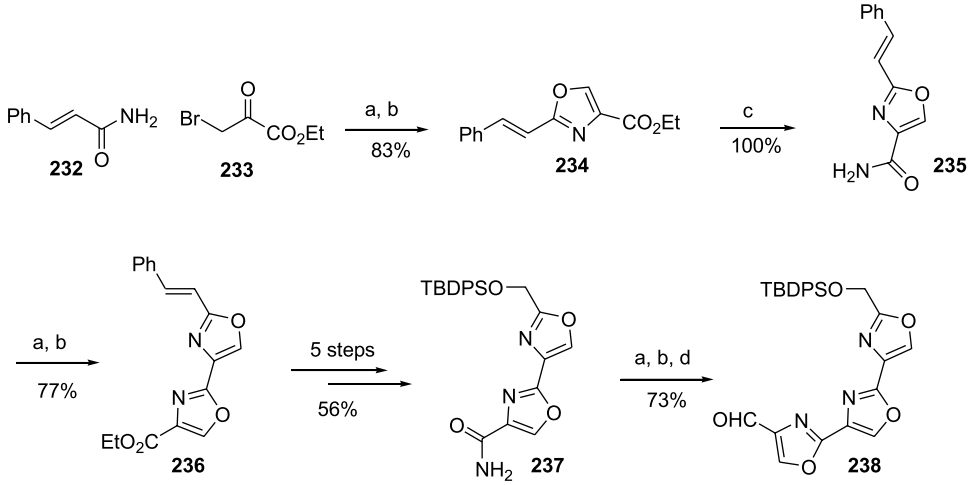


Scheme 48.

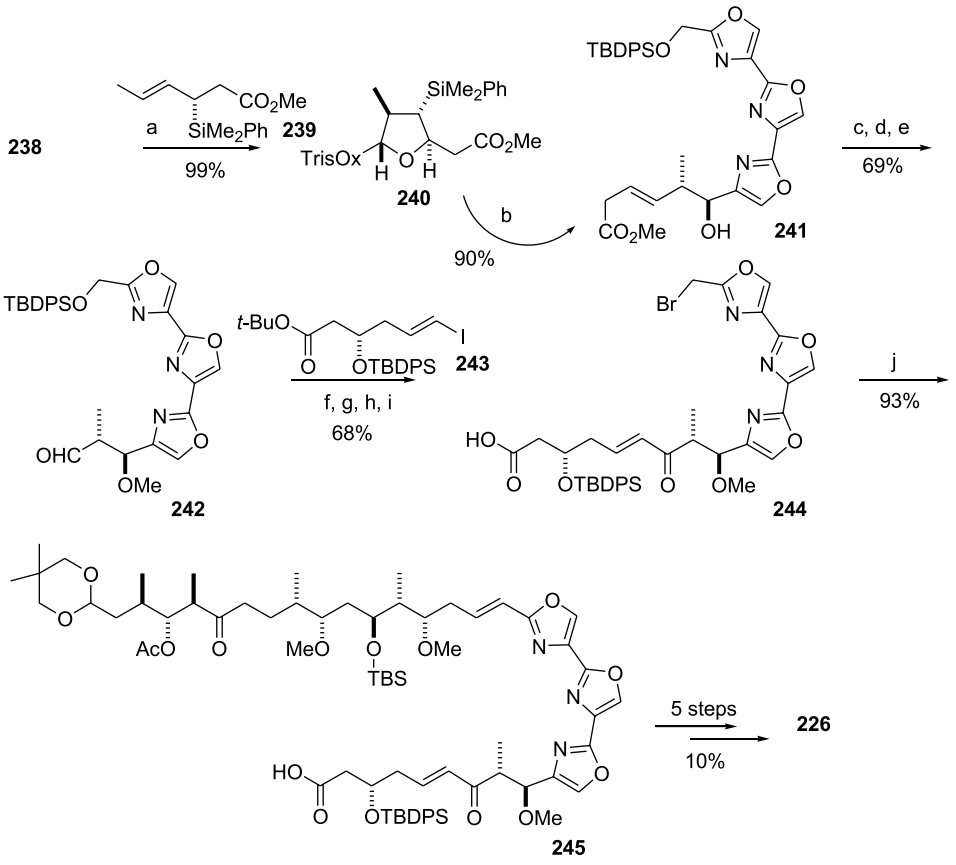
2.9.1. Panek's total synthesis of (−)-mycalolide A (226).[111] The main synthetic strategy for mycalolide A (**226**) is outlined in Scheme 48. Disconnections were made at the C1 ester, the C19–C20 olefin and C6–C7 which gave polypropionate fragment **229**, trisoxazole fragment **231** and ester **230**. For the synthesis of **229**, Panek and co-workers showcased the utility of their asymmetric crotylation methodology.[114] Fragments **230** and **231** were brought together by $CrCl_2/NiCl_2$-mediated Kishi–Nozaki reaction.[115]

For the synthesis of the trisoxazole fragment **238**, a Hantzsch-type methodology[116,110h] was employed in an iterative sequence to generate all three oxazole rings. The Hantzsch oxazole synthesis involves the treatment of an amide with an α-halo ketone to generate a 2,4-disubstituted oxazole in an efficient one pot process. This method avoids the more common peptide based two-step cyclization–oxidation sequence which often suffers from low yields due to a competing elimination pathway or difficult aromatization step.[110a,d] The sequence was initiated by heating cinnamamide **232** and ethyl bromopyruvate **233** in $NaHCO_3$ buffer to give an intermediate hydroxyl oxazoline which was dehydrated in situ with trifluoroacetic anhydride to afford oxazole **234** in 83% yield (Scheme 49). Ethyl ester **234** was converted to the corresponding amide **235** by treatment with NH_4OH, and **235** underwent a second Hantzsch reaction to give bis-oxazole **236** in 77% yield. The olefin was then oxidatively cleaved, and further elaborated into amide **237** (56% yield, five steps). A final application of the Hantzsch reaction followed by DIBAL-H reduction gave the targeted trisoxazole aldehyde **238** in good overall yield (73% yield, three steps).

Homologation from aldehyde **238** required the construction of C8–C9 stereogenic centers with an *anti*-relationship. This was achieved with the application of the chiral (*E*)-crotylsilane technology developed by Panek and co-workers.[114] Bidentate Lewis acid ($TiCl_4$) promoted addition of silane (*S*)-**239** to trisoxazole aldehyde **238**



Scheme 49. (a) **233**, $NaHCO_3$, THF. (b) TFAA. (c) NH_4OH. (d) DIBAL-H.

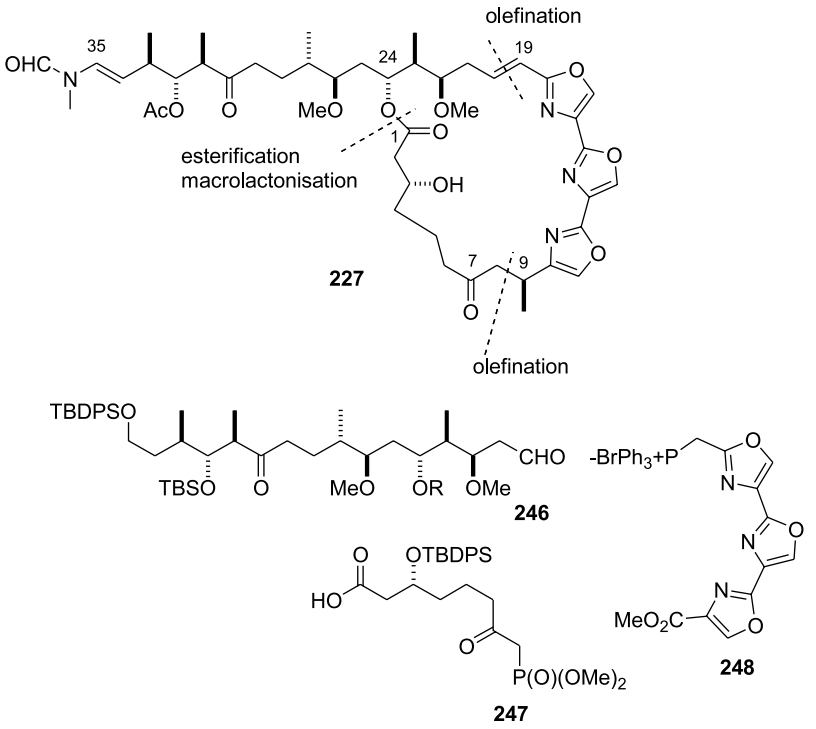


Scheme 50. (a) $TiCl_4$, CH_2Cl_2. (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2. (c) Ag_2O, MeI. (d) OsO_4, TMANO. (e) $Pb(OAc)_4$. (f) $NiCl_2/CrCl_2$, THF/DMF. (g) Dess–Martin. (h) TBAF; CBr_4, PPh_3. (i) TFA. (j) Et_3P, DMF; DBU, **229**.

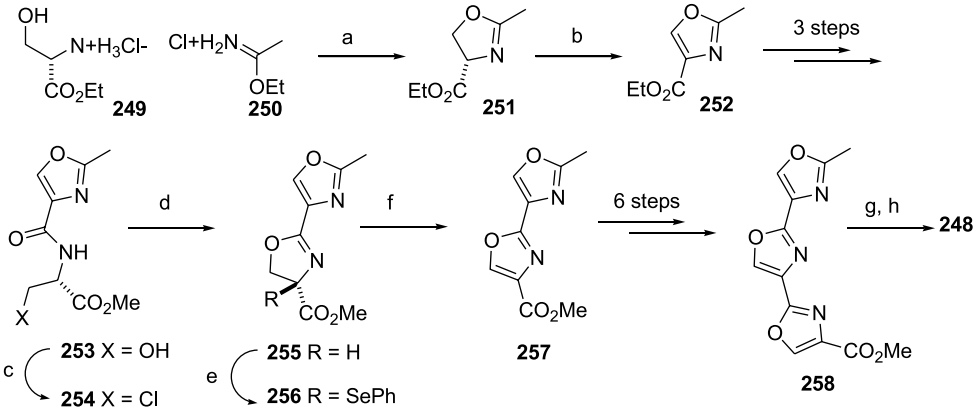
gave an excellent yield (99%) of a tetrahydrofuran product **240** which could be ring-opened to the corresponding homoallyl alcohol **241** by treatment with $BF_3{\cdot}OEt_2$ (Scheme 50). Methylation of the C9 alcohol followed by oxidative cleavage of the olefin, gave aldehyde **242** (69% yield, three steps). Aldehyde **242** was coupled with the C1–C6 unit **243** via a $NiCl_2/CrCl_2$ promoted Kishi–Nozaki reaction.[115] Dess–Martin oxidation of the resulting allylic alcohol gave an enone which was further elaborated into bromide **244** via routine transformations (68% yield, four steps). The crucial fragment coupling was achieved by a Witting reaction between aldehyde **229** and phosphonium salt generated in situ from bromide **244** and Et_3P using DBU as base. The coupled product **245** was isolated in 93% yield as a single olefin isomer. The natural product (**226**) was completed in five additional steps which included Yamaguchi macrolactonization, formation of the terminal *N*-methylformamide, and global deprotection (10% from **245**). The synthesis of mycalolide A (**226**) demonstrated the utility of the Hantzsch reaction in iterative oxazole formation, and the efficiency of chiral (*E*)-crotylsilanes in the construction of contiguous stereogenic centers.

2.9.2. Pattenden's synthesis of a diastereomer of ulapualide A (227).[112] Ulapualide A (**227**), a member of the trisoxazole marine macrolides which include the aforementioned mycalolide A (**226**), was isolated by Scheuer et al. in 1986 from the egg masses of nudibranch *Hexabranchus sanguineus* (Fig. 9).[T03] Ulapualide A differs from mycalolide A in the oxidation patterns and methyl substitutions found in the aliphatic portion of their structures. It was postulated by Pattenden and co-workers that these trisoxazole marine natural products may behave as ionophores. The authors have proposed the relative stereochemistry of ulapualide as shown in (**227**) based on a molecular mechanics study on a metal chelated ulapualide.[117] Although the total synthesis achieved by Pattenden and co-workers on the structure of **227** turned out to be that of a diastereomer of natural ulapualide A, substantial contributions to the chemistry of these complex natural products were made through their study.[112] The disconnections were made at the C1 lactone, C8–C9 carbons and the C19–C20 olefin affording three building blocks, aldehyde **246**, acid **247**, and trisoxazole **248** (Scheme 51). Trisoxazole **248** was coupled with the aliphatic aldehyde **246** via a Wittig reaction. The macrocycle was formed by two approaches: (i) acylation of the C24 hydroxyl group with carboxylic acid **247** followed by macrocylization via an intramolecular Wadsworth–Emmons olefination, or (ii) olefination at C8–C9 followed by macrolactonization.

The trisoxazole synthesis began with the condensation of serine ethylester hydrochloride **249** and ethyl acetimidate hydrochloride **250** to give oxazoline **251** (Scheme 52). Dehydrogenation with nickel peroxide according to Meyers et al.[25] gave oxazole **252** (yields were not provided in the paper). The ester was hydrolyzed and converted into serine amide **253** which, upon treatment with thionyl chloride,



Scheme 51.



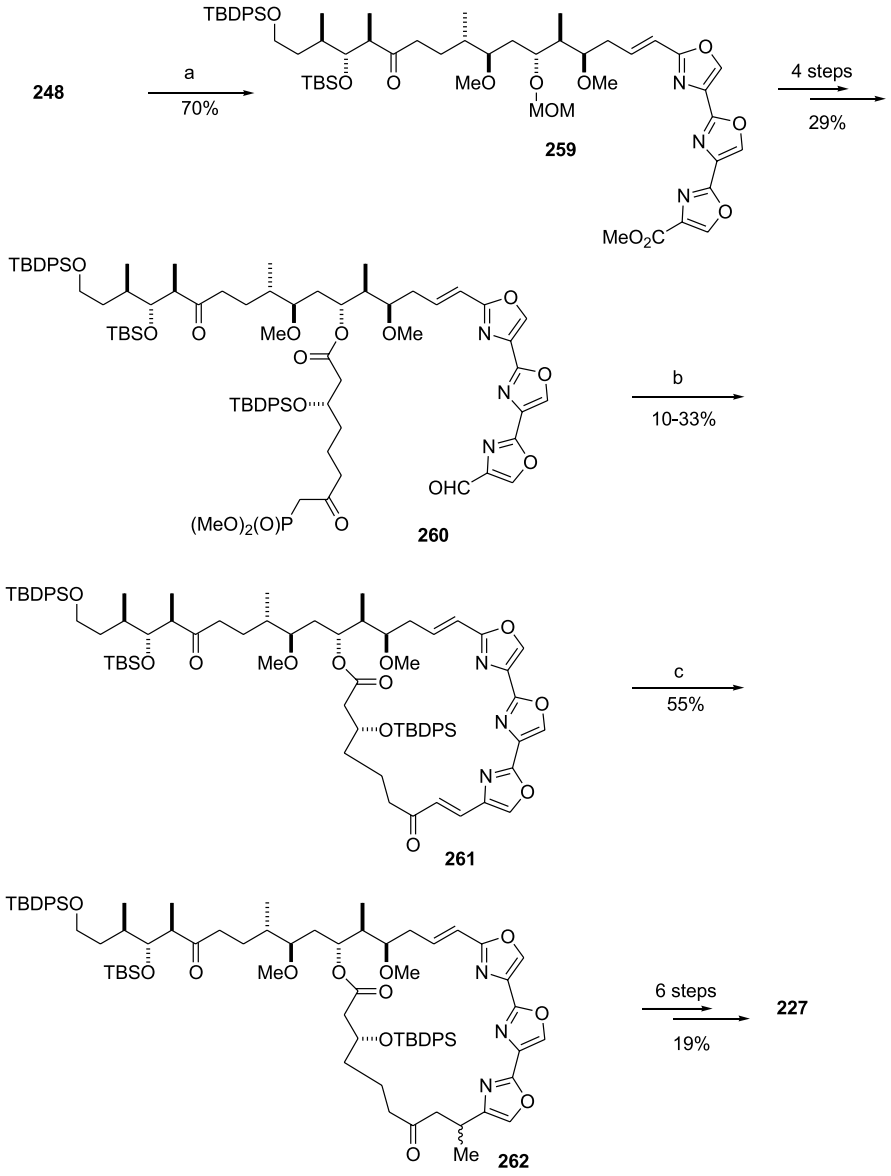
Scheme 52. (a) Et_3N. (b) Nickel peroxide. (c) Thionyl chloride. (d) Ag(OTf). (e) LDA, $(PhSe)_2$. (f) From **255**: nickel peroxide or NBS, *hν*, from **256**; H_2O_2. (g) NBS, AIBN. (h) Ph_3P.

gave the corresponding chloride **254**. The cyclization was promoted by silver triflate which produced oxazoline **255**. Oxidation of **255** using nickel peroxide gave bisoxazole **257** in low yields. Higher yields in the oxidation step were achieved either by using *N*-bromosuccinimide with irradiation from a sun lamp,[118] or converting **255** into phenylselenide **256** followed by H_2O_2 induced elimination of phenylselenic acid. The same protocol was repeated for the formation of the third oxazole ring to afford **258** which was then brominated and converted into the phosphonium salt **248**.

Wittig reaction between phosphonium salt **248** and aliphatic aldehyde **246** gave the coupled product in 70% yield as a single *E*-isomer (Scheme 53). In four steps (29% yield), the ester group in **259** was converted into an aldehyde and the C28 alcohol was acylated with β-keto ester **247** to afford ester **260**. Intramolecular Wadsworth–Emmons olefination using K_2CO_3 in the presence of 18-crown-6 produced the macrolide enone **261** in 30% yield. Alternatively, the reverse of the above reaction sequence, i.e. olefination followed by macrolactonization, afforded **261** in only 10% yield. The C9 methyl group was introduced by addition of dimethylcuprate to macrolide enone **261** to give a 3:2 separable mixture of epimers **262**. The total synthesis of the ulapualide A isomer (**227**) was completed in six additional steps (19% from **262**).

2.10. Phorboxazoles

Phorboxazole A (**263**) and its C13 epimer phorboxazole B (**264**) are marine macrolides isolated by Molinski and co-workers from the marine sponge *Phorbas* sp. found in the Indian Ocean.[119] The structure, and the relative and



Scheme 53. (a) *n*-BuLi, **246**, THF. (b) K_2CO_3, 18-C-6, tol. (c) Me_2CuLi, Et_2O.

absolute configuration, were based on from extensive 2D NMR spectroscopy, derivatization, and degradation-correlation studies.[119,120] Their unique structures feature an unprecedented array of pyran, oxazole, and polyene moieties organized in a macrolide (C1–C26) and a side-chain (C26–C46) (Fig. 10). Both phorboxazole A and B exhibit exceptional antifungal activities against *Candidas albicans*. Furthermore, both natural products have been selected by the National Cancer Institute for in vivo antitumor trials due to their potent cytostatic activity against 60 tumor cell lines.[120] It appears that phorboxazoles induce cell arrest at the S phase of the cell cycle and showed neither inhibition of tubulin polymerization nor interference with the microtubules. Due to the biological activity of these natural products and their unprecedented structure, many synthetic research groups have pursued their total synthesis.[121] The first total synthesis of phorboxazole A (**263**) was achieved by Forsyth and co-workers.[122] Evans et al. completed the first synthesis of phorboxazole B (**264**).[123] Shortly after, Smith et al. also reported the completion of (**263**).[124] Most recently, Pattenden's[125] and Williams'[126] groups have reported simultaneously the latest total syntheses of **263**, which are important contributions to the science of complex natural product synthesis. However, due to the similarities in the

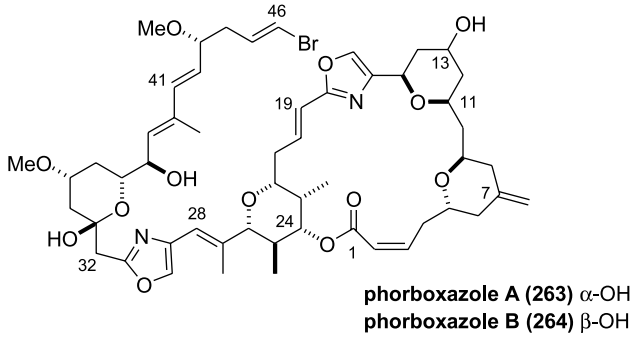
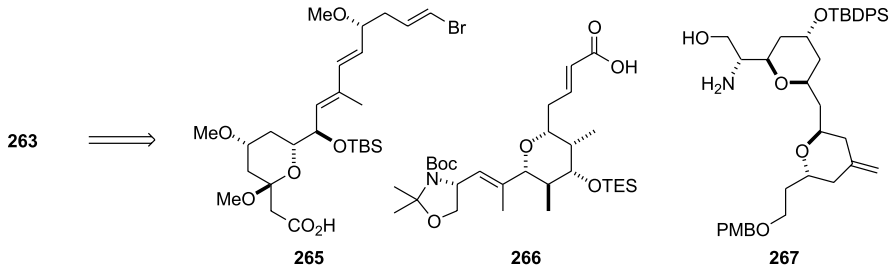


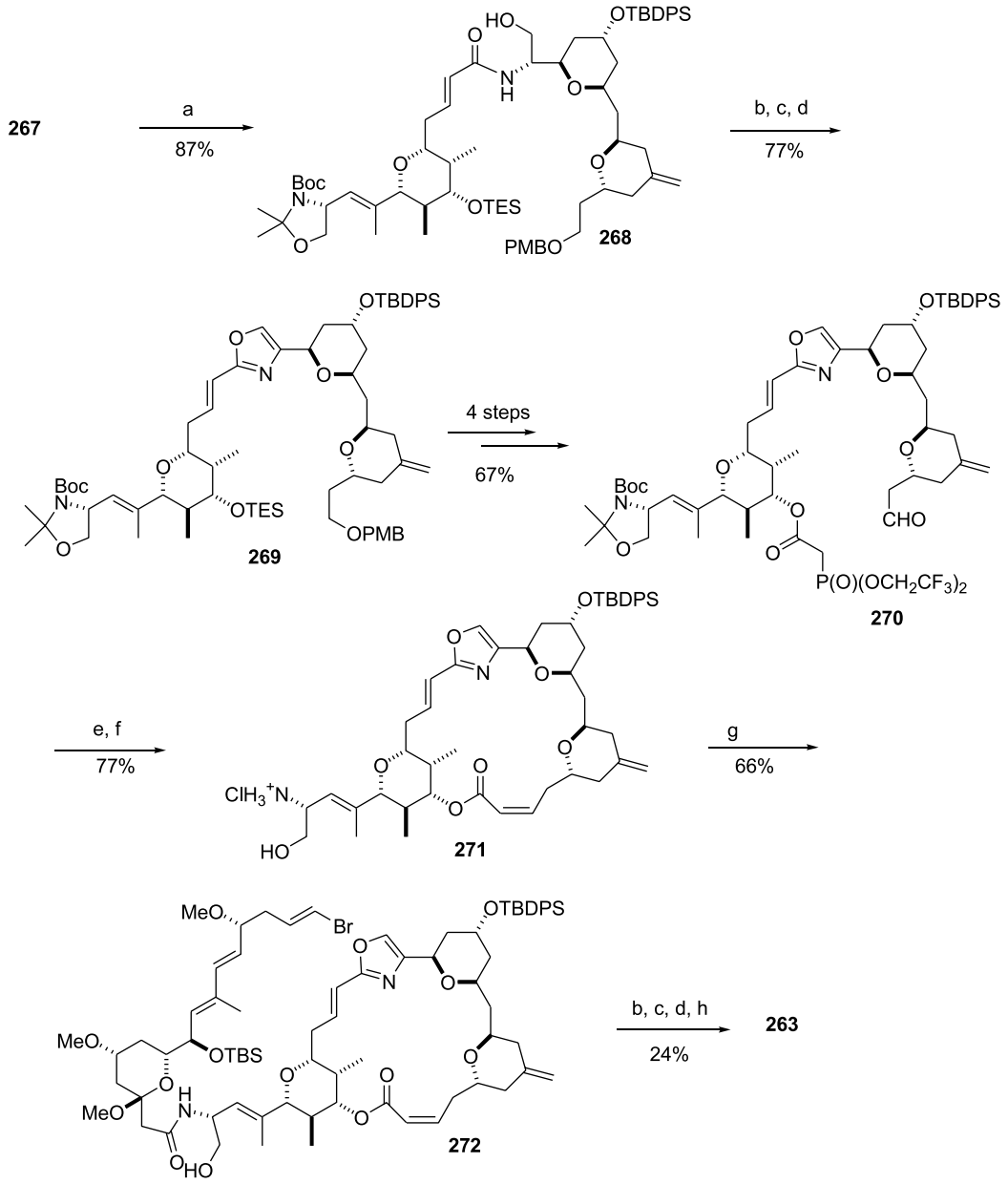
Figure 10.



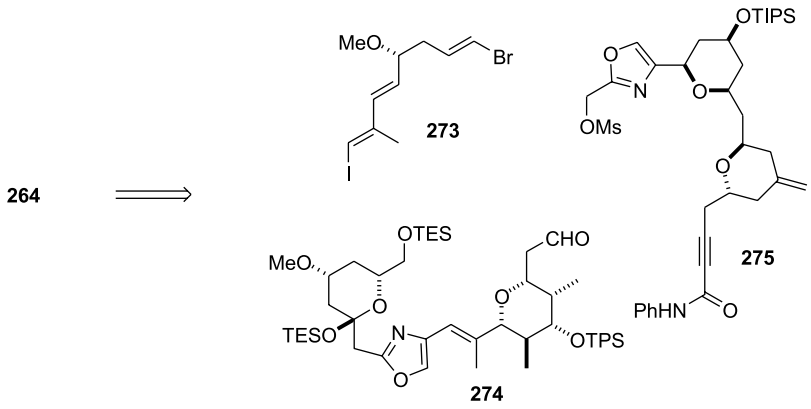
Scheme 54.

oxazole synthesis strategies in comparison to the earlier syntheses, their works have not been included in the following discussion.

2.10.1. Forsyth's synthesis of phorboxazole A (263).[122] Forsyth and co-workers reported the first synthesis of phorboxazole A (**263**) in 1998.[122] Their highly convergent synthesis involved dissecting the natural product into three main fragments representing C31–C46 **265**, C18–C30 **266**, and C3–C17 **267** (Scheme 54). The most remarkable feature of their synthesis is the biomimetic assembly of fragments at the two oxazole rings.



Scheme 55. (a) EDCI, HOBt. (b) Dess–Martin. (c) $(BrCCl_2)_2$, Ph_3P, 2,6-di-*t*-Bu-4-Me-pyr, CH_2Cl_2. (d) DBU, CH_3CN. (e) K_2CO_3, 18-C-6, tol. (f) TsOH, MeOH; HCl, dioxane. (g) **265**, EDCI, DMAP, *i*-Pr_2NEt, CH_2Cl_2. (h) TBAF; HCl.

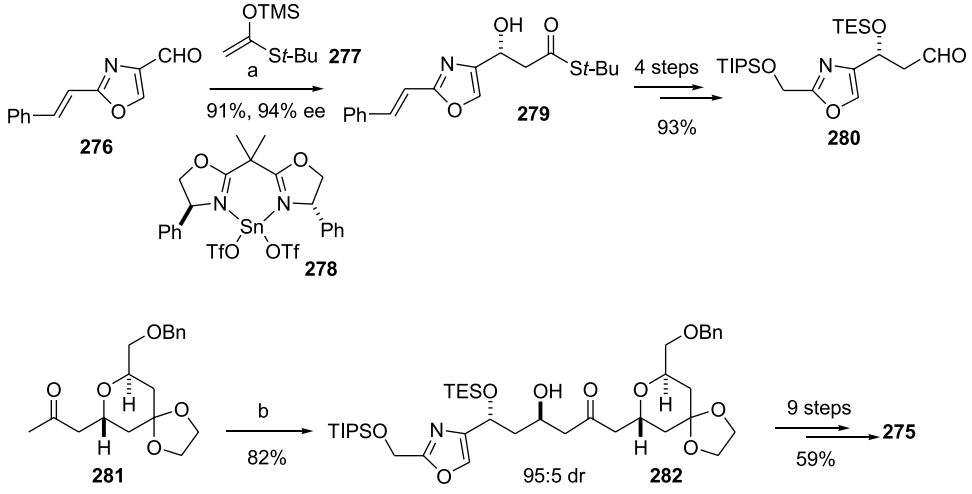


Scheme 56.

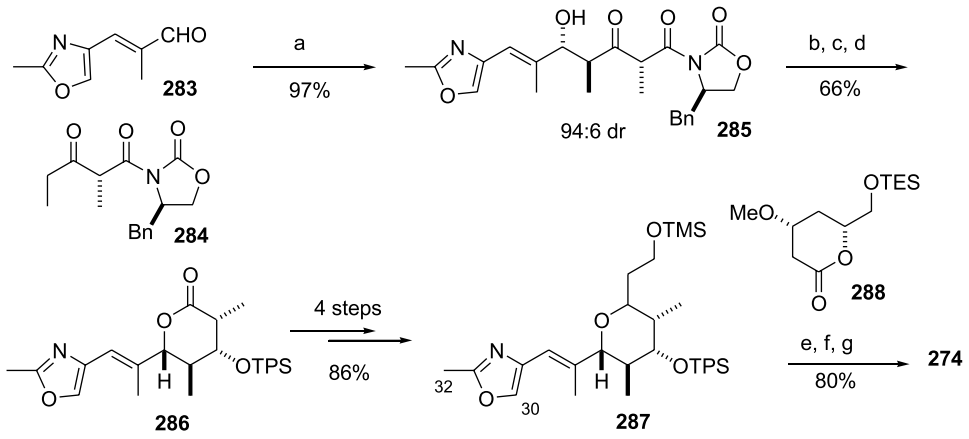
The C3–C17 bis-oxane fragment **267** was attached to the C18–C30 fragment **266** through acylation to give the coupled product **268** in 87% yield (Scheme 55). Application of Wipf's oxidation–cyclodehydration procedure[60] for the formation of 2,4-disubstituted oxazoles commenced with oxidation of **268** with Dess–Martin periodinane, followed by formation of an intermediate bromooxazoline using a phosphorous based dehydration reagent. Subsequent dehydrobromination with DBU gave the oxazole **269** in 77% overall yield from **268**. Compound **269** was further elaborated into macrocylization precursor **270** in four steps (67% from **269**). The *Z*-C2–C3 olefin was installed by intramolecular Still–Gennari olefination which formed the macrocycle in 77% and 4:1 ratio of *Z* vs *E* olefin geometry. After the removal of the nitrogen protecting groups, amine **271** was joined with the C31–C46 side-chain fragment **265** via EDCI promoted acylation. Formation of the second oxazole using the same oxidation–cyclodehydration protocol gave a modest yield (33%) of the desired product, which after global deprotection, afforded synthetic phorboxazole A (**263**).

2.10.2. Evans' synthesis of phorboxazole B.[123] (264) In Evans and co-workers' synthesis of phorboxazole B (**264**), the natural product was divided into two major fragments of similar complexity, C1–C19 **275** and C20–C38 **274**, in addition to the C39–C46 side chain **273** which was appended late in the synthesis (Scheme 56). The two major fragments were assembled via an *E*-selective oxazole-stabilized Wittig olefination to generate the C19–C20 double bond akin to the total synthesis of (+)-calyculin carried out earlier in Evans' laboratory.[19] The macrocycle was formed by Yamaguchi macrolactonization, and the required (*Z*)-alkene was generated by Lindlar hydrogenation of the corresponding acetylene. Following the completion of the macrocycle, the side chain was appended by chelation-controlled nucleophilic addition of a vinylmagnesium bromide derived from **273** to a C38 aldehyde to complete the carbon framework of phorboxazole B (**264**). In contrast to Forsyth's synthesis of **263** which featured oxazole construction as key steps in fragment assembly, Evans' synthesis exploited the inherent reactivity of the intact oxazole to participate in fragment couplings.

A number of catalytic enantioselective aldol reactions developed in the Evans' laboratory were utilized for the construction of key building blocks.[127] Addition of silylketene acetal **277** to aldehyde **276** was catalyzed by 10 mol % of chiral Lewis acid **278**[127] to give adduct **279** in excellent yield and selectivity (91%, 94% ee) (Scheme 57). Compound **279** was elaborated into aldehyde **280**, (73% yield, four steps) which was then coupled with pyran **281** using a highly diastereoselective aldol reaction (95:5 dr). The 1,5-anti induction was controlled by the C9



Scheme 57. (a) 10 mol% **278**, CH_2Cl_2. (b) *n*-Bu_2BOTf, *i*-Pr_2NEt, **280**, CH_2Cl_2.



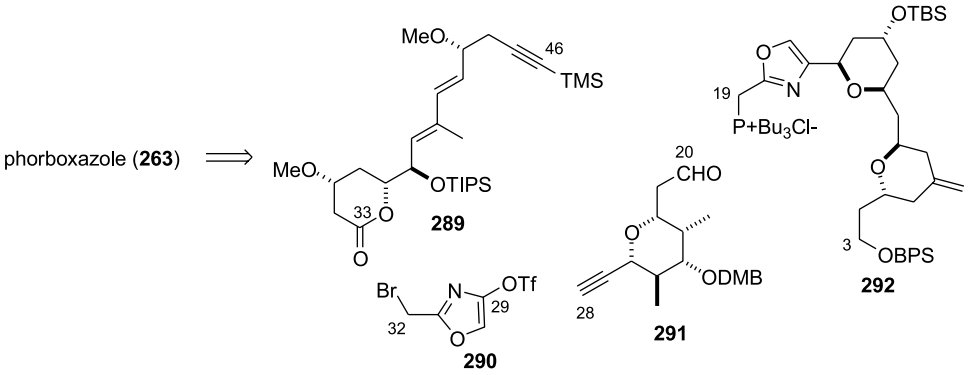
Scheme 58. (a) $(Cy)_2BCl$, $EtNMe_2$, Et_2O. (b) $Me_4NBH(OAc)_3$, AcOH. (c) DBU, CH_2Cl_2. (d) TPSCl, imidazole. (e) $LiNEt_2$, THF, then **288**. (f) TESOTf, pyr; $NaHCO_3$, MeOH. (g) Dess–Martin.

stereogenic center.[128] Aldol adduct **282** was converted into **275** in nine additional steps (59% from **282**).

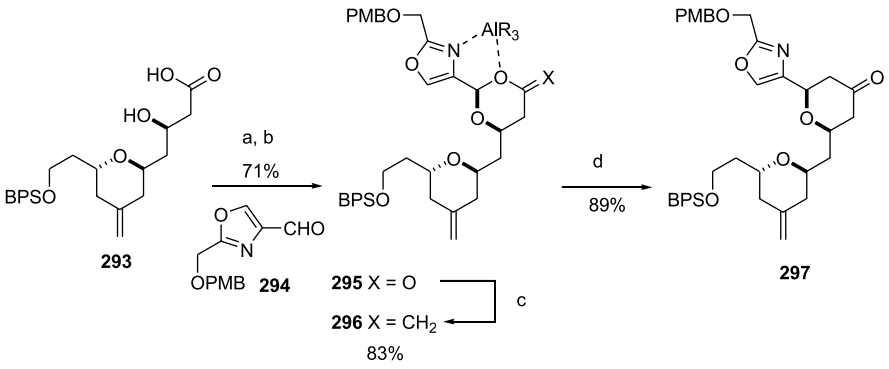
The synthesis of the C20–C38 unit **274** began with a highly diastereoselective aldol reaction between β-ketoimide **284**[129] and aldehyde **283** (97% yield, 94:6 dr) (Scheme 58).[130] Hydroxy-directed reduction of the C24 ketone afforded an 1,3-anti diol, which cyclized to give lactone **286** under basic conditions (DBU, CH_2Cl_2) (66% yield, three steps). The lactone **286** was then extended by two carbons to provide **287** (86%, four steps). Regioselective metallation at C32 of the oxazole methyl group was achieved using the non-hindered, strong base $LiNEt_2$, and the lithiated oxazole added smoothly to lactone **288** to give fragment **274** after lactol protection and oxidation of the primary alcohol (80% yield, three steps). The regioselectivity of the lithiation step was extensively studied.[121] It was found that, in the presence of amine, an equilibrium exists between 5-lithiooxazole (C30) and the more thermodynamically stable 2-lithiomethyloxazole. With a less sterically encumbered amine, such as diethylamine, the equilibration is rapid and favors the latter isomer. With access to the key fragments, phorboxazole B (**274**) was assembled through the aforementioned sequence.

2.10.3. Smith's synthesis of phorboxazole A (263).[124] The key disconnections made in Smith et al.'s synthesis of phorboxazole A (**263**) were at C2–C3, C19–C20, and C28–C29 which divided the natural product into fragments of similar complexity (Scheme 59). The fragment assembly required the use of an oxazole-stabilized Wittig reaction for the formation of the C19–C20 double bond similar to Evans' synthesis of phorboxazole B,[123] and the application of an intramolecular Still–Gennari olefination to close the macrocycle. For the construction of the C29–C48 side chain, a novel oxazole triflate **290** linchpin strategy was utilized to join lactone **289** with the rest of the macrolide. An elegant application of the Petasis–Ferrier rearrangement[131] was utilized in the construction of the two *cis*-substituted tetrahydropyrans.

The C5–C9 *trans*-substituted tetrahydropyran **293** was built using a catalytic asymmetric hetero-Diels–Alder reaction between a protected aldehyde and Danishefsky's diene, followed by 1,4-vinyl cuprate addition, hydroboration and formation of the *exo*-methylene group. A two carbon extention using the Nagao aldol reaction gave **293**.[132] Condensation of **293** and oxazole aldehyde **294** gave dioxanone **295** (71% yield, two steps) which was reacted with the Petasis–Tebbe reagent[133] to give enol acetal **296**, (83% yield) the substrate for the Petasis–Ferrier rearrangement (Scheme 56). Treatment of **296** with Me_2AlCl resulted in clean formation of the desired *cis*-tetrahedropyran **297** in excellent yield (89%). The authors have shown that the oxazole nitrogen played an important role in directing the rearrangement since a 1,3-transposed analog of enol ether



Scheme 59.



Scheme 60. (a) HMDS. (B) TMSOTf. (c) Cp_2TiMe_2. (d) Me_2AlCl.

296 resulted in none of the desired rearrangement. Compound **297** was converted into fragment **292** through some functional group manipulations (Scheme 60).

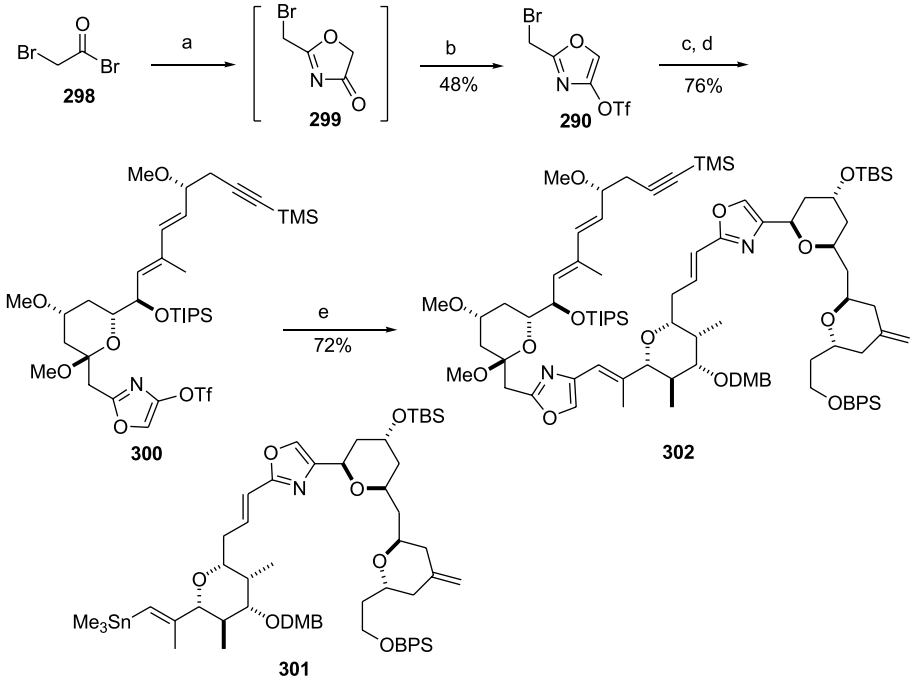
Another example of novel oxazole chemistry in Smith's synthesis is the use of the difunctional oxazole **290** as the linchpin for bidirectional assembly of the side chain. Using a modified procedure of a method developed by Sheehan in 1949,[134] the authors treated bromoacetyl bromide **298** with silver isocyanate and diazomethane to give an intermediate oxazolone **299**, which was then converted into triflate **290** (48% yield, two steps) (Scheme 61). Generation of an organomagnesium reagent via Grignard exchange[135] in the presence of lactone **289** afforded the coupled product **300** in 76% yield as a single isomer. The triflate **300** was then efficiently coupled with stannane **301** by a Stille reaction to produce the macrolide precursor **302** (72% yield). The completion of the natural product was achieved by the formation of the macrolide using an intramolecular Still–Gennari olefination to form the (*Z*)-C2–C3 olefin.

2.11. Promothiocin A

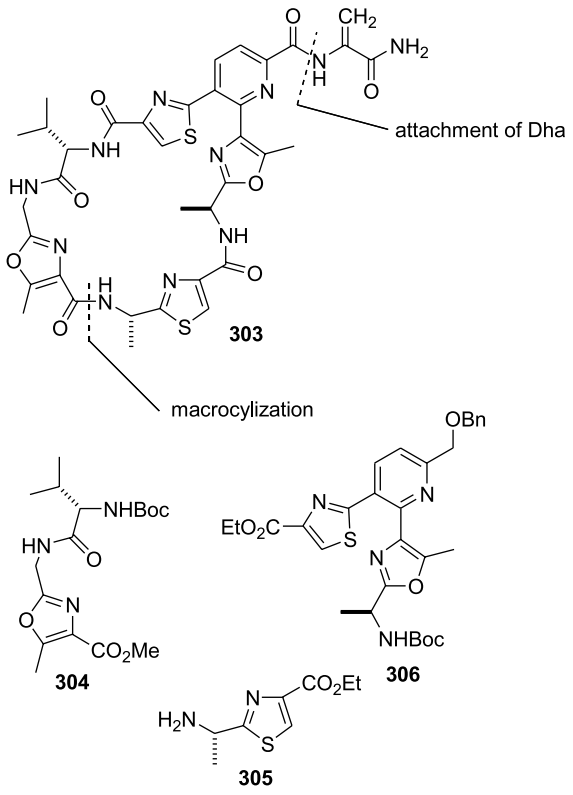
Promothiocin A (**303**)[136] belongs to a class of sulfur-containing highly modified cyclic peptide antibiotics called thiopeptides which also include thiosptrepton,[137] nosiheptide,[138] micrococcin,[139] and amythiamycin.[140] The structural features that are shared among these natural products are the central tri- or tetrasubstituted pyridine cores surrounded by oxazole or thiazole heterocycles and dehydroamino acids. Most thiopeptides inhibit bacterial growth by binding to the complex of 23SrRNA with ribosomal protein L11.[141] Prior to the completion of **303** by Moody et al. in 2000,[142] the only other reported thiopeptide total synthesis was that of micrococcin.[143] Fragment syntheses of nosiheptide,[144] berninamycin,[145] micrococcin,[146] sulfomycin,[147] A10255,[148] and GE 2270A[149] have also been reported.

2.11.1. Moody's synthesis of promothiocin A (303).[142]

The total synthesis of promothiocin A (**303**) by Moody et al.



Scheme 61. (a) AgOCN, Et_2O, CH_2N_2. (b) Et_3N, Tf_2O, THF. (c) *i*-PrMgCl, **289**. (d) *p*-TSA, MeOH. (e) **301**, $Pd(PPh_3)_4$, LiCl, dioxane.



Scheme 62.

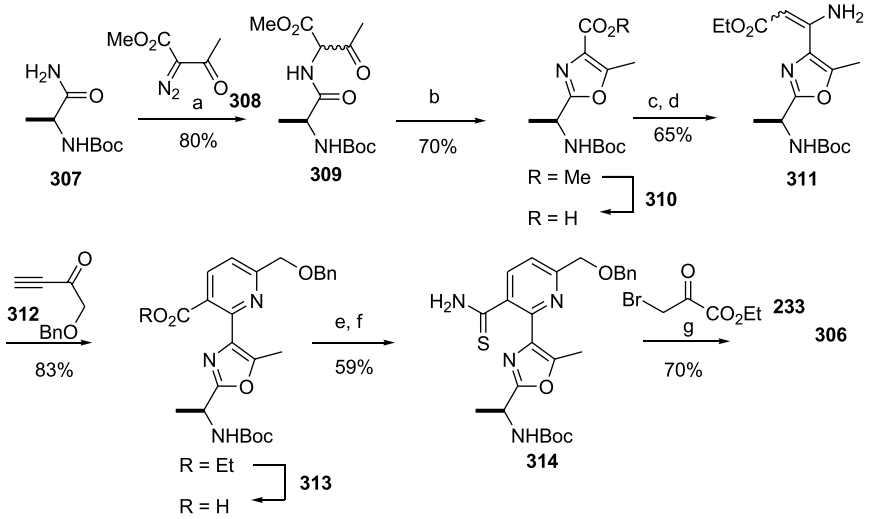
featured efficient couplings of peptide fragments derived from natural amino acids, macrocyclization, and final attachment of the dehydroalanine (Dha) side chain (Scheme 62).[142]

The synthesis of the pyridine core **306** highlighted the application of a new oxazole assembly method developed by the authors based on rhodium carbenoid N–H insertion.[150] Treatment of diazoacetoacetate **308** with dirhodium(II) acetate in the presence of alaninamide **307** gave ketoamide **309** in 80% yield (Scheme 63). Cyclodehydration of **309**, using Wipf's procedure,[48] gave oxazole **310** in good yield (70%) without racemization of the stereogenic center. Compound **310** was converted into enamine **311** by homologation of the corresponding acid with magnesium ethyl malonate[151] through the mixed anhydride followed by treatment with ammonium acetate. The de novo pyridine formation was achieved by heating enamine **311** and ynone **312**, a method developed by Bohlmann and Rahtz which gave pyridine **313** in 83% yield.[152] The thiazole ring at the 3-position of the pyridine core was installed by first converting ester **313** into thioamide **314**. Then, treatment with bromopyruvate **233** in the presence of $KHCO_3$ and dehydration with trifluoroacetic anhydride gave the core fragment **306** in good overall yields (41% yield, three steps).[116]

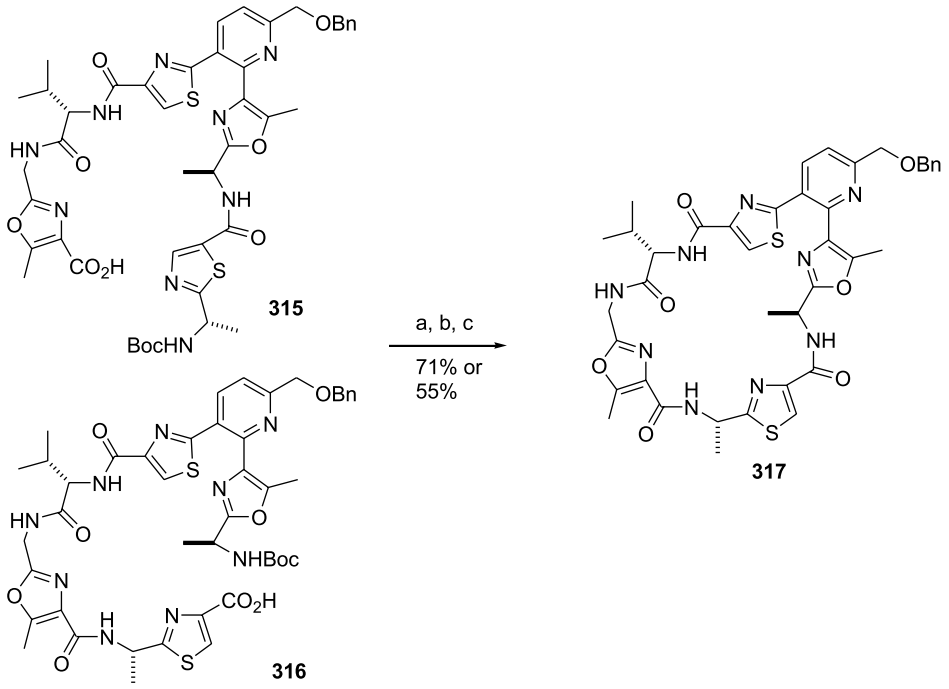
After the completion of the pyridine core **306**, the remainder of the fragments were joined by mixed anhydride mediated peptide coupling to give two macrocycle precursors **315** and **316** (Scheme 64). Schmidt's pentafluorophenyl ester protocol[153] was utilized in the cyclization step with **315** giving 71% overall yield, and **316** giving 55% overall yield. With the macrocycle in place, the completion of the natural product was subsequently achieved in six additional steps. These included deprotection of the benzylic alcohol and oxidation to the carboxylic acid, acylation of serine amide, and dehydration to install the desired dehydroalanine side chain.

2.12. Thiangazole

(−)-Thiangazole (**318**) was isolated by Jansen and co-workers from the gliding bacterium *Polyangium spec*. in 1992.[154] Oxazole (**318**), along with structurally related tantazole B (**319**)[155] and mirabazole B (**320**) (Fig. 11),[156] constitute a unique family of cytotoxic poly-heterocyclic natural products which feature successive 2,4-disubstituted thiazoline-oxazole rings. In the original report by Jansen, thiangazole was shown to be highly active against HIV-1 but exhibited low cell toxicity even at millimolar levels.[157] Due to the bioactivity and unique structural challenges, significant synthetic work has been accomplished by several



Scheme 63. (a) $Rh_2(OAc)_4$, $CHCl_3$. (b) Ph_3P, I_2, Et_3N, CH_2Cl_2. (c) EtO_2CCl, Et_3N; Mg ethyl malonate. (d) NH_4OAc, AcOH. (e) EtO_2CCl, Et_3N, NH_4OH. (f) Lawesson's reagent. (g) $KHCO_3$.



Scheme 64. (a) C_6F_5OH, EDCI, CH_2Cl_2. (b) HCl, dioxane. (c) $KHCO_3$, aq. $CHCl_3$.

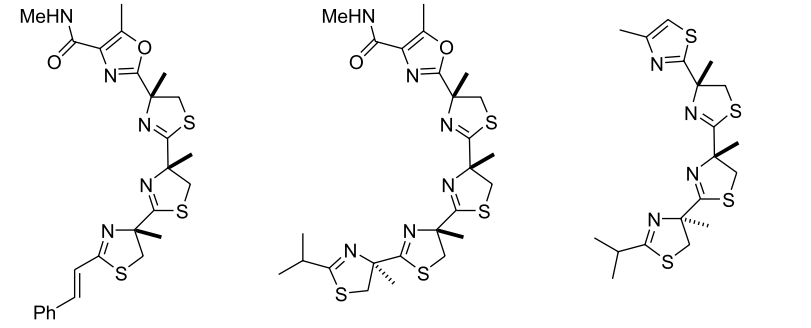
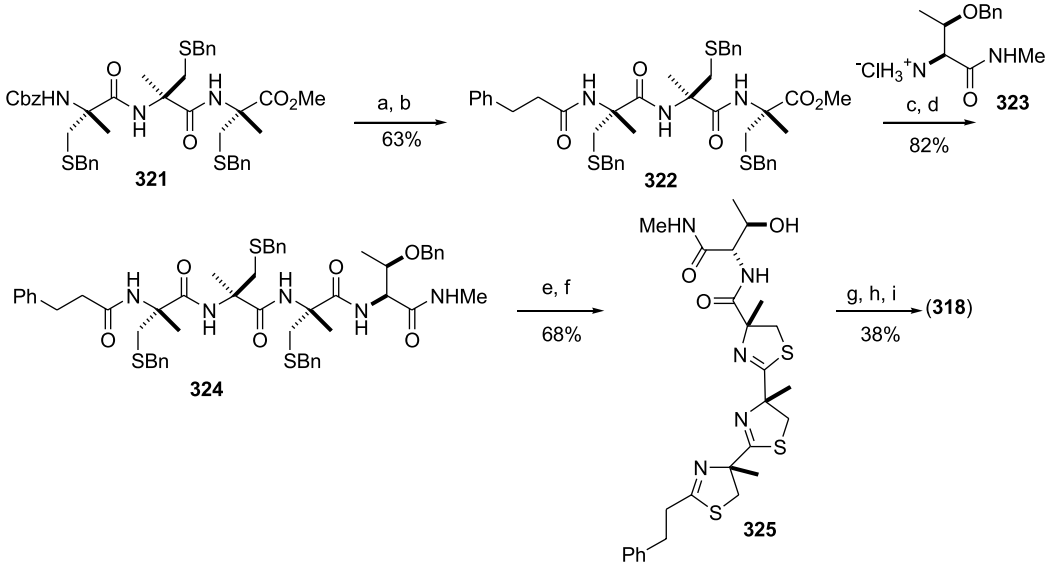
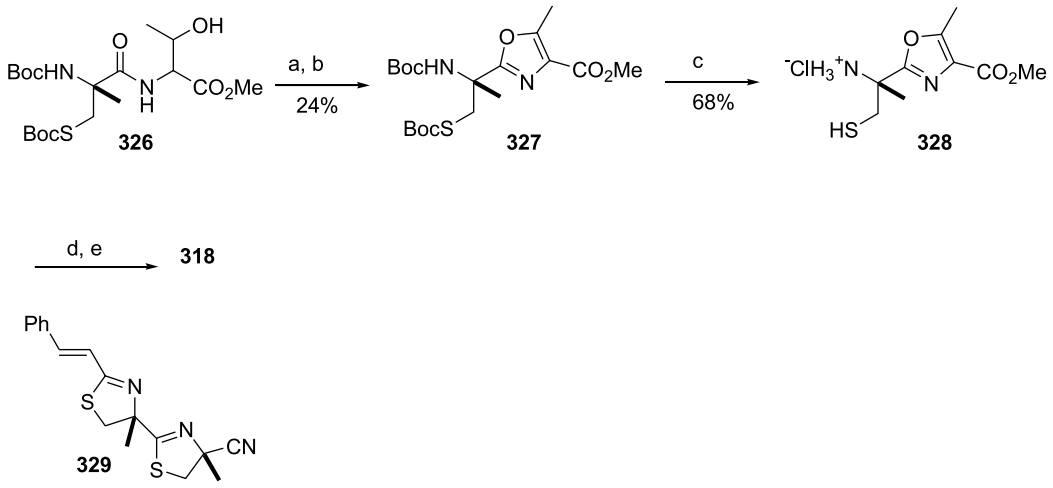


Figure 11.



Scheme 65. (a) HBr, HOAc, thioanisole. (b) $PhCH_2CH_2COCl$, DMAP, *i*-Pr_2NEt. (c) NaOH. (d) PyBrop, DMAP, *i*-Pr_2NEt, CH_2Cl_2. (e) Na, NH_3, THF. (f) $TiCl_4$, CH_2Cl_2. (g) Dess–Martin period. (h) *p*-TsOH, C_6H_6. (i) DDQ.



Scheme 66. (a) Burgess reagent, THF. (b) *t*-BuO_3Cph, Cu(I)Br, C_6H_6. (c) HCl, Et_2O. (d) Et_3N, MeOH. (e) $MeNH_2$, EtOH.

research groups on this family of natural products.[158] Total syntheses of thiangazole have been reported by Heathcock,[159] Ehrler,[160] Pattenden,[161] Wipf,[162] and Akaji.[163] The strategies utilized in Ehrler's and Akaji's syntheses are similar to the other reported syntheses, and are therefore, not included in the following discussion. An independent biological evaluation performed by Wipf and co-workers on synthetic (**318**) and related analogues has failed to confirm the originally reported antiviral activity from the original report.

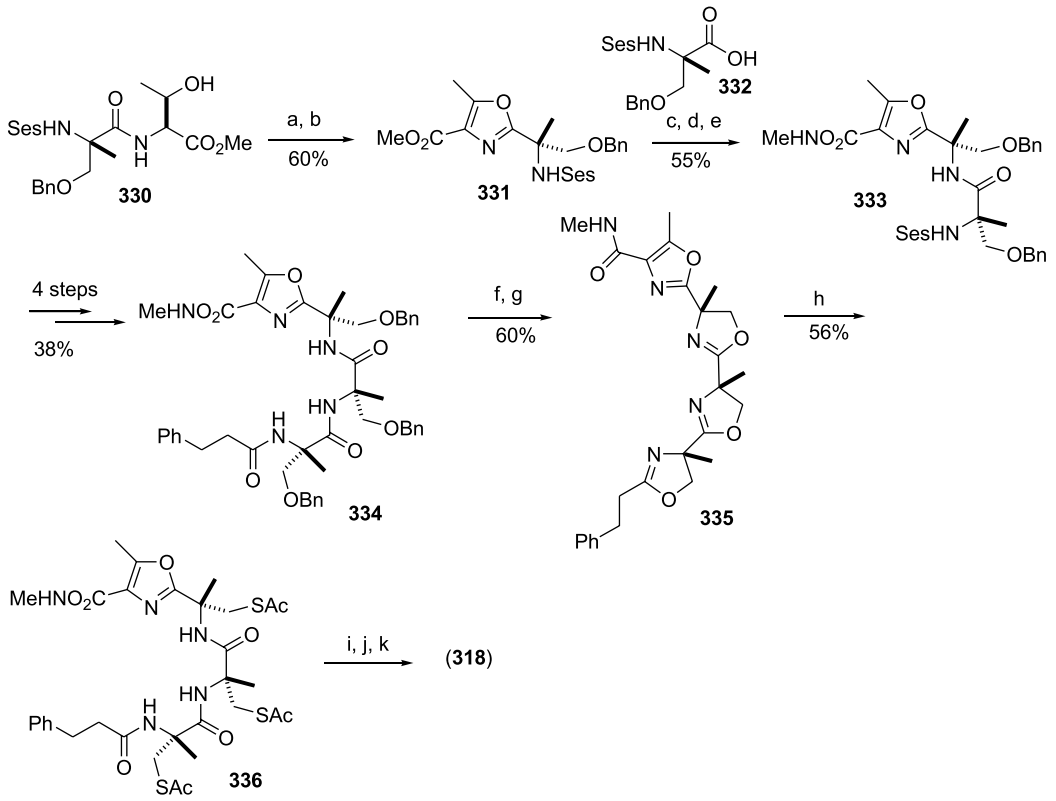
2.12.1. Heathcock's synthesis of (−)-thiangazole (318).[159] The key transformation in Parson and Heathcock's synthesis of (−)-thiangazole (**318**) is the simultaneous construction of the three thiazoline rings via a $TiCl_4$-mediated cyclodehydration of thiol residues onto a peptide amide backbone.[159] The same strategy was utilized by Akaji and Kiso in their synthesis of thiangazole.[163] The synthesis began with the elaboration of the known tripeptide **321**[164] by successive acylation of the amino terminus with dihydrocinnamoyl chloride and coupling with protected threonine derivative **323** at the carboxylic acid terminus to give tetrapeptide **324** (Scheme 65). After reductive debenzylation, the crude polythiol was treated with $TiCl_4$ to give the corresponding tri-thiazoline **325** in an excellent 68% yield. After oxidation of alcohol **325** to the corresponding ketone, the terminal oxazole was constructed by an acid-catalyzed Robinson–Gabriel cyclodehydration (38% yield, three steps). Other methods of cyclodehydration examined such as triphenylphosphine and iodine failed, due to the presence of sensitive thiazoline moieties.

2.12.2. Pattenden's synthesis of (−)-thiangazole (318).[161] The synthesis of (−)-thiangazole (**318**) reported by Pattenden and co-workers relied on the convergent cyclocondensation of nitrile **329** and oxazole **328** (Scheme 66).[161] The oxazole fragment was synthesized from cyclodehydration of 2-methylcysteine-threonine amide **326** with the Burgess reagent to afford an intermediate oxazoline which was oxidized with *t*-butylperoxybenzoate and copper(I) bromide[93] to give oxazole **327** in 24% yield from **326**. Deprotection of **327** with HCl gave ammonium salt **328**. Triethylamine induced cyclocondesation of fragments **328** and **329** gave the full carbon framework of the natural product which upon treatment with methylamine gave synthetic (**318**).

2.12.3. Wipf's synthesis of (−)-thiangazole (318).[162] The unique feature of Wipf and Venkatraman's synthesis of thiangazole (**318**) is a triple oxazoline-thiazoline transformation which allowed the authors to efficiently access oxazoline analogues of the natural product for biological testing.[162] Oxazole **331** was synthesized by Dess–Martin oxidation of D-threonine amide **330** followed by cyclodehydration with triphenylphosphine and iodine (Scheme 67).[48] The remaining amino acid building blocks were linearly assembled to give the tetrapeptide **334** using PyBrop as coupling reagent. After removal of the benzyl protecting groups, a triple cyclization mediated by the Burgess reagent gave the trisoxazoline analogue of thiangazole **335** in 60% yield. The oxazolines were transformed into thiazolines by first refluxing **335** in thioacetic acid to give **336**, followed by aminolysis and $TiCl_4$-induced cyclodehydration according to Heathcock's procedure.[159]

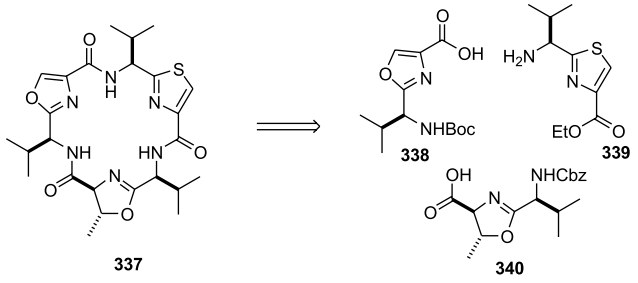
2.13. Oxazole-containing marine cyclic peptides

A large number of oxazole and thiazole-containing cyclic peptide natural products have been isolated from cyanobacteria, marine sponges and ascidians (sea squirts).[165] Many of these natural products exhibit significant biological activities including cytotoxic, antibacterial, and antiviral activities,[165] and some have also been found to act as antineoplastic agents.[166] Moreover, these cyclic peptides have been studied as ionophores.[167] A large body of synthetic work on these marine cyclic peptides has appeared in the literature and has been summarized in a 1995 review by Wipf.[168] Since the review, elegant total syntheses have been accomplished on bistratamides,[169] ceratospongamide,[170] cyclodidemnamide,[171] dendroamide,[172] dolastatin E,[173] leucamide A,[174] lissoclinamides,[175] nostocyclamide,[176] and trunkamide.[177] Three examples from the recent literature have been chosen to illustrate some of the general approaches to these natural products.



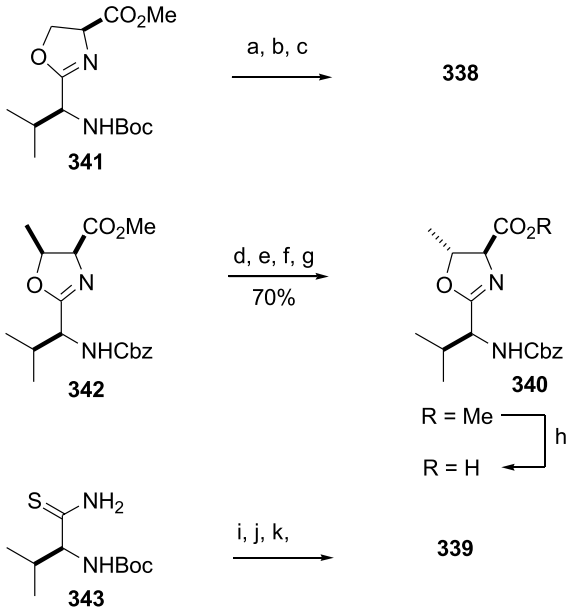
Scheme 67. (a) Dess–Martin. (b) Ph_3P, I_2, Et_3N. (c) $MeNH_2$, MeOH. (d) TBAF. (e) **332**, PyBrop, DMAP, CH_2Cl_2. (f) $Pd(OH)_2$, H_2, MeOH. (g) Burgess reagent, THF. (h) AcSH. (i) NH_3, MeOH. (j) $TiCl_4$, CH_2Cl_2. (k) $PhSeO_2H$, C_6H_6.

2.13.1. Meyers' synthesis of bistratamide D (337).[169] Bistratamide D (**337**) was isolated from *ascidian Lissoclinum bistratum* and was found to be highly cytotoxic.[178] The retrosynthetic approach utilized by Meyers et al. disconnected all of the amide bonds to break the molecule down into three key building blocks (Scheme 68).



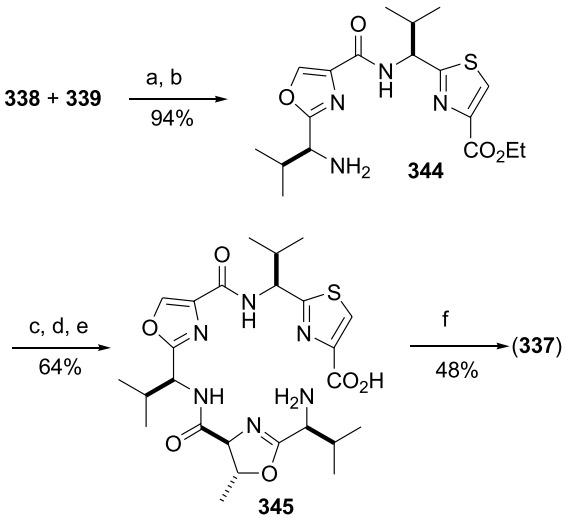
Scheme 68.

This was similar to an approach used in Meyers' bistratamide C synthesis.[169b] Oxazole **338** was obtained from aromatization of valine–serine-derived oxazoline **341** via two methods (Scheme 69). Radical oxidation using copper(I) bromide, copper(II) acetate, and *t*-butylperoxybenzoate, developed by Meyers et al. gave **338** in 67% yield on multigram scale.[179] Alternatively, when Williams' protocol[54] ($BrCCl_3$ and DBU) was utilized, oxazole **338** was obtained in quantitative yield. The oxazoline fragment **340** was derived from epimerization of the more readily available *cis*-oxazoline **342** based on conditions reported by Wipf.[180] Sequential treatment of valine–threonine derived



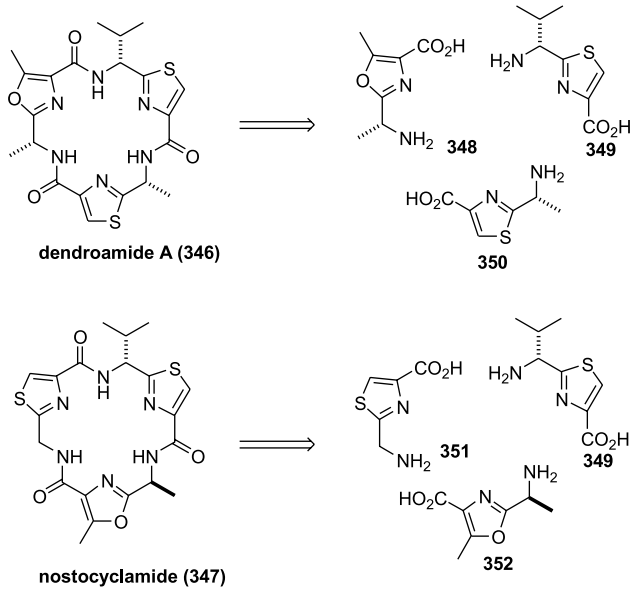
Scheme 69. (a) CuBr, $Cu(OAc)_2$, $PhCO_3tBu$, 67%. (b) $BrCCl_3$, DBU. quant. (c) LiOH, MeOH, H_2O, 99%. (d) HCl. (e) K_2CO_3. (f) Al_2O_3, MeOH. (g) Burgess reagent. (h) 2 M NaOH, MeOH (i) $KHCO_3$, $BrCH_2COCO_2Et$. (j) TFAA, 2,6-lutidine. (k) AcCl, EtOH.

oxazoline **342** with HCl, K_2CO_3, and basic Al_2O_3 in refluxing MeOH produced a dipeptide with *allo*-threonine configuration which was re-cyclized with the Burgess reagent[29] to provide *trans*-oxazoline **340**. The thiazole fragment was synthesized from thioamide **343** using Holzapfel's modified Hantzsch procedure.[181]



Scheme 70. (a) EDCI, HOBt, DMF. (b) AcCl, EtOH. (c) **340**, EDCI, HOBt, DMF. (d) H_2, Pd, Et_3N, EtOH. (e) LiOH. (f) HATU, *i*-Pr_2NEt, DMF.

Fragments **338** and **339** were effectively joined by treatment with EDCI in the presence of HOBt to give the corresponding coupled product in 94% yield which, after protecting group removal, gave amine **344** (Scheme 70).



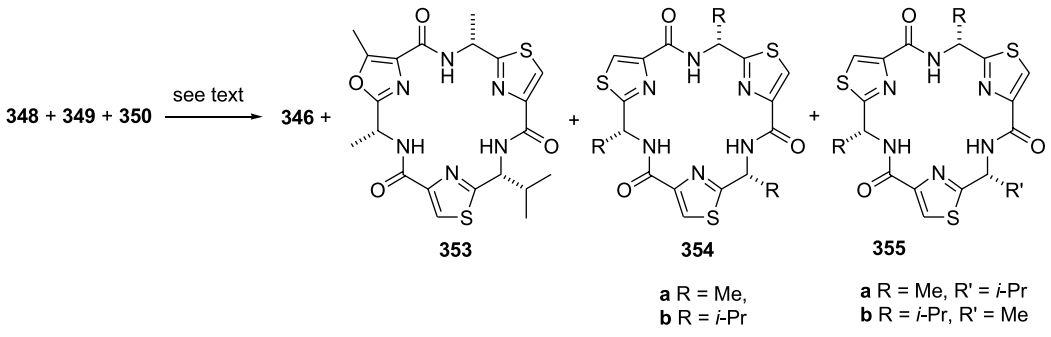
Scheme 71.

Oxazoline **340** was attached to **344** by EDCI mediated acylation and, after removal of the Cbz group and hydrolysis of the ester, the macrocyclization precursor **345** was obtained. The removal of the Cbz group under standard hydrogenolysis conditions (Pd/C, 1 atm H_2) proved challenging due to catalyst poisoning from the sulfur-containing thiazole group. However, the crucial deprotection was ultimately achieved using Pd black as the catalyst under high pressure (100 psi) in EtOH/Et_3N solvent. Macrocyclization was induced by O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uronium hexafluorophosphate (HATU) giving **337** in 48% yield.

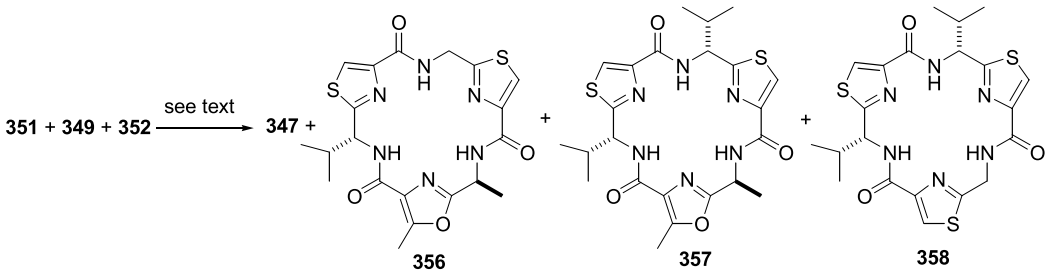
2.13.2. Pattenden's synthesis of dendroamide A (346) and nostocyclamide (347).[172a] Dendroamide A (**346**) and nostocyclamide (**347**) are two structurally similar oxazole- and thiazole-containing marine cyclic peptides isolated from cyanobacteria *Stigonema dendroideum*[182] and *Nostoc* sp.,[183] respectively. Both natural products have been synthesized previously using linear assembly of amino acid fragments followed by macrocyclization.[172b,176b] Bertram and Pattenden have investigated the syntheses of these natural products based on the one step cyclooligomerization of oxazole and thiazole amino acid building blocks (**348** to **352**) with or without metal ions as templates (Scheme 71).[172a] Cyclooligomerization strategies of macrocyclic peptide assembly have also been investigated by Pattenden,[184] Rebek,[185] and Wipf[180] on related systems.

For the synthesis of dendroamide A (**346**), mixing equimolar amounts of building blocks **348**, **349** and **350** in the presence of diisopropylethylamine (DIPEA) and pentafluorophenyl diphenyl phosphinate (FDPP) in acetonitrile gave dendroamide A (**346**) in 23% yield along with positional isomer **353** (22%) and four tris-thiazoles **354**-**355** (30% combined) (Scheme 72). In the presence of various metal ions it was found that only **354b** and **346** were formed in different ratios. For example, in the presence of $AgBF_4$, **354b** was the only product formed, albeit in a low yield (13%). When $Ca(BF_4)_2$ was used as a template, the ratio of **346** to **354b** was 2.3:1.

Cyclooligomerization of equimolar amounts of **351**, **349** and **352** in the presence of FDPP-DIPEA gave equal amounts of nostocyclamide (**347**) and cyclic peptide trimer analogues **356**, **357** and **358** in a combined yield of 65% (Scheme 73). It was found that in the presence of Zn, Na, or K, isomer **357** was preferentially formed. In the presence of



Scheme 72.

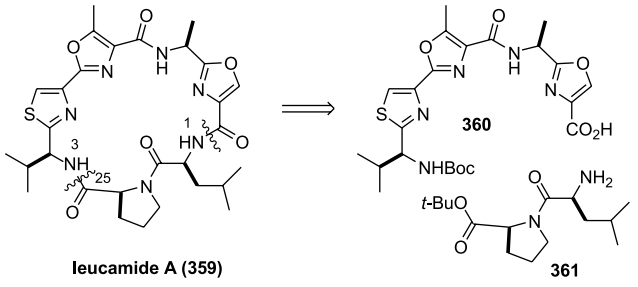


Scheme 73.

$AgBF_4$, **357** was the exclusive product. Although the study demonstrated the ability of metal ions to act as templates for cyclooligomerization, the factors governing the selectivity are still not understood.

2.13.3. Nan's synthesis of Leucamide A (359).[174]

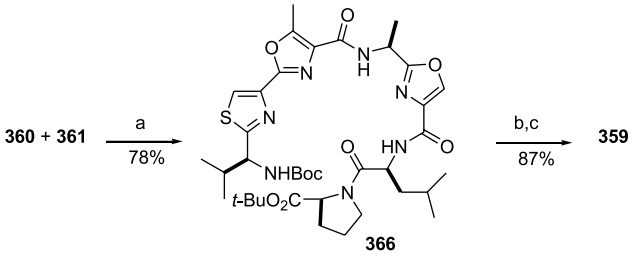
Leucamide A (**359**) is a moderately cytotoxic cyclic peptide isolated from marine sponge *Leucetta microraphis* which features a unique tandem 2,4-disubstituted methyloxazole–thiazole subunit.[186] The retrosynthesis involved dissecting the molecule into tricyclic fragment **360** and dipeptide **361** (Scheme 74). The macrocycle was formed in the final step of the synthesis at the N3–C25 amide to give the desired *trans*-geometry of the proline amide bond.[187]



Scheme 74.

The synthesis of the heterocyclic portion of the natural product began with the known valine-derived thiazole **362** previously reported by Meyers.[169b] Acylation of acid **362** with L-threonine methyl ester gave amide **363**. DAST-mediated cyclodehydration gave an intermediate oxazoline which was oxidized using bromotrichloromethane and DBU to provide oxazole **364** in 51% yield from amide **363** (Scheme 75). The reliability of the two step protocol developed by Wipf and Williams[53c] for the synthesis of oxazoles was once again demonstrated in this synthesis.

Having completed the bis-azole fragment **360**, the alanine–serine derived oxazole **365** was then attached by EDCI-mediated acylation to give **360**. The natural product was completed by joining **360** with dipeptide **361** followed by HATU-mediated macrocyclization of **366** to give leucamide A (**359**) in a remarkable yield of 87% for the cyclization step (Scheme 76).



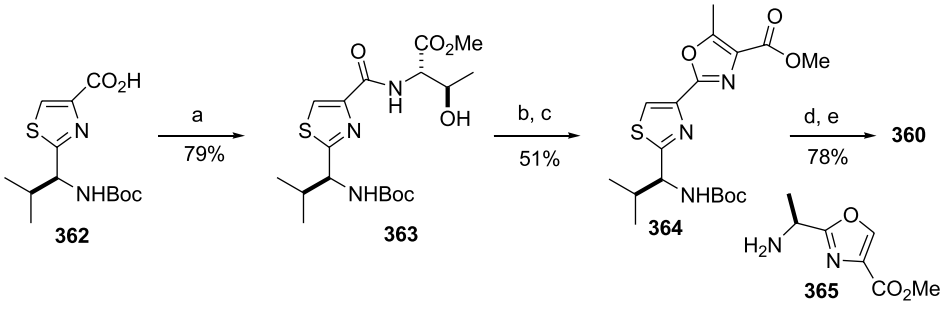
Scheme 76. (a) EDCI, HOBt. (b) TFA, CH_2Cl_2. (c) HATU, *i*-Pr_2NEt, DMF.

3. Summaries and conclusions

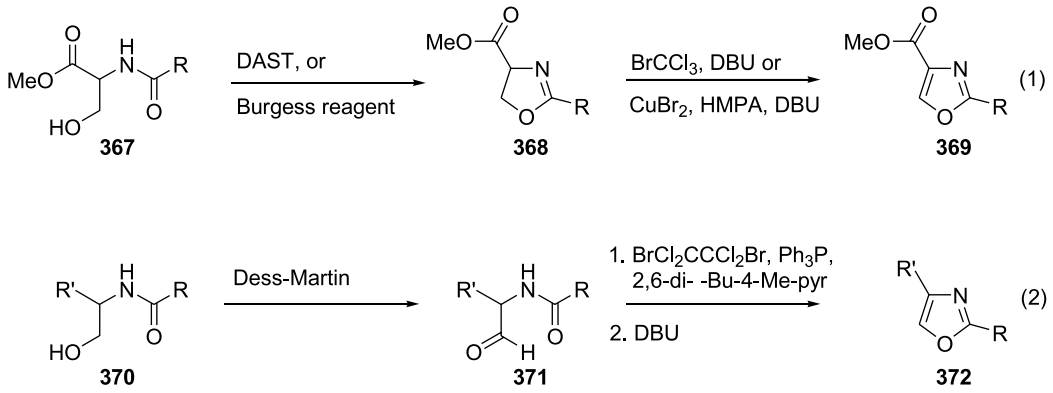
The literature survey on the total syntheses of oxazole-containing natural products shows two favored methods. These methods are desirable for natural product synthesis due to their mild conditions and selectivity, and are summarized below in Schemes 77 and 78. In addition, there are several unique oxazole synthesis methods worthy of additional attention from synthetic chemists. Two of these are summarized below in Scheme 79.

3.1. Oxazole synthesis from peptide precursors

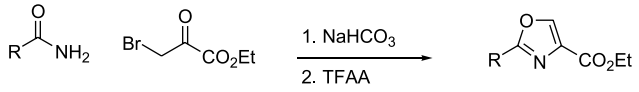
Synthesis of the oxazole moiety from a peptide precursor is by far the most utilized amongst the natural product syntheses featured in this review. As shown in Eq. (1) of Scheme 77, serine amide **367** can be dehydrated with either DAST or the Burgess reagent to obtain the intermediate



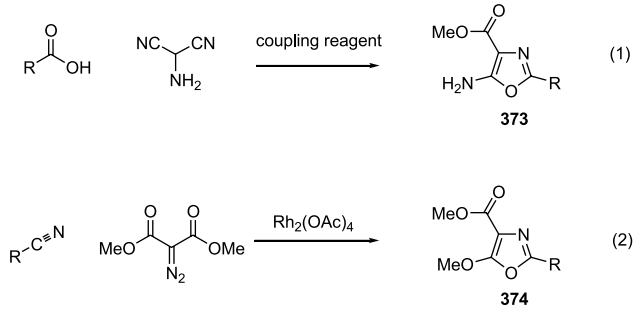
Scheme 75. (a) *i*-BuOCOCl, NMM; L-threonine Me ester·HCl. (b) DAST, then K_2CO_3. (c) DBU, $BrCCl_3$. (d) LiOH. (e) EDCI, HOBt, **365**.



Scheme 77.



Scheme 78.



Scheme 79.

oxazoline **368**, which can be oxidized with either $BrCCl_3$ or $CuBr_2$ to give oxazole **369**. This method may be desirable when an ester handle on the 4 position of the oxazole is needed for further elaboration. The reagents and conditions used in this sequence are mild and the yield of the desired oxazole are generally high as seen in a number of syntheses discussed in the review (Sections 2.2.3, 2.4.1, 2.5.2, and 2.13.3). Alternatively, an oxazole can be derived through oxidation from an amide of amino alcohol such as **370** to give the intermediate aldehyde **371** and then dehydrated to give 2,4-di-substituted oxazole **372** (Scheme 77, Eq. (2)). This method is advantageous since it allows the coupling of two fragments through an amide formation which renders a synthesis more convergent (See: Sections 2.5.1, 2.8.1, and 2.10.1). The degree of success of this strategy may depend on the stability of the aldehyde intermediate. If the intermediate aldehyde is not stable then it might result in a lower yield of the oxazole product.

3.2. Hantzsch oxazole synthesis

A 2,4-disubstituted oxazole can also be efficiently generated from a two-steps, one pot reaction procedure between an amide and an α-halo ketone followed by dehydration as shown in Scheme 78 (Sections 2.2, 2.4 and 9.1). This process is advantageous for large scale synthesis of oxazole since the starting materials and reagents involved are readily available and the reaction sequence is high yielding.

3.3. Other oxazole synthesis methods

Most of the methodologies featured in this review have focused on the assembly of 2,4-disubstitited or 2,4,5-trisubstituted oxazoles derived from serine or threonine peptide precursors. However, methods that allow functionalization on the 5 position may be desirable for exploration of the structure-activity relationships around the oxazole moiety. Scheme 79 highlights two such methods. Eq. (1) shows coupling of an acid and aminomalononitrile to generate an aminooxazole **373**, and the amino group can be further functionalized as seen in Section 3.1. Eq. (2) depicts cycloaddition between a nitrile and a diazomalonate to give a methoxyoxazole **374** as seen in Section 2.6.4. The methoxy group can also be a handle for further elaboration.

Nature continues to be an inexhaustible source of novel molecular structures. Many natural products containing oxazole moieties presented here in this review have attracted the attention of synthetic chemists due to their biological activities. Over the course of synthetic studies, many ingenious oxazole syntheses methodologies have been developed, and it can be expected that these advances will be incorporated as an essential part of the methodological repertoire for medicinal and synthetic chemists.

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Biographical sketch

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Stereoselective synthesis of procyanidin B3-3-*O*-gallate and 3,3″-di-*O*-gallate, and their abilities as antioxidant and DNA polymerase inhibitor☆

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Abstract—A simple method for the synthesis of procyanidin B3 substituted with a galloyl group at the 3 and 3″ position is described. Condensation of a benzylated catechin-3-*O*-gallate electrophile with a nucleophile, catechin and catechin-3-*O*-gallate, proceeded smoothly and stereoselectively to afford the corresponding dimer gallates, procyanidin B3-3-*O*-gallate and procyanidin B3-3,3″-di-*O*-gallate, in good yields. Further, their antioxidant activities on UV-induced lipid peroxide formation, DPPH radical scavenging activity and inhibitory activity of DNA polymerase were also investigated. Among three procyanidin B3 congeners (procyanidin B3, 3-*O*-gallate and 3,3″-di-*O*-gallate), the 3,3″-di-*O*-gallate derivative showed the strongest antioxidant and radical scavenging activity. Interestingly, the 3-*O*-gallate derivative was the strongest inhibitor of mammalian DNA polymerase α with IC_{50} value of 0.26 μM, although it showed the weakest antioxidant and radical scavenging activity. It became apparent that the presence of a galloyl group at the C-3 position in the proanthocyanidin oligomer was very important for biological activity, however, the antioxidant activity of these compounds was not parallel to the DNA polymerase inhibitory activity.

1. Introduction

Proanthocyanidins, condensed tannins and/or oligomeric flavonoids,[2,3] are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers, and bark. They react with one-electron oxidants, resulting in powerful antioxidant activity (free-radical scavenging activity).[4] Numerous other biological activities have been reported for proanthocyanidins; for example, anti-bacterial,[5] antiviral,[6] antimutagenic,[7] anti-inflammatory,[8] hypotensive,[9] and reduction of the risk of heart diseases.[10] In addition, they have been found to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase.[11] The structure–activity relationship of proanthocyanidin oligomers is most important; however, it has not been proved yet, because a large number of similar isomers in the plants makes it very difficult to purify individual compounds and thus to supply extremely pure compounds necessary for biological assay. Another problem in the investigation of proanthocyanidins is that there are so many oligomers[2,3] substituted with a methyl group, a galloyl group, sugar, etc., in plants.[2,3] Many reports[12] on the isolation and semi-synthesis of procyanidin oligomers have been published thus far, but few studies concerning substituted oligomers have appeared. We previously reported a stereoselective

☆ See Ref. 1.

Keywords: Proanthocyanidin; Procyanidin B3; Galloyl ester; Antioxidant activity; DPPH radical scavenging activity; DNA polymerase inhibitor; Stereoselective synthesis.
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synthesis of procyanidin dimers[1a,13] and trimers[1b,14] consisting of (+)-catechin and (−)-epicatechin, both of which have two hydroxyl groups on the B ring. In this report, we undertook a stereoselective synthesis of procyanidin B3 derivatives substituted with a galloyl group at the C-3 and 3″ position (**2** and **3**). The bioactivities, antioxidant activities on UV-induced lipid peroxide formation, DPPH radical scavenging activity and inhibitory activity of DNA polymerases, of three procyanidin B3 congeners (procyanidin B3, 3-*O*-gallate and 3,3″-di-*O*-gallate) were investigated, and the details of their results were described (Figs. 1 and 2).

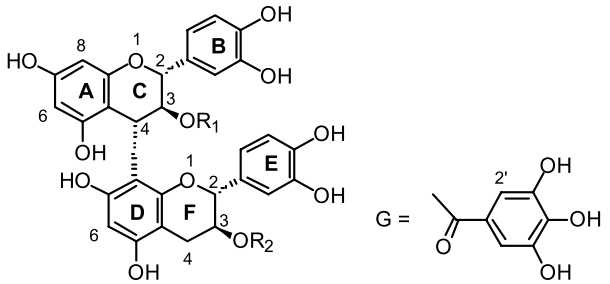


Figure 1. Structures of procyanidin B3 and its derivatives.

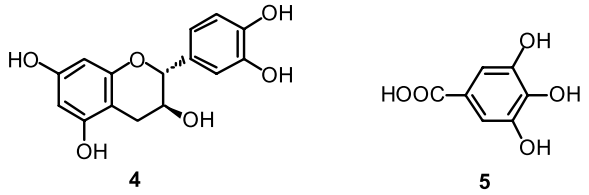


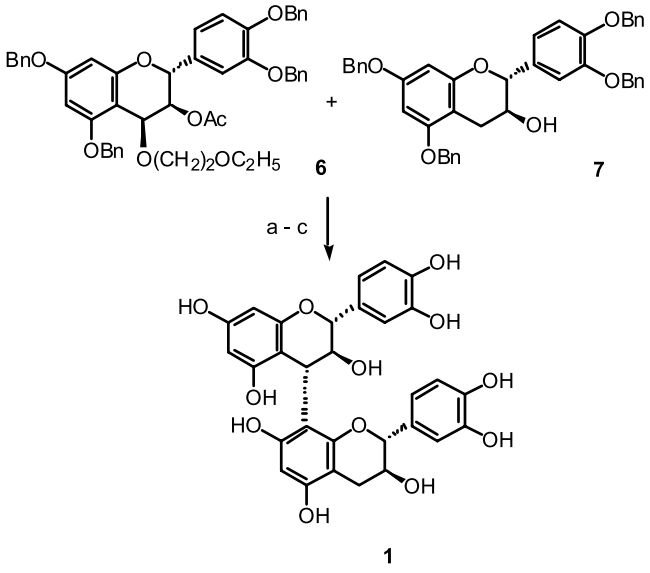
Figure 2. Structures of catechin **4** and gallic acid **5**.

2. Results and discussion

2.1. Stereoselective synthesis of procyanidin B3 substituted with a galloyl group

Many research groups have recently reported the isolation of proanthocyanidin oligomers substituted with a galloyl group and their bioactivities.[15] However, there is no systematic study of the bioactivity of various galloyl oligomers, because it is very difficult to separate purely individual structural analogues from the plant. In 1999, Tückmantel et al.[16] reported a synthesis of procyanidin B2-3,3″-di-*O*-gallate from octa-*O*-benzylprocyanidin B2 and its bioactivities. These current works stimulated us to start to find a simple systematic synthetic method for the gallate oligomers, 3-*O*-gallate and 3,3″-di-*O*-gallate derivatives.

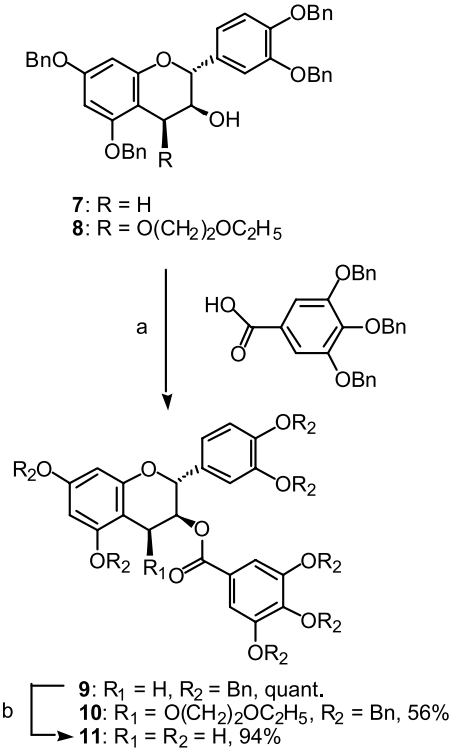
A stereoselective synthesis of procyanidin B3, (+)-catechin-(4α-8)-(+)-catechin dimer, was reported by us as shown in Scheme 1.[13a] The method is that the catechin electrophile **6** and nucleophile **7** derived from (+)-catechin **4** were condensed in the presence of TMSOTf (trimethylsilyl triflate) as a catalyst at −78 °C in CH_2Cl_2. Following deprotection and purification yielded pure procyanidin B3 **1**



Scheme 1. Synthesis of procyanidin B3. Reagents: (a) TMSOTf, CH_2Cl_2, −78 °C; (b) DIBAL, CH_2Cl_2; (c) $Pd(OH)_2/C$, H_2,THF/MeOH/H_2O.

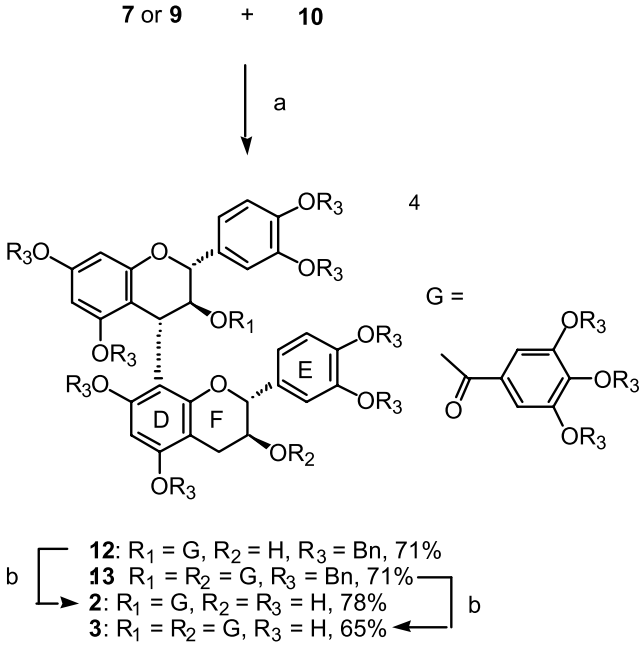
in good yield without contamination by related compounds. This stereoselective condensation reaction by the neighboring group participation effect of 3-*O*-acetate **6** prompted us to synthesize the 3-*O*-galloyl and 3,3′-di-*O*-galloyl substituted procyanidin B3.

As shown in Scheme 2, nucleophile **9** and electrophile **10** with a substituted galloyl group at the C-3 position were prepared by condensation of **7** and **8** with tri-*O*-benzyl gallic acid derived from gallic acid **5** in a quantitative yield and 56% yield, respectively. Benzylated catechin-3-*O*-gallate **9**



Scheme 2. Synthesis of 3-*O*-galloyl catechin derivatives. Reagents: (a) DCC, DMAP, CH_2Cl_2; (b) $Pd(OH)_2/C$, H_2,THF/MeOH/H_2O.

was hydrogenated with $Pd(OH)_2/C$ under hydrogen atmosphere to give (+)-catechin-3-*O*-gallate **11**. The electrophile **10** was condensed with nucleophile **7** and **9** in the presence of TMSOTf to give dimer **12** (71% yield) and **13** (71% yield)[17] and subsequent deprotection of these compounds yielded procyanidin B3-3-*O*-gallate **2** and 3,3″-di-*O*-gallate **3** in 78% and 65% yield, respectively (Scheme 3). The mono-galloyl compound **2** is reported as a natural product isolated from *Sanguisorba officinalis*.[18] The spectral data and optical rotation value of the synthetic **2** were identical with those of the natural product. Since this method is applicable to the synthesis of various galloyl oligomers, synthetic studies of other dimers consisting of (+)-catechin and (−)-epicatechin as structural components are under way. The new di-galloyl compound **3** gave satisfactory NMR and IR data together with HRMS.



Scheme 3. Synthesis of 3-*O*-galloyl procyanidin B3 derivatives. Reagents: (a) TMSOTf, CH_2Cl_2, −78 °C; (b) $Pd(OH)_2/C$, H_2,THF/MeOH/H_2O.

2.2. Antioxidant activity and DPPH radical scavenging activity

Proanthocyanidins are known as a strong antioxidant and radical scavenger as described above. In our previous research,[1b] we investigated the antioxidant activity of dimers and trimers, and it became apparent that antioxidant activity was not influenced by the length of the oligomer chain.[19] Then, we examined the effect of the galloyl moiety on antioxidant and radical scavenging activity. The antioxidant activity[1b] of compound **1**, **2**, **3**, **4**, **5**, **11** and DL-α-tocopherol on UV-induced lipid peroxide formation using the TBA method is shown in Table 1.

The IC_{50} values (concentration of 50% inhibitory activity) of these compounds were 21, 57, 18, 37, 200, 22 and 580 μM, respectively. On the other hand, the SC_{50} values (concentration of 50% scavenging activity) of the DPPH radical scavenging activity[20] were 1.3, 3.2, 1.1, 2.6, 2.4, 1.7 and 17 μM, respectively. The tendency of antioxidant activity was similar to that of DPPH radical scavenging activity. The strongest activity was revealed by **3**, procyanidin B-3,3″-di-*O*-gallate, and the weakest activity was that of **2**, procyanidin B3 3-*O*-gallate, in both experiments. Surprisingly, the activity of dimeric 3-*O*-gallate **2** was lower than that of monomeric 3-*O*-gallate **11** and (+)-catechin **4**. These results substantiated the data obtained in our previous experiment using oligomers and monomers with no ester linkage at the C-3 position.[1b]

Table 1. Inhibitory activity of synthetic proanthocyanidins on lipid peroxidation by the TBA and the DPPH method

Entry	Compound	IC_{50} (μM) by TBA method	SC_{50} (μM) by DPPH method
1	**1**	21	1.3
2	**2**	57	3.2
3	**3**	18	1.1
4	**4**	37	2.6
5	**5**	200	2.4
6	**11**	22	1.7
7	DL-α-Tocopherol	580	17

2.3. Effects of galloyl-substituted compounds on the inhibitory activities of mammalian DNA polymerase α and β

Monomeric flavan-3-*O*-gallates, (−)-epicatechin-3-*O*-gallate, (−)-epigallocatechin-3-*O*-gallate, etc., that occur in green tea, are known as inhibitors of DNA and RNA polymerases,[21] and it was apparent that a galloyl group is essential for the inhibitory effect, because flavan-3-ols without galloyl group were not effective for these inhibitory activities. DNA polymerases, especially DNA polymerase α, are regarded as the target of some anticancer drugs because DNA polymerases play central roles in DNA replication which is indispensable for the proliferation of cancer cells. These facts allowed us to expect galloyl-substituted procyanidin dimers to be inhibitors of DNA polymerases.

Table 2 shows the IC_{50} values of catechin-induced compounds (compounds **1**, **2**, **3** and **11**) against calf DNA polymerase α and rat DNA polymerase β. DNA polymerase α and β are replicative and repair-related DNA polymerases in nuclei, respectively.[22] These compounds did not inhibit DNA polymerase β activity, but inhibited DNA polymerase α activity. The inhibition by each compound was dose-dependent. Interestingly, compound **2** with the weakest antioxidant and radical scavenging activity, is the strongest inhibitor of DNA polymerase α. Further, compound **3** and **11** were more effective for inhibiting DNA polymerase α

Table 2. IC_{50} values of enzymatic inhibition against mammalian DNA polymerase α and β

Entry	Compound	DNA polymerase α, IC_{50} (μM)	DNA polymerase β, IC_{50} (μM)
1	**1**	36.4	>100
2	**2**	0.26	>100
3	**3**	8.1	>100
4	**4**	>100	>100
5	**5**	>100	>100
6	**11**	13.8	>100

activity than compound **1**. These results obtained from the inhibitory activity experiment using DNA polymerases suggest that (1) galloyl catechin is effective for the selective inhibition against DNA polymerase α; (2) galloyl group is important for their inhibitory activity; (3) the inhibitory activity is independent of their antioxidant activity and radical scavenging activity.

3. Conclusion

We have developed an efficient synthetic method for the galloyl-substituted procyanidin B3 at the 3 and 3″ position. Their antioxidant activity, DPPH radical scavenging activity and DNA polymerase inhibitory activity were investigated. From the results of these activity tests, it became apparent that antioxidant activity and DPPH radical scavenging activity were not influenced by the oligomer length, the presence of a galloyl group in proanthocyanidin was important for their bioactivity and antioxidant activity which were independent of DNA polymerase inhibition. Systematic synthesis of other structurally related compounds and their bioactivity tests is now under way.

4. Experimental

4.1. Synthesis

Optical rotation was measured with a Horiba SEPA-300 spectrometer. IR spectra were measured with a Shimadzu OR-8000 spectrometer. ^{1}H NMR spectra were measured with JEOL JNMLA400 spectrometer at rt, and MS spectra were recorded with a JEOL JMS-AX500 instrument. HPLC purification was carried out on a Mightysil® RP-18 GP column (Kanto Chemical Co. Inc, Japan; 250×20 mm, 5 μm) using the solvents (A) 0.05% CF_3CO_2H in CH_3CN and (B) 0.05% CF_3CO_2H in H_2O. Elusion was done with a linear gradient 5 to 100% A in 40 min (flow rate, 3.0 mL/min).

4.1.1. (2*R*,3*S*)-5,7,3′,4′-Tetra-*O*-benzylflavan-3-yl (3″,4″,5″-tri-*O*-benzyl)gallate (9). To a solution of (2*R*,3*S*)-5,7,3′,4′-tetra-*O*-benzylflavan-3-ol (**7**)[13a] (499 mg, 0.77 mmol) and 3,4,5-tri-*O*-benzylgallic acid (675 mg, 1.53 mmol) in CH_2Cl_2 (60 ml) was added DCC (315 mg, 1.53 mmol) and DMAP (5.0 mg). After stirring for 12 h at rt, the reaction mixture was quenched with water, and extracted with CH_2Cl_2. The organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and silica gel column chromatography (benzene/EtOAc, 20/1) afforded 825 mg (0.77 mmol, 100%) of **9** as a pale yellow amorphous powder; $[\alpha]_D^{24}=+38.2$ (*c* 0.98, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.42–7.16 (37H, m), 7.00 (1H, d, *J*=1.5 Hz), 6.90 (1H, dd, *J*=1.5, 8.3 Hz), 6.85 (1H, d, *J*=8.3 Hz), 6.30 (1H, d, *J*=1.5 Hz), 6.29 (1H, d, *J*=1.5 Hz), 5.45 (1H, dd, *J*=5.4, 8.1 Hz), 5.14–4.97 (15H, m), 3.04 (1H, dd, *J*=5.4, 16.8 Hz), 2.83 (1H, dd, *J*=8.1, 16.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 165.1, 158.9, 157.7, 156.0, 152.3, 149.0, 148.9, 142.5, 137.4, 137.1, 136.9, 136.8, 136.7, 136.5, 131.1, 128.6–127.2 (C×18), 125.0, 120.0, 114.8, 113.4, 109.1, 101.4, 94.3, 93.7, 78.3, 75.1, 71.3, 71.13, 71.08, 70.1, 69.9 (×2), 24.2; IR (neat, cm^{-1}) 3090 (w), 3065 (w), 3032 (m), 2866 (m), 2361 (w), 2342 (w), 1952 (w), 1873 (w), 1811 (w), 1699 (m), 1593 (s), 1504 (m), 1454 (m), 1385 (s), 1126 (s), 1041 (m), 917 (w), 887 (w), 758 (m); FAB-MS (*m/z*) 1097 (4.4), 1096 (8.3), 1095 ([M+Na]$^+$, 24), 1075 (6.7), 1074 (16), 1073 ([M+H]$^+$, 24), 724 (13), 723 (31), 722 (38), 634 (28), 633 (76), 632 (100), 631 (30); FAB-HRMS calcd for $C_{71}H_{61}O_{10}$ [M+H]$^+$, 1073.4265; found:1073.4260.

4.1.2. (2*R*,3*S*,4*S*)-5,7,3′,4′-Tetra-*O*-benzyl-4-(2″-ethoxyethoxy)flavan-3-yl (3″,4″,5″-tri-*O*-benzyl)gallate (10). To a solution of (2*R*,3*S*,4*S*)-5,7,3′,4′-tetra-*O*-benzyl-4-(2″-ethoxyethoxy)flavan-3-ol (**8**)[13a] (251 mg, 0.34 mmol) and 3,4,5-tri-*O*-benzylgallic acid (299 mg, 0.68 mmol) in CH_2Cl_2 (30 ml) was added DCC (140 mg, 0.68 mmol) and DMAP (5.00 mg). After stirring for 12 h at rt, the reaction mixture was quenched with water, and extracted with CH_2Cl_2. The organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and silica gel column chromatography (benzene/EtOAc, 20/1) afforded 217 mg (0.19 mmol, 56%) of **10** as a white powder, and 67 mg (27%) of the starting material **8** was recovered; $[\alpha]_D^{25}=+101.1$ (*c* 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.44–7.21 (37H, m), 7.08 (1H, d, *J*=1.7 Hz), 6.98 (1H, dd, *J*=1.7, 8.3 Hz), 6.83 (1H, d, *J*=8.3 Hz), 6.28 (1H, d, *J*=2.0 Hz), 6.18 (1H, d, *J*=2.0 Hz), 5.42 (1H, d, *J*=10.8 Hz), 5.36 (1H, dd, *J*=2.9, 10.8 Hz), 5.09–4.98 (15H, m), 3.86–3.81 (1H, m), 3.76–3.71 (1H, m), 3.46–3.42 (2H, m), 3.38–3.28 (2H, m), 1.04 (3H, t, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 164.9, 161.0, 158.6, 155.8, 152.5, 149.3, 149.1, 142.7, 137.5, 137.1, 137.0, 136.6, 136.5, 130.6, 128.6–127.5 (C×19), 124.6, 121.4, 114.7, 114.4, 109.1, 103.8, 94.3, 93.9, 75.1, 74.3, 73.7, 71.4, 71.21 (×2), 71.17, 70.4, 70.1, 69.9, 68.7, 66.4, 15.2; IR (neat, cm^{-1}) 3065 (w), 3032 (m), 2928 (m), 2870 (m), 1717 (s), 1617 (s), 1592 (s), 1455 (s), 1374 (s), 1335 (s), 1266 (s), 1152 (s), 1115 (s), 1028 (s), 911 (w), 857 (w), 814 (w), 754 (s), 696 (s); FAB-MS (*m/z*) 1184 (3.1), 1183 ([M+Na]$^+$, 4.1), 1161 ([M+H]$^+$, 3.0), 1160 (2.7), 1073 (4.4), 1072 (5.9), 783 (6.1), 782 (11), 633 (12), 632 (31), 631 (50), 424 (29), 423 (100); FAB-HRMS calcd for $C_{75}H_{69}O_{12}$ [M+H]$^+$, 1161.4789; found:1161.4771.

4.1.3. (+)-Catechin-3-*O*-gallate (11). A solution of **9** (90 mg, 0.084 mmol) in 22 mL of THF/MeOH/H_2O, 20/1/1 was hydrogenated over 20% $Pd(OH)_2$/C (5 mg) for 3 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (EtOH) and HPLC purification to give 35 mg (0.079 mmol, 94%) of **11** as a colorless amorphous solid; $[\alpha]_D^{24}=+52.7$ (*c* 0.46, Me_2CO); ^{1}H NMR (400 MHz, CD_3OD) 6.95 (2H, s), 6.82 (1H, s), 6.71 (2H, s), 5.95 (1H, d, *J*=2.2 Hz), 5.93 (1H, d, *J*=2.2 Hz), 5.36 (1H, dt, *J*=5.1, 5.9 Hz), 5.05 (1H, d, *J*=5.9 Hz), 2.80 (1H, dd, *J*=5.1, 16.6 Hz), 2.70 (1H, dd, *J*=5.9, 16.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) 167.5, 158.1, 157.6, 156.5, 146.4 (×2), 146.3, 146.2, 131.5, 121.4, 119.2, 116.2, 114.4, 110.1, 99.6, 96.4, 95.6, 79.3, 71.1, 24.3; IR (neat, cm^{-1}) 3350 (br s), 2979 (m), 1695 (m), 1612 (s), 1520 (m), 1453 (s), 1390 (m), 1318 (m), 1240 (s), 1198 (m), 1142 (s), 1109 (m), 1065 (m), 1036 (s), 984 (w), 876 (w), 822 (w), 766 (w); FAB-MS (*m/z*) 466 (14), 465 ([M+Na]$^+$, 30), 464 (12), 444 (13), 443 ([M+H]$^+$, 31), 442 (6.1), 441 (6.4), 331

(5.8), 330 (20), 275 (20), 274 (69), 273 (100), 272 (31); FAB-HRMS calcd for $C_{22}H_{19}O_{10}$ $[M+H]^+$, 443.0978; found:443.0972.

4.1.4. [4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-Octa-*O*-benzyl-bi-(+)-catechin-3-*O*-(tri-*O*-benzyl)gallate (12). To a solution of **7** (117 mg, 0.18 mmol) and **10** (52 mg, 0.045 mmol) in CH_2Cl_2 (30 ml) was added dropwise TMSOTf (0.09 ml, 0.045 mmol, 0.5 M solution in CH_2Cl_2) at −20 °C. After stirring for 5 min, the pale yellow reaction mixture was quenched with sat. sodium hydrogen carbonate. The aq. solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and preparative silica gel TLC purification (hexane/EtOAc/$CHCl_3$, 6/1/5) afforded 55 mg (0.032 mmol, 71%) of **12** as a colorless oil; $[\alpha]_D^{25}=-155.6$ (*c* 0.44, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 0.6: 0.4 mixture of rotational isomers) major isomer: 7.45–6.65 (37.2H, m), 6.46 (0.6H, dd, *J*=1.7, 8.3 Hz), 6.24 (0.6H, d, *J*=2.2 Hz), 6.12 (0.6H, d, *J*=2.2 Hz), 8.05 (0.6H, t, *J*=9.5 Hz), 5.87 (0.6H, s), 5.12–4.66 (13.8H, m), 4.44 (0.6H, d, *J*=11.7 Hz), 4.39 (0.6H, d, *J*=11.7 Hz), 3.95–3.85 (0.6H, m), 2.90 (0.6H, dd, *J*=5.4, 16.6 Hz), 2.73 (0.6H, dd, *J*=7.4, 16.6 Hz), 1.60–1.20 (0.6H, m, OH); minor isomer: 7.45–6.65 (24.0H, m), 6.59 (0.4H, d, *J*=8.3 Hz), 6.56 (0.4H, d, *J*=1.7 Hz), 6.35 (0.4H, dd, *J*=1.7, 8.3 Hz), 6.26 (0.4H, s), 6.19 (0.4H, d, *J*=2.2 Hz), 6.14 (0.4H, d, *J*=2.2 Hz), 5.95 (0.4H, t, *J*=9.8 Hz), 5.12–4.66 (9.2H, m), 4.57 (0.4H, d, *J*=11.4 Hz), 3.35–3.28 (0.8H, m), 2.89–2.84 (0.4H, m), 2.35–2.25 (0.4H, m), 1.60–1.20 (0.4H, m, OH); ^{13}C NMR (100 MHz, $CDCl_3$, 0.6: 0.4 mixture of rotational isomers) major isomer: 164.4, 158.2, 157.8, 156.8, 156.7, 155.9, 152.6, 152.3, 152.2, 148.9, 148.82, 148.80 (C×2), 148.6, 142.2, 142.1, 137.7–136.5 (C×16), 131.5, 130.9, 128.6–126.7 (C×22), 125.7, 125.2, 120.6, 120.2, 114.9, 114.5, 113.8, 113.7, 109.2, 108.8, 108.8, 102.4, 94.9, 94.5, 91.2, 80.8, 79.7, 75.1, 75.0, 71.4–69.7 (C×7), 68.0, 35.4, 26.8; minor isomer: 164.2, 158.1, 157.7, 156.8, 155.7, 155.6, 153.9, 152.3, 152.2, 149.1, 149.0, 148.9, 148.6, 147.9, 142.4, 141.9, 137.7–136.5 (C×16), 131.1, 131.0, 128.6–126.7 (C×22), 126.0, 124.7, 120.9, 119.4, 115.7, 114.8, 114.4, 113.7, 111.3, 110.4, 108.6, 102.2, 94.9, 94.4, 91.2, 80.4, 79.9, 71.4–69.7 (C×9), 68.1, 35.1, 28.0; IR (neat, cm^{-1}) 3519 (br), 3090 (m), 3065 (m), 3033 (m), 2928 (m), 2870 (m), 1954 (w), 1877 (w), 1813 (w), 1721 (m), 1605 (s), 1514 (s), 1499 (s), 1455 (s), 1428 (s), 1381 (s), 1331 (s), 1264 (s), 1113 (s), 1065 (s), 911 (w), 853 (w), 812 (w), 737 (s), 696 (s); FAB-MS (*m*/*z*) 1281 (100), 1746 (11), 1745 (14), 1744 ($[M+Na]^+$, 7), 1724 (7), 1723 (10), 1722 ($[M+H]^+$, 9); FAB-HRMS calcd for $C_{114}H_{97}O_{26}$ $[M+H]^+$, 1721.6777; found:1721.6879.

4.1.5. [4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-Octa-*O*-benzyl-bi-(+)-catechin-3,3″-di-*O*-(tri-*O*-benzyl)gallate (13). To a solution of **9** (418 mg, 0.39 mmol) and **10** (110 mg, 0.067 mmol) in CH_2Cl_2 (50 ml) was added dropwise TMSOTf (0.19 ml, 0.095 mmol, 0.5 M solution in CH_2Cl_2) at -20 °C. After stirring for 5 min, the pale yellow reaction mixture was quenched with sat. sodium hydrogen carbonate. The aq. solution was extracted with $CHCl_3$ and the organic phase was washed with water and brine, and dried (Na_2SO_4). Filtration, concentration and preparative silica gel column chromatography (benzene) afforded 144 mg (0.067 mmol, 71%) of **13** as a white powder; $[\alpha]_D^{22}=-43.9$ (*c* 0.42, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 0.66: 0.34 mixture of rotational isomers) major isomer: 7.40–6.62 (50.82H, m), 6.76 (0.66H, d, *J*=1.7 Hz), 6.54 (0.66H, d, *J*=8.3 Hz), 6.44 (0.66H, dd, *J*=1.7, 8.3 Hz), 6.23 (0.66H, d, *J*=2.2 Hz), 6.06 (0.66H, d, *J*=2.2 Hz), 5.96 (0.66H, s), 6.12–5.97 (0.66H, m), 5.38 (0.66H, ddd, *J*=5.6, 7.1, 7.3 Hz), 5.15 (0.66H, d, *J*=7.3 Hz), 5.13–4.53 (18.48H, m), 4.44 (0.66H, d, *J*=11.5 Hz), 4.38 (0.66H, d, *J*=11.5 Hz), 3.13 (0.66H, dd, *J*=5.6, 16.8 Hz), 2.90 (0.66H, dd, *J*=7.1, 16.8 Hz); minor isomer: 7.40–6.53 (26.86H, m), 6.50 (0.34H, dd, *J*=1.7, 8.3 Hz), 6.29 (0.34H, s), 6.18 (0.34H, d, *J*=2.2 Hz), 6.14 (0.34H, d, *J*=2.2 Hz), 6.12–5.97 (0.34H, m), 5.13–4.53 (10.54H, m), 3.63 (0.34H, d, *J*=9.3 Hz), 3.15–3.11 (0.34H, m), 2.48 (0.34H, dd, *J*=10.0, 17.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer: 165.3, 164.3, 158.3, 157.5, 157.1, 156.6, 155.5, 152.5 (×2), 152.1 (×2), 148.93, 148.88, 148.6, 142.6, 142.1, 137.5–136.5 (C×19), 131.3, 131.1, 128.6–126.7 (C×27), 125.2, 125.1, 120.4, 120.3, 114.83, 114.75, 114.2, 113.8, 113.7, 111.2, 109.1, 108.7, 108.2, 101.9, 94.8, 94.6, 90.9, 79.7, 78.2, 77.2, 75.13, 75.01, 71.6–69.8 (C×12), 35.2, 24.9; minor isomer: 165.1, 164.6, 158.1, 157.6, 156.9, 155.9, 153.8, 152.6, 152.5, 152.42, 152.36, 149.1, 148.8, 147.8, 142.5, 142.4, 137.6–136.5 (C×19), 131.1, 131.0, 128.6–126.7 (C×27), 125.5, 125.0, 120.9, 119.7, 115.9, 114.8, 114.1, 113.8, 113.7, 111.5, 109.2, 109.1, 108.7, 101.7, 94.9, 94.3, 91.3, 80.0, 78.3, 77.6, 75.1, 75.0, 71.6–69.8 (C×12), 35.1, 26.6; IR (neat, cm^{-1}) 3090 (m), 3032 (m), 2930 (m), 2870 (m), 1954 (w), 1811 (w), 1717 (s), 1592 (s), 1514 (s), 1454 (s), 1430 (s), 1375 (s), 1215 (s), 1113 (s), 1028 (s), 910 (w), 856 (w), 810 (w), 754 (s); FAB-MS (*m*/*z*) 2145 (0.1), 2144 ($[M+H]^+$, 0.1).

4.1.6. Procyanidin B3-3-*O*-gallate (2). A solution of **12** (200 mg, 0.12 mmol) in 22 mL of THF/MeOH/H_2O, 20/1/1 was hydrogenated over 20% $Pd(OH)_2$/C (5 mg) for 8 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (EtOH) and HPLC purification to give 70 mg (0.096 mmol, 78%) of procyanidin B3-3-*O*-gallate **2** as a colorless amorphous solid; $[\alpha]_D^{25}=-180.7$ (*c* 0.28, Me_2CO) {lit.[18a] $[\alpha]_D^{25}=-170.1$ (*c* 0.72, Me_2CO)}; ^{1}H NMR (400 MHz, 10% D_2O in CD_3COCD_3, 0.6: 0.4 mixture of rotational isomers) major isomer: 6.86 (1.2H, s), 6.79 (0.6H, d, *J*=1.7 Hz), 6.72 (0.6H, d, *J*=1.7 Hz), 6.69 (0.6H, d, *J*=8.3 Hz), 6.58 (0.6H, d, *J*=8.3 Hz), 6.49 (0.6H, dd, *J*=1.7, 8.3 Hz), 6.33 (0.6H, dd, *J*=1.7, 8.3 Hz), 6.19 (0.6H, dd, *J*=8.6, 10.0 Hz, C3), 6.02 (0.6H, s, D6), 5.95 (0.6H, d, *J*=2.2 Hz, C8), 5.86 (0.6H, d, *J*=2.2 Hz, C6), 4.75 (0.6H, d, *J*=7.7 Hz, F2), 4.72 (0.6H, d, *J*=8.6 Hz, C2), 4.62 (0.6H, d, *J*=10.0 Hz, C4), 3.84 (0.6H, ddd, *J*=5.6, 7.7, 8.8 Hz, F3), 2.71 (0.6H, dd, *J*=5.6, 16.3 Hz, F4), 2.52 (0.6H, dd, *J*=8.8, 16.3 Hz, F4); minor isomer: 7.00 (0.4H, d, *J*=1.7 Hz), 6.98 (0.8H, s), 6.90–6.62 (1.6H, m), 6.65 (0.4H, d, *J*=8.3 Hz), 6.16 (0.4H, dd, *J*=8.8, 10.3 Hz), 6.13 (0.4H, s), 5.87 (0.4H, d, *J*=2.2 Hz), 5.86–5.84 (0.4H, m), 4.75–4.73 (0.4H, m), 4.66 (0.4H, d, *J*=10.3 Hz), 4.43 (0.4H, d, *J*=8.8 Hz), 3.64–3.59 (0.4H, m), 2.85 (0.4H, dd, *J*=5.9, 16.1 Hz), 2.53–2.48 (0.4H, m); ^{13}C NMR (100 MHz, 10% D_2O in CD_3COCD_3, 0.6: 0.4 mixture of rotational isomers) major isomer: 165.5, 158.0, 157.6, 156.9, 155.8, 154.8, 154.7, 145.7–145.6 (C×4), 144.91, 144.85, 131.5, 130.6,

121.6, 120.4, 119.2, 115.8, 115.6, 115.4, 114.9, 109.7, 105.4, 105.3, 101.6, 97.2, 96.5, 95.7, 81.9 (C2), 81.2 (F2), 73.1 (C3), 68.1 (F3), 35.9 (C4), 29.4 (F4); minor isomer: 165.1, 156.0, 157.3, 157.0, 155.7, 155.4, 154.6, 145.7–145.6 (C×4), 145.3, 145.1, 131.5, 130.6, 121.9, 120.5, 120.0, 116.2, 116.0, 115.6, 115.2, 110.0, 105.9, 105.7, 100.3, 97.3, 97.1, 96.0, 83.3 (C2), 81.4 (F2), 73.6 (F3), 68.5 (C3), 35.7 (C4), 28.4 (F4); IR (neat, cm^{-1}) 3360 (br s), 2979 (m), 2928 (m), 1693 (m), 1613 (s), 1522 (m), 1453 (s), 1370 (s), 1285 (m), 1238 (s), 1146 (m), 1103 (m), 1038 (s), 876 (w), 818 (w), 779 (w); FAB-MS (*m/z*) 755 (21), 754 (37), 753 ($[M+Na]^+$, 47), 752 (19), 733 (26), 732 (27), 731 ($[M+H]^+$, 30), 730 (24), 614 (34), 613 (48), 482 (100); FAB-HRMS calcd for $C_{37}H_{31}O_{16}$ $[M+H]^+$, 731.1612; found:731.1600.

4.1.7. Procyanidin B3-3,3″-di-*O*-gallate (3). A solution of **13** (90 mg, 0.042 mmol) in 22 mL of THF/MeOH/H_2O, 20/1/1 was hydrogenated over 20% $Pd(OH)_2$/C (5 mg) for 5 h at rt. Filtration and concentration afforded a pale brown solid, which was purified by Sephadex® LH-20 column chromatography (MeOH) and HPLC purification to give 24 mg (0.027 mmol, 65%) of procyanidin B3-3,3″-di-*O*-gallate **3** as a colorless amorphous solid; $[\alpha]_D^{23}=-209.7$ (*c* 1.00, Me_2CO); ^1H NMR (400 MHz, 10% D_2O in CD_3COCD_3, 0.75: 0.25 mixture of rotational isomers) major isomer: 7.12 (1.5H, s, 2′), 6.95–6.93 (0.75H, m, B6), 6.90 (1.5H, s, 2′), 6.82 (0.75H, d, *J*=1.7 Hz, B2), 6.65 (0.75H, d, *J*=1.7 Hz, E2), 6.57 (0.75H, dd, *J*=1.7, 8.3 Hz, E6), 6.50 (0.75H, d, *J*=8.3 Hz, B5), 6.47 (0.75H, d, *J*=8.3 Hz, E5), 6.19 (0.75H, dd, *J*=7.1, 10.0 Hz, C3), 5.99 (0.75H, s, D6), 5.95 (0.75H, d, *J*=2.2 Hz, A8), 5.75 (0.75H, d, *J*=2.2 Hz, A6), 5.32 (0.75H, ddd, *J*=4.6, 5.1, 5.6 Hz, F3), 5.21 (0.75H, d, *J*=4.6 Hz, F2), 4.77 (0.75H, d, *J*=7.1 Hz, C2), 4.51 (0.75H, d, *J*=10.0 Hz, C4), 2.61 (0.75H, dd, *J*=5.6, 17.1 Hz, F4), 2.53 (0.75H, dd, *J*=5.1, 17.1 Hz, F4); minor isomer: 7.12–6.51 (2.5H, m), 6.19–5.81 (1.0H, m), 5.00–4.85 (0.5H, m), 4.74 (0.25H, d, *J*=8.3 Hz, C2), 4.59 (0.25H, d, *J*=10.0 Hz, C4), 2.95–2.90 (0.25H, m, F4), 2.63–2.48 (0.25H, m); ^{13}C NMR (100 MHz, 10% D_2O in CD_3COCD_3, 0.75: 0.25 mixture of rotational isomers) major isomer: 167.0, 166.2, 158.5, 157.4, 156.9, 155.4, 154.5, 153.7, 145.9, 145.8, 145.6, 145.1 (×2), 145.0, 138.9, 138.7, 130.44, 130.36, 121.2, 121.1, 120.1, 118.5, 116.1, 115.7, 115.4, 113.5, 109.9, 109.8, 106.7, 105.8, 100.1, 97.4, 96.5, 95.7, 81.5 (C2), 78.4 (F2), 75.6 (C3), 69.7 (F3), 35.3 (C4), 23.8 (F4); minor isomer: 166.1, 165.2, 158.1, 157.0, 155.8, 155.3, 154.2, 153.7, 145.9, 145.8, 145.6, 145.1 (×2), 145.0, 138.9, 138.7, 130.7, 130.6, 121.8, 121.2, 120.5, 119.2, 116.3, 115.6, 115.2, 115.1, 110.0 (×2), 106.7, 105.8, 99.0, 97.4, 96.5, 96.0, 81.5 (C2), 79.9 4 (F2), 73.7 (C3), 71.3 (F3), 35.7 (C4), 26.5 (F4); IR (neat, cm^{-1}) 3350 (br s), 2977 (s), 2910 (s), 1684 (s), 1622 (s), 1520 (m), 1456 (s), 1383 (s), 1244 (s), 1146 (m), 1090 (s), 986 (w), 878 (m), 818 (w), 768 (w); FAB-MS (*m/z*) 517 (100), 561 (83), 882 (19), 883 ($[M+H]^+$, 37), 884 (20), 903 (33), 904 (64), 905 ($[M+Na]^+$, 86), 906 (38); FAB-HRMS calcd for $C_{44}H_{34}O_{20}Na$ $[M+Na]^+$, 905.1541; found: 905.1578.

4.2. The measurement of antioxidant activity and DPPH radical scavenging activity

All of the assay samples were HPLC pure. Antioxidant activity was measured with the general TBA method.[1b] And DPPH radical scavenging activity was measured as follows:[20] A solution of DPPH radical in EtOH (30 μM, 1.0 ml) was added to 1 μl of the test sample in DMSO, and incubated at 30 °C for 30 min. The scavenging activity was estimated by measuring the absorption of the reaction mixture at 517 nm with the microplate reader (Model 3550, BIO-RAD).

4.3. DNA polymerase assays

All of the assay samples were HPLC pure. DNA polymerase α was purified from calf thymus by immuno-affinity column chromatography as described previously.[23] Recombinant rat DNA polymerase β was purified from *E. coli* JMpβ5 as described by Date et al.[24] The reaction mixtures for DNA polymerase α and β were described previously.[25] The substrates of DNA polymerases used poly(d*A*)/oligo(d*T*)$_{12-18}$ and deoxythymidine triphosphates (dTTP) as template-primer DNA and nucleotide substrate, respectively. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Four μl of the sonicated samples were mixed with 16 μl of each enzyme (final 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM EDTA, and kept at 0 °C for 10 min. These inhibitor–enzyme mixtures (8 μl) were added to 16 μl of each of the enzyme standard reaction mixtures, and incubation was carried out at 37 °C for 60 min. The activity without the inhibitor was considered to be 100%, and the remaining activities at each concentration of inhibitor were determined as percentages of this value. One unit of each DNA polymerase activity was defined as the amount of enzyme that catalyzes the incorporation of 1 nmol of deoxyribonucleotide triphosphates (i.e. dTTP) into synthetic template-primers (i.e. poly(d*A*)/oligo(d*T*)$_{12-18}$, *A*/*T*=2/1) in 60 min at 37 °C under the normal reaction conditions for each enzyme.[25]

Acknowledgements

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Heterogeneous organocatalysis for the asymmetric desymmetrization of *meso*-cyclic anhydrides using silica gel-supported bis-cinchona alkaloids

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Abstract—The silica gel-supported bis-cinchona alkaloid **1a** was prepared and found to be an efficient heterogeneous chiral organocatalyst with high catalytic activities, enantioselectivities (up to 92% ee), and recyclability for the asymmetric desymmetrization of *meso*-cyclic anhydrides with alcoholysis.

1. Introduction

Enantioselective desymmetrization of *meso* compounds using enzymatic[1] and non-enzymatic[2] methods has proven to be a powerful synthetic means of preparing enantiomerically enriched products where multiple stereocenters can be introduced in one step, enabling the conversion of cheap starting materials into more expensive ones. The non-enzymatic method reported by Oda[3] and Aitken[4] employed a catalytic amount of inexpensive and readily available cinchona alkaloids for the asymmetric methanolysis of *meso*-cyclic anhydrides to afford chiral hemiesters in good to excellent yields and moderate enantiomeric excesses. Based on the findings of Oda and Aitken groups, Bolm and co-workers developed a more enantioselective methanolysis of *meso*-cyclic anhydrides by using a stoichiometric quantity of cinchona alkaloids.[5]

Recently, Deng and co-workers found that commercially available modified cinchona alkaloids are able to function as effective chiral Lewis-base/nucleophilic organic catalysts.[6] These organocatalysts allow desymmetrization and (dynamic) kinetic resolution of cyclic anhydrides, cyanation of ketones, and conjugate addition of thiols to cyclic enones with high enantioselectivity. Among them, a highly enantioselective organocatalytic desymmetrization of prochiral *meso*-cyclic anhydrides with methanolysis is achieved by using the commercially available bis-cinchona alkaloids such as 1,4-bis(dihydroquinidinyl)anthraquinone $(DHQD)_2AQN$ **1** (Scheme 1).[6c] For the first time, this method overcomes the frequently encountered problem of the high loading of cinchona alkaloids to obtain high enantioselectivity in such reactions.

Very recently, we reported the immobilization of the bis-cinchona alkaloid, 1,4-bis(dihydroquininyl)anthraquinone $(DHQ)_2AQN$ **2**, onto silica gel and its use for the asymmetric desymmetrization of *meso*-cyclic anhydrides (Fig. 1).[7] The resulting heterogeneous chiral organocatalyst **2a** gave moderate enantioselectivities (up to 84% ee) in those reactions. Reuse of this heterogeneous organocatalyst invariably gave a small reduction in ee values and conversions and thereby showed some stability under the reaction conditions. Although the results obtained using organocatalyst **2a** were somewhat satisfactory, a similar study using its counterpart **1a** would offer an opportunity to compare their enantioselectivity for the desymmetrization of *meso*-cyclic anhydrides and thus to observe a true diastereomeric effect stemming from the pseudo-enantiomeric alkaloid **1a**.

Here, we report the preparation of the silica gel-supported organocatalyst, SGS-$(DHQD)_2AQN$ **1a**, and its use for the asymmetric desymmetrization of *meso*-cyclic anhydrides. For comparison studies, the more flexible organocatalysts **1b** and **2b** were also prepared, where only one of dihydroquinidine (DHQD) or dihydroquinine (DHQ) moieties in **1** and **2** was tethered to silica gel by use of their derivatives **8** and **9** containing quinidine (QD) and quinine (QN),

Keywords: Asymmetric organocatalysis; Heterogeneous chiral organocatalyst; Desymmetrization; Cinchona alkaloid; Cyclic anhydride.
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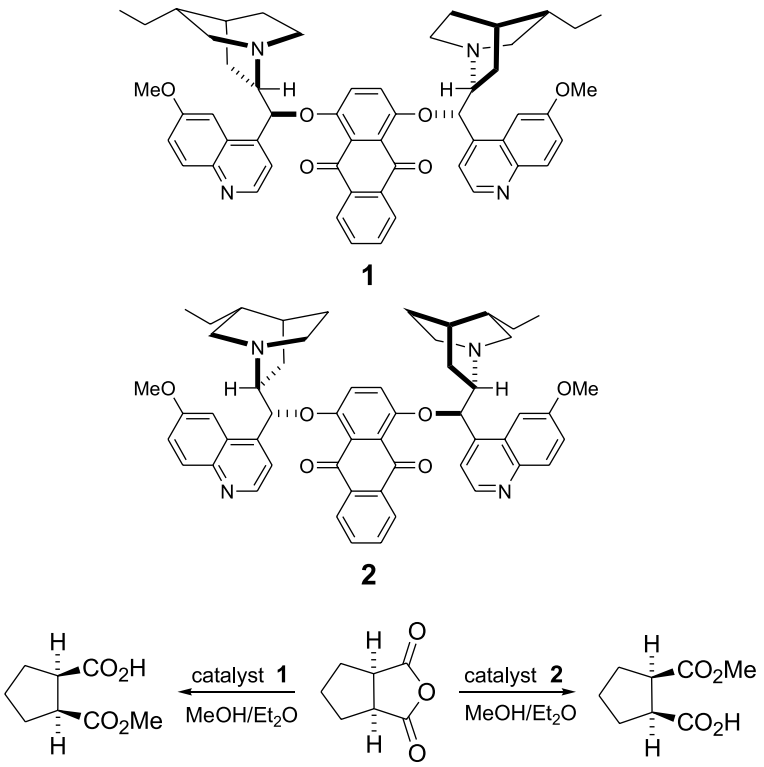


Scheme 1. Structures of the bis-cinchona alkaloids, $(DHQD)_2AQN$ **1** and $(DHQ)_2AQN$ **2**, and their use for the asymmetric desymmetrization reaction of *meso*-cyclic anhydrides with methanol.

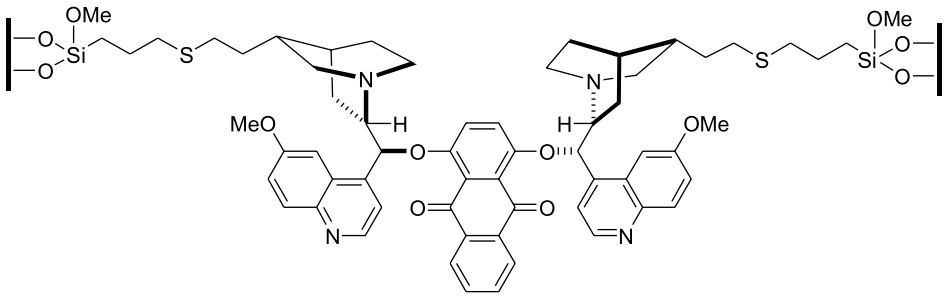
respectively. We found that **1a** showed higher enantioselectivities compared to **2a** and their flexible derivatives **1b** and **2b**. In addition, organocatalyst **1a** could be recycled five times without any significant loss of catalytic activity and enantioselectivity.

2. Results and discussion

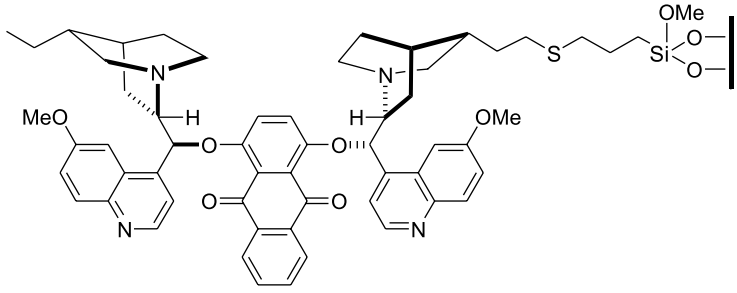
To synthesize the silica gel-supported organocatalyst **1a**, we started with 1,4-bis(quinidinyl)anthraquinone $(QD)_2AQN$ **4**, a homogeneous analogue of **1**. Alkaloid **4** was prepared by nucleophilic substitution of 1,4-difluoroanthraquinone (**3**) with the lithium salt of quinidine in THF at room temperature (Scheme 2).[8] The desired silica gel-supported bis-cinchona alkaloid **1a** was prepared by reacting chiral monomer **4** with mercaptopropylsilanized silica gel in the presence of α,α'-azoisobutyronitrile (AIBN) as a radical initiator in $CHCl_3$.[9] The nitrogen analysis of **1a** confirmed 6.09 wt% incorporation of monomeric alkaloid **4** onto silica gel (0.0711 mmol/g). Organocatalysts **1b**, **2a**, and **2b** were prepared in a similar manner.

In a first series of experiments, we examined the desymmetrization of *meso*-cyclic anhydride **10a** in various solvents using **1a** as an organocatalyst and methanol as a nucleophile under heterogeneous conditions (Scheme 3). Our results are summarized in Table 1. To optimize the reaction conditions, the influence of catalyst amount, the nucleophile to solvent ratio (MeOH/solvent), and temperature on the efficiency of the process was investigated, with particular regard to enantioselectivity. The reaction was performed at -30 °C for 72 h because the higher temperature resulted in a decrease in optical yield and the lower temperature slowed down the reaction rate. In reactions with organocatalyst **1a** (5 mol%), the best enantioselectivity (88% ee) was attained by using the 0.05:1 mixture of methanol and toluene/CCl_4 (1:1) at -30 °C (Table 1, entry 3). In particular, SGS-$(DHQD)_2AQN$ **1a** was superior to SGS-$(DHQ)_2AQN$ **2a** for the asymmetric desymmetrization of **10a** (Table 1, entries 1–3 vs 7–9). These results are consistent with those obtained by Oda,[3b] Aitken,[4b] Bolm,[5c] Bigi[10] who pointed out that diastereomeric quinidine, a pseudo-enantiomer of quinine, afforded the ring opening product with slightly higher enantioselectivity. A comparison between rigid and flexible organocatalysts (**1a**, **2a** vs **1b**, **2b**) shows that the rigidity of the active site seems to be crucial to the enantioselectivity and stability of the catalytic system (Table 1, entries 1, 7 vs 10, 11).

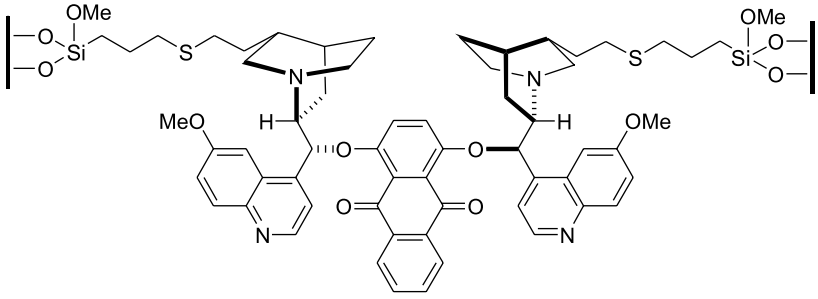
The highest enantioselectivity (92% ee) was obtained by a one-pot conversion of *meso*-cyclic anhydride **10b** with organocatalyst **1a** (20 mol%) into the corresponding desymmetrized mono ester acid **11b** in the 0.1:1 mixture of methanol and diethyl ether at -10 °C (Table 2, entry 2). In contrast, *meso*-cyclic anhydrides **10c–e** afforded very low conversions despite excellent ee values: **10c** gave **11c** in 9% conversion and 83% ee; **10d** gave **11d** in 8% conversion and 77% ee. The low reactivity of *meso*-cyclic anhydrides **10c–e** could probably be due to their steric effects in the heterogeneous asymmetric desymmetrization reactions. We also investigated the effect of various nucleophiles on the asymmetric desymmetrization reactions by replacing methanol with ethanol or 2-propanol. As a result, ethanol



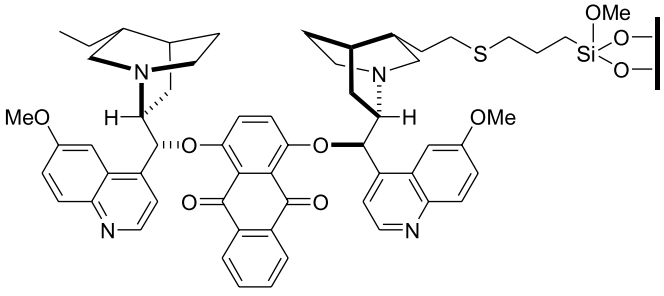
1a



1b



2a



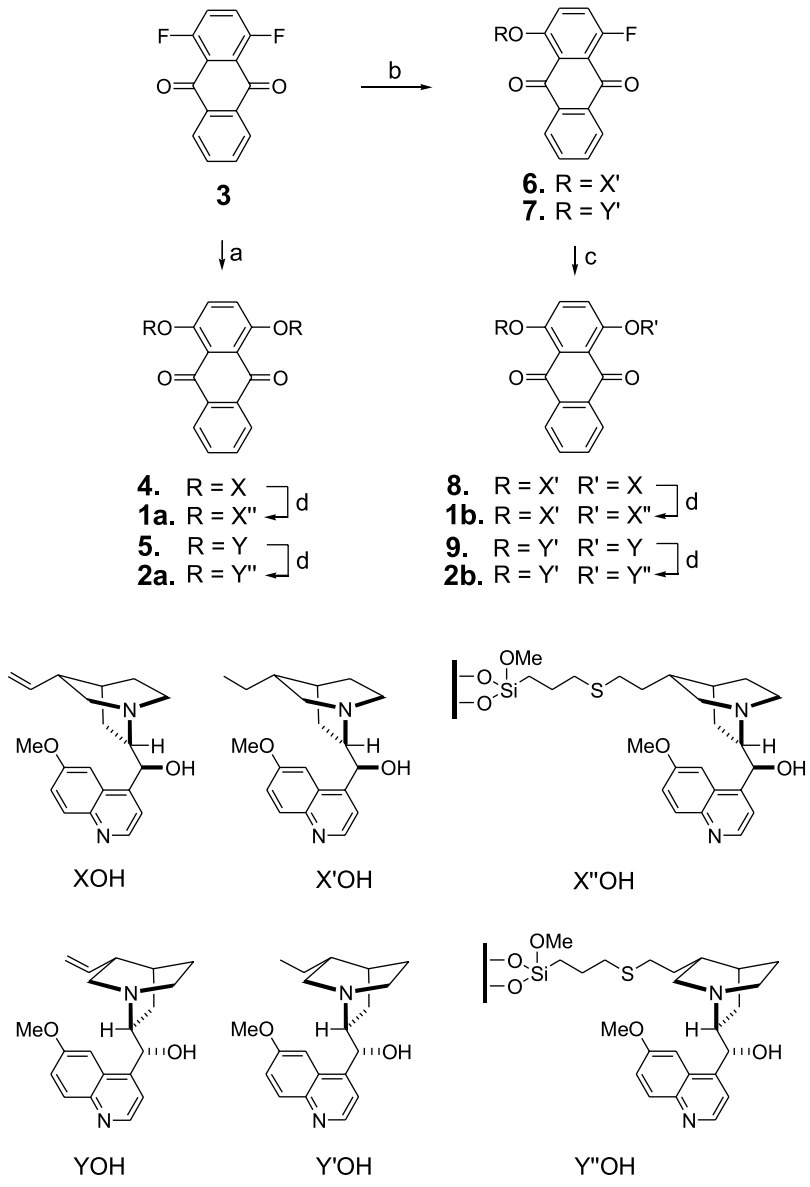
2b

Figure 1. Structures of the silica gel-supported bis-cinchona alkaloids, SGS-$(DHQD)_2$AQN **1a** and **1b** and SGS-$(DHQ)_2$AQN **2a** and **2b**.

and 2-propanol as nucleophiles exhibited lower reactivity and enantioselectivity compared to methanol (Table 2, entries 1 vs 6, 7).

Finally, the recyclability of silica gel-supported organocatalysts was also examined by carrying out the reaction with **1a** (20 mol%) in the 0.1:1 mixture of methanol and diethyl ether at -10 °C for 72 h (Table 3). To our delight, excellent enantioselectivity was retained throughout the successive recycling of organocatalysts. Organocatalyst **1a** could be separated from the reaction mixture by simple filtration and reused for five consecutive reactions without any significant decrease in enantioselectivity (92–89% ee) and catalytic activity (73–70% conversion). A gradual decrease in the enantioselectivity and catalytic activity of the silica gel-supported organocatalyst **1a** with repetitive use was likely attributed to its slight solubility in methanol and thereby somewhat leaching from the reaction mixture (ca. 1% for each cycle).

In summary, we succeeded in a heterogeneous organocatalytic asymmetric methanolysis of various *meso*-cyclic anhydrides in diethyl ether using the silica gel-supported chiral organocatalyst **1a** to afford the corresponding chiral



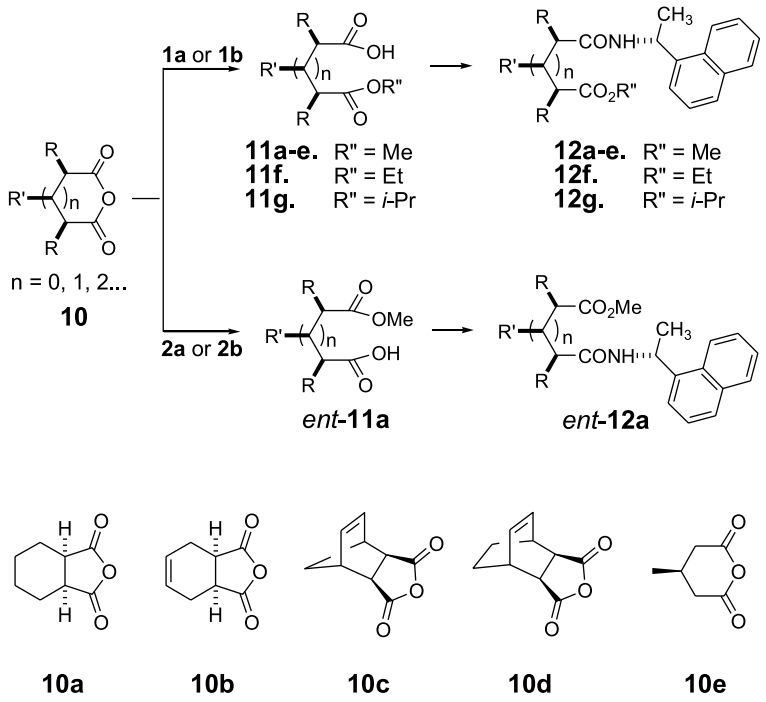
Scheme 2. Synthesis of SGS-$(DHQD)_2$AQN **1a** and **1b** and SGS-$(DHQ)_2$AQN **2a** and **2b**: (a) quinidine (XOH) or quinine (YOH), *n*-BuLi, THF, −50 °C to rt, (**4**, 71%; **5**, 76%), or XOH, NaH, DMF, rt, (**4**, 72%); (b) hydroquinidine (X′OH) or hydroquinine (Y′OH), NaH, DMF, rt, (**6**, 45%; **7**, 68%); (c) XOH or YOH, NaH, DMF, rt, (**8**, 77%; **9**, 98%); (d) mercaptopropylsilanized silica gel (SGS-SH), AIBN, $CHCl_3$, 80 °C, (**1a**, 0.0711 mmol/g; **2a**, 0.0733 mmol/g; **1b**, 0.0604 mmol/g; **2b**, 0.0630 mmol/g).

hemiesters with excellent enantioselectivities in moderate conversions. In such reactions, the rigidity of the organocatalyst appears to be an important parameter. Furthermore, the immobilized chiral organocatalyst **1a** could be reused several times without any significant decrease in catalytic activity and enantioselectivity. Our process therefore retains the ease of catalyst removal/recycling as well as the efficient reaction protocol.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 or a Varian Mercury 300 NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with reference to tetramethylsilane or solvent and coupling constants (J) are reported in hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-AM505WA mass spectrometer using a fast atom bombardment (FAB) technique. Optical rotations (α) were determined on a Rudolph AUTOPOL III automatic polarimeter. Elemental analysis was performed on a CE EA1110 elemental analyzer. GC analysis was performed on a Younglin Acme 6000 GC system. HPLC analysis was performed on a Waters 600 HPLC system equipped with a 2487 dual λ absorbance detector. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates (0.25 mm thickness, Merck). Flash chromatography was carried out on silica gel 60 (230–400 mesh, Merck). Bis-cinchona alkaloids **4**, **5**, **8**, and **9** were prepared by



Scheme 3. Desymmetrization of *meso*-cyclic anhydrides **10a–e** with alcoholysis in diethyl ether using **1a**, **1b**, **2a**, or **2b** as a organocatalyst followed by conversion of the resulting hemiesters **11a–g** and *ent*-**11a** into the corresponding amide–esters **12a–g** and *ent*-**12a**, respectively.

Table 1. Effects of various heterogeneous organocatalysts and solvents on conversions and ee values in the desymmetrization reaction of *cis*-1,2-cyclohexanedicarboxylic anhydride **10a** with methanol[a]

Entry	Anhydride	Product	Catalyst[b] (mol%)	Solvent	Conversion[c] (%)	ee[d] (%)	Configuration[e]
1	**10a**	**11a**	**1a** (5)	Diethyl ether	76	80	1*R*,2*S*
2	**10a**	**11a**	**1a** (5)	THF	44	88	1*R*,2*S*
3	**10a**	**11a**	**1a** (5)	Toluene/CCl_4 (1:1)	78	88	1*R*,2*S*
4	**10a**	**11a**	**1a** (5)	Toluene	9	67	1*R*,2*S*
5	**10a**	**11a**	**1a** (5)	EtOAc	5	70	1*R*,2*S*
6	**10a**	**11a**	**1a** (5)	*t*-Butyl methyl ether	47	86	1*R*,2*S*
7	**10a**	*ent*-**11a**	**2a** (5)	Diethyl ether	65	64	1*S*,2*R*
8	**10a**	*ent*-**11a**	**2a** (5)	THF	16	63	1*S*,2*R*
9	**10a**	*ent*-**11a**	**2a** (5)	Toluene/CCl_4 (1:1)	67	46	1*S*,2*R*
10	**10a**	**11a**	**1b** (5)	Diethyl ether	58	67	1*R*,2*S*
11	**10a**	*ent*-**11a**	**2b** (5)	Diethyl ether	45	43	1*S*,2*R*

[a] MeOH (nucleophile)/solvent (ca. 0.05:1 (v/v)), reaction temperature (−30 °C), reaction time (72 h).
[b] SGS-$(DHQD)_2$AQN **1a** and **1b**, SGS-$(DHQ)_2$AQN **2a** and **2b**.
[c] Determined by GC analysis of an enantiomeric mixture for **11a** or *ent*-**11a** on a Chiraldex G-TA column (30 m×0.25 mm).
[d] Determined by HPLC analysis of a diastereomeric mixture for **12a** or *ent*-**12a** on a Hypersil silica column (4.6×200 mm, 5 μm).[6c,7]
[e] The absolute configuration of **11a** and *ent*-**11a** was determined as described.[5,7]

Table 2. Desymmetrization of *meso*-cyclic anhydrides **10a-e** with alcoholysis in diethyl ether using heterogeneous organocatalyst **1a**[a]

Entry	Anhydride	Product	Catalyst[b] (mol%)	Time (h)	Conversion[c,d] (%)	ee[e] (%)	Configuration[f]
1	**10a**	**11a**	**1a** (20)	48	82	89	1*R*,2*S*
2	**10b**	**11b**	**1a** (20)	72	73	92	1*R*,2*S*
3	**10c**	**11c**	**1a** (20)	72	9	83	2*R*,3*S*
4	**10d**	**11d**	**1a** (20)	72	8	77	2*R*,3*S*
5	**10e**	**11e**	**1a** (20)	72	22	43	3*S*
6	**10a**	**11f**[g]	**1a** (20)	72	7	82	1*R*,2*S*
7	**10a**	**11g**[h]	**1a** (20)	72	6	53	1*R*,2*S*

[a] MeOH (nucleophile)/Et_2O (solvent) (ca. 0.1:1 (v/v)), reaction temperature (−10 °C).
[b] SGS-$(DHQD)_2$AQN **1a**.
[c] Determined by GC analysis of an enantiomeric mixture for **11a**, **11f**, or **11g** on a Chiraldex G-TA column (30 m×0.25 mm).
[d] Determined by GC analysis of an enantiomeric mixture for each of **11b–e** on a HP-1 column (30 m×0.32 mm×0.25 μm).
[e] Determined by HPLC analysis of a diastereomeric mixture for each of **12a–g** on a Hypersil silica column (4.6×200 mm, 5 μm).[6c,7]
[f] The absolute configuration of **11a–g** was determined as described.[5,7]
[g] EtOH instead of MeOH as a nucleophile.
[h] *i*-PrOH instead of MeOH as a nucleophile.

Table 3. The recyclability of the heterogeneous bis-cinchona alkaloid-based organocatalyst **1a** in the asymmetric desymmetrization reaction of **10b** with methanol[a]

ee (%) with consecutive use of recycled organocatalyst **1a**					
Recycle	1st	2nd	3rd	4th	5th
ee (%)	92	91	93	86	89
Conversion (%)	73	72	70	71	70

[a] Asymmetric desymmetrization reaction using 20 mol% silica gel-supported chiral organocatalyst **1a** was carried out in the 0.1:1 mixture of methanol and diethyl ether at −10 °C for 72 h.

modified procedures.[8] Reagent-grade chemicals were purchased from Aldrich, Fluka, Junsei, and TCI and used as received unless otherwise specified.

3.1.1. Mercaptopropylsilanized silica gel (SGS-SH). Dried silica gel 60 (230–400 mesh, 14.0 g) was treated with (3-mercaptopropyl)trimethoxysilane (61.3 mL) in anhydrous pyridine/toluene (1:1) (59.0 mL). After stirring at 90 °C for 24 h, the slurry was cooled to room temperature, filtered, washed with MeOH and $CHCl_3$, and dried in vacuo for 24 h to afford derivatized silica gel (15.6 g) containing 3.59 wt% S, corresponding to 1.12 mmol of S per g of derivatized silica gel. Element analysis (wt%): C 6.81, H 1.60, S 3.59.

3.1.2. SGS-(DHQD)$_2$AQN 1a. To a suspension of SGS-SH (2.64 g, 2.95 mmol) in $CHCl_3$ (60 mL) was added **4** (1.20 g, 1.41 mmol) and α,α'-azoisobutyronitrile (AIBN, 120 mg, 0.731 mmol). After stirring at reflux for 48 h under Ar, the slurry was cooled to rt, filtered, exhaustively washed with MeOH and CH_2Cl_2, and dried in vacuo to give **1a** (2.74 g). Element analysis (wt%) of **1a**: C 10.00, H 1.82, N 0.40, S 3.44.

3.1.3. SGS-(DHQD)$_2$AQN 1b. To a suspension of SGS-SH (1.31 g, 1.46 mmol) in $CHCl_3$ (60 mL) was added **8** (1.50 g, 1.75 mmol) and AIBN (125 mg, 0.760 mmol). After stirring at reflux for 48 h under Ar, the slurry was cooled to rt, filtered, exhaustively washed with MeOH and CH_2Cl_2, and dried in vacuo to give **1b** (1.33 g). Element analysis (wt%) of **1b**: C 9.45, H 1.81, N 0.34, S 3.54.

3.1.4. SGS-(DHQ)$_2$AQN 2a. To a suspension of SGS-SH (2.09 g, 2.34 mmol) in $CHCl_3$ (60 mL) was added **5** (1.0 g, 1.17 mmol) and AIBN (100 mg, 0.609 mmol). After stirring at reflux for 48 h under Ar, the slurry was cooled to rt, filtered, exhaustively washed with MeOH and CH_2Cl_2, and dried in vacuo to give **2a** (2.2 g). Element analysis (wt%) of **2a**: C 10.18, H 1.69, N 0.41, S 3.44.

3.1.5. SGS-(DHQ)$_2$AQN 2b. To a suspension of SGS-SH (1.31 g, 1.46 mmol) in $CHCl_3$ (60 mL) was added **9** (1.50 g, 1.75 mmol) and AIBN (125 mg, 0.760 mmol). After stirring at reflux for 48 h under Ar, the slurry was cooled to rt, filtered, exhaustively washed with MeOH and CH_2Cl_2, and dried in vacuo to give **2b** (1.35 g). Element analysis (wt%) of **2b**: C 10.15, H 1.78, N 0.35, S 3.03.

3.2. General procedure for the asymmetric methanolysis of *meso*-cyclic anhydrides 10a–e

Described for the reaction of *cis*-1,2-cyclohexanedicarboxylic anhydride **10a** in the mixture of methanol (ca. 60 equiv) and diethyl ether (5 mL per 0.1 mmol anhydride) at an approximate ratio of ca. 0.05:1 (v/v).

After a suspension containing *cis*-1,2-cyclohexanedicarboxylic anhydride **10a** (12 mg, 0.0778 mmol) and SGS-(DHQD)$_2$AQN **1a** (54.7 mg, 5 mol%) in dry diethyl ether (3.9 mL) at −30 °C was stirred for 10 min under Ar, dry MeOH (195 μL, 4.81 mmol) was added. After stirring at −30 °C for 72 h, the reaction mixture was filtered, and then the filtrate was concentrated in vacuo. The crude residue was purified by flash chromatography (EtOAc/*n*-hexane = 1:2) to afford an enantiomeric mixture for **11a** as a colorless oil. For determining conversion efficiency, GC analysis of an enantiomeric mixture for **11a** was performed prior to work-up. The filtrate for GC analysis was prepared by filtering the reaction mixture followed by washing with EtOAc.

GC analysis of an enantiomeric mixture for **11a**, **11f**, or **11g** obtained by use of **1a** was performed on a Chiraldex G-TA column (Advanced Separation Technology, 30 m × 0.25 mm) under the condition: initial temperature, 130 °C; initial time, 10.0 min; 2.0 °C/min gradient; final temperature, 170 °C, 17 psi. Retention time (min): **11a**, t_R = 32.44, t_R = 32.58 (major); **11f**, t_R = 30.26; **11g**, t_R = 30.27. GC analysis of an enantiomeric mixture for each of **11b–e** was performed on a HP-1 column (Hewlett Packard, 30 m × 0.32 mm × 0.25 μm) under the condition: initial temperature, 50 °C; initial time, 5.0 min; 15.0 °C/min gradient; final temperature, 170 °C, 17 psi. Retention time (min): **11b**, t_R = 9.75; **11c**, t_R = 10.78; **11d**, t_R = 10.83; **11e**, t_R = 10.77.

3.3. General procedure for the ee determination of hemiesters 11a–g (described for *cis*-1,2-cyclohexanedicarboxylic acid monomethyl ester 11a)

The enantiomeric excess of the product was determined by HPLC analysis of a diastereomeric mixture for the corresponding amide–ester **12a** prepared from an enantiomeric mixture for hemiester **11a** according to the literature procedure.[6c,7]

To a filtrate containing an enantiomeric mixture for *cis*-1,2-cyclohexanedicarboxylic acid monomethyl ester **11a** (36.4 mg, 0.195 mmol) in CH_2Cl_2 (9.8 mL) at room temperature was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI, 44.9 mg, 0.234 mmol). After stirring for 10 min, 4-(dimethylamino)pyridine (DMAP, 7.2 mg, 58.6 μmol) and (*R*)-(+)-1-(1-naphthyl)-ethylamine (34.7 μL, 0.215 mmol) were added to the mixture. After stirring at room temperature for 5 h, the reaction mixture was extracted with CH_2Cl_2/H_2O. The combined organic layers were dried over Na_2SO_4 and then concentrated in vacuo. The crude residue was purified by flash chromatography (EtOAc/*n*-hexane = 1:2) to afford a diastereomeric mixture for **12a** as a yellow oil. For determining the ee value, HPLC analysis of a diastereomeric mixture for **12a** was performed prior to column

purification. The diastereomeric mixture was dissolved in EtOAc and diluted with *n*-hexane for HPLC analysis.

HPLC analysis of a diastereomeric mixture for each of **12a**–**g** was performed on a Hypersil silica column (Thermo, 4.6×200 mm, 5 μm) with UV monitoring at 280 nm and a flow rate of 1.0 mL/min under isocratic conditions: **12a**, *n*-hexane/2-propanol=97/3, t_R=8.20 (major), t_R=11.09; **12b**, *n*-hexane/2-propanol=97/3, t_R=10.74 (major), t_R=13.89; **12c**, *n*-hexane/2-propanol=97/3, t_R=16.75 (major), t_R=22.47; **12d**, *n*-hexane/2-propanol=97/3, t_R=14.86 (major), t_R=18.32; **12e**, *n*-hexane/2-propanol=96/4, t_R=24.54, t_R=26.80 (major); **12f**, *n*-hexane/2-propanol=97/3, t_R=6.98 (major), t_R=8.67; **12g**, *n*-hexane/2-propanol=97/3, t_R=8.52 (major), t_R=11.30.

Acknowledgements

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Unprecedented cyclisations of calix[4]arenes under the Mitsunobu protocol. Part 3: Thiacalix[4]crowns versus dimers

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Abstract—Intermolecular couplings versus intramolecular ring closures were observed in the reaction of *p-tert*-butylthiacalix[4]arene and diethylene glycols affording dimers **2** and/or the inherently chiral 1,2-thiacalix[4]crown-3 derivatives **5** under the Mitsunobu protocol. The enantiomeric separation of **5a** was achieved by chiral HPLC. The reaction of thiacalix[4]monocrowns **1** with diethylene glycols failed to give crowned thiacalix-tubes **7**, instead biscrowns **8** were formed. Partially alkylated double thiacalix[4]arenes **10**, **11** were obtained via the base promoted alkylations of a thiacalixarene dimer **2a** containing diethyleneoxy linkers.

1. Introduction

In a recent paper we have reported the unexpectedly selective diametrical ring closure of thiacalix[4]arene (TCA) with oligoethylene glycols under Mitsunobu conditions.[1] With the aid of this simple and mild method 1,3-thiacalixcrown-5 and -6 derivatives **1** were accessible in yields of 40–50%, which are comparable with those of the classical templated procedures.

The results obtained with oligoethylene glycol homologues suggested that the intra versus intermolecular reaction pathway was mainly controlled by the chain length of glycols. Whereas tri-, tetra- and pentaethylene glycols preferred 1,3-intramolecular coupling leading to **1** (A= $(OCH_2CH_2)_{1-3}O$), the short chained diethylene glycol (DEG) gave dimer **2a** in an intermolecular reaction.[1] It should be noted that vicinal glycols effected the so far unprecedented O,S-cycloalkylation affording the unique sulfonium phenoxide betaines **3a**,**b** (Fig. 1).[2]

Double- and multi-calixarenes have attracted great interest for years.[3] In particular, calix[4]tubes comprised of 2–4 CA units connected with glycolic linkers seem to be important synthetic mimetics in modelling ion channels.[4] These molecules allow metal cation tunneling across the π-basic tube of the 1,3-*alt* calixarene units.[5–8] Calix[4]tubes and semitubes comprised of two *conic* calix[4]arene cores connected with alkylene linkers have also been utilised in the preparation of ionophores displaying exceptional cesium,[9] rubidium[9] and potassium selectivities.[10–15] None of these systems have yet been described in thiacalixarene chemistry (apart from dimer **2a**[1]), and herein our efforts to explore their synthetic availability are reported.

2. Results and discussion

The thorough analysis of the reaction leading to dimer **2a** (molar ratio of TCA/diethylene glycol/(TPP/DEAD)= 1:2.2:3, toluene, rt)[1] revealed, that a competitive reaction also took place in an intramolecular pathway affording the tethered 1,2-crown-3 derivative **5a** in a yield of 34%. This observation prompted us to continue our studies in this field. Therefore, we have further investigated the Mitsunobu reaction of TCA with thia- and aza-analogues of DEG following the procedure described for **2a**.[1] With thiodiethylene glycol (TDEG), dimer **2b** was the only product isolated in high yield, and 1,2-coupled molecule relating to **5a** was not detected. *N*-Phenyl-iminodiethanol **4** gave also dimer **2c**, although in low yield, but using a larger excess of **4** resulted in exclusively a 2:1 coupled product according to the FAB-MS molecular peak ($[M+H^+]=1029$).

Keywords: Thiacalix[4]arenes; Cyclisations; Thiacalix[4]crowns; Dimers; Mitsunobu reaction.
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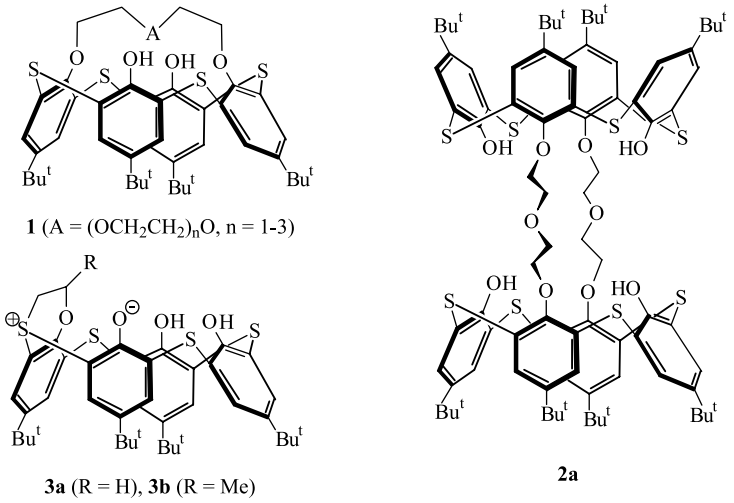


Figure 1. Survey of products obtained from TCA and oligoethylene glycols under the Mitsunobu protocol.[1,2]

Comprehensive NMR analysis of **5b** supported a conic trisubstituted structure similarly to **5a** (Fig. 2).

Proton and carbon NMR spectra of **5a**,**b** are quite complex due to the asymmetric structure, which is mainly reflected by the ^{1}H NMR spectra. For example, **5a** displays four singlets for the Bu^{t} protons, a complex pattern for the bridging CH_2O in the range 5.10–3.70 ppm and four pairs of doublets for the aromatic protons. The absence of symmetry elements in **5a**,**b** is further corroborated by their ^{13}C NMR spectra, which, at least in case of **5a**, exhibit the expected 20 four-line pattern for the aromatic carbons of the thiacalixarene skeleton, dispersed in the range 160–121 ppm.

The enantiomeric resolution of the inherently chiral **5a**,**b** racemates was attempted by direct HPLC separation using a chiral stationary phase. The Chiralpak AD (amylose tris-(3,5-dimethylphenylcarbamate) column was proposed for the chromatographic resolution of chiral calixcrown analogues,[16,17] and it was also successfully applied in our cases for the detection of enantiomers. Figure 3 shows typical chromatograms for the resolution of racemic **5a**,**b**.

The difference in retention times for the enantiomers of **5b** ($\Delta t = 8$ min) made their separation feasible on a semipreparative scale. Repeated 20 µl injections of the racemic **5b** (2 mg/ml) and collection of the eluates of the two chromatographic peaks afforded two samples whose CD spectra are mirror images of each other, confirming their enantiomeric nature (Fig. 4).

To provide chemical evidence for the structures of **5a**,**b**, they were subjected to a repeated Mitsunobu cyclisation at 80 °C, and 1,2-bis(crown-3) compounds **6a** and **6b** were

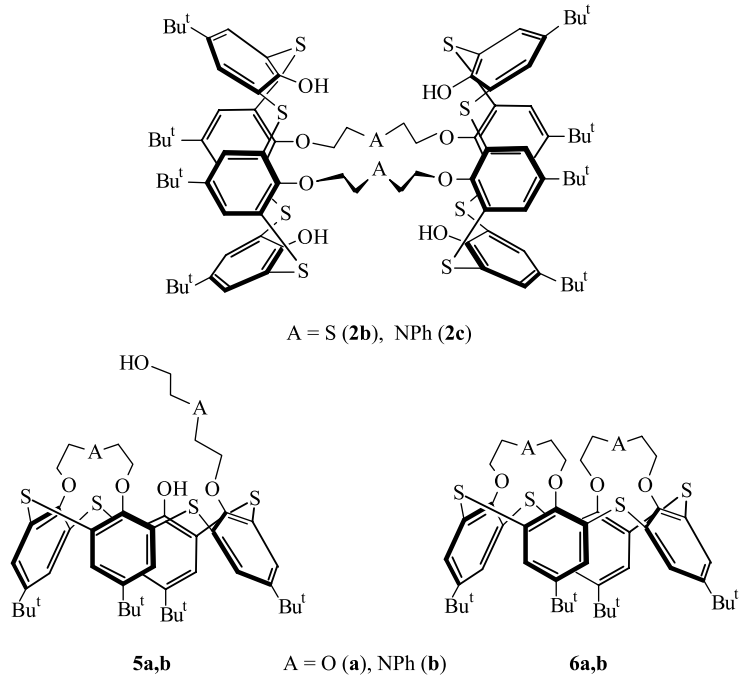


Figure 2. Dimers and 1,2-thiacalix[4]crown-3 molecules.

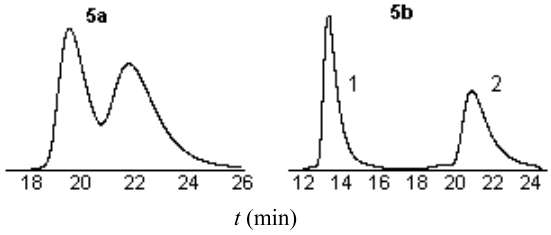


Figure 3. HPLC separation of **5a**,**b** on Chiralpak AD (mobile phase *n*-hexane/2-propanol = 95:5 at 0.5 ml/min).

obtained (Fig. 2). The C_2 symmetric *conic* conformation of these molecules was proven by the simple ^{1}H NMR spectra displaying two aromatic and one Bu^t signals.

Dimers **2a–c** offered the possibility to access to thiacalixtubes such as **7** for the first time in thiacalixarene chemistry. These ligands would be useful models to study how the alkali metal ion tunneling across the π-basic tube is affected by the central O, S or N atom in the linkers.

First, our Mitsunobu reaction mediated cyclisation of TCA to 1,3-thiacalix[4]monocrowns[1] was adapted for the synthesis of thiacalix[4]tubes (Scheme 1). Thus, it was attempted to couple dimers **2a**,**b** with different oligoethylene glycols, but no reaction was observed at room temperature. At 80 °C using a 2.5-fold excess of tri- or tetraethylene glycol and a 5-fold excess of coupling agents an intractable mixture of products was formed.

In contrast, under these conditions dimer **2b** was cyclised, at least partly, with DEG and TDEG resulting in thiacalix[4]-half-crowned dimers **8a**,**b**. In these molecules the conformation of the crowned thiacalix unit changed to 1,3-*alt*, whilst that of the unaffected part retained *cone* (Fig. 5).

Subsequently, thiacalixcrowns **1a** (n=2) and **1b** (A = OCH_2CH_2O–1,2-C_6H_4–OCH_2CH_2O in Fig. 1) were treated with a 2-fold excess of DEG and a 4-fold excess of the coupling agents TPP/DEAD in toluene at 80 °C. An analogous experiment was carried out with **1a** and TDEG. We were disappointed again, 1:1 intramolecular coupling

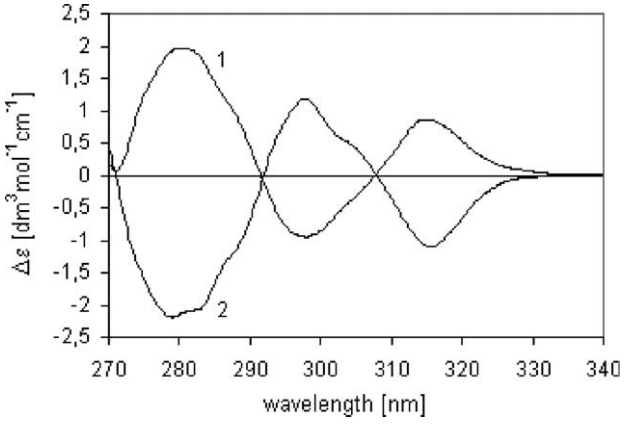
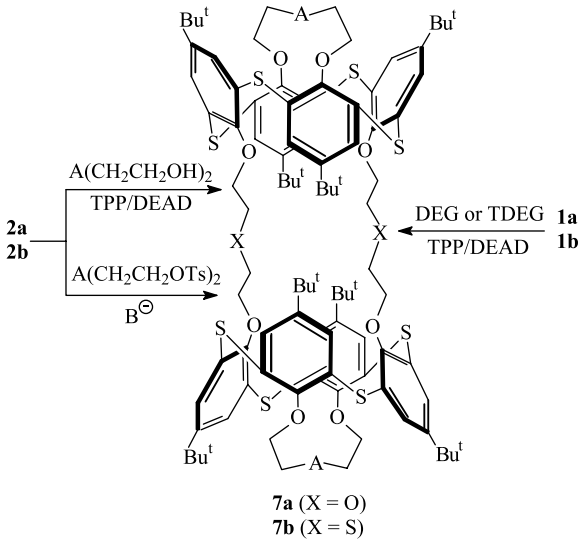


Figure 4. CD spectra (MeCN) of the enantiomers of **5b** obtained from the first (1) and the second eluted peak (2).



Scheme 1. Synthetic approaches to thiacalix[4]tubes **7**.

took place furnishing biscrowns **9a–c** instead of thiacalixtubes **7** (Fig. 6).

The nearly symmetric arrangement of the two dibenzocrown units in **9a–c** is reflected by only one pair of the ^{1}H and ^{13}C NMR signals assigned to the CCH_3 groups (e.g., for **9a** 1.32/31.5 and 1.39/31.6 ppm). The NOESY proximities measured between the methyl, methylene and aromatic protons are in accord with the 1,3-*alt* conformation. The results depicted in Figures 5 and 6 were quite surprising since as shown, at room temperature the short chain of diethylene glycols was not able to link the distal OH groups of the parent TCA, rather the formation of dimer **2a**[1] or **2b** and proximally bridged **5a** was preferred. Probably, at 80 °C the conformational mobility of the free phenolic moieties in dimer **2b** and in monocrowns **1a**,**b** was significantly increased allowing the bridging of the distal OH groups.

To achieve our initial goal, there remained nothing but the traditional base-promoted cycloalkylation. The reactions of **2a** conducted in MeCN with glycol ditosylates/Cs_2CO_3

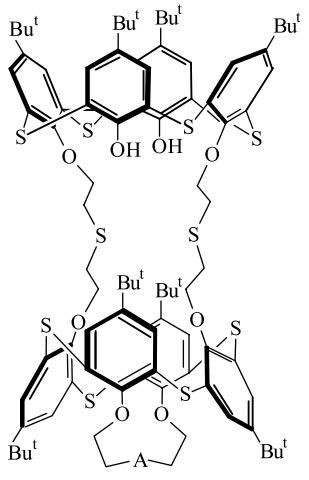
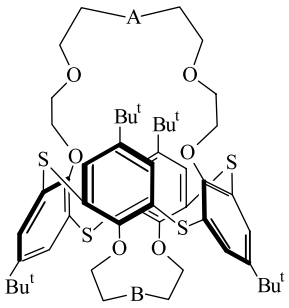


Figure 5. Half-crowned thiacalix[4]dimers.



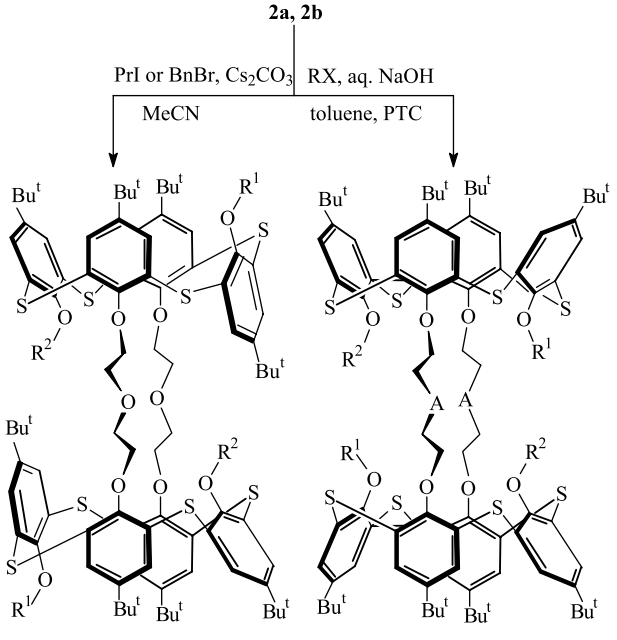
9a A, B = O; **9b** A = O, B = S
9c A = O(1,2-C_6H_4)O, B = O

Figure 6. Thiacalix[4]biscrowns **9a–c**.

gave only open chained intermediates, while on use of NaH/DMF, cleavage of the linkers occurred.

The failure of the three approaches aroused the necessity of studying the reactivity of the OH groups in dimer **2a**. Therefore, exhaustive alkylations were performed with Me_2SO_4, PrI and BnBr under two different conditions: (1) PrI, BnBr/Cs_2CO_3, MeCN, 80 °C, (2) RX, 50% aq NaOH/toluene, PTC, 100 °C.

The common feature of these reactions was the formation of doubly alkylated products **10** and **11** in moderate to good yields but in different conformations (Scheme 2). As expected, compounds **10** obtained with Cs_2CO_3 exist in *paco*, while the PTC alkylation products **11**, in *cone* conformation. The former is reflected by the significant upfield shifts of the propyl CH_3 (−0.93 ppm) and CH_2 (0.83 ppm) protons in the spectrum of *paco* **10a**, which are



10a (R^1 = Pr, R^2 = H)
10b (R^1 = Bn, R^2 = H)

11a (R^1 = Me, R^2 = H, A = O)
11b (R^1 = Pr, R^2 = H, A = O)
11c (R^1 = Bn, R^2 = H, A = O)
12 (R^1, R^2 = Bn, A = S)

Scheme 2. Alkylation of **2a**,**b** under different conditions.

attributed to the steric proximity of the *tert*-butyl groups. The same chemical shifts of *cone* **11b**, where this shielding effect does not occur, are 1.12 and 1.97 ppm, respectively.

The unsuccessful exhaustive alkylations of **2a** may be attributed to the formation of insoluble alkali–phenolate complexes of the dialkylated intermediates, which precipitate from the solvents and prevented further reaction. This observation may explain the failure of ring closures of dimer **2a**. In contrast, the thioanalogue **2b** did not show precipitation under similar conditions and, in fact, it could be completely benzylated under PTC conditions to afford tetraether **12** (Scheme 2). However, the Cs_2CO_3 promoted alkylations of **2b** gave inseparable mixtures and we were unable to recover any single product to be identified.

Recently, Beer et al. have found that ionophores derived from the less rigid calix[4]semitubes[15] exhibit much faster complexation kinetics than the respective cryptand-type calix[4]tubes,[12] whilst retaining the exceptional K^+ selectivities. Although, the rather large, central crown-6 core in our semitubes **10** and **11** did not seem to be properly preorganised for binding, alkali picrate extraction experiments were carried out in CH_2Cl_2/water biphasic system to assess the cation extractabilities.

In fact, at neutral pH the conic ligands **11a–c** scarcely complexed any alkali cations (5–10%), but the *paco* **10a** displayed moderate extractabilities for Cs^+ (30%) and Rb^+ (20%). The latter result may be due to the contribution of the two aromatic rings in *anti* position to the complexation via π–cation interaction. Obviously, this effect would be more pronounced with peralkylated dimers in double 1,3-*alt* conformation, but presently these ionophores could not be prepared.

The explanation for the partial alkylation of **2a** implied the formation of alkali phenolate complexes, therefore, the biphasic extraction experiments were repeated with the most lipophilic ligands **10b** and **11c** under strongly basic conditions. At pH 12 (adjusted by the addition of the appropriate alkali hydroxide to the picrate solution) the cation extractabilities significantly changed. The K^+ and Rb^+ extraction capacity of the conic ligand **11c** increased to 56 and 58%, respectively, indicating the formation of phenolate anions stabilising the complexed cations by additional ion-pair interactions. In contrast, the *paco* counterpart **11b** extracted none of cations, since its phenolate salts precipitated from the biphasic system.

3. Conclusions

In the Mitsunobu reaction of *p-tert*-butylthiacalix[4]arene and diethylene glycols intermolecular coupling versus intramolecular ring closure were observed. Depending on the glycol used, dimers **2** and/or the inherently chiral 1,2-thiacalix[4]crown-3 derivatives **5** were formed. The enantiomeric separation of **5b** was achieved by chiral HPLC and the CD spectrum confirmed the enantiomeric nature of the eluted fractions. Efforts have been made to synthesise thiacalix[4]tubes **7**, that is, dimers containing terminal crown rings. All attempts with the Mitsunobu coupling and

with base-mediated cyclisations of dimers **2a**,**b** or monocrowns **1a**,**b** failed, instead half-crowned dimers **8** or 1,3-*alt* biscrowns **9** were formed. The base-promoted alkylations of oxadimer **2a** revealed that complete reaction could not be attained, only partially alkylated molecules were formed in each case. In contrast, thiadimer **2b** could be exhaustively benzylated under PTC conditions. The diethyleneoxy linkers in the partially alkylated oxadimers **10** and **11** are not properly preorganised for alkali cation binding, as inferred by the low extractabilities at neutral pH. Under strongly basic conditions the binding capacity of conic **11a** was greatly increased due to the formation of complexed cation–phenolate ion pairs.

4. Experimental

4.1. General

Melting points are uncorrected. NMR spectra were recorded in $CDCl_3$ at 500/125 MHz on a Bruker Avance DRX-500 spectrometer. FAB mass spectra were recorded (frequently in the presence of a mixture of alkali picrates) on a Finigan MAT 8430 instrument (matrix: m-NBA, gas: xenon, accelerating voltage: 9 kV). Precoated silica gel plates (Merck 60 F_{254}) were used for analytical TLC and Kieselgel 60 for column chromatography. All chemicals were reagent grade and used without further purification. *n*-Hexane and 2-propanol (HPLC grade) were purchased from Merck. TCA[18] and DEAD[19] were synthesised as described in the literature. (Caution! DEAD may explode if exposed to shock, friction or heating.)

The HPLC measurements were performed on a JASCO liquid chromatograph (pump 1580) with UV spectrophotometric detector (UV-1575) operating at 256 nm. The column (250×4.6 mm) was packed with Chiralpak AD coated on 10 μm silicagel (Daicel, Tokyo). The CD spectra were recorded on a JASCO 800 spectropolarimeter.

4.2. Synthesis of dimers 2a–c, 1,2-monocrowns 5a,b and 1,2-biscrowns 6a,b

To the stirred solution of TCA (0.72 g, 1 mmol), TPP (0.80 g, 3 mmol) and glycol (1 mmol) in 20 ml toluene, a 40% toluene solution of DEAD (1.3 ml, 3 mmol) was added at room temperature and allowed to react for 1 h, then worked up.

Oxadimer **2a** precipitated from the solution was filtered off (after washing with MeOH and drying it is recovered in analytically pure form),[1] and the filtrate was evaporated to dryness. The residue was simply dissolved in hot MeCN (15 ml) and upon cooling **5a** was precipitated as microcrystals.

In the other two cases the solvent was removed in vacuo, thereafter the solid residue was recrystallised from MeCN (**2b**) or chromatographed on silica with hexane/EtOAc=9:1 eluent (**2c**). The main fractions were collected, evaporated and washed with MeOH to remove the insoluble unreacted TCA. Compound **2c** is recovered from the filtrate in essentially pure form.

For the preparation of **5b** the molar ratios were enhanced to TCA/**4**/(TPP/DEAD)=1:5:5 following the procedure above. The reaction mixture was evaporated to dryness and chromatographed on silica with hexane/EtOAc=9:1 eluent to give **5b**. All materials were obtained as white solids.

4.2.1. Thiadimer 2b. Yield: 90%, mp 297–298 °C; ^{1}H NMR δ 8.06 (s, 4H, O*H*), 7.59 (s, 8H, Ar*H*), 6.89 (s, 8H, Ar*H*), 4.94 (t, 8H, J=7.4 Hz, OCH_2), 3.39 (t, 8H, J=7.4 Hz, SCH_2), 1.30 (s, 36H, Bu^t), 0.76 (s, 36H, Bu^t); ^{13}C NMR δ 156.1, 155.7, 148.1, 142.6, 135.0, 132.8, 129.6, 122.4 (Ar), 72.5 (O*C*H$_2$), 34.3, 34.2 (*C*(CH$_3$)$_3$), 31.8, 31.0 (C(*C*H$_3$)$_3$), 30.6 (S*C*H$_2$); FAB-MS *m*/*z* (%): 1613 [M+H]$^+$ (100), 1611 [M−H]$^-$ (100). Anal. Calcd for $C_{88}H_{108}O_8S_{10}$ (1614.42): C, 65.47; H, 6.74; S, 19.86. Found: C, 65.20; H, 6.72; S, 19.67%.

4.2.2. *N*-Phenylazadimer 2c. Yield: 33%, mp 265–266 °C; ^{1}H NMR δ 8.04 (s, 4H, O*H*), 7.58 (s, 8H, Ar*H*), 7.13 (t, 4H, J=8.0 Hz, Ar*H*), 7.00 (d, 4H, J=8.0 Hz, Ar*H*), 6.88 (s, 8H, Ar*H*), 6.62 (t, 2H, J=8.0 Hz, Ar*H*), 4.89 (t, 8H, J=7.5 Hz, OCH_2), 4.12 (t, 8H, J=7.5 Hz, NCH_2), 1.37 (s, 36H, Bu^t), 0.80 (s, 36H, Bu^t); ^{13}C NMR δ 156.2, 155.9, 155.6, 142.6, 136.4, 134.9, 132.7, 129.5, 129.3, 122.3, 120.6, 113.14 (Ar), 71.3 (O*C*H$_2$), 50.3 (N*C*H$_2$), 34.5, 34.3 (*C*(CH$_3$)$_3$), 31.9, 31.1 (C(*C*H$_3$)$_3$); 1734 [M+H]$^+$ (80), 1732 [M−H] (20). Anal. Calcd for $C_{100}H_{118}N_2O_8S_8$ (1732.52): C, 69.33; H, 6.86; N, 1.62; S, 14.80. Found: C, 69.17; H, 6.82; N, 1.61; S, 14.92%.

4.2.3. 1,2-Thiacalix[4]crown 5a. Yield: 34%, mp 147–150 °C; ^{1}H NMR δ 8.74 (s, 1H, O*H*), 7.80 (d, 1H, J=1.5 Hz, Ar*H*), 7.72 (d, 1H, J=1.5 Hz, Ar*H*), 7.66 (d, 1H, J=1.5 Hz, Ar*H*), 7.61 (d, 1H, J=1.5 Hz, Ar*H*), 7.37 (d, 1H, J=1.5 Hz, Ar*H*), 7.26 (d, 1H, J=1.5 Hz, Ar*H*), 7.59 (d, 1H, J=1.5 Hz, Ar*H*), 6.57 (d, 1H, J=1.5 Hz, Ar*H*), 5.10 (d, 1H, J=7.5 Hz, OCH_2), 4.74 (t, 1H, J=7.5 Hz, OCH_2), 4.41–4.31 (m, 4H, OCH_2), 4.19–4.10 (m, 4H, OCH_2), 3.92 (t, 2H, J=4.5 Hz, OCH_2), 3.88 (t, 2H, J=9.0 Hz, OCH_2), 3.78 (br s, 1H, OCH_2), 3.72 (t, 1H, J=4.5 Hz, OCH_2), 1.35 (s, 9H, Bu^t), 1.33 (s, 9H, Bu^t), 1.02 (s, 9H, Bu^t), 0.55 (s, 9H, Bu^t); ^{13}C NMR δ 159.6, 156.6, 156.3, 156, 147.9, 147.1, 146.3, 142.3, 136.9, 136.3, 135.4, 135.3, 134.8, 133.7, 131.7, 131.2, 130.8, 130.4, 130, 129.4, 129.3, 127.9, 123.4, 121.3 (Ar), 77.6, 75.6, 74.9, 74, 72.8, 71.3, 70.6, 62.2 (O*C*H$_2$), 34.8, 34.5, 34.4, 33.9 (*C*(CH$_3$)$_3$), 31.8, 31.7, 31.3, 30.9 (C(*C*H$_3$)$_3$); FAB-MS *m*/*z* (%): 900.9 [M+Na]$^+$ (100). Anal. Calcd for $C_{48}H_{62}O_7S_4$ (879.25): C, 65.57; H, 7.11; S, 14.59. Found: C, 65.33; H, 6.93; S, 14.46%.

4.2.4. 1,2-Thiacalix[4]-*N*-phenylazacrown 5b. Yield: 25%, mp 152–153 °C; ^{1}H NMR δ 8.71 (s, 1H, O*H*), 7.75 (d, 1H, J=2.5 Hz, Ar*H*), 7.72 (d, 1H, J=2.5 Hz, Ar*H*), 7.71 (d, 1H, J=2.4 Hz, Ar*H*), 7.63 (d, 1H, J=2.4 Hz, Ar*H*), 7.51 (d, 1H, J=2.4 Hz, Ar*H*), 7.45 (d, 1H, J=2.4 Hz, Ar*H*), 7.27 (t, 2H, J=8.0 Hz, Ar*H*), 7.19 (t, 2H, J=8.0 Hz, Ar*H*), 7.00 (d, 2H, J=8.0 Hz, Ar*H*), 6.77 (t, 1H, J=7.2 Hz, Ar*H*), 6.68 (t, 1H, J=7.2 Hz, Ar*H*), 6.44 (d, 2H, J=8.0 Hz, Ar*H*), 6.36 (d, 1H, J=2.3 Hz, Ar*H*), 6.25 (d, 1H, J=2.3 Hz, Ar*H*), 4.71 (m, 2H, OCH_2), 4.66 and 4.56 (t, 1+1H, J=6.5, 7.0 Hz, OCH_2), 4.54 and 4.36 (m+d, 1+1H, J=8.3 Hz, OCH_2), 4.11 and 3.97 (dt+m, 1+1H, J=6.5, 15.1 Hz, NCH_2), 3.98 (m, 2H, OCH_2), 3.96 and 3.76 (m+m, 1+1H, NCH_2), 3.92

and 3.79 (m+m, 1+1H, NCH_2) 3.74 (m, 2H, NCH_2), 1.37 (s, 9H, Bu^t), 1.33 (s, 9H, Bu^t), 1.14 (s, 9H, Bu^t), 0.41 (s, 9H, Bu^t); ^{13}C NMR δ 160.1, 157.3, 156.2, 155.4, 149.0, 148.7, 147.6, 147.5, 146.2, 142.6, 132.4, 131.1, 131.1, 130.1, 129.7, 127.8, 124.2, 120.7 (Ar), 78.1, 74.6, 70.7, 60.9 (OCH_2), 64.9, 54.2, 53.3, 51.9 (NCH_2), 34.6, 34.6, 34.4, 33.6 (C(CH$_3$)$_3$), 31.7, 31.6, 31.2, 30.7 (C(CH$_3$)$_3$); FAB-MS *m/z* (%): 1029 [M+H]$^+$ (100), 1027 [M−H]$^-$ (100). Anal. Calcd for $C_{60}H_{72}N_2O_5S_4$ (1029.48): C, 70.00; H, 7.05; N, 2.72; S, 12.46. Found: C, 69.87; H, 7.10; N, 2.73; S, 12.55%.

4.3. Ring closure of the tethered monocrowns 5a and 5b

To the mixture of **5a** or **5b** (0.25 mmol), TPP (0.13 g, 0.5 mmol) in toluene (10 ml), a 40% toluene solution of DEAD (0.22 ml, 0.5 mmol) was added and stirred at 80 °C for 1 h. The solvent was then evaporated and the residue was chromatographed on silica with hexane/EtOAc=9:1 eluent to give **5a**,**b** as white solids.

4.3.1. 1,2-Thiacalix[4]bis(crown-3) 6a. Yield: 76%, mp 248–250 °C; ^{1}H NMR δ 7.44 (d, 4H, J=2.5 Hz, ArH), 7.33 (d, 4H, J=2.5 Hz, ArH), 4.50 (t, 8H, J=3.2 Hz, OCH_2), 4.30 (dt, 4H, J=13.2, 3.3 Hz, OCH_2), 4.06 (dm, 4H, J=13.2 Hz, OCH_2), 1.09 (s, 36H, Bu^t); ^{13}C NMR δ 158.6, 146.9, 135.5, 135.1, 131.3, 130.2 (Ar), 76.2, 72.5 (OCH_2), 34.4 (C(CH$_3$)$_3$), 31.4 (C(CH$_3$)$_3$); FAB-MS *m/z* (%): 861 [M+H]$^+$ (30), 859 [M−H]$^-$ (20). Anal. Calcd for $C_{48}H_{60}O_6S_4$ (861.24): C, 66.94; H, 7.02; S, 14.89. Found: C, 66.72; H, 7.12; S, 15.01%.

4.3.2. 1,2-Thiacalix[4]bis(*N*-phenylazacrown-3) 6b. Yield: 93%, mp 134–136 °C; ^{1}H NMR δ 7.33 (d, 4H, J=2.5 Hz, ArH), 7.30 (d, 4H, J=2.5 Hz, ArH), 7.17 (t, 4H, J=7.8 Hz, N–Ph$_m$), 6.61 (m, 4+2H, N–Ph$_{o,p}$), 4.58 and 4.41 (br t, 4+4H, OCH_2), 4.57 (m, 4H, OCH_2), 4.41 (m, 4H, OCH_2), 3.85 (m, 8H, NCH_2), 1.06 (s, 36H, Bu^t); ^{13}C NMR δ 159.5, 146.8, 135.2, 135.1, 130.8, 130.6 (Ar), 148.1, 129.5, 116.1, 111.7 (N–Ph), 75.7 (OCH_2), 53.0 (NCH_2), 34.4 (C(CH$_3$)$_3$), 31.4 (C(CH$_3$)$_3$); FAB-MS *m/z* (%):1011 [M+H]$^+$ (100). Anal. Calcd for $C_{60}H_{70}N_2O_4S_4$ (1011.46): C, 71.25; H, 6.98; N, 2.77; S, 12.68. Found: C, 71.43; H, 7.04; N, 2.70; S, 12.77%.

4.4. Synthesis of half-crowned thiadimers 8a,b

To the stirred solution of dimer **2b** (0.5 mmol), TPP (1.31 g, 5 mmol) and DEG or TDEG (2.5 mmol) in 20 ml toluene, a 40% toluene solution of DEAD (2.25 ml, 5 mmol) was added at 80 °C and kept at this temperature for 2 h. The solvent was then removed under reduced pressure and the residue was chromatographed on silica (eluent: hexane/EtOAc=9:1) to give white solids.

4.4.1. Compound 8a. Yield: 25%, mp 270–272 °C; ^{1}H NMR δ 7.81 (s, 2H, OH), 7.68 (s, 4H, ArH), 7.43 (s, 4H, ArH), 7.32 (s, 4H,), 6.98 (s, 4H, ArH), 4.67 (t, 4H, J=6.0 Hz, OCH_2), 4.02 (t, 4H, J=8.0 Hz, OCH_2), 3.64 (t, 4H, J=6.5 Hz, OCH_2), 3.16 (t, 4H, J=6.0 Hz, SCH_2), 2.78 (t, 4H, J=8.0 Hz, OCH_2), 2.15 (t, 4H, J=6.5 Hz, SCH_2), 1.36 (s, 18H, Bu^t), 1.30 (s, 18H, Bu^t), 1.23 (s, 18H, Bu^t), 0.82 (s, 18H, Bu^t); ^{13}C NMR δ 157.1, 156.5, 156.2, 155.2, 148.3, 147.1, 146.2, 142.8, 134.5, 133.2, 128.9, 129.1, 128.6, 127.3, 127.0, 122.3 (Ar), 71.5, 70.9, 69.3, 69.0 (OCH_2), 34.6, 34.6, 34.4, 34.4 (C(CH$_3$)$_3$), 33.5 (SCH_2), 31.7, 31.4, 31.4 (C(CH$_3$)$_3$), 31.1 (SCH_2), 31.0 (C(CH$_3$)$_3$); FAB-MS *m/z* (%): 1685 [M+H]$^+$ (100). Anal. Calcd for $C_{92}H_{114}O_9S_{10}$ (1684.51): C, 65.60; H, 6.82; S, 19.03. Found: C, 65.49; H, 6.96; S, 18.91%.

4.4.2. Compound 8b. Yield: 30%, mp 264–268 °C; ^{1}H NMR δ 7.81 (s, 2H, OH), 7.68 (s, 4H, ArH), 7.53 (s, 4H, ArH), 7.32 (s, 4H,), 7.01 (s, 4H, ArH), 4.67 (t, 4H, J=6.5 Hz, OCH_2), 3.97 (t, 4H, J=8.0 Hz, OCH_2), 3.66 (t, 4H, J=6.0 Hz, OCH_2), 3.20 (t, 4H, J=6.5 Hz, SCH_2), 2.11 (t, 4H, J=8.0 Hz, SCH_2), 1.95 (t, 4H, J=6.0 Hz, SCH_2), 1.36 (s, 18H, Bu^t), 1.32 (s, 18H, Bu^t), 1.26 (s, 18H, Bu^t), 0.83 (s, 18H, Bu^t); ^{13}C NMR δ 158.4, 156.7, 156.3, 154.1, 148.4, 147.6, 146.4, 142.8, 134.6, 133.4, 130.5, 130.3, 129.0, 127.3, 126.6, 122.2 (Ar), 74.8, 73.4, 69.3 (OCH_2), 34.7, 34.5, 34.4, 34.3 (C(CH$_3$)$_3$), 33.5, 33.1 (SCH_2), 31.7, 31.5, 31.5, 31.0 (C(CH$_3$)$_3$), 30.8 (SCH_2); FAB-MS *m/z* (%): 1701 [M+H]$^+$ (100). Anal. Calcd for $C_{92}H_{114}O_8S_{11}$ (1700.57): C, 64.98; H, 6.76; S, 20.74. Found: C, 64.87; H, 6.69; S, 20.83%.

4.5. General synthesis of 1,3-*alt*-thiacalix[4]biscrowns 9a–c

To the stirred solution of monocrowns **1a** or **1b** (0.5 mmol), TPP (0.53 g, 2 mmol) and DEG or TDEG (1 mmol) in 20 ml toluene, a 40% toluene solution of DEAD (0.9 ml, 2 mmol) was added at room temperature and refluxed 12 h. The solvent was then removed under reduced pressure and the residue was chromatographed on silica (eluent: hexane/EtOAc=9:1) to give white solids.

4.5.1. Biscrown 9a. Yield: 42%, mp >350 °C; ^{1}H NMR δ 7.42 (s, 4H, *ArH*), 7.31 (s, 4H, ArH), 3.91 (dd, 4H, J=8.4, 8.0 Hz, OCH_2), 3.63 (t, 4H, J=5.3 Hz, OCH_2), 3.61 (dd, 4H, J=4.1, 4.0 Hz, OCH_2), 3.40 (dd, 4H, J=4.0, 4.1 Hz, OCH_2), 3.03 (dd, 4H, J=8.3, 8.1 Hz, OCH_2), 2.76 (t, 4H, J=5.3 Hz, OCH_2), 1.39 (s, 18H, Bu^t), 1.32 (s, 18H, Bu^t); ^{13}C NMR δ 156.6, 155.8, 147.0, 146.1, 128.8, 127.7, 127.5, 127.2 (Ar), 73.8, 71.7, 71.2, 70.7, 69.0, 66.6 (OCH_2), 34.6, 34.5 (C(CH$_3$)$_3$), 31.6, 31.5 (C(CH$_3$)$_3$); FAB-MS *m/z* (%): 949 [M+H]$^+$ (69), 986.1 [M+K]$^+$ (22), 1032.2 [M+Rb]$^+$ (15). Anal. Calcd for $C_{52}H_{68}O_8S_4$ (949.34): C, 65.79; H, 7.22; S, 13.51. Found: C, 79.34; H, 7.23; S, 13.64%.

4.5.2. Biscrown 9b. Yield: 44%, mp >350 °C; ^{1}H NMR δ 7.51 (s, 4H, *ArH*), 7.31 (s, 4H, ArH), 3.85 (br s, 4H, OCH_2), 3.64 (br s, 4H, OCH_2), 3.60 (br s, 4H, OCH_2), 3.39 (br s, 4H, OCH_2), 2.97 (br s, 4H, OCH_2), 1.91 (br s, 4H, SCH_2), 1.37 (s, 18H, Bu^t), 1.31 (s, 18H, Bu^t); ^{13}C NMR δ 157.3, 153.9, 146.8, 145.5, 129.7, 129.3, 126.9, 126.3 (Ar), 73.5, 73.0, 71.4, 69.7, 66.2 (OCH_2), 34.4, 34.2 (C(CH$_3$)$_3$), 32.7 (SCH_2), 31.3, 31.2 (C(CH$_3$)$_3$); FAB-MS *m/z* (%): 1003.5 [M+K]$^+$ (26); 1049.5 [M+Rb]$^+$ (15). Anal. Calcd for $C_{52}H_{68}O_7S_5$ (965.40): C, 64.70; H, 7.10; S, 16.60. Found: C, 64.21; H, 6.97; S, 16.52%.

4.5.3. Biscrown 9c. Yield: 40%, mp >350 °C; ^{1}H NMR δ 7.38 (s, 4H, *ArH*), 7.32 (s, 4H, ArH), 6.88–6.91 (m, 4H, ArH), 3.98 (br s, 4H, OCH_2), 3.94 (t, 4H, J=8.0 Hz, OCH_2), 3.65 (br s, 4H, OCH_2), 3.58 (br s, 4H, OCH_2), 2.96 (t, 4H,

J=7.0 Hz, OCH_2), 2.75 (br s, 4H, OCH_2), 1.29 (s, 18H, Bu^t), 1.22 (s, 18H, Bu^t); ^{13}C NMR δ 156.3, 155.5, 149.3, 146.8, 146.1, 128.5, 127.8, 127.6, 127.4, 122.2, 116.5 (Ar), 70.8, 70.6, 69.6, 69.2, 67.9 (OCH_2), 34.7, 34.6 (C(CH$_3$)$_3$), 31.6, 31.5 (C(CH_3)$_3$); FAB-MS m/z (%): 1041 [M+H]$^+$ (100). Anal. Calcd for $C_{58}H_{72}O_9S_4$ (1041.44): C, 66.89; H, 6.97; S, 12.31. Found: C, 66.51; H, 6.90; S, 12.42%.

4.6. Alkylations of dimers 2a,b

1. Cs_2CO_3 promoted alkylation. The mixture of **2a** (0.36 g, 0.25 mmol) and Cs_2CO_3 (1.63 g, 5 mmol) in MeCN (20 ml) was refluxed under stirring for 2 h, then the alkylating agent (PrI, BnBr: 5 mmol each) was added and further refluxed for 48 h. After removal of the solvent, the residue was dissolved in $CHCl_3$, washed with dilute aq HCl, water and dried. After removal of the solvent, the crude products were triturated with MeOH to give pure **9** as white solids.

4.6.1. Dipropyl oxadimer 10a (*paco*). Yield: 48%, mp 375–377 °C; ^{1}H NMR δ 8.32 (s, 2H, OH), 7.61 (s, 8H, ArH), 7.56 (s, 4H, ArH), 7.42 (s, 4H, ArH), 4.37–4.36 (br s, 4H, OCH_2), 4.04–4.03 (br s, 4H, OCH_2), 3.92–3.91 (br s, 4H, OCH_2), 3.75–3.74 (br s, 4H, OCH_2), 3.11 (t, 4H, J=7.4 Hz, OCH_2), 1.30 (s, 18H, Bu^t), 1.28 (s, 18H, Bu^t), 1.23 (s, 36H, Bu^t), 0.83 (q, 4H, J=7.5 Hz, CH_2), −0.93 (t, 6H, J=7.4 Hz, CH_3); ^{13}C NMR δ 158.8, 158.5, 157.3, 156.8, 147.1, 146.5, 142.1, 134.8, 134.1, 130.8, 129.9, 129.8, 128.1, 121.2 (Ar), 71.7, 71.0, 68.9, (OCH_2), 34.4 (C(CH$_3$)$_3$), 34.3 (C(CH$_3$)$_3$), 33.8 (C(CH$_3$)$_3$), 31.4 (C(CH$_3$)$_3$), 31.2 (C(CH$_3$)$_3$), 22.3 (CH$_2$), 9.2 (CH$_3$); FAB-MS m/z (%): 1664.5 [M+H]$^+$ (100), 1663 [M−H]$^-$ (100). Anal. Calcd for $C_{94}H_{120}O_{10}S_8$ (1666.46): C, 67.75; H, 7.26; S, 15.39. Found: C, 67.43; H, 7.22; S, 15.45%.

4.6.2. Dibenzyl oxadimer 10b (*paco*). Yield: 48%, mp >360 °C; ^{1}H NMR δ 8.60 (s, 2H, OH), 7.72 (s, 4H, ArH), 7.53 (s, 4H, ArH), 7.40 (d, 4H, J=2.5 Hz, ArH), 7.02 (d, 4H, J=2.5 Hz, ArH), 6.69 (t, 2H, J=7.5 Hz, Bn–ArH), 6.38 (t, 4H, J=7.5 Hz, Bn–ArH), 5.88 (d, 4H, J=7.5 Hz, Bn–ArH), 4.77 (s, 4H, Bn–CH_2O), 4.37 and 4.17 (m+dd, 4+4H, J=15.5 Hz, OCH_2), 3.91 and 3.71 (dt+m, 4+4H, J=10.3 Hz, OCH_2), 1.37 (s, 18H, Bu^t), 1.36 (s, 18H, Bu^t), 0.81 (s, 36H, Bu^t); ^{13}C NMR δ 158.2, 156.7, 156.1, 148.1, 142.5, 137.7, 135.5, 134.8, 129.5, 129.1, 128.4, 128.3, 128.0, 121.8 (Ar), 127.6, 125.7, 124.3 (Bn–Ar), 70.6, 68.9, (OCH_2), 66.8 (Ph–CH_2), 35.1, 34.4, 34.2 (C(CH$_3$)$_3$), 31.8, 30.9 (C(CH$_3$)$_3$); FAB-MS m/z (%): 1764.5 [M+H]$^+$ (100), 1763 [M−H]$^-$ (100). Anal. Calcd for $C_{102}H_{120}O_{10}S_8$ (1762.54): C, 69.51; H, 6.86; S, 14.55. Found: C, 69.68; H, 6.89; S, 14.43%.

2. PTC alkylation procedure. The mixture of **2a** or **2b** (0.25 mmol), alkylating agent (Me_2SO_4, PrBr, BnBr:5 mmol each), 50% aq NaOH (4 ml), TBAB catalyst (0.1 g) and toluene (20 ml) were agitated at 80 °C for 12 h. The organic phase was then evaporated, the aqueous residue was acidified with dilute HCl, extracted with $CHCl_3$ and washed with water. After removal of the solvent, the crude product thus obtained was purified by trituration with MeOH and filtered to give white solids.

4.6.3. Dimethyl oxadimer 11a (*cone*). Yield: 32%, mp 318–321 °C; ^{1}H NMR δ 8.23 (s, 2H, OH), 7.50 (s, 4H, ArH), 7.41 (s, 8H, ArH), 7.05 (s, 4H, ArH), 5.14 (br s, 4H, OCH_2), 3.86 (br s, 6H, OCH_3), 3.59 (br s, 8H, OCH_2), 3.37 (br s, 4H, OCH_2), 1.40 (s, 18H, Bu^t), 1.32 (s, 18H, Bu^t), 0.99 (s, 36H, Bu^t); ^{13}C NMR δ 156.2, 146.1, 141.1, 134.2, 132.8, 131.1, 130.0, 122.2 (Ar), 71.3, (OCH_3), 34.8 (C(CH$_3$)$_3$), 34.4 (C(CH$_3$)$_3$), 34.3 (C(CH$_3$)$_3$), 32.0 (C(CH$_3$)$_3$), 31.3 (C(CH$_3$)$_3$); FAB-MS m/z (%): 1609.6 [M+H]$^+$ (80). Anal. Calcd for $C_{90}H_{112}O_{10}S_8$ (1610.35): C, 67.13; H, 7.01; S, 15.93. Found: C, 66.74; H, 6.96; S, 15.79%.

4.6.4. Dipropyl oxadimer 11b (*cone*). Yield: 90%, mp 309–310 °C; ^{1}H NMR δ 8.38 (s, 2H, OH), 7.36 (d, 4H, J=2.3 Hz, ArH), 7.35 (d, 4H, J=2.3 Hz, ArH), 7.32 (s, 4H, ArH), 7.05 (s, 4H, ArH), 4.81 (m, 4H, OCH_2), 4.74 (m, 4H, OCH_2), 4.30 (br, 4H, OCH_2), 4.18 (t, 4H, J=7.5 Hz, OCH_2), 1.97 (q, 4H, J=7.5 Hz, CH_2), 1.15 (s, 18H, Bu^t), 1.12 (t, 6H, J=7.5 Hz, CH_3), 1.10 (s, 36H, Bu^t), 0.91 (s, 18H, Bu^t); ^{13}C NMR δ 158.8, 157.8, 157.1, 146.8, 146.4, 146.2, 141.5, 135.1, 134.6, 134.2, 133.1, 131.3, 130.6, 130.4, 129.2, 128.4, 125.5, 121.7 (Ar), 77.8, 74.0, 70.7 (OCH_2), 34.4 (C(CH$_3$)$_3$), 34.1 (C(CH$_3$)$_3$), 34.05 (C(CH$_3$)$_3$), 31.5 (C(CH$_3$)$_3$), 31.4 (C(CH$_3$)$_3$), 31.5 (C(CH$_3$)$_3$), 23.1 (CH$_2$), 10.8 (CH$_3$); FAB-MS m/z (%): 1664.5 [M+H]$^+$ (100), 1663 [M−H]$^-$ (100). Anal. Calcd for $C_{94}H_{120}O_{10}S_8$ (1666.46): C, 67.75; H, 7.26; S, 15.39. Found: C, 67.51; H, 7.31; S, 15.47%.

4.6.5. Dibenzyl oxadimer 11c (*cone*). Yield: 80%, mp 293–295 °C; ^{1}H NMR δ 8.48 (s, 2H, OH), 7.74 (d, 4H, J=7.5 Hz, ArH), 7.65 (d, 4H, J=2.2 Hz, ArH), 7.63 (d, 4H, J=2.2 Hz, ArH), 7.39 (t, 4H, J=7.5 Hz, ArH), 7.36 (t, 2H, J=7.5 Hz, ArH), 7.20 (s, 4H, ArH), 6.70 (s, 4H, ArH), 5.06 (s, 4H, Bn–CH_2O), 4.60 (m, 8H, OCH_2), 3.85 (m, 8H, OCH_2), 1.28 (s, 36H, Bu^t), 1.04 (s, 18H, Bu^t), 0.68 (s, 18H, Bu^t); ^{13}C NMR δ 160.0, 157.1, 156.2, 146.8, 146.4, 141.5, 137.9, 135.4, 135.1, 131.9, 131.7, 131.0, 130.2, 129.4, 128.5, 128.3, 121.23 (Ar), 78.6, 74.2, 70.0 (OCH_2), 34.5 (C(CH$_3$)$_3$), 34.0 (C(CH$_3$)$_3$), 33.9 (C(CH$_3$)$_3$), 31.6 (C(CH$_3$)$_3$), 31.3 (C(CH$_3$)$_3$), 31.1 (C(CH$_3$)$_3$); FAB-MS m/z (%): 1764.5 [M+H]$^+$ (100), 1763 [M−H]$^-$ (100). Anal. Calcd for $C_{102}H_{120}O_{10}S_8$ (1762.54): C, 69.51; H, 6.86; S, 14.55. Found: C, 69.01; H, 6.81; S, 14.62%.

4.6.6. Tetrabenzyl thiadimer 12 (*cone*). Yield: 38%, mp 240–243 °C; ^{1}H NMR δ 7.69 (s, 8H, ArH), 7.36 (t, 8H, J=7.5 Hz, Bn–ArH), 7.35 (t, 4H, J=7.5 Hz, Bn–ArH), 7.30 (d, 8H, J=7.5 Hz, Bn–ArH), 6.80 (s, 8H, ArH), 5.20 (s, 8H, Ph–CH_2O), 4.63 (dd, 8H, J=9.3, 7.4 Hz, OCH_2), 3.50 (dd, 8H, J=9.3, 7.4 Hz, SCH_2), 1.35 (s, 36H, Bu^t), 0.81 (s, 36H, Bu^t); ^{13}C NMR δ 160.1, 156.6, 146.4, 146.2, 135.5, 132.3, 131.5, 129.5 (Ar), 136.6, 130.1, 128.4, 128.3 (Bn–Ar), 79.2 (Ph–CH_2), 75.0 (OCH_2), 32.8 (SCH_2), 34.6, 34.1 (C(CH$_3$)$_3$), 31.7, 31.1 (C(CH$_3$)$_3$). Anal. Calcd for $C_{116}H_{132}O_8S_{10}$ (1974.91): C, 70.55; H, 6.74; S, 16.23. Found: C, 70.25; H, 6.80; S, 16.35%.

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Novel examples of the *N*-methyl effect on cyclisations of *N*-Boc derivatives of amino alcohols. A theoretical study

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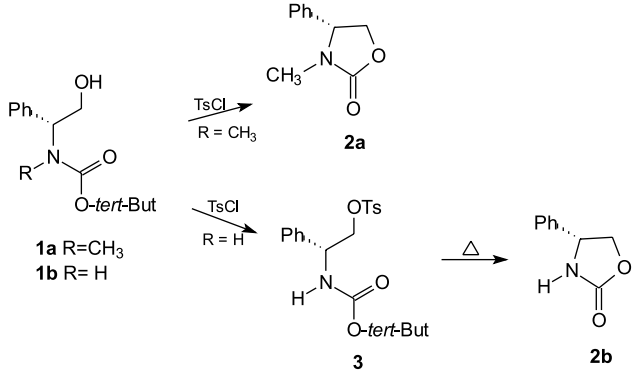
Abstract—New examples of the *N*-methyl effect on the cyclisation of *N-tert*-butoxycarbonyl derivatives of amino alcohols are reported. Ab initio studies for the displacement step with formation of the five-membered heterocycle indicate that the increase of the nucleophile character of the carbonyl oxygen of the carbamate group with the *N*-methyl substitution is responsible for the acceleration of the cyclisation step.

1. Introduction

The acceleration of cyclisation rates by the presence of *gem*-dialkyl groups located between the interacting groups was described by Thorpe and Ingold.[1] They assumed that the increase in the bond angle by mutual repulsion between alkyl groups would be transferred in a scissor-like deformation to the opposite bond angle favouring cyclisation processes. Different hypothesis[2] have been proposed for the explanation of this effect and studied theoretically.

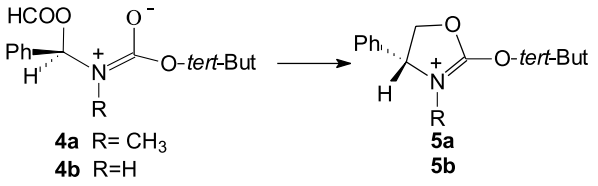
The enhancement of cyclisation rates was also observed in reactions involving carbamates (*N-tert*-butoxycarbonyl (*N*-Boc) group).[3] The beneficial outcome of the *N*-methyl substitution was illustrated for example by the different behaviour of the *N*-Boc *N*-methyl derivatives of (*R*)-phenylglycinol **1a** and its nor-analogue **1b** (Scheme 1). The **a** and **b** acronyms will be related to the *N*-methyl and nor-analogue structures, respectively. Reaction of the substrate **1a** with *p*-toluenesulfonyl chloride (TsCl) at 0 °C directly leads to the oxazolidinone **2a**. However, its nor-analogue **1b** yields the tosylate **3** and subsequent heating at 60 °C is required to give the oxazolidinone **2b**.

The *N*-methyl effect on cyclisation was studied[3] by means of AM1 calculations performed on model reactions with



Scheme 1.

molecules **4a** and **4b** (Scheme 2). The calculations showed that the possibility of a higher population of the '*syn* rotamer', for the explanation of the observed reactivity, should be discarded because the *E* and *Z* structures for molecules **4a** and **4b** were not significant for the differences in reactivity. The authors reported that the only significant



Scheme 2.

Keywords: Cyclocarbamation; Amino alcohols; Thorpe–Ingold effect.
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result was a compression of the C2–N=C4 valence angle NH 122.6° *N*-Me 120.6°, and such effect was indirectly related to the Thorpe–Ingold effect.

In this work new examples of the *N*-methyl effect on the cyclisation of *N*-Boc derivatives of amino alcohols will be reported. Ab initio calculations for the displacement step with formation of these heterocycles have been performed in order to get a better understanding of the *N*-methyl effect.

2. Results and discussion

In the course of our work we have found novel examples of the *N*-methyl effect in the acceleration of cyclisation reactions (Scheme 3). The comparison of the results of the reactions of the *N*-Boc-3-methylamino-1,2-diol **6a** and the nor-analogues **6b** and **7** shows clearly the mentioned effect. In the case of the *N*-Boc aminodiol **7**, (R=H) the tosylation reaction at room temperature yields the expected tosylate **8**.[4] However, the tosylation of the 3-methylamino-1,2-diol **6a** yields the oxazinone **9**. Formation of the oxazinone **9** is achieved by a domino tosylation/cyclisation reaction.

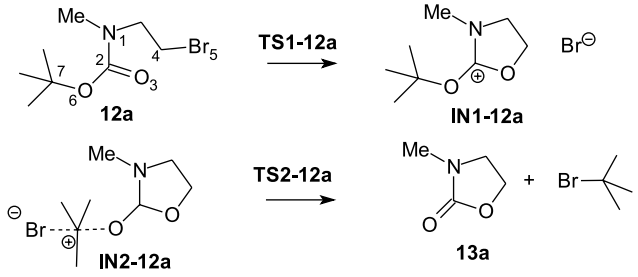
Other example of the *N*-methyl effect is the reaction of the *N*-Boc aminodiol **6a** with DEAD-Ph_3P. The nor-analogue **6b** with DEAD-Ph_3P affords the epoxide **10**,[5], however, the same reaction on the 3-methylamino-1,2-diol **6a** affords the oxazolidinone **11**. The formation of both heterocyclic compounds **9** and **11** can be explained by an increase of the nucleophilicity of the *N*-methyl carbamate that favours the subsequent cyclisation step.

The structure of the new compounds **9** and **11** were determined spectroscopically. The ^{1}H NMR of the compound **9** shows chemical shift values for the vicinal protons H-4 and H-5 appears at δ 4.57 and 4.01 ppm, respectively. The two protons of the methylene group are clearly differentiated at δ 4.17 and 4.27 ppm. It is worth mentioning a long range coupling observed between the proton H-4 and one of the protons of the methylene group that appears at δ 4.17 ppm. This coupling suppose a conformation of the six membered ring with H-4 in equatorial disposition. The coupling constant of 1.5 Hz for $^3J_{4,5}$ is in agreement with this conformation, where phenyl and hydroxyl groups are arranged in *trans* diaxial position.

In the ^{1}H NMR of compound **11**, the signals of the hydrogen atoms of the methylene group were also differentiated and appeared both as double of doublets (J=12, 3.3 Hz) at δ 3.87 and 3.61 ppm. The coupling constant between the protons 4-H and 5-H was 7 Hz, similar to other *trans*-4,5-disubstituted oxazolidin-2-ones.[6] The *trans* disposition of the substituents was confirmed by ^{1}H NOE experiments. On irradiation of the proton 4-H, no significant NOE was observed on the proton H-5. However, on irradiation at the phenyl group protons a positive NOE was measured for the proton H-5. Similarly the proton H-4 gave positive NOE on irradiation of the methylene group protons.

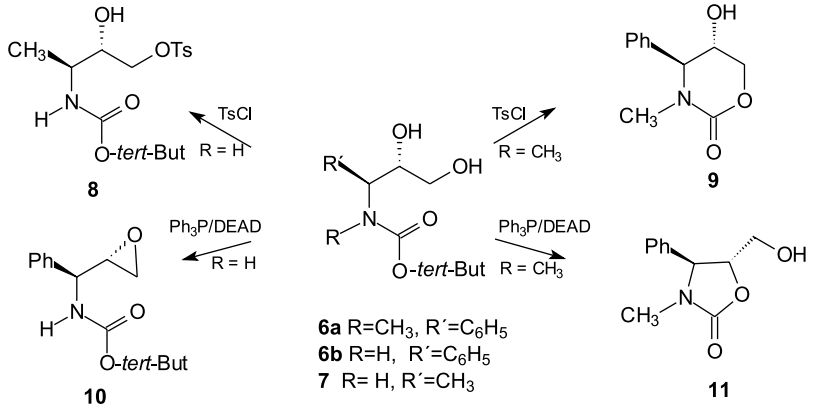
2.1. Theoretical study of the *N*-methyl effect

The *N*-methyl effect on the cyclisation of these *N*-*tert*-butoxycarbonyl derivatives was studied using ab initio methods. This study comprises two parts: in the first one the mechanism of the reaction of the *N*-(2-bromoethyl)-*N*-methyl carbamate **12a** with formation of the oxazolidinone **13a** will be presented (see Scheme 4). In the second part the *N*-methyl effect on the formation of these heterocycles will be studied by comparing the cyclisation step of the *N*-methyl derivative **12a** and its nor-analogue **12b** (see Scheme 5). The role of ring-size and the leaving group will be also considered.



Scheme 4.

2.1.1. Study of the formation of the oxazolidinone 13a from the *N*-(2-bromoethyl)-*N*-methyl derivative 12a. The first step of the mechanism of the transformation of the *N*-(2-bromoethyl)-*N*-methyl derivative **12a** in the oxazolidinone **13a** is the intramolecular displacement of the bromide ion on **12a** to give the five-membered heterocyclic intermediate **IN1**-**12a** (see Scheme 4). The second step is



Scheme 3.

the extrusion of the *tert*-butyl framework on this intermediate to give the oxazolidinone **13a**. This extrusion was modelled by capture of the *tert*-butyl cation by the bromide on the ion-pair **IN2-12a** to yield *tert*-butyl bromide.

It is well-known that the necessity to use diffuse functions in order to describe those chemical processes, where some negative charge is involved, but this demand is an additional computational cost. The Menshutkin reaction of amine bases with methyl halides, including bromide atom, has been widely studied at different computational levels using a variety of basis set, some of them including diffuse sp shell to halide atom in order to describe the charge separation problem.[7] Recently Paneth et al.[8] have used calculation at the HF/6-31G* level of theory augmented by the PCM continuum solvent model for studying substituent and solvent effects on the kinetic isotope effects of Menshutkin reactions. To validate the this level of theory on which the discussion is based, the cyclisation step of **12a** and its nor-analogue **12b** were studied at the HF/6-31G* and HF/6-31+G* levels. The energetic results are summarised in Table S1 in supplementary material. With the inclusion of diffuse functions the total energies of the stationary points involved in the cyclisation step decrease between 16.7 and 17.7 kcal/mol; however, this stabilisation has a minor incidence on the relative energies. The activation energies for the cyclisation step decrease by 0.4 (**TS1-12a**) and 1.2 (**TS1-12b**) kcal/mol, while the methyl effect measured by the difference between the activation energies see later, increases by 0.8 kcal/mol. This comparative analysis allows to establish the HF/6-31G* level as a reasonable level for the study of the methyl substitution on these cyclisation reactions.

The thermodynamic parameters for the reaction of **12a** are given in Table 1. The first step with formation of the five-membered intermediate **IN1-12a**, presents an activation enthalpy of 33.2 kcal/mol, and it is endothermic in 28.4 kcal/mol. The subsequent extrusion of the *tert*-butyl framework was modelled by capture of the *tert*-butyl cation by the bromide ion. This capture requires the formation of the intermediate **IN2-12a** in which the bromide is forming an ion-pair with the *tert*-butyl framework. The ion-par **IN2-12a** is ca. 4.5 kcal/mol higher in energy than **IN1-12a**. However, the subsequent extrusion of the *tert*-butyl group with formation of the 1,3-oxazolidin-2-one **13a** has not an appreciable barrier. The gas-phase calculations afforded similar relative enthalpies for **TS1-12a**, 33.2 kcal/mol, associated to the cyclisation step, and for **TS2-12a**, 33.0 kcal/mol, associated to the *tert*-butyl extrusion; however, inclusion of the entropy to the free energies makes the first step ca. 4 kcal/mol higher than the second.

Table 1. Thermodynamic data[a] (relative enthalpies, ΔH, and free energies, ΔG, in kcal/mol, and relative entropies, ΔS, in kcal/mol K) computed at 298.15 K for the formation of 1,3-oxazolidin-2-one **13a**

	ΔH	ΔS	ΔG
TS1-12a	33.2	−2.9	34.1
IN1-12a	28.4	−0.5	28.6
IN2-12a	33.0	10.3	29.9
TS2-12a	33.0	11.2	29.7
13a+*tert*-ButBr	−0.8	15.0	−5.3

[a] Energies relative to **12a**.

Note that while the cyclisation step has a negative activation entropy, −2.9 cal/mol K, this value for the extrusion of the *tert*-butyl group is positive in ca. 11 cal/mol K. Therefore, the first is the rate-limiting step of the overall process. The existence of an alternative mechanism for the *tert*-butyl extrusion, including formation of isobutene with lower free activation energy does not modify our hypothesis for the first rate-limiting step.

The geometries of the transition structures **TS1-12a** and **TS2-12a** are represented in Figure 1. The lengths of the O3–C4 forming bond and C4–Br5 breaking bond at **TS1-12a** are 1.822 and 2.628 Å, respectively. The O3–C4–Br5 bond angle at this transition structure (TS), 2.0 degrees, indicates that these atoms are in a line. Inclusion of diffuse functions at the HF/6-31+G* level does not modify these geometrical parameter, the lengths of the O3–C4 forming bond and C4–Br5 breaking bond at **TS1-12a** are 1.824 and 2.647 Å, respectively. At **TS2-12a**, the lengths of the O6–C7 breaking bond and the C7–Br5 forming bond are 2.636 and 3.175 Å, respectively. The large O6–C7 distance indicates that the O6–C7 breaking bond is very advanced. The O6–C7 and C7–Br5 distances at the intermediate **IN2-12a**, 2.482 and 3.365 Å, respectively, are closer to those found at **TS2-12a**. In this intermediate, the *tert*-butyl group presents a near planar arrangement stabilised by both carbonyl oxygen and bromide ion. The closer geometry of the intermediate **IN2-12a** to **TS2-12a** together with the strong exothermic character of the process are in agreement with the Hammond postulate.[9]

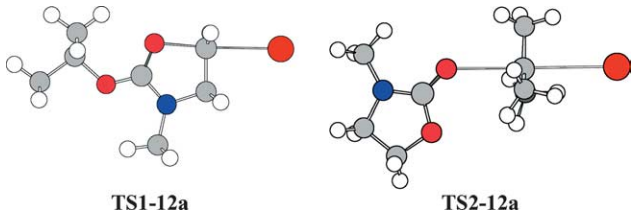
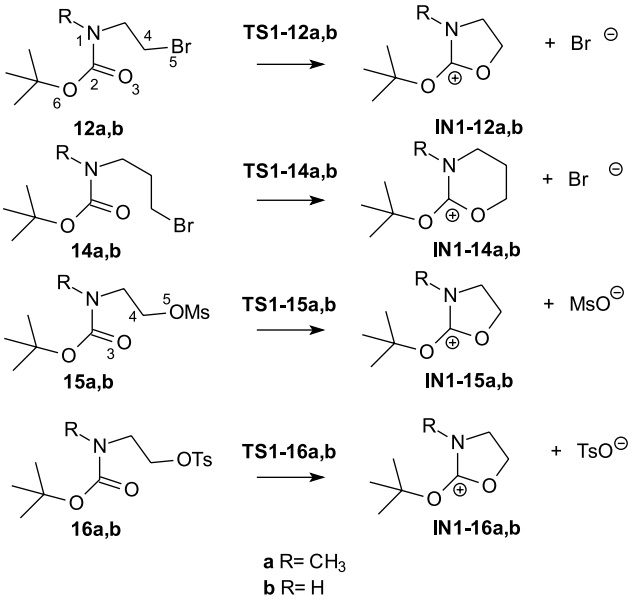


Figure 1. Optimised geometry of **TS1-12a** and **TS2-12a**.

Since some species on the reaction path have an ionic character and solvent effects can stabilise them, they were taken into account by optimisation of the gas-phase structures using the PCM model. The energetic results are given in Table 2. Solvent effects stabilise all stationary points between 2 and 21 kcal/mol. The most stabilised species are the TSs and the intermediates due to their ionic nature. In condensed phase **TS2-12a** is 4.2 kcal/mol more stable than **TS1-12a**. In consequence, solvent effects states the cyclisation step as the rate-determining of the overall process. Inclusion of solvent effects on the geometry optimisation increases slightly the O3–C4 forming bond at **TS1-12a**, 0.1 Å, while the C4–Br5 breaking bond decreases in the same extension. In chloroform **TS1-12a** is slightly more advanced as a consequence of the stabilisation of the charge separation that takes place along the cyclisation step.

2.1.2. Study of the *N*-methyl effect on the cyclisation step. The *N*-methyl effect on the formation of five-membered heterocycles was studied by comparing the activation entalphies for the cyclisation step of the *N*-methyl derivative **12a** and its nor-analogue **12b** (see Scheme 5). The



Scheme 5.

Table 2. Relative energies in chloroform (ΔE_{sol}, in kcal/mol) of the stationary points involved at the cyclisation reactions of **12a** and **12b**

	ΔE_{sol}
TS1-12a	26.8
IN1-12a	11.8
IN2-12a	15.5
TS2-12a	22.6
13a	−3.1
TS1-12b	30.5
IN1-12b	17.1

Energies relative to **12a** and **12b**.

geometries of the TSs are given in Figure 2, while the thermodynamic data are summarised in Table 3. The intramolecular displacement of bromide anion with formation of the five-membered ring at nor-derivative **12b** presents an activation enthalpy of 37.8 kcal/mol. This barrier is 4.6 kcal/mol higher than that obtained for the *N*-methyl derivative **12a**, 33.2 kcal/mol. Therefore, the *N*-methyl effect has a significant incidence on the rate of the cyclisation step in clear agreement with the experiments.

The calculations show that the *N*-methyl intermediate **IN1-12a** is 5.9 kcal/mol more stable than the nor-analogue **IN1-12b**. This larger stabilisation can be rationalised taking into account the carbocationic nature of these intermediates, where the positive charge is mainly located at the carboxyl

Table 3. Thermodynamic data (relative enthalpies, ΔH, and free energies, ΔG, in kcal/mol, and relative entropies, ΔS, in kcal/mol K) computed at 298.15 K for the TSs and intermediates involved at the cyclisation step

	ΔH	ΔS	ΔG
TS1-12a	33.2	−2.9	34.1
IN1-12a	28.4	−0.5	28.6
TS1-12b	37.8	−3.2	38.7
IN1-12b	34.3	−8.9	34.6
TS1-14a	36.9	−3.3	37.9
IN1-14a	13.2	−3.0	16.2
TS1-14b	38.5	−4.3	39.8
IN1-14b	17.6	−10.7	20.6
TS1-15a	34.1	1.3	33.7
IN1-15a	11.0	−3.9	12.2
TS1-15b	36.9	1.7	36.4
IN1-15b	26.3	−0.9	26.5
TS1-16a	35.0	4.2	33.8
IN1-16a	22.8	5.0	21.3
TS1-16b	37.2	−8.3	39.7
IN1-16b	27.5	0.3	27.4

Energies relative to the 3-amino-1,2-diols.

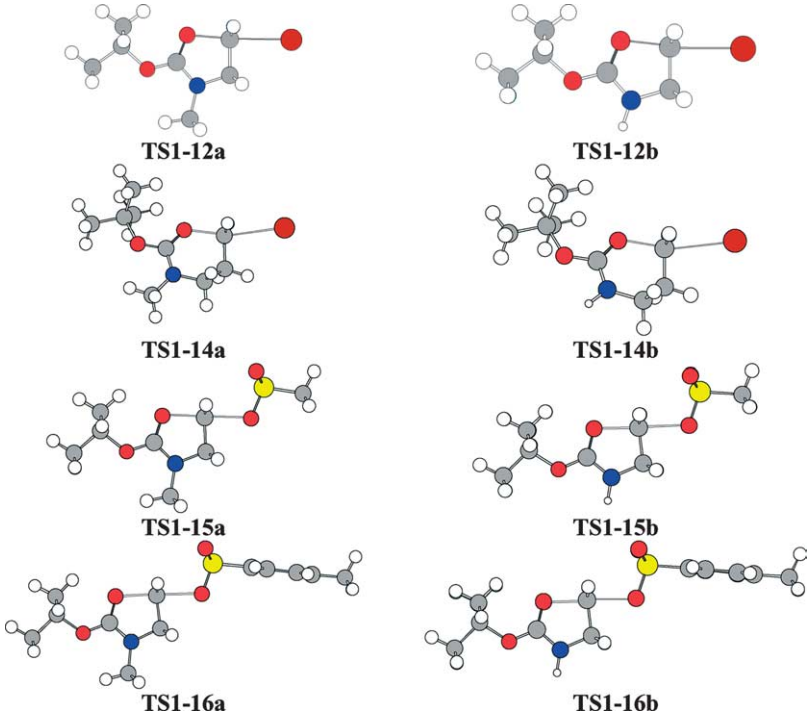
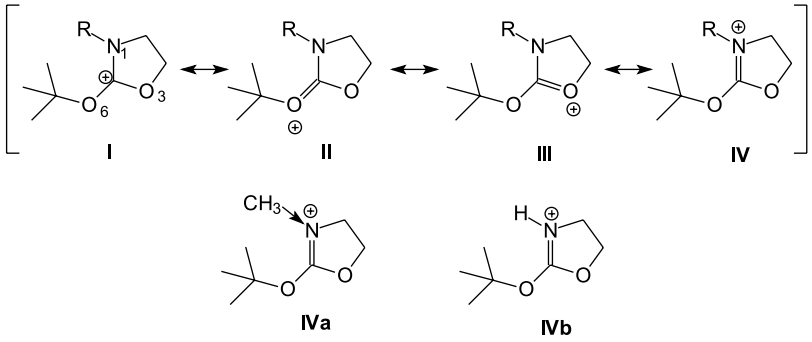


Figure 2. Optimised geometries of the transition structures involved in the cyclisation step.



Scheme 6.

C2 carbon atom. These species are stabilised by delocalisation of the lone-pairs of the N1 nitrogen and O3 and O6 oxygen atoms on the carbocationic C2 carbon (see the corresponding Lewis structures in Scheme 6). The electron-releasing character of the methyl group present on the nitrogen atom causes a larger stabilisation of the Lewis structure **IVa** relative to **IVb**. In consequence **IVa** has a large contribution to the resonant structure of the **IN1-12a**, it being more stabilised than its nor-analogue intermediate **IN1-12b**. This electronic effect that is also present at the corresponding TSs is responsible for the acceleration found with the *N*-methyl substitution. In consequence, the *N*-methyl effect can be rationalised as an increase of the nucleophilicity of the carbamate framework that facilitates the displacement step.

Inclusion of solvent effects produces a larger reduction of the activation energy for **TS1-12b**, 26.8 kcal/mol, than for **TS1-12a**, 30.5 kcal/mol (see Table 2). In consequence, solvent effects diminish the *N*-methyl effect to 3.7 kcal/mol as a consequence of a larger stabilisation of **TS1-12b**. Note that while inclusion of diffuse functions increases the *N*-methyl effect in 0.8 kcal/mol, the inclusion of solvent effects by the PCM model decreases it in 0.9 kcal/mol.

The activation enthalpy for the formation of the six-membered heterocycle at **14a** is ca. 4 kcal/mol lower than that for **12a** (see Table 3). The larger activation enthalpy found at the formation five-membered heterocycle can be related with the strain associated to the five membered ring. Now, the *N*-methyl effect decreases the activation enthalpy in 1.6 kcal/mol. In consequence, the *N*-methyl effect has a larger incidence in the formation of the five-membered ring than in the six one.

Finally, the displacement of the methanosulfonyl and *p*-toluensulfonyl groups was also studied in order to state the role of the leaving group (Scheme 5). The activation enthalpies associated to **TS1-15a** and **TS1-16a** are slightly larger than that associated to **TS1-12a** (see Table 3). For the nor-analogue derivatives the activation enthalpies are ca. 2.8 kcal/mol larger than that for the *N*-methyl derivatives. This energy difference that is lower than that for the bromide derivative indicates that the nature of the leaving group appears to have some incidence in the *N*-methyl effect.

The geometries of the transition structures associated to these displacement reactions are given in Figure 2. The more relevant lengths and theirs corresponding bond order[10] (BO) are summarised in Table 4. For the TSs associated to the bromide displacement the lengths of the O3–C4 forming bond and the C4–Br5 breaking bond are in the range: 1.80–1.84 Å, and 2.63–2.75 Å, respectively. For the TSs associated to the mesylate and tosylate displacement the lengths of the O3–C4 forming bond and the C4–O5 breaking bond are in the range: 1.87–1.88 Å and 2.07–2.10 Å. The O3–C4 forming bonds at the *N*-methyl derivatives are slightly larger than those at the nor-analogue derivatives, indicating that these TSs are more delayed.

3. Conclusions

Two new examples of the *N*-methyl effect on the cyclisation of *N*-Boc carbamates with formation of five and six-membered heterocycles are reported. Ab initio calculations for the cyclisation step show that the *N*-methyl substitution decreases the activation energy associated to the

Table 4. Selected lengths, l in Å, and bond order, BO, of the TS involved at the cyclisation step

	O3–C4		C4–X5 (Br or O)		C2–O6		N1–C2	
	l	BO	l	BO	l	BO	l	BO
TS1-12a	1.822	0.32	2.628	0.21	1.239	1.14	1.319	1.06
TS1-12b	1.804	0.33	2.655	0.20	1.239	1.14	1.317	1.06
TS1-14a	1.835	0.30	2.734	0.17	1.236	1.16	1.328	1.05
TS1-14b	1.833	0.31	2.752	0.16	1.234	1.16	1.325	1.06
TS1-15a	1.882	0.28	2.069	0.19	1.232	1.17	1.325	1.04
TS1-15b	1.872	0.29	2.096	0.18	1.231	1.17	1.324	1.04
TS1-16a	1.880	0.28	2.069	0.19	1.233	1.16	1.325	1.04
TS1-16b	1.869	0.29	2.096	0.18	1.232	1.17	1.323	1.05

intramolecular displacements between 1.6 and 4.5 kcal/mol. While the nature of the leaving group, bromide, mesylate or tosylate, appears to have some incidence, formation of the six membered ring presents a remarkable decreasing of the *N*-methyl effect. The *N*-methyl effect can be understood as a larger stabilisation of the transition structures associated to the cyclisation step of the methyl derivative as a consequence of the electron-releasing effect of the methyl group that stabilised the positive charge that is developing at the carbonyl carbon atom of the Boc along the nucleophilic displacement.

4. Experimental

4.1. General

Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. Solvents were distilled prior to use. Thin-layer chromatography was performed on Merck $^{60}F_{254}$ sheets. Preparative column chromatography was performed on Merck Kieselgel 60 (230–240 mesh) silica gel. IR spectra were recorded on a FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Avance DPX Bruker 500 MHz or an Avance 400 MHz Bruker or an Avance DRX Bruker 300 MHz spectrometers, in $CDCl_3$ solutions. Chemical shifts were recorded in parts per million (ppm), downfield from internal Me_4Si. High-resolution mass spectral data were obtained on a VG Autospec, TRIO 1000 (Fisons) instrument. The ionisation mode used in mass spectra were electron impact (EI), or chemical ionisation (CI) at 70 eV.

4.2. Computational details

Ab initio calculations were performed at the HF/6-31G* and HF/6-31+G* theory levels.[11] The optimisations were carried out using the Berny analytical gradient optimisation method.[12] The stationary points were characterised by frequency calculations in order to verify that the TSs have one and only one imaginary frequency. Thermal corrections to enthalpy and entropy values were evaluated at 298.15 K. The computed values of enthalpies energies were estimated by means of the HF/6-31G* potential energy barriers, along with the gas-phase harmonic frequencies.[11] The solvent effect, chloroform, was considered by HF/6-31G* geometry optimisation of the stationary points involved on the reaction using a relatively simple self-consistent reaction field (SCRF)[13] based on the polarisable continuum model (PCM)[14] of the Tomasi's group. All calculations were carried out with the Gaussian 98 suite of programs.[15]

4.2.1. 3-[(*tert*-Butoxycarbonyl)(methyl)amino]-3-phenyl-1,2-propanediol (6a). To a solution of 3-methylamino-3-phenyl-1,2-propanediol[16] (0.3 g, 2 mmol) in chloroform (2 mL), a solution of di-*tert*-butyl dicarbonate (0.44 g, 2 mmol) in chloroform (1 mL) was added drop wise. After stirring at room temperature for 24 h, the solvent was concentrated to dryness. The residue was chromatographed on triethylamine-pretreated silica gel (2.5% v/v), eluting with 3:2 hexane/ethyl acetate mixture to afford the amino alcohol **6a** (83%). Colourless oil. IR (KBr): ν_{max} 3402, 1665 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.39 (s, 3H), 3.64 (m, 2H), 4.13 (m, 1H), 5.19 (m, 1H), 7.28 (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 28.20 (q), 29.97 (q), 58.56 (d), 63.13 (t), 69.09 (d), 80.56 (s), 127.47 (d), 128.25 (d), 129.03 (d), 136.73 (s), 156.99 (s); HRCI-MS *m*/*z* calcd for $[M+H]^+$ $C_{15}H_{24}NO_4$: 282.1705, found: 282.1716.

4.2.2. 5-Hydroxy-3-methyl-4-phenyltetrahydro-1,3-oxazin-2-one (9). *p*-Toluensulfonyl chloride (0.52 g, 2.2 mmol) was added to a solution of the amino alcohol **6a** (0.7 g, 2.5 mmol) in pyridine (5 mL) at 0 °C. The mixture was stirred at 0 °C for 4 h and kept at 4 °C for 48 h and then stirred 24 h at room temperature. The reaction mixture was quenched with addition of H_2O (9 mL) and extracted with dichloromethane (3×15 mL). The combined organic layers were washed with 2 M aqueous HCl, then saturated solution of $NaHCO_3$, dried (Na_2SO_4) and concentrated to dryness. The residue was chromatographed on triethylamine-pretreated silica gel (2% v/v), eluting with 1:1 hexane/ethyl acetate mixtures to afford the 1,3-oxazin-2-one **9** (60%). White solid. Mp 155–157 °C. IR (KBr): ν_{max} 3257, 1655 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.98 (s, 3H), 4.01 (bs, 1H, H-5), 4.17 (ddd, 1H, J=11.8, 2.4, 1.5 Hz, H-6eq), 4.19 (bs, 1H, OH), 4.27 (dd, 1H, J=11.8, 1.5 Hz, H-6ax), 4.57 (bs, 1H, H-4), 7.29 (m, 2H), 7.37 (m, 1H), 7.45 (m, 2H); ^{13}C NMR (75.4 MHz, CD_3OD) δ 37.1 (q), 68.5 (t), 68.6 (d), 69.8 (d), 127.9 (d), 129.8 (d), 130.7 (d), 139.8 (s), 156.6 (s); HREI-MS *m*/*z* calcd for $[M]^+$ $C_{11}H_{13}NO_3$: 207.0895, found: 207.0922.

4.2.3. 5-Hydroxymethyl-3-methyl-4-phenyl-1,3-oxazolidin-2-one (11). A mixture of the amino alcohol **6b** (0.52 g, 1.87 mmol), triphenylphosphine (0.52 g, 1.96 mmol) and diethyl azodicarboxylate (0.37 g, 1.96 mmol) in chloroform (16 mL) was refluxed for 36 h. After elimination of the solvent at reduced pressure, the residue was chromatographed on triethylamine-pretreated silica gel (2% v/v), eluting with 2:3 hexane/ethyl acetate mixture afforded the oxazolidin-2-one **11** (80%). White solid. Mp 102–103 °C (hexane/chloroform). IR (KBr): ν_{max} 3383, 1738 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (s, 3H), 3.61 (dd, 1H, J=12.4, 3.3 Hz, CH_2O), 3.87 (dd, 1H, J=12.4, 3.3 Hz, CH_2O), 3.90 (bs, 1H), 4.22 (dt, 1H, J=7, 3.3 Hz, H-5), 4.57 (d, 1H, J=7 Hz, H-4), 7.31 (m, 2H), 7.39 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 29.5 (c), 61.3 (t), 63.2 (d), 82.4 (d), 127.2 (d), 129.1 (d), 129.5 (d), 137.8 (s), 158.6 (s); HREI-MS *m*/*z* calcd for $[M]^+$ $C_{11}H_{13}NO_3$: 207.0895, found: 207.0888.

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Supplementary data

Supplementary data associated with this article can be found at 10.1016/j.tet.2004.10.038

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Microwave accelerated Pictet–Spengler reactions of tryptophan with ketones directed toward the preparation of 1,1-disubstituted indole alkaloids

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Abstract—Using the Pictet–Spengler reactions of tryptophan with aldehydes under acidic conditions at ambient temperature, diastereoisomers of 1,3-disubstituted-1,2,3,4-tetrahydro-β-carbolines could readily be furnished in short time (0.5–4 h) with good to excellent yields (50–98%). Though intrinsically slow in reaction rates, ketone reactions can be accelerated (from days to minutes) using microwaves in open vessels with high isolated yields (67–99%), making those carbolines feasible reaction intermediates for the synthesis of both natural and unnatural indole alkaloids. Preparation of two indole alkaloids, tetrahydro-β-carbolinediketopiperazines and tetrahydro-β-carbolinehydantoins, were briefly discussed.

1. Introduction

Since its discovery, the Pictet–Spengler reaction has been extensively studied and continues to be a focus of research in areas including the preparation of new heterocycles for combinatorial applications and its incorporation in total synthesis of natural and unnatural products.[1] For example, Katzenellenbogen recently reported a vinylogous Pictet–Spengler cyclization as the key step aiming to prepare breast tumor imaging agents.[2] Though broadly useful, a vast of the literature concerning the Pictet–Spengler reactions so far were most with aldehydes or activated ketones such as 1,2-dicarbonyl compounds. The reactions with ketones were, however, known to be either far less reactive at room temperature (typical reaction time in days) or sluggish under reflux conditions. Some arylketones even do not react at all with, for example, tryptophan and consequently these ketone reactions have seldom been addressed.[3]

The low reactivity from ketones may be attributed to, at first, the slow imine formation and thus a sterically sensitive, if not totally prohibited, cyclization of the resulting imine to produce a congested tetrasubstituted C-1 carbon of the tetrahydro-β-carbolines or tetrahydroisoquinolines. For ketone reactions previously reported in literature, most results either required long reaction times or were with low reaction yields. Only few gave satisfactory results.[2,3] Owing to its intrinsically slow reaction, the development of rapid and convenient methods would therefore be of value to speed up the optimization process of the ketone reactions. Of these, microwaves can offer its great potential in expediting synthetic organic reactions.[4] We report here that the Pictet–Spengler reaction of tryptophan **1** with ketones **2** by microwaves proceeds readily to produce 1,1-disubstituted tetrahydro-β-carbolines **3** in much shorter reaction times with good to excellent isolated yields (Scheme 1). Most significantly, this microwave-accelerated Pictet–Spengler reaction is clean and the tetrahydro-β-carboline adducts are only products under our experimental conditions.

2. Results and discussion

The Pictet–Spengler reaction is an acid-catalyzed intramolecular cyclization of the intermediate imine of tryptophan, formed by condensation with a carbonyl compound, to give 1,2,3,4-tetrahydro-β-carbolines **3**.[1] In our early investigation of this reaction,[3c,5e] we utilized a conventional Pictet–Spengler synthetic protocol of refluxing the tryptophan and the carbonyl compound in a solvent such as toluene to speed up the reaction. We found that, although aldehydes cyclized readily, ketones often gave product mixtures contaminated with the failed-to-cyclize imine intermediates and other side-products of unknown

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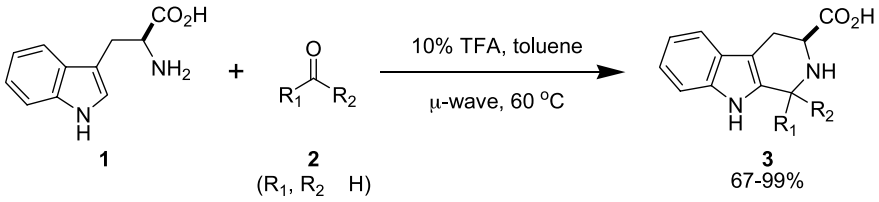


Scheme 1. The Pictet–Spengler reaction.

structures. Only upon extended heating at lower temperature, we were able to obtain clean reaction with good product yield. We thus turned our attention to alternative means known to accelerate organic reactions. We, and others, have recently reported that microwaves readily facilitated the Pictet–Spengler reaction of tryptophan with both aliphatic and aryl aldehydes.[5] For example, under our previously established experimental condition, tryptophan completed the Pictet–Spengler reaction with benzaldehyde in toluene within only 15 s with an 83% isolated yield.[5e] In this paper, we demonstrate that ketone reactions with tryptophan could also be effectively accelerated using microwaves.

As shown in Table 1 for the reactions with L-tryptophan, ketones give the slowest reaction rates at ambient temperature (13 h–76.5 days, entries 9–12, compounds **3i**–**3l**) and all aliphatic aldehydes studied complete the Pictet–Spengler reaction in 1 h (entries 1–6, compounds **3a**–**3f**) using 4 equiv of carbonyl compounds. Although they were significantly different in reaction rates, both ketones and aldehydes were furnished with good to excellent yields (50–98%). Except for acetaldehyde, aliphatic aldehydes with enolizable protons, in general, tended to produce less Pictet–Spengler adducts, presumably due to competitive aldol reactions under experimental conditions (50–68%, entries 2–6, compounds **3b**–**3f**). In all aldehyde cases, the carboline products were isolated as mixtures of diastereoisomers. The assignment for *cis*/*trans*-tetrahydro-β-carbolines was based on a detailed study of the ^{13}C NMR spectroscopy well-established by Cook.[1] Thus, the signals for C-1 and C-3 in the *trans*-isomer appeared at higher field in the carbon spectrum than the analogous carbons of the corresponding *cis*-isomer, due to the 1,3-interactions present in the *trans*-isomer. Using benzaldehyde and *p*-tolualdehyde as representative examples, reactions from aromatic aldehydes were often completed in 3–4 h with high isolated yields (entries 7 and 8, compounds **3g** and **3h**). With L-tryptophan, acyclic ketones at ambient temperature slowly underwent the Pictet–Spengler reaction and eventually produced the corresponding adducts with excellent yields (entries 9 and 10, compounds **3i** and **3j**). The reactions of the cyclic ketones, cyclohexanone and cyclopentanone, yielded the corresponding 1,1-spirocycloalkylated tetrahydro-β-carbolines with exceedingly different rates (entries 11 and 12, compounds **3k**–**3l**). This result suggested that the Pictet–Spengler cyclization, as anticipated, is sensitive to the steric congestion.[3j] Moreover, reactions of D-tryptophan with aldehydes or ketones expectedly gave similar, if not identical, results in reaction time, yield, and product diastereoisomeric ratio (data not shown in Table 1, but provided in Section 4).

Because the slow ketone reactions presented in Table 1 were totally impractical for use in the library preparation of 1,1-disubstituted natural and unnatural products, we subsequently decided to employ large excess (12 equiv) of inexpensive and readily available ketones to further expedite the Pictet–Spengler reactions. Besides it can shorten the reaction time, the employment of excessive ketone may also compensate for competitive side reactions such as aldol condendsation with the aim that low reactivity of ketone will not plaque the reaction. We were pleased to find that at ambient temperature the rates of the

Table 1. Synthesis of tetrahydro-β-carbolines from aldehydes and ketones with L-tryptophan at ambient temperature[a]

Entry	Product	R_1COR_2	Tryptophan[b]	Reaction time	Yield (%)[c]	Diastereomeric ratio[d]
1	**3a**	Acetaldehyde	L-form	30 min	93	53:47
2	**3b**	Propionaldehyde	L-form	1 h	68	50:50
3	**3c**	Butyraldehyde	L-form	1 h	51	50:50
4	**3d**	Valeraldehyde	L-form	1 h	58	50:50
5	**3e**	Capronaldehyde	L-form	1 h	50	42:58
6	**3f**	Phenylacetaldehyde	L-form	40 min	56	27:73
7	**3g**	Benzaldehyde	L-form	3 h	95	61:39
8	**3h**	*p*-Tolualdehyde	L-form	4 h	78	62:38
9	**3i**	2-Butanone	L-form	16.5 days	87	58:42
10	**3j**	3-Pentanone	L-form	76.5 days	88	—
11	**3k**	Cyclohexanone	L-form	13 h	84	—
12	**3l**	Cyclopentanone	L-form	15.5 days[e]	74[f]	—

[a] The reaction condition: tryptophan, 255 mg (1.25 mmol); aldehyde or ketone, 5.0 mmol; TFA, 10% (v/v); dichloromethane, 12.5 mL.
[b] Tryptophan of both forms were used in this study. Only results from L-tryptophan were shown in the table. Results of D-tryptophan were provided in Section 4.
[c] Isolated yield unless otherwise mentioned. The tetrahydro-β-carbolines were only products.
[d] The stereochemistry of the diastereomers obtained from aldehyde reaction was readily determined by ^{13}C NMR developed by Cook (Ref. 1). For the given pair of diastereomers derived from aldehyde reaction, the second number represents the *trans* isomer in this table and its diastereomeric ratio was measured by C18-HPLC. In a pair of diastereomers derived from 2-butanone reaction, the second number was arbitrarily assigned as the *trans* isomer in this table.
[e] Time required to completely consume the starting tryptophan.
[f] Analytical yield. The Pictet–Spengler adduct was contaminated with the failed-to-cyclize imine intermediate.

Table 2. Cyclization of L-tryptophan with ketones at ambient temperature[a]

Entry	Product	R_1COR_2	[L-Tryptophan]/[R_1COR_2]					
			1:4			1:12		
			Reaction time	Yield[b] (%)	Diastereomeric ratio[c]	Reaction time	Yield[b] (%)	Diastereomeric ratio[c]
1	**3m**	Acetophenone	116 days	85[d]	23:77	40 days	62	22:78
2	**3i**	2-Butanone[e]	16.5 days	87	58:42	2.5 days	93[f]	47:53
3	**3n**	3-Methyl-2-butanone	58 days	99	56:44	12.5 days	99	67:32
4	**3j**	3-Pentanone[e]	76.5 days	88	—	5.5 days	99	—
5	**3k**	Cyclohexanone	13 h	84	—	3.5 h	89	—
6	**3l**	Cyclopentanone	15.5 days	74 [g]	—	1 day	89	—

[a] The reaction condition: tryptophan, 255 mg (1.25 mmol); ketone, 5.0 mmol; (4 equiv) or 15.0 mmol (12 equiv); TFA, 10% (v/v); dichloromethane, 12.5 mL.
[b] Isolated yield unless otherwise mentioned. The tetrahydro-β-carbolines were only products.
[c] The diastereomeric ratio was measured by C18-HPLC. In a pair of diastereomers, the second number was arbitrarily assigned as the *trans* isomer in this table.
[d] The degree of reaction conversion. The tetrahydro-β-carbolines were only products and the progress of the reaction was analysis by C18-HPLC.
[e] Under the conditions of neat TFA or 50% TFA, the progress of the Pictet–Spengler reaction was negligible: 2-butanone in neat TFA, 2% reaction conversion was detected after 4.5 days; 2-butanone in 50% TFA, only 5% conversion was monitored after 7.5 days; 3-pentanone in neat TFA, 5% reaction conversion was measured after 16.5 days.
[f] In case of [L-tryptophan]/[2-butanone]=1:8, quantitative yield was isolated after 4.5 days.
[g] Analytical yield. The Pictet–Spengler adduct was contaminated with the failed-to-cyclize imine intermediate.

Pictet–Spengler reactions with ketones were indeed improved (Table 2). Results presented in Table 2 clearly show that all ketones studied completed the Pictet–Spengler reactions not only in shorter reaction times but also with better yields. Most significantly, cyclopentanone did not complete its reaction after 15.5 days when employing 4 equiv of ketone, but using 12 equiv of ketone the reaction was complete in 1 day with an 89% isolated yield (entry 6 in Table 2). These results set the stage for ultimate improvement of the Pictet–Spengler reactions of tryptophan with ketones by microwaves.

Using large excess of ketones in toluene, we utilized microwaves to accelerate the synthesis of 1,1-disubstituted tetrahydro-β-carbolines and were pleased to find that in the presence of microwaves the Pictet–Spengler reaction proceeds remarkably well and cleanly with both aliphatic and aromatic ketones, though the latter are much less reactive. The details of the investigation are given in Table 3. Under our experimental conditions, the microwaves-accelerated Pictet–Spengler reaction appears to conduct superior with cyclic ketones than acyclic ketones (entries 5 and 6 in Table 3, compounds **3k** and **3l**). Specifically, under the condition of microwave irradiation (60 °C and 150 W), cyclic ketones such as cyclohexanone and cyclopentanone reacted cleanly with tryptophan to yield the corresponding spiro-fused tetrahydro-β-carbolines in 10 and 20 min, respectively, with quantitative isolated yields. Both cyclohexanone and cyclopentanone reactions if carried out at ambient temperature required, however, much longer reaction times (6 h and 2.5 days, respectively) with less yields (Table 3). Since the Pictet–Spengler reaction is sensitive to steric hindrance at the site of cyclization, among acyclic ketones investigated 2-butanone gave the fastest rate (20 min using microwaves) and 3-methyl-2-butanone was far less reactive (15 h by microwaves) as expected (entry 2 vs entry 3 in Table 3, compound **3i** vs compound **3n**). The preparation of the tetrahydro-β-carboline obtained from acetophenone reaction with tryptophan deserves additional comment (compound **3m** in Table 3). Using HPLC to monitor the progress of the acetophenone reaction, we found that it gave an incomplete reaction even after 49 days at ambient temperature (Table 3). When we attempted to shorten the reaction time by increasing the temperature, we observed that, in our case, harsh conditions such as conventional reflux or microwaves at higher temperatures (e.g., 100 °C) gave complete conversion of the starting tryptophan to produce, however, complicated and degraded

Table 3. Microwave-accelerated Pictet–Spengler reaction of L-tryptophan with ketones[a]

Entry	Product	R_1COR_2	Room temperature			Heated at 60 °C[b]			Microwaves[c]		
			Reaction time	Yield[d] (%)	dr[e]	Reaction time	Yield[d] (%)	dr[e]	Reaction time	Yield[d] (%)	dr[e]
1	**3m**	Acetophenone	49 days[f]	90[f]	20:80	95 h	96	25:75	40 h	67	23:77
2	**3i**	2-Butanone	18 h	74	51:49	3.5 h	99	55:45	20 min	96	50:50
3	**3n**	3-Methyl-2-butanone	11.5 days	87	68:32	62 h	86	60:40	15 h	76	55:45
4	**3j**	3-Pentanone	5.5 days	96	—	52 h	99	—	3.3 h	91	—
5	**3k**	Cyclohexanone	6 h	68	—	75 min	99	—	10 min	99	—
6	**3l**	Cyclopentanone	2.5 days	95	—	55 min	99	—	20 min	99	—

[a] The reaction condition: L-tryptophan, 51 mg (0.25 mmol); ketone, 3.0 mmol; (12 equiv); TFA, 10% (v/v); toluene, 2.5 mL.
[b] The reaction was carried out using a conventional heating device with temperature controlled at 60 °C.
[c] The microwave-assisted Pictet–Spengler reaction was carried out in an open vessel and temperature-controlled at 60 °C using a commercial instrument (300 W, Synthewave 402, Prolabo). A 50% microwave power was applied throughout the reaction period to avoid formation of side products of unknown structures.
[d] Isolated yield unless otherwise mentioned. The tetrahydro-β-carbolines were only products.
[e] The diastereomeric ratio (dr) was measured by C18-HPLC. In a pair of diastereomers, the second number was arbitrarily assigned as the *trans* isomer in this table.
[f] The Pictet–Spengler reaction was incomplete under the experimental condition. The number indicated its percentage of reaction conversion after 49 days.

reaction mixtures. Under our optimized and milder condition (60 °C and 150 W), we were pleased that, despite an extended reaction time, microwave irradiation at lower temperature could achieve clean tetrahydro-β-carboline product with acceptable isolated yield (67%) (entry 1 in Table 3). Instead of toluene solvent used in this work, we also employed dimethylformamide, an efficient coupler of microwaves, for the microwave-assisted reaction and found that it took a rather longer time to carry out the desired Pictet–Spengler reaction; for example, only 38% conversion, instead of quantitative conversion in toluene, was observed if the microwave-mediated tryptophan reaction with 2-butanone was carried out at 100 °C for 15 min. Moreover, Table 3 shows that, although the heating with temperature controlled at the same 60 °C using standard laboratory setups undoubtedly facilitated the progress of the Pictet–Spengler reaction, the microwave heating is evidently far better than conventional heating in terms of reaction acceleration. This rate enhancement by microwaves is likely due to the fact that microwaves transfer heat efficiently.[4] Our results presented in Table 3 clearly demonstrate that the presence of microwaves greatly improves the rate of chemical reactions, which can be critical to high throughput library synthesis, not only with a large reduction of reaction time (mostly days at ambient temperature down to hours or even minutes under microwaves) but also with improvement of, in our case, the final yield.

To further demonstrate the usefulness of 1,1-disubstituted tetrahydro-β-carbolines **3** and application of this microwave methodology toward the preparation of natural and unnatural indole alkaloids, we carried out the multistep synthesis of new, ketone-derived demethoxyfumitremorgin C analog **4** and tetrahydro-β-carbolinehydantoins **5a–c** (Scheme 2). Without attempting to optimize reaction conditions and isolated yields, our preliminary result indicated that both classes of compounds can be prepared from 1,1-disubstituted tetrahydro-β-carbolines **3**. For example, starting from L-tryptophan methyl ester, an overall isolated yield of 11% for compound **4** via a three-step synthesis (the Pictet–Spengler reaction, the Schotten–Baumann acylation, and the deprotection and intramolecular cyclization) was obtained (Scheme 2). In addition, good overall yields were achieved for compounds **5a–c** (50–70%) via a two-step synthesis: the Pictet–Spengler reaction and the subsequent hydantoin-forming reaction (Scheme 2). Using 'microwaves in organic synthesis', we are currently conducting the combinatorial preparation of the ketone-based tetrahydro-β-carbolinediketopiperazines **4** as well as tetrahydro-β-carbolinehydantoins **5**. These classes of compounds may possess valuable biological activities.

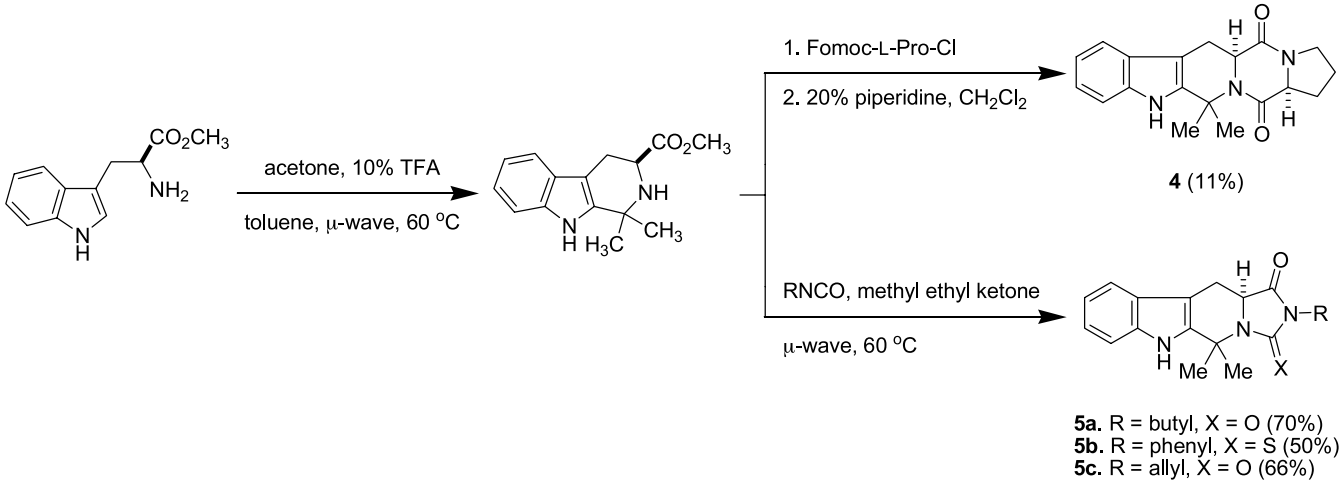
3. Conclusion

In this investigation, we demonstrate that the slow Pictet–Spengler condensation of tryptophan with ketones can be smoothly and cleanly accelerated using microwaves. Because of the rigid heterocyclic skeleton of the 1,1-disubstituted tetrahydro-β-carboline pharmacophore and the vast Pictet–Spengler cyclization literature, our results reported here present an initially impractical but now feasible opportunity for further chemical manipulation of conformationally constrained peptidomimetics, as well as combinatorial preparation of natural and unnatural indole alkaloids.

4. Experimental

4.1. General experimental section

Flash chromatography was performed on silica gel (230–400 mesh). TLC was carried out on aluminum-backed silica plates precoated with silica (0.2 mm), which were developed using standard visualizing agents such as UV fluorescence and ninhydrin. Analytical HPLC was performed on an Agilent 1100 series equipped with a diode array detector, using a C_{18} column (ChemcoPak Chemcosorb 5-ODS-H, 5 μm, 4.6×250 mm, flow rate: 1.0 mL/min). Eluant solvent system: (i) 0–10 min; water, (ii) 10–40 min; acetonitrile/water (0:100 to 100:0) eluant linear gradient over 30 min, then (iii) 40–50 min; 100% acetonitrile for additional 10 min, both solvents contain 0.1% TFA. Compound purity and the *cis*/*trans* diastereoisomeric ratio were measured from integrated peak areas of HPLC chromatographs generated at 280 nm. Unless



Scheme 2. The preparation of demethoxyfumitremorgin C analog **4** and tetrahydro-β-carbolinehydantoins **5a–c**.

otherwise indicated, all reactions were carried out without the aid of dry nitrogen or argon. NMR spectra were recorded on a Bruker AVANCE DPX 400 at 400 MHz (^{1}H) in D_2O and 100.6 MHz (^{13}C) in DMSO-d_6 unless otherwise stated. Chemical shifts were quoted in parts per million (ppm). ^{13}C NMR spectral data included the signals for both *cis* and *trans* diastereoisomers. Melting points were determined on a Fargo MP-2D apparatus (Taiwan, ROC) and are uncorrected. Solvents, reagents, and tryptophan of L- and D-forms were obtained from commercial sources and were used without further purification.

4.2. General procedure for the synthesis of tetrahydro-β-carbolines at ambient temperature using the Pictet–Spengler reaction

In a typical reaction, L- or D-tryptophan (255 mg, 1.25 mmol) was dissolved in dichloromethane (12.5 mL) and trifluoroacetic acid (10%, v/v). The aldehyde or ketone (5.0 mmol) was added in one portion to the stirred mixture at room temperature. The reaction was allowed to proceed until tryptophan was completely consumed as monitored by TLC using the ninhydrin test (0.5–1 h for aliphatic aldehydes; 3–4 h for aromatic aldehydes; 12 h–76.5 days for ketones). Upon completion of the reaction, the reaction mixture was concentrated to dryness under reduced pressure to obtain a residue which was dissolved in dichloromethane, then extracted with acidic water (3×), and lyophilized. In cases that the product was slightly soluble in dichloromethane could result in lower yields. Products were obtained in excellent purity as determined by NMR and HPLC.

4.2.1. Compound 3a. [L-Tryptophan reaction] off-white solid (93% yield, 53:47 diastereomeric mixture); mp 140–148 °C; R_f=0.71 (Butanol/HOAc/H_2O=10:1:1); ^{1}H NMR (400 MHz, D_2O) δ 1.75 (d, J=2.92 Hz, *trans* CH_3, 3H), 1.77 (d, J=2.96 Hz, *cis* CH_3, 3H), 3.13 (dd, J=2.36, 14.18 Hz, *trans* Trp-NCH_2, 1H), 3.23 (dd, J=1.16, 8.42 Hz, *cis* Trp-NCH_2, 1H), 3.45 (dt, J=5.68, 15.28 Hz, *cis/trans* Trp-NCH_2, 2H), 4.43 (dd, J=5.42, 12.1 Hz, *cis* Trp-CHN, 1H), 4.61 (q, J=5.72 Hz, *trans* Trp-CHN, 1H), 4.80 (q, J=6.72 Hz, *trans* MeCHN, 1H), 4.93–5.12 (m, *cis* MeCHN, 1H), 7.04–7.08 (m, *cis/trans* ArH, 2H), 7.15 (qt, J=1.24, 8.24 Hz, *cis/trans* ArH, 2H), 7.35 (dt, J=0.76, 10.7 Hz, *cis/trans* ArH, 2H), 7.48 (dd, J=2.68, 7.82 Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.5, 17.9, 18.7, 22.0, 22.4, 47.4, 49.5, 51.0, 54.9, 103.7, 104.7, 111.5, 118.2, 119.1, 119.2, 119.8, 121.9, 125.6, 130.9, 131.1, 136.3, 136.4, 170.3; FAB-HRMS m/z $[M+H]^+$ calcd 231.1134, obsd 231.1136.

[D-Tryptophan reaction] off-white solid (96% yield, 59:41 diastereomeric mixture); R_f=0.79 (Butanol/HOAc/H_2O=10:1:1); mp 142–151 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.74 (d, J=6.88 Hz, *trans* CH_3, 3H), 1.78 (d, J=4.64 Hz, *cis* CH_3, 3H), 3.13 (dd, J=2.48, 14.14 Hz, *trans* Trp-NCH_2, 1H), 3.24 (dd, J=1.16, 7.48 Hz, *cis* Trp-NCH_2, 1H), 3.46 (dt, J=5.34, 15.62 Hz, *cis/trans* Trp-NCH_2, 2H), 4.46 (dd, J=5.24, 12.16 Hz, *cis* Trp-CHN, 1H), 4.62 (q, J=5.68 Hz, *trans* Trp-CHN, 1H), 4.63–4.95 (m, *cis/trans* MeCHN, 2H), 7.06–7.08 (m, *cis/trans* ArH, 2H), 7.16 (qt, J=5.52, 7.04 Hz, *cis/trans* ArH, 2H), 7.35 (dt, J=0.76, 8.32 Hz, *cis/trans* ArH, 1H), 7.47–7.51 (m, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.4, 17.9, 21.9, 22.4, 47.3, 49.5, 51.0, 54.8, 103.7, 104.7, 111.4, 118.2, 119.1, 119.2, 121.9, 125.6, 130.8, 131.0, 136.2, 136.4, 170.3; FAB-HRMS m/z $[M+H]^+$ calcd 231.1134, obsd 231.1140.

4.2.2. Compound 3b. [L-Tryptophan reaction] off-white solid (68% yield, 50:50 diastereomeric mixture); R_f=0.71 (Butanol/HOAc/H_2O=10:1:1); mp 97–103 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.18 (t, J=2.96 Hz, CH_3, 3H), 1.21 (t, J=4.44 Hz, CH_3, 3H), 2.05–2.07 (m, *cis* $MeCH_2$, 2H), 2.09–2.16 (m, *trans* $MeCH_2$, 2H), 3.14 (ddd, J=1.32, 2.40, 14.18 Hz, Trp-NCH_2, 1H), 3.40–3.51 (m, *cis/trans* Trp-NCH_2, 3H), 4.37 (dd, J=5.12, 12.12 Hz, *cis* Trp-CHN, 1H), 4.62 (q, J=6.16 Hz, *trans* Trp-CHN, 1H), 4.69–4.71 (m, *trans* EtCHN, 1H), 4.78 (t, J=6.60 Hz, *cis* EtCHN, 1H), 7.01–7.08 (qt, J=0.76, 9.76 Hz, *cis/trans* ArH, 2H), 7.15 (t, J=7.12 Hz, *cis/trans* ArH, 2H), 7.36 (dd, J=4.96, 8.14 Hz, *cis/trans* ArH, 2H), 7.48 (d, J=7.88 Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 9.4, 10.0, 21.8, 22.3, 23.9, 25.4, 51.8, 52.6, 54.7, 55.2, 104.2, 105.3, 111.5, 118.1, 119.2, 122.0, 125.6, 129.7, 129.8, 136.3, 136.5, 170.3; FAB-HRMS m/z $[M+H]^+$ calcd 245.1290, obsd 245.1294.

[D-Tryptophan reaction] off-white solid (69% yield, 49:51 diastereomeric mixture); R_f=0.75 (Butanol/HOAc/H_2O=10:1:1); mp 95–100 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.19 (t, J=2.96 Hz, *trans* CH_3, 3H), 1.21 (t, J=4.48 Hz, *cis* CH_3, 3H), 2.05–2.26 (m, *cis/trans* $MeCH_2$, 4H), 3.14 (ddd, J=2.44, 14.06, 14.3 Hz, Trp-NCH_2, 1H), 3.25–3.32 (m, Trp-NCH_2, 1H), 3.40–3.49 (m, Trp-NCH_2, 2H), 4.36 (dd, J=5.12, 12.6 Hz, *cis* Trp-CHN, 1H), 4.63 (d, J=6.04 Hz, *trans* Trp-CHN, 1H), 4.65 (m, *trans* EtCHN, 1H), 4.79 (t, J=6.64 Hz, *cis* EtCHN, 1H), 7.05 (qt, J=1.04, 7.72 Hz, *cis/trans* ArH, 2H), 7.15 (dd, J=7.28, 5.74 Hz, *cis/trans* ArH, 2H), 7.36 (dd, J=5.20, 8.14 Hz, *cis/trans* ArH, 2H), 7.48 (d, J=7.84 Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, D_2O–MeOH) δ 9.6, 10.2, 23.1, 23.6, 25.8, 27.1, 53.9, 54.7, 56.7, 57.4, 105.7, 106.9, 112.4, 112.5, 119.0, 120.6, 120.7, 123.6, 127.1, 130.0, 130.0, 138.4, 138.6, 171.3; FAB-HRMS m/z $[M+H]^+$ calcd 245.1290, obsd 245.1291.

4.2.3. Compound 3c. [L-Tryptophan reaction] off-white solid (51% yield, 50:50 diastereomeric mixture); R_f=0.82 (Butanol/HOAc/H_2O=10:1:1); mp 97–106 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.06 (t, J=7.36 Hz, *trans* CH_3, 3H), 1.12 (t, J=4.56 Hz, *cis* CH_3, 3H), 1.60–1.65 (m, *cis/trans* CH_2, 4H), 1.89–2.01 (m, *cis/trans* CH_2, 2H), 2.08–2.15 (m, CH_2, 1H), 2.23–2.34 (m, CH_2, 1H), 3.12 (ddd, J=2.44, 14.16, 28.24 Hz, Trp-NCH_2, 1H), 3.26–3.34 (m, Trp-NCH_2, 1H), 3.45 (ddd, J=0.76, 5.46, 16.78 Hz, *cis/trans* Trp-NCH_2, 2H), 4.36 (dd, J=5.04, 12.08 Hz, *cis* Trp-CHN, 1H), 4.61 (q, J=6.16 Hz, *trans* Trp-CHN, 1H), 4.73–4.75 (m, *trans* PrCHN, 1H), 4.82–4.94 (m, *cis* PrCHN, 1H), 7.06 (dt, J=0.84, 7.54 Hz, *cis/trans* ArH, 2H), 7.15 (t, J=7.28 Hz, *cis/trans* ArH, 2H), 7.35 (t, J=6.56 Hz, *cis/trans* ArH, 2H), 7.48 (dd, J=0.6, 7.84 Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.8, 13.9, 17.9, 18.4, 21.8, 22.4, 33.0, 34.3, 51.1, 51.9, 53.2, 55.3, 104.1, 105.2, 111.5, 118.6, 119.2, 120.5, 122.0, 125.7, 130.0, 130.1, 136.4, 136.6,

170.3, 170.4; FAB-HRMS *m/z* $[M+H]^+$ calcd 259.1447, obsd 259.1449.

[D-Tryptophan reaction] off-white solid (65% yield, 54:46 diastereomeric mixture); $R_f=0.82$ (Butanol/HOAc/H_2O=10:1:1); mp 90–98 °C; ^1H NMR (400 MHz, D_2O) δ 1.06 (t, $J=7.68$ Hz, *trans* CH_3, 3H), 1.11 (t, $J=5.40$ Hz, *cis* CH_3, 3H), 1.61–1.65 (m, *cis/trans* CH_2, 3H), 1.89–2.01 (m, *cis/ trans* CH_2, 3H), 2.08–2.15 (m, CH_2, 1H), 2.23–2.34 (m, CH_2, 1H), 3.13 (ddd, $J=2.48$, 14.12, 28.18 Hz, Trp-NCH_2, 1H), 3.23–3.34 (m, Trp-NCH_2, 1H), 3.46 (ddd, $J=0.92$, 6.00, 19.34 Hz, *cis/trans* Trp-NCH_2, 2H), 4.40 (dd, $J=5.04$, 12.10 Hz, *cis* Trp-CHN, 1H), 4.65 (q, $J=6.08$ Hz, *trans* Trp-CHN, 1H), 4.74–4.76 (m, *trans* PrCHN, 1H), 4.80–4.91 (m, *cis* PrCHN, 1H), 7.06 (t, $J=6.8$ Hz, *cis/trans* ArH, 2H), 7.15 (dt, $J=1.00$, 7.62 Hz, *cis/trans* ArH, 2H), 7.36 (dd, $J=$ 7.22, 7.10 Hz, *cis/trans* ArH, 2H), 7.49 (d, $J=7.48$ Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.5, 14.6, 18.7, 19.2, 22.6, 23.1, 33.8, 35.1, 51.9, 52.7, 54.1, 56.0, 104.9, 105.9, 112.3, 119.0, 120.0, 122.8, 122.8, 126.5, 130.8, 130.9, 137.2, 137.4, 171.0, 171.1; FAB-HRMS *m/z* $[M+H]^+$ calcd 259.1447, obsd 259.1441.

4.2.4. Compound 3d. [L-Tryptophan reaction] off-white solid (58% yield, 50:50 diastereomeric mixture); $R_f=0.83$ (Butanol/HOAc/H_2O=10:1:1); mp 114–122 °C; ^1H NMR (200 MHz, D_2O) δ 0.99 (t, $J=7.32$ Hz, *trans* CH_3, 3H), 1.02 (t, $J=7.28$ Hz, CH_3, 3H), 1.45–1.61 (m, *cis/trans* CH_2CH_2, 8H), 1.97–2.05 (m, *cis/trans* CH_2, 2H), 2.13–2.20 (m, CH_2, 1H), 2.35–2.41 (m, CH_2, 1H), 3.11 (ddd, $J=2.36$, 14.14, 28.26 Hz, Trp-NCH_2, 1H), 3.25–3.32 (m, Trp-NCH_2, 1H), 3.43 (dd, $J=6.00$, 21.1 Hz, *cis/trans* Trp-NCH_2, 2H), 4.25 (dd, $J=7.04$, 12.12 Hz, *cis* Trp-CHN, 1H), 4.52 (q, $J=$ 1.08 Hz, *trans* Trp-CHN, 1H), 4.63–4.82 (m, BuCHN, 2H), 7.05 (qt, $J=0.8$, 7.50 Hz, *cis/trans* ArH, 2H), 7.15 (t, $J=$ 7.76 Hz, *cis/trans* ArH, 2H), 7.48 (d, $J=7.84$ Hz, *cis/trans* ArH, 2H), 7.47–7.49 (m, *cis/trans* ArH, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.8, 22.1, 22.1, 22.5, 26.6, 27.1, 30.8, 32.0, 51.1, 52.3, 53.3, 55.8, 104.5, 105.6, 111.5, 118.1, 119.5, 121.8, 125.8, 130.3, 130.4, 136.4, 136.4, 136.6, 170.4, 170.5; FAB-HRMS *m/z* $[M+H]^+$ calcd 273.1603, obsd 273.1605.

[D-Tryptophan reaction] off-white solid (76% yield, 54:46 diastereomeric mixture); $R_f=0.85$ (Butanol/HOAc/H_2O=10:1:1); mp 110–118 °C; ^1H NMR (200 MHz, D_2O) δ 0.99 (t, $J=4.88$ Hz, *trans* CH_3, 3H), 1.03 (t, $J=3.24$ Hz, *cis* CH_3, 3H), 1.45–1.59 (m, *cis/trans* CH_2CH_2, 8H), 1.97–2.05 (m, *cis/trans* CH_2, 2H), 2.13–2.20 (m, CH_2, 1H), 2.35–2.41 (m, CH_2, 1H), 3.13 (dt, $J=2.24$, 13.48 Hz, Trp-NCH_2, 1H), 3.26–3.32 (m, Trp-NCH_2, 1H), 3.45 (dt, $J=6.00$, 19.76 Hz, *cis/trans* Trp-NCH_2, 2H), 4.35 (dd, $J=5.04$, 12.06 Hz, *cis* Trp-CHN, 1H), 4.60 (t, $J=6.24$ Hz, *trans* Trp-CHN, 1H), 4.72–4.84 (m, *cis/trans* BuCHN, 2H), 7.06 (t, $J=7.16$ Hz, *cis/trans* ArH, 2H), 7.15 (t, $J=7.72$ Hz, *cis/trans* ArH, 2H), 77.36 (dd, $J=5.08$, 8.04 Hz, *cis/trans* ArH, 2H), 7.48 (d, $J=7.72$ Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.8, 13.8, 22.1, 22.2, 22.7, 26.6, 27.1, 30.9, 32.0, 51.0, 52.6, 53.3, 56.2, 104.7, 105.8, 111.5, 118.1, 119.0, 119.1, 121.8, 125.8, 130.5, 130.6, 136.4, 136.6, 170.4, 170.6; FAB-HRMS *m/z* $[M+H]^+$ calcd 273.1603, obsd 273.1600.

4.2.5. Compound 3e. [L-Tryptophan reaction] off-white solid (50% yield, 42:58 diastereomeric mixture); $R_f=0.85$ (Butanol/HOAc/H_2O=10:1:1); mp 114–122 °C; ^1H NMR (400 MHz, D_2O) δ 0.93 (t, $J=7.16$ Hz, *trans* CH_3, 3H), 0.96 (t, $J=5.72$ Hz, *cis* CH_3, 3H), 1.40–1.45 (m, *cis/trans* CH_2CH_2, 8H), 1.61–1.63 (m, *cis/trans* CH_2, 4H), 1.97–2.05 (m, *cis/trans* CH_2, 2H), 2.13–2.20 (m, CH_2, 1H), 2.35–2.41 (m, CH_2, 1H), 3.07 (ddd, $J=2.36$, 14.1, 28.36 Hz, Trp-NCH_2, 1H), 3.25–3.35 (m, Trp-NCH_2, 1H), 3.38 (dd, $J=$ 6.00, 16.48 Hz, Trp-NCH_2, 1H), 3.45 (dd, $J=1.24$, 16.2 Hz, Trp-NCH_2, 1H), 4.12 (dd, $J=5.00$, 21.52 Hz, *cis* Trp-CHN, 1H), 4.37 (t, $J=6.12$ Hz, *trans* Trp-CHN, 1H), 4.66–4.68 (m, *trans* $C_5H_{11}CHN$, 1H), 4.76–4.79 (m, *cis* $C_5H_{11}CHN$, 1H), 7.05 (qt, $J=0.84$, 7.42 Hz, *cis/trans* ArH, 2H), 7.14 (dt, $J=1.04$, 8.10 Hz, *cis/trans* ArH, 2H), 7.34 (t, $J=$ 6.24 Hz, *cis/trans* ArH, 2H), 7.48 (d, $J=7.88$ Hz, *cis/trans* ArH, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.1, 22.0, 22.1, 22.6, 24.3, 24.8, 31.1, 31.3, 31.3, 32.4, 51.5, 52.3, 53.7, 55.7, 104.3, 105.4, 111.7, 118.3, 118.8, 119.3, 119.3, 122.0, 122.1, 122.5, 125.9, 130.3, 130.4, 136.6, 136.8, 170.4, 170.5; FAB-HRMS *m/z* $[M+H]^+$ calcd 287.1760, obsd 287.1757.

[D-Tryptophan reaction] off-white solid (50% yield, 42:58 diastereomeric mixture); $R_f=0.87$ (Butanol/HOAc/H_2O=10:1:1); mp 107–118 °C; ^1H NMR (200 MHz, D_2O) δ 0.94 (t, $J=7.12$ Hz, *trans* CH_3, 3H), 0.96 (t, $J=6.92$ Hz, *cis* CH_3, 3H), 1.28–1.60 (m, *cis/trans* CH_2CH_2, 8H), 1.61–1.62 (m, *cis/trans* CH_2, 4H), 1.97–2.05 (m, *cis/trans* CH_2, 2H), 2.13–2.20 (m, CH_2, 1H), 2.35–2.41 (m, CH_2, 1H), 3.07 (ddd, $J=2.48$, 14.2, 28.26 Hz, Trp-NCH_2, 1H), 3.23–3.28 (m, Trp-NCH_2, 1H), 3.33–3.48 (m, *cis/trans* Trp-NCH_2, 2H), 4.12 (dd, $J=5.00$, 12.10 Hz, *cis* Trp-CHN, 1H), 4.37 (t, $J=6.16$ Hz, *trans* Trp-CHN, 1H), 4.66–4.68 (m, *trans* $C_5H_{11}CHN$, 1H), 4.79–4.81 (m, *cis* $C_5H_{11}CHN$, 1H), 7.04–7.07 (m, *cis/trans* ArH, 2H), 7.14 (dt, $J=1.04$, 7.62 Hz, *cis/trans* ArH, 2H), 7.34 (t, $J=7.24$ Hz, *cis/trans* ArH, 2H), 7.48 (d, $J=7.72$ Hz, *cis/trans* ArH, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.9, 21.9, 22.6, 24.1, 24.6, 31.2, 31.2, 51.0, 52.4, 55.9, 104.5, 105.6, 111.4, 118.0, 118.9, 121.7, 125.7, 130.4, 130.5, 136.3, 136.4, 170.28, 170.4; FAB-HRMS *m/z* $[M+H]^+$ calcd 287.1760, obsd 287.1761.

4.2.6. Compound 3f. [L-Tryptophan reaction] off-white solid (56% yield, 27:73 diastereomeric mixture); $R_f=0.83$ (Butanol/HOAc/H_2O=10:1:1); mp 132– 141 °C; ^1H NMR (400 MHz, D_2O) δ 3.11–3.22 (m, *cis/trans* Trp-NCH_2, 2H), 3.28–3.34 (m, phCH_2, 2H), 3.44–3.48 (m, *cis/trans* Trp-NCH_2, 1H), 3.55 (dd, $J=5.68$, 14.38 Hz, phCH_2, 2H), 3.63–3.71 (m, Trp-NCH_2, 1H), 4.04–4.07 (m, *cis/trans* Trp-CHN, 2H), 4.91–4.93 (m, *cis* BzCHN, 1H), 5.17 (t, $J=6.28$ Hz, *trans* BzCHN, 1H), 7.04–7.52 (m, *cis/trans* ArH, 18H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.8, 22.5, 37.0, 38.0, 52.4, 54.7, 55.7, 104.9, 105.4, 111.6, 118.3, 119.2, 119.3, 122.1, 125.7, 127.4, 128.8, 129.3, 129.6, 129.7, 129.8, 135.3, 135.6, 136.4, 136.5, 170.1, 170.6; FAB-HRMS *m/z* $[M+H]^+$ calcd 307.1447, obsd 307.1446.

[D-Tryptophan reaction] off-white solid (63% yield, 26:74 diastereomeric mixture); $R_f=0.81$ (Butanol/HOAc/H_2O=10:1:1); mp 131–140 °C; ^1H NMR (400 MHz, D_2O) δ 3.12–3.29 (m, *cis/trans* Trp-NCH_2, 2H), 3.30 (m, *trans* phCH_2, 2H), 3.46–3.49 (m, Trp-NCH_2, 1H), 3.57 (dd, $J=5.80$,

14.40 Hz, *cis* $PHCH_2$, 2H), 3.80 (dd, $J=3.92$, 14.58 Hz, Trp-NCH_2, 1H), 4.17–4.21 (m, *cis/trans* Trp-CHN, 2H), 4.94–4.97 (t, *cis* BzCHN, 1H), 5.19 (t, $J=6.16$ Hz, *trans* BzCHN, 1H), 7.07–7.48 (m, *cis/trans* ArH 18H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.9, 22.5, 37.1, 38.0, 52.3, 54.6, 55.7, 105.0, 105.5, 111.6, 118.2, 119.2, 119.3, 122.1, 125.7, 127.3, 128.8, 128.8, 129.5, 129.6, 129.8, 135.3, 135.8, 136.4, 136.5, 170.3, 170.7; FAB-HRMS *m/z* $[M+H]^+$ calcd 307.1447, obsd 307.1452.

4.2.7. Compound 3g. [L-Tryptophan reaction] off-white solid (95% yield, 61:39 diastereomeric mixture); $R_f=0.79$ (Butanol/HOAc/H_2O=10:1:1); mp 175–182 °C; ^{1}H NMR (400 MHz, D_2O) δ 3.34–3.41 (m, *cis/trans* Trp-NCH_2, 2H), 3.55–3.61 (m, *cis/trans* Trp-NCH_2, 2H), 4.42 (q, $J=$ 6.96 Hz, *trans* Trp-CHN, 1H), 4.60 (dd, $J=5.16$, 12.02 Hz, *cis* Trp-CHN, 1H), 5.90 (s, *cis* PhCHN, 1H), 6.05 (s, *trans* PhCHN, 1H), 7.10–7.56 (m, *cis/trans* ArH, 18H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 22.0, 22.3, 52.3, 55.5, 57.0, 58.4, 106.9, 107.4, 111.8, 118.7, 120.2, 123.2, 125.5, 127.4, 128.5, 129.5, 129.7, 129.9, 130.7, 130.9, 132.7, 133.3, 136.9, 171.8, 172.1; FAB-HRMS *m/z* $[M+H]^+$ calcd 293.1290, obsd 293.1284.

[D-Tryptophan reaction] off-white solid (98% yield, 64:36 diastereomeric mixture); $R_f=0.78$ (Butanol/HOAc/H_2O= 10:1:1); mp 163–174 °C; ^{1}H NMR (200 MHz, D_2O) δ 3.31–3.42 (m, *cis/trans* Trp-NCH_2, 2H), 3.59 (dd, $J=4.94$, 16.2 Hz, *cis/trans* Trp-NCH_2, 2H), 4.45 (q, $J=5.80$ Hz, *trans* Trp-CHN, 1H), 4.61 (dd, $J=5.12$, 12.10 Hz, *cis* Trp-CHN, 1H), 5.59 (s, *cis* PhCHN, 1H), 6.06 (s, *trans* PhCHN, 1H), 7.14–7.58 (m, *cis/trans* ArH, 18H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 22.5, 51.8, 54.9, 55.9, 57.9, 106.4, 107.2, 111.9, 114.2, 118.5, 119.5, 120.1, 122.3, 125.8, 128.2, 128.9, 129.2, 130.2, 130.6, 129.9, 134.1, 134.6, 137.0, 137.1, 170.0, 170.2; FAB-HRMS *m/z* $[M+H]^+$ calcd 293.1290, obsd 293.1284.

4.2.8. Compound 3h. [L-Tryptophan reaction] off-white solid (76% yield, 62:38 tereomeric mixture); $R_f=0.87$ (Butanol/HOAc/H_2O=10:1:1); mp 140–151 °C; ^{1}H NMR (400 MHz, D_2O) δ 2.37 (s, *trans* CH_3, 3H), 2.41 (s, *cis* CH_3, 3H), 3.29–3.34 (m, *cis/trans* Trp-NCH_2, 2H), 3.54–3.59 (m, *cis/trans* Trp-NCH_2, 2H), 4.40 (q, $J=5.76$ Hz, *trans* Trp-NCH, 1H), 4.55 (dd, $J=5.16$, 13.18 Hz, *cis* Trp-NCH, 1H), 5.84 (s, *cis* ArCHN, 1H), 6.00 (s, *trans* ArCHN, 1H), 7.10–7.56 (m, *cis/trans* ArH, 16H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.8, 20.9, 22.3, 51.3, 54.4, 55.5, 57.5, 106.1, 106.8, 111.7, 118.3, 118.4, 119.2, 120.2, 122.0, 122.3, 125.5, 125.6, 128.2, 129.2, 130.4, 130.9, 131.5, 136.7, 136.9, 139.5, 139.6, 170.0, 170.1; FAB-HRMS *m/z* $[M+H]^+$ calcd 307.1447, obsd 307.1444.

[D-Tryptophan reaction] off-white solid (81% yield, 58:42 diasteromeric mixture); $R_f=0.87$ (Butanol/HOAc/H_2O= 10:1:1); mp 136–146 °C; ^{1}H NMR (400 MHz, D_2O) δ 2.37 (s, *trans* CH_3, 3H), 2.41 (s, *cis* CH_3, 3H), 3.31–3.40 (m, *cis/trans* Trp-NCH_2, 2H), 3.54–3.60 (m, *cis/trans* Trp-NCH_2, 2H), 4.39 (q, $J=5.76$ Hz, *trans* Trp-CHN, 1H), 4.57 (dd, $J=5.20$, 12.02 Hz, Trp-CHN, 1H), 5.84 (s, *cis* ArCHN, 1H), 6.00 (s, *trans* ArCHN, 1H), 7.08–7.87 (m, *cis/trans* ArH, 16H); ^{13}C NMR (50 MHz, DMSO–d_6) δ 20.8, 20.9, 22.2, 22.4, 51.4, 54.4, 55.6, 57.5, 106.1, 106.8, 111.6, 111.7, 118.2, 118.3, 119.2, 120.2, 122.0, 122.2, 125.5, 125.6, 128.4, 129.2, 129.6, 129.8, 130.2, 130.3, 131.1, 131.8, 135.9, 136.7, 136.8, 139.4, 139.6, 170.0, 170.2; FAB-HRMS *m/z* $[M+H]^+$ calcd 307.1447, obsd 307.1440.

4.2.9. Compound 3i. [D-Tryptophan reaction] off-white solid (95% yield, 58:42 diasteromeric mixture); $R_f=0.79$ (Butanol/HOAc/H_2O=10:1:1); mp 121–131 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.09 (t, $J=7.60$ Hz, CH_3, 3H), 1.12 (t, $J=7.48$ Hz, CH_3, 3H), 1.74 (s, CH_3, 3H), 1.83 (s, CH_3, 3H), 1.98–2.08 (m, CH_2, 1H), 2.11–2.23 (m, CH_2, 2H), 2.32–2.39 (m, CH_2, 1H), 3.12 (ddd, $J=2.64$, 7.86, 23.48 Hz, Trp-NCH_2, 2H), 3.45–3.52 (m, Trp-NCH_2, 2H), 4.51 (dd, $J=5.12$, 11.90 Hz, Trp-CHN, 1H), 4.65 (dd, $J=$ 5.72, 11.80 Hz, Trp-CHN, 1H), 7.05 (t, $J=7.16$ Hz, ArH, 2H), 7.15 (dt, $J=1.16$, 8.82 Hz, ArH, 2H), 7.35 (t, $J=$ 7.12 Hz, ArH, 2H), 7.48 (dd, $J=4.24$, 7.84 Hz, ArH, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 7.5, 8.9, 22.0, 22.3, 22.9, 30.0, 31.2, 51.0, 51.9, 59.7, 59.7, 103.7, 103.9, 111.4, 118.2, 119.1, 121.9, 125.4, 125.4, 133.2, 134.4, 136.2, 136.3, 170.3, 170.5; FAB-HRMS *m/z* $[M+H]^+$ calcd 259.1447, obsd 259.1445.

4.2.10. Compound 3j. [D-Tryptophan reaction] off-white solid (96% yield); $R_f=0.69$ (Butanol/HOAc/H_2O=10:1:1); mp 89–97 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.03 (t, $J=$ 7.56 Hz, CH_3, 3H), 1.08 (t, $J=7.56$ Hz, CH_3, 3H), 2.02–2.20 (m, CH_2, 3H), 2.39 (h, $J=7.44$ Hz, CH_2, 1H), 3.14 (dd, $J=12.0$, 16.24 Hz, Trp-NCH_2, 1H), 3.47 (dd, $J=5.6$, 16.4 Hz, Trp-NCH_2, 1H), 4.56 (dd, $J=12.0$, 5.6 Hz, Trp-CHN, 1H), 7.05 (t, $J=7.36$ Hz, ArH, 1H), 7.14 (t, $J=$ 7.24 Hz, ArH, 1H), 7.37 (d, $J=8.15$ Hz, ArH, 1H), 7.47 (d, $J=7.84$ Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 7.2, 8.9, 22.3, 27.5, 28.2, 51.6, 62.9, 104.5, 111.5, 118.3, 119.1, 122.0, 125.5, 132.7, 136.4, 170.5; FAB-HRMS *m/z* $[M+H]^+$ calcd 273.1603, obsd 273.1604.

4.2.11. Compound 3k. [D-Tryptophan reaction] off-white solid (82% yield); $R_f=0.81$ (Butanol/HOAc/H_2O=10:1:1); mp 158–166 °C; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ 1.51–2.14 (m, Ch, 8H), 2.29–2.36 (m, Ch, 2H), 3.10 (dd, $J=$ 11.76, 16.26 Hz, Trp-NCH_2, 1H), 3.46 (dd, $J=5.36$, 16.28 Hz, Trp-NCH_2, 1H), 4.40 (dd, $J=5.36$, 11.62 Hz, Trp-CHN, 1H), 7.05 (dt, $J=1.08$, 7.41 Hz, ArH, 1H), 7.15 (dt, $J=1.08$, 7.66 Hz, ArH, 1H), 7.38 (d, $J=8.08$ Hz, 1H), 7.48 (d, $J=7.88$ Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.7, 23.8, 24.5, 33.7, 34.6, 52.3, 57.0, 104.9, 111.6, 118.1, 119.0, 121.6, 125.5, 125.8, 136.0, 136.3, 136.7, 171.8; FAB-HRMS *m/z* $[M+H]^+$ calcd 285.1603, obsd 285.1608.

4.2.12. Compound 3l. [D-Tryptophan reaction] off-white solid (72% yield); $R_f=0.75$ (Butanol/HOAc/H_2O=10:1:1); mp 128–136 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.98–2.16 (m, Cp, 4H), 2.31 (p, $J=5.68$ Hz, Cp, 2H), 2.49 (p, $J=8.00$ Hz, Cp, 1H), 3.12 (dd, $J=12.0$, 16.12 Hz, Trp-NCH_2, 1H), 3.47 (dd, $J=5.36$, 16.12 Hz, Trp-NCH_2, 1H), 4.45 (dd, $J=5.16$, 12.06 Hz, Trp-CHN, 1H), 7.05 (dt, $J=0.96$, 7.52 Hz, ArH, 1H), 7.15 (dt, $J=1.16$, 7.66 Hz, ArH, 1H), 7.36 (d, $J=$ 4.12 Hz, ArH, 1H), 7.48 (d, $J=7.88$ Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 22.7, 24.9, 25.0, 37.4, 53.2, 59.4, 61.6, 65.9, 103.8, 106.5, 111.5, 114.2, 118.1, 118.8, 119.2, 120.5, 122.0, 121.9, 125.4, 127.4, 134.5, 134.7,

136.6, 136.9, 170.5, 170.7; FAB-HRMS *m/z* $[M+H]^+$ calcd 271.1447, obsd 271.1456.

4.3. General procedure for the microwave-accelerated Pictet–Spengler reaction of L-tryptophan with ketones

In a typical reaction, L-tryptophan (51 mg, 0.25 mmol) was dissolved in toluene (2.5 mL) and trifluoroacetic acid (10%, v/v). The ketone (3.0 mmol) was added in one portion to the mixture. The microwave-assisted Pictet–Spengler reaction was carried out in an open vessel and temperature-controlled at 60 °C using a commercial microwave oven (300 W, Synthewave 402, Prolabo). A 50% microwave power was applied throughout the reaction period to avoid formation of side products of unknown structure. The reaction was allowed to proceed until tryptophan was completely consumed as monitored by TLC using the ninhydrin test or HPLC at 280 nm (10 min–40 h). Upon completion of the reaction, the reaction mixture was concentrated to dryness under reduced pressure to obtain a residue which was dissolved in toluene, then extracted with acidic water (3×), and lyophilized. Products were obtained in excellent purity as determined by NMR and HPLC.

4.3.1. Compound 3i. [L-Tryptophan reaction] off-white solid (87% yield, 58:42 diasteromeric mixture); R_f=0.75 (Butanol/HOAc/H_2O=10:1:1); mp 122–130 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.06–1.14 (m, CH_3, 6H), 1.74 (s, CH_3, 3H), 1.83 (s, CH_3, 3H), 2.00–2.06 (m, CH_2, 1H), 2.12–2.21 (m, CH_2, 2H), 2.26–2.33 (m, CH_2, 1H), 3.10 (dd, *J*=12.84, 15.94 Hz, Trp-NCH_2, 2H), 3.43–3.51 (m, Trp-NCH_2, 2H), 4.40 (dd, *J*=3.84, 11.82 Hz, Trp-CHN, 1H), 4.53 (q, *J*=3.84 Hz, *trans* Trp-CHN, 1H), 7.03–7.07 (m, ArH, 2H), 7.12–7.17 (m, ArH, 2H), 7.32–7.36 (m, ArH, 2H), 7.45–7.49 (m, ArH, 2H); ^{13}C NMR (50 MHz, D_2O–MeOH) δ 6.8, 8.2, 22.0, 22.1, 22.6, 31.7, 51.9, 52.7, 60.4, 60.6, 104.0, 104.2, 111.7, 113.6, 118.5, 119.4, 120.1, 123.0, 125.3, 132.7, 133.7, 136.5, 136.6, 171.5, 171.7; FAB-HRMS *m/z* $[M+H]^+$ calcd 259.1447, obsd 259.1445.

4.3.2. Compound 3j. [L-Tryptophan reaction] off-white solid (88% yield); R_f=0.67 (Butanol/HOAc/H_2O=10:1:1); mp 83–92 °C; ^{1}H NMR (200 MHz, D_2O) δ 1.04 (t, *J*=7.56 Hz, CH_3, 3H), 1.08 (t, *J*=7.52 Hz, CH_3, 3H), 2.07–2.20 (m, CH_2, 3H), 2.36–2.44 (m, CH_2, 1H), 3.14 (dd, *J*=11.84, 16.24 Hz, Trp-NCH_2, 1H), 3.49 (dd, *J*=5.56, 16.28 Hz, Trp-NCH_2, 1H), 4.61 (dd, *J*=5.40, 11.84 Hz, Trp-CHN, 1H), 7.05 (t, *J*=7.08 Hz, ArH, 1H), 7.15 (dt, *J*=1.12, 8.00 Hz, ArH, 1H), 7.36 (d, *J*=8.12 Hz, ArH, 1H), 7.49 (d, *J*=7.88 Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 7.1, 8.8, 22.2, 27.4, 28.2, 51.5, 62.9, 104.4, 111.4, 118.2, 119.0, 121.9, 125.4, 132.6, 136.3, 170.4; FAB-HRMS *m/z* $[M+H]^+$ calcd 273.1603, obsd 273.1595.

4.3.3. Compound 3k. [L-Tryptophan reaction] off-white solid (84% yield); R_f=0.77 (Butanol/HOAc/H_2O=10:1:1); mp 160–168 °C; ^{1}H NMR (400 MHz, CD_3OD-d_4) δ 1.51–2.14 (m, Ch, 8H), 2.29–2.36 (m, Ch, 2H), 3.10 (dd, *J*=11.76, 16.26 Hz, Trp-NCH_2, 1H), 3.46 (dd, *J*=5.36, 16.28 Hz, Trp-NCH_2, 1H), 4.40 (dd, *J*=5.36, 11.62 Hz, Trp-CHN, 1H), 7.05 (dt, *J*=1.08, 7.41 Hz, ArH, 1H), 7.15 (dt, *J*=1.08, 7.66 Hz, ArH, 1H), 7.38 (d, *J*=8.08 Hz, 1H), 7.48 (d, *J*=7.88 Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.6, 23.6, 24.3, 30.8, 33.6, 34.4, 52.0, 56.8, 57.2, 104.6, 105.4, 110.9, 111.5, 117.3, 118.1, 118.7, 119.0, 120.8, 121.6, 125.7, 127.5, 135.2, 136.2, 136.4, 170.9, 171.7; FAB-HRMS *m/z* $[M+H]^+$ calcd 285.1603, obsd 285.1608.

4.3.4. Compound 3l. [L-Tryptophan reaction] off-white solid (81% yield); R_f=0.81 (Butanol/HOAc/H_2O=10:1:1); mp 132–143 °C; ^{1}H NMR (400 MHz, D_2O) δ 1.99–2.17 (m, Cp, 4H), 2.77 (p, *J*=6.56 Hz, Cp, 1H), 2.50 (p, *J*=8.04 Hz, Cp, 1H), 3.12 (dd, *J*=12.6, 16.16 Hz, Trp-NCH_2, 1H), 3.48 (dd, *J*=5.28, 15.56 Hz, Trp-NCH_2, 1H), 4.47 (q, *J*=5.32, 11.98 Hz, Trp-CHN, 1H), 7.06 (dt, *J*=1.72, 8.48 Hz, ArH, 1H), 7.15 (dt, *J*=1.12, 13.64 Hz, ArH, 1H), 7.37 (d, *J*=7.36 Hz, ArH, 1H), 7.47 (d, *J*=7.88 Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 22.6, 24.8, 25.0, 37.4, 38.3, 53.1, 66.0, 103.7, 111.5, 118.2, 119.2, 122.0, 125.4, 134.7, 136.6, 170.5; FAB-HRMS *m/z* $[M+H]^+$ calcd 271.1447, obsd 271.1437.

4.3.5. Compound 3m. [L-Tryptophan reaction] off-white solid (62% yield, 22:78 diasteromeric mixture); R_f=0.80 (Butanol/HOAc/H_2O=10:1:1); mp 154–159 °C; ^{1}H NMR (200 MHz, D_2O) δ 2.14 (s, CH_3, 3H), 2.25 (s, CH_3, 3H), 3.05–3.10 (m, Trp-NCH_2, 2H), 3.30–3.41 (m, Trp-NCH_2, 2H), 3.73–3.78 (m, Trp-CHN, 1H), 4.47–4.51 (m, Trp-CHN, 1H), 7.13–7.58 (m, ArH, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.5, 23.0, 23.7, 24.8, 52.3, 52.9, 61.6, 61.9, 106.2, 106.3, 111.8, 111.9, 118.7, 118.9, 119.5, 122.7, 125.7, 125.7, 128.2, 128.7, 128.8, 129.0, 129.5, 129.7, 132.5, 133.8, 136.9, 138.8, 170.2, 170.3; FAB-HRMS *m/z* $[M+H]^+$ calcd 307.1447, obsd 307.1441.

4.3.6. Compound 3n. [L-Tryptophan reaction] off-white solid (99% yield, 67:32 diasteromeric mixture); R_f=0.73 (Butanol/HOAc/H_2O=10:1:1); mp 88–93 °C; ^{1}H NMR (200 MHz, D_2O) δ 0.94 (d, *J*=7.04 Hz, CH_3, 3H), 1.05 (dd, *J*=8.48, 8.00 Hz, CH_3, 6H), 1.23 (d, *J*=7.00 Hz, CH_3, 3H), 1.70 (s, CH_3, 3H), 1.75 (s, CH_3, 3H), 2.18 (p, *J*=6.84 Hz, $(CH_3)_2CH$, 1H), 2.55 (p, *J*=7.04 Hz, $(CH_3)_2CH$, 1H), 3.11–3.22 (m, Trp-NCH_2, 2H), 3.38–3.45 (m, Trp-NCH_2, 2H), 4.35 (dd, *J*=4.80, 11.9 Hz, *cis* Trp-CHN, 1H), 4.51 (t, *J*=5.62 Hz, *trans* Trp-CHN, 1H), 7.03 (t, *J*=7.04 Hz, ArH, 1H), 7.13 (t, *J*=6.16 Hz, ArH, 1H), 7.37 (d, *J*=8.20 Hz, ArH, 1H), 7.43 (d, *J*=7.64 Hz, ArH, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 16.6, 17.2, 17.5, 18.4, 22.2, 22.7, 34.6, 35.7, 51.0, 52.8, 62.5, 63.5, 103.7, 105.1, 111.5, 118.2, 119.1, 121.9, 125.5, 134.2, 136.2, 136.6, 169.9, 170.3; FAB-HRMS *m/z* $[M+H]^+$ calcd 273.1603, obsd 273.1601.

4.4. Procedures for the preparation of demethoxyfumitremorgin C analog 4 and tetrahydro-β-carbolinehydantoins 5a–c

The experimental procedures for preparing compounds **4** and **5** were not optimized. Using the protocol described in Section 4.3, L-tryptophan methyl ester (50 mg, 0.23 mmol) was, first, reacted with acetone (0.3 mL) in the presence of microwaves (60 °C, 60 W) to form the corresponding 1,1-dimethyl 1,2,3,4-tetrahydro-β-carboline. In the case of compound **4** synthesis, after the acylation reaction with the acid chloride (5 equiv) formed from Fmoc-protected

L-proline (5 equiv) with excessive thionyl chloride, the Fmoc-deprotection and subsequent cyclization to form the diketopiperazine ring was achieved by piperidine (20% in methylene chloride). Without attempting to optimize its overall yield, compound **4** was isolated as white solid in >95% purity as a single stereoisomer after the aqueous wash and silica gel column chromatography. In cases of preparing compounds **5a–5c**, 1,1-dimethyl 1,2,3,4-tetrahydro-β-carboline from the Pictet–Spengler reaction was mixed with butyl isocyanate, phenyl isothiocyanate, or allyl isocyanate (1.1 equiv) in methyl ethyl ketone, and the reaction was carried out at 60 °C under low-power microwave condition (60 W). The progress of the reaction was monitored using TLC (typically 2 min for all three reactions). Upon the completion of the reactions, the solution mixtures containing the desired tetrahydro-β-carbolinehydantoins **5a–5c** were evaporated in vacuo and purified by silica gel column chromatography (ethyl acetate/hexane=1:4). The products were afforded as off-white solid.

4.4.1. Compound 4. White solid (8.1 mg, 11%); mp 194 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88–2.13 (m, Pro-CH_2CH_2, 3H), 1.90 (s, CH_3, 3H), 1.95 (s, CH_3, 3H), 2.42–2.48 (m, Pro-CH_2CH_2, 1H), 2.93 (dd, J=11.6, 16.1 Hz, Trp-CH_2, 1H), 3.49–3.63 (m, Pro-NCH_2, 1H), 3.70–3.78 (m, Pro-NCH_2 (1H), Trp-NCH_2 (1H), 2H), 4.09–4.13 (m, Pro-CHN, 1H), 4.26 (dd, J=2.9, 10.6 Hz, Trp-CHN, 1H), 7.13–7.22 (m, ArH, 4H), 7.36 (d, J=7.9 Hz, ArH, 1H), 7.58 (d, J=7.7 Hz, ArH, 1H), 8.01 (br s, indole NH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1, 23.8, 24.9, 29.8, 30.3, 45.5, 57.2, 59.7, 59.8, 105.7, 110.9, 118.6, 120.0, 122.3, 126.2, 136.1, 138.3, 164.4, 168.8.; FAB-HRMS calcd for $C_{19}H_{22}N_3O_2$ ($[M+H]^+$) 324.1712, found 324.1719.

4.4.2. Compound 5a. Off-white solid (89 mg, 70% yield); mp 192–193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, J=7.4 Hz, CH_3, 3H), 1.25–1.40 (m, CH_2, 2H), 1.58–1.68 (m, CH_2, 2H), 1.74 (s, CH_3, 3H), 2.03 (s, CH_3, 3H), 2.80 (dd, J=3.4, 11 Hz, Trp-CH_2, 1H), 3.38 (dd, J=4.5, 10.3 Hz, Trp-CH_2, 1H), 3.55 (t, J=7.4 Hz, NCH_2, 2H), 4.22 (dd, J=4.5, 6.8 Hz, Trp-CHN, 1H), 7.15 (dt, J=0.7, 7 Hz, Trp-ArH, 1H), 7.21 (dt, J=1.1, 7.0 Hz, Trp-ArH, 1H), 7.35 (d, J=8.0 Hz, Trp-ArH, 1H), 7.50 (d, J=7.72 Hz, Trp-ArH, 1H), 8.34 (s, indolyl NH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 20.1, 22.8, 26.0, 28.1, 30.2, 55.3, 56.0, 105.3, 111.0, 118.3, 120.0, 122.4, 126.1, 136.1, 138.6, 154.6, 171.9; FAB-HRMS for $C_{19}H_{24}N_3O_2$: ($[M+H]^+$) calcd 326.1869, found 326.1873.

4.4.3. Compound 5b. Off-white solid (47 mg, 50% yield); mp 197 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04 (s, CH_3, 3H), 2.33 (s, CH_3, 3H), 2.99 (dd, J=3.6, 11.4 Hz, Trp-CH_2, 1H), 3.53 (dd, J=4.4, 10.6 Hz, Trp-CH_2, 1H), 4.52 (dd, J=4.4, 7.0 Hz, Trp-CHN, 1H), 7.15–7.21 (m, Trp-ArH, 2H), 7.34–7.40 (m, Trp-ArH(2H), Ph(1H), 3H), 7.43–7.56 (m, Ph, 4H), 7.91 (s, indole NH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.0, 23.2, 28.7, 59.9, 60.3, 103.8, 104.2, 111.4, 111.4, 118.4, 119.1, 121.7, 125.8, 126.0, 128.8, 128.9, 129.2, 129.5, 133.6, 133.7, 134.2, 136.4, 136.5, 139.9, 171.4, 173.0, 179.6, 179.8; FAB-HRMS for $C_{21}H_{20}ON_3S$: $(M+H)^+$ calcd 362.1327, found 362.1335.

4.4.4. Compound 5c. Off-white solid (80 mg, 66% yield); mp 232–236 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (s, CH_3, 3H), 2.03 (s, CH_3, 3H), 2.82 (dd, J=2.5, 11.4 Hz, Trp-CH_2, 1H), 3.39 (dd, J=4.5, 10.4 Hz, Trp-CH_2, 1H), 4.18 (d, J=5.7 Hz, NCH_2, 2H), 4.27 (dd, J=4.5, 6.9 Hz, Trp-CHN, 1H), 5.21 (dt, J=1.1, 3.4 Hz, CH=CH_2, 2H), 5.8–5.9 (m, CH=CH_2(1H), 2H), 7.21–7.23 (m, Trp-ArH, 2H), 7.33 (d, J=11.0 Hz, Trp-ArH, 1H), 7.50 (d, J=7.6 Hz, Trp-ArH, 1H), 8.46 (s, indole, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8, 25.9, 28.1, 40.6, 55.5, 56.1, 105.1, 111.1, 116.1, 118.1, 118.3, 119.8, 122.4, 126.1, 131.2, 136.1, 138.6, 154.1, 171.5; FAB-HRMS for $C_{18}H_{20}O_2N_3$ ($[M+H)^+$] calcd 310.1556, found 310.1562.

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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.2004.10.025

References and notes

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Enantioselective inclusion of (*R*)-phenylglycyl-(*R*)-phenylglycine with benzyl methyl sulfoxides

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Abstract—A crystalline dipeptide, (*R*)-phenylglycyl-(*R*)-phenylglycine (*RR*-**1**), recognized *p*-halobenzyl methyl sulfoxides with high *R*-enantioselectivity (86–99% ee) to form inclusion compounds. The single-crystal X-ray analyses showed that *RR*-**1** molecules are arranged in parallel and zigzags via hydrogen bonding to construct a pleated sheet. The guest molecules that form hydrogen bond with $^{+}NH_3$ of *RR*-**1** are accommodated in the channel cavity between the layers. In contrast to the inclusion crystals of parent benzyl methyl sulfoxide, in which a rectangular cavity is formed, the cavity including *p*-halobenzyl methyl sulfoxides becomes rhomboidal. We also examine the guest exchange in these inclusion compounds and it was found that the guest exchanges occur when the host structure changes.

1. Introduction

Many kinds of organic host molecules that construct a three-dimensional rigid network or a two-dimensional framework in a crystal have been designed to include guest molecules.[1,2] However, there are only a few reports on the organic host molecules that form a precise two-dimensional network (sheet structure) followed by the inclusion of a guest molecule.[3] Ward and his co-workers developed pillared two-dimensional hydrogen-bonded networks comprising guanidinium ions and disulfonate ions, in which the disulfonate ions act as pillars that connect opposing hydrogen-bonded sheets with adjustable porosity.[4] About ten years ago,[5] we found that a simple dipeptide, (*R*)-phenylglycyl-(*R*)-phenylglycine (*RR*-**1**), forms an inclusion crystal with a sheet structure. The straight glycylglycine backbones of dipeptide (*RR*-**1**) are arranged in parallel to construct a two-dimensional flat layer by means of intermolecular salt formation between COOH and NH_2 group (Fig. 1). The phenyl groups stand perpendicular to the layer to form a chiral cavity for molecular recognition.

It was also reported by us that *RR*-**1** included several sulfoxides, which have a chiral center on the sulfur atom, with high enantioselectivity.[6] Based on their single-crystal X-ray analyses, we elucidated the sheet structure of *RR*-**1**, the host-guest interactions, and the origin of the chiral discrimination. In the inclusion crystals of aryl methyl sulfoxides, the size and the shape of the included sulfoxides induced several motifs of benzene–benzene interaction of *RR*-**1** to create the cavity suitable for one enantiomer of the

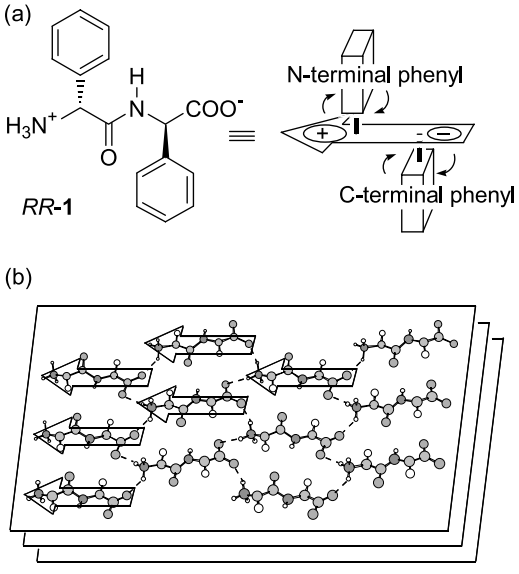

Figure 1. (a) Structure of (*R*)-phenylglycyl-(*R*)-phenylglycine (*RR*-**1**). (b) Sheet structure of dipeptide backbone.

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sulfoxide by altering the phenyl conformation.[6a] As a typical example, the structure of benzyl methyl sulfoxide-included crystal of *RR*-**1** is shown in Figure 2.[6b] In this inclusion compound, two conformers (vide infra) of *RR*-**1** are present; consequently the cavities on the upper and lower sides of the sheet are suitable for the (*S*)-enantiomer and (*R*)-enantiomer, respectively. Since both enantiomers of benzyl methyl sulfoxide were simultaneously included, the stereochemistry of recognition became totally nonenantioselective.

As seen from Figure 2, benzyl methyl sulfoxide is fit in size enough to fill the rectangular cavities above and below the flat sheet structure of *RR*-**1**. If a halogen atom is substituted at the *para*-position of the benzyl group, it would receive steric hindrance and electrostatic repulsion from the phenyl groups of *RR*-**1** to distort the inclusion cavity for the inclusion of *p*-halobenzyl methyl sulfoxides. With this consideration in mind, we investigated the formation of inclusion crystals from *RR*-**1** and *p*-halobenzyl methyl sulfoxides. Here, we report that the *p*-halobenzyl methyl sulfoxides are included in novel cavities, which are different from those of benzyl methyl sulfoxide-included crystals, with high *R*-enantioselectivity. We also report whether the guest molecules are exchanged in these inclusion compounds.

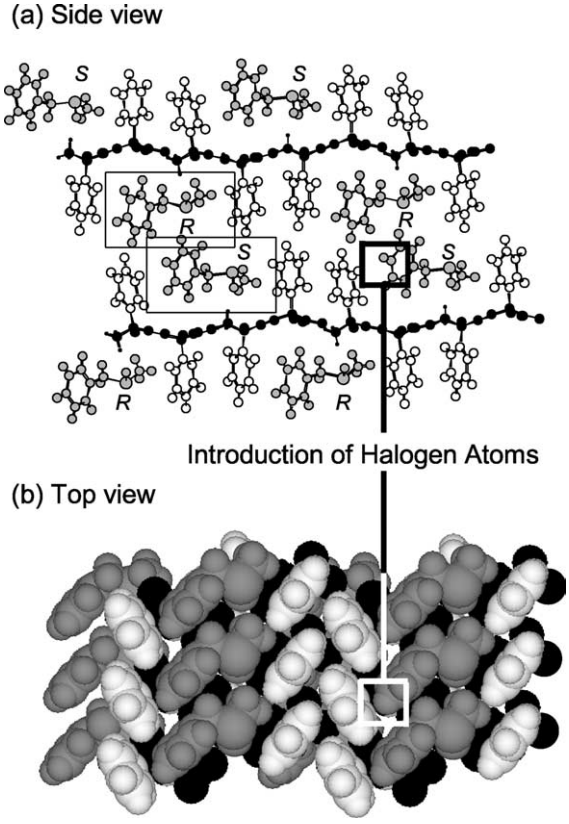


Figure 2. Inclusion compound of *RR*-**1** (host: backbones in black and phenyl groups in white) and *racemic* benzyl methyl sulfoxide (guest in gray). (a) Side view of layer structure with rectangular cavity (ball and stick model). (b) Top view of sheet structure (space-filling model).

2. Results and discussion

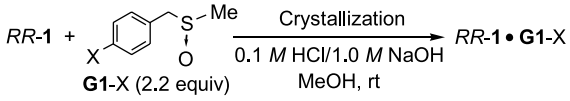
2.1. Enantioselective inclusion of *p*-substituted benzyl methyl sulfoxides

Since (*R*)-phenylglycyl-(*R*)-phenylglycine (*RR*-**1**) is insoluble in water and common organic solvents, we obtained an aqueous solution of *RR*-**1** by carefully neutralizing its acidic solution (see Section 4). After a methanolic solution of *p*-halobenzyl methyl sulfoxide (**G1**-X, X=F, Cl, Br, or I; 2.2 equiv) was added, the resulting mixture was allowed to stand for several days. The deposited inclusion crystals, *RR*-**1**·**G1**-F, *RR*-**1**·**G1**-Cl, *RR*-**1**·**G1**-Br, and *RR*-**1**·**G1**-I, were collected by filtration and washed with chloroform and water. The results are summarized in Table 1, which shows van der Waals radii[7] of the halogen substituent, enantioselectivity, inclusion efficiency (Ef.), and decomposition temperature (Dec.) measured by TG-DTA. The inclusion efficiency means the molar ratio of the guest molecule to the *RR*-**1** molecule in the inclusion compound determined by ^{1}H NMR. In all cases examined here, the inclusion efficiencies were 1.00, meaning that a 1:1 inclusion compound was formed. Enantiomeric excess of the sulfoxide was measured by chiral HPLC analysis. In any case, the included *p*-halobenzyl methyl sulfoxide showed good to high *R*-enantioselectivity (86–99% ee). It is noteworthy that among the inclusion compounds *RR*-**1**·**G1**-F showed the highest decomposition temperature (190 °C). This is most likely attributable to significant intermolecular interactions between the guest molecule and the host framework, which are disclosed by its crystal structure (vide infra).

2.2. Crystal structures of inclusion compounds of *p*-halobenzyl methyl sulfoxides

In the present work, we obtained three inclusion compounds, *RR*-**1**·**G1**-F, *RR*-**1**·**G1**-Br and *RR*-**1**·**G1**-I, as single crystals suitable for X-ray crystallographic analysis. Inclusion crystals of *RR*-**1**·**G1**-F has a space group $P2_12_12_1$, but those of *RR*-**1**·**G1**-Br and *RR*-**1**·**G1**-I are isostructural and their space groups are $P2_1$. Their top views of sheet structures show that *p*-halobenzyl methyl sulfoxides (guests) were accommodated in the channel between the walls that are constructed by the stacked benzene rings of *RR*-**1** (Fig. 3).

Table 1. Enantioselective inclusion of benzyl methyl sulfoxides by *RR*-**1**



Entry	**G1**-X X=	VDW of X[a] (Å)	*RR*-**1**·**G1**-X Ef.[b] (%)	ee (%)	Dec.[c] (°C)
1	H	1.20	100	*Rac.*[d]	177
2	F	1.47	100	99 (*R*)	190
3	Cl	1.75	100	86 (*R*)	173
4	Br	1.85	100	96 (*R*)	179
5	I	1.98	100	95 (*R*)	188

[a] Ref. 7.
[b] Efficiency means mol% of the guest based on the dipeptide in the inclusion compound.
[c] Dec. is a decomposition temperature measured by TG-DTA.
[d] *Rac.* means enantioselectivity within 10%.

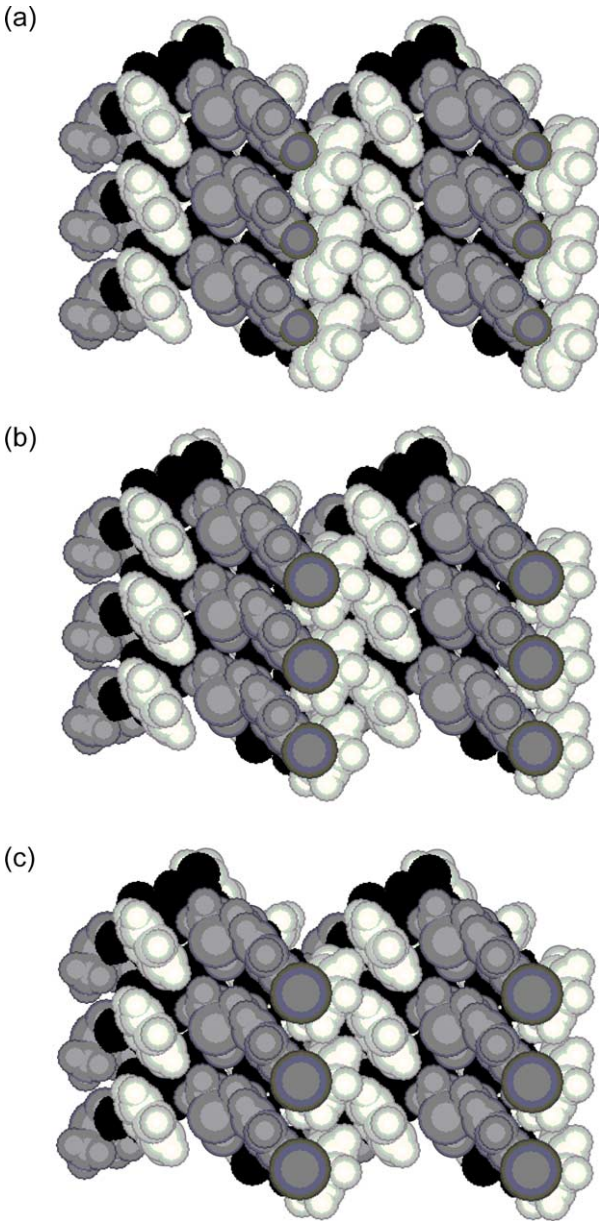
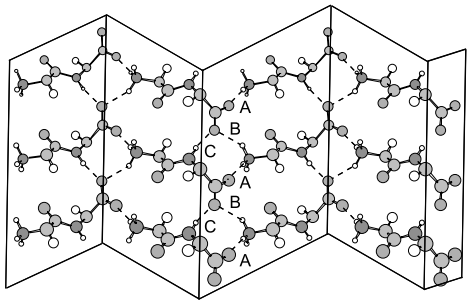


Figure 3. Top views of sheet structure of inclusion crystals (CPK model). (a) *RR*-**1**·**G1**-F. (b) *RR*-**1**·**G1**-Br. (c) *RR*-**1**·**G1**-I.

Interestingly, *RR*-**1** molecules constructed a pleated sheet in all of the inclusion crystals. The detailed description of the hydrogen bonding is given in Figure 4. As shown in Figure 4, the glycylglycine backbone of dipeptide as the hosting frame arranged in parallel and zigzags to construct a novel pleated sheet. Intermolecular distances of hydrogen bonding are comparable in three *RR*-**1**·**G1**-F, *RR*-**1**·**G1**-Br and *RR*-**1**·**G1**-I. These sheets involve ionic pairing of carboxyl and amino groups via hydrogen bonding network (hydrogen bonding A and B in Figure 4): one terminal COO^- contacts with two $^+NH_3$ of adjacent dipeptides at both oxygen sites and, inversely, the $^+NH_3$ are also bound to two adjacent COO^- groups (O···N). This hydrogen bonding mode of *RR*-**1** is also seen in the flat sheet of *RR*-**1** inclusion crystals of alkyl phenyl sulfoxide[5,6] as well as the ether-included (*R*)-naphthylglycyl-(*R*)-phenylglycine crystals.[8] It is noteworthy that the present pleated sheet structure by zigzag arrangement of *RR*-**1** makes it possible



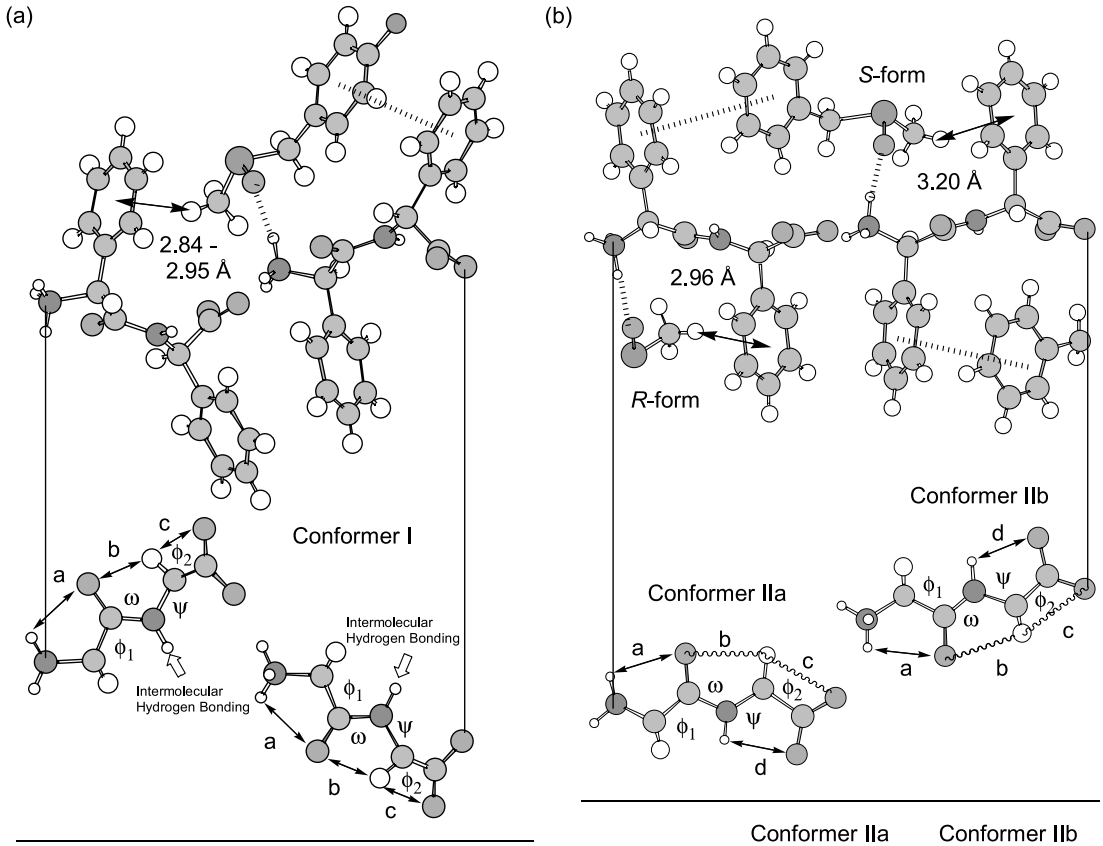
	host-host / Å			host-guest / Å
	A N---O	B N---O	C N---O	N--O(**G1**-X)
RR-1 • **G1**-F	2.80	2.82	3.07	2.78
RR-1 • **G1**-Br	2.77	2.79	3.01	2.82
RR-1 • **G1**-I	2.78	2.82	3.03	2.80

Figure 4. Dipeptide backbone and atomic distances of intermolecular hydrogen bonds.

to form another important intermolecular hydrogen bond (C in Fig. 4), as described below.

Figure 5 shows the included sulfoxide molecule surrounded by *RR*-**1** molecules. All of *RR*-**1** molecules in the present inclusion crystals with *p*-halobenzyl methyl sulfoxides adopt the similar conformations (conformer I), which are quite different from those (conformers IIa and IIb) in *RR*-**1**·**G1**-H (also see Fig. 2). In both of conformer I and conformer II, the conformations of the *N*-terminal phenylglycyl moiety are similar; the amide oxygen contacts with the ammonium hydrogen to provide a well-known five-membered ring.[9,10a] In conformer I, the *C*-terminal α-hydrogen is close to the amide oxygen and the carboxylate oxygen. This type of intramolecular C–H···O hydrogen bonding was often found in the crystals of amino acids and peptides.[10] As a result, the amide hydrogen is free from the intramolecular hydrogen bonding and contributes to the intermolecular hydrogen bonding (Bond C in Fig. 4). In other words, the hydrogen bonding (C) observed in conformer I compensates the loss of contact between hydrogen of the amide linkage and the oxygen of the carboxylate that is seen in conformer II. From the space-filling models of the inclusion crystals (Fig. 3), it is apparent that the phenyl groups of *RR*-**1** stack each other in an edge-to-face manner to construct a wall on the glycylglycine pleated sheet. These benzene–benzene interactions are seen in natural proteins[11] as well as artificial supramolecular aggregates.[12]

As mentioned above, *p*-halobenzyl methyl sulfoxides were recognized in the rhomboidal cavities of *RR*-**1** (also see Fig. 6). The guest molecules were linked to $^+NH_3$ of *RR*-**1** via hydrogen bonding and accommodated between the adjacent walls of phenyl groups of *RR*-**1** on the pleated layer. The distances between the ammonium nitrogen and the sulfinyl oxygen are listed in the table of Figure 4. Besides hydrogen bonding, benzene–benzene edge-to-face interaction plays an essential role in the inclusion of the



	Conformer I			Conformer IIa	Conformer IIb
	G1-F	**G1**-Br	**G1**-I		
	Atomic distances / Å			Atomic distances / Å	
a(NH-–O)	2.48	2.49	2.48	2.53	2.52
b(CαH-–O)	2.28	2.33	2.30	2.65	2.58
c(CαH-–O)	2.47	2.42	2.41	2.61	2.50
	Torsion angles / °			Torsion angles / °	
ϕ_1 (N-C-C-N)	147.0	154.8	152.8	141.5	139.0
ω (C-C-N-C)	170.2	167.1	166.1	177.0	178.2
ψ (C-N-C-C)	101.7	106.1	106.4	155.2	144.2
ϕ_2 (N-C-C-O)	128.1	133.7	134.4	155.1	152.3

Figure 5. Crystal structure of recognition sites. The list of selected atomic distances and torsion angles of *RR*-**1**. (a) *RR*-**1**·**G1**-X (X=F, Br, I). (b) *RR*-**1**·**G1**-H.

guest molecules. These interactions are shown in dotted lines in Figure 5. The methyl hydrogen of the guest approaches to the center of another benzene ring of the host to make weak contact between them as well as *RR*-**1**·**G1**-H.

Here, we wish to define the direction of the pleated sheet by the direction of the arranged *RR*-**1** molecules from their *N*-terminal to *C*-teminal. Interestingly, the sheets in *RR*-**1**·**G1**-Br and *RR*-**1**·**G1**-I are arranged in a parallel fashion, while those of *RR*-**1**·**G1**-F are in anti-parallel (Fig. 6). These sheet arrangements are reflected in the space groups ($P2_1$ and $P2_12_12_1$, respectively) of the crystals. The anti-parallel arrangement of the sheets in *RR*-**1**·**G1**-F crystals makes two **G1**-F molecules on the faced sheets be paired in an anit-parallel mode. This realizes the edge-to-face benzene–benzene interaction between the paired **G1**-F molecules (shown by a circle in Fig. 6). Furthermore, the fluorine atom is close (2.59 Å) to the *para*-hydrogen of the *RR*-**1** phenyl group of the faced sheet. This distance is smaller than the sum of van der Waals radii of the hydrogen and fluorine atoms (1.20 and 1.47 Å, respectively). These interactions seem to contribute to the stability of the *RR*-**1**·**G1**-F crystals.[13]

In contrast, *RR*-**1**·**G1**-Br and *RR*-**1**·**G1**-I inclusion crystals do not exhibit so intimate C–H···halogen interaction; the

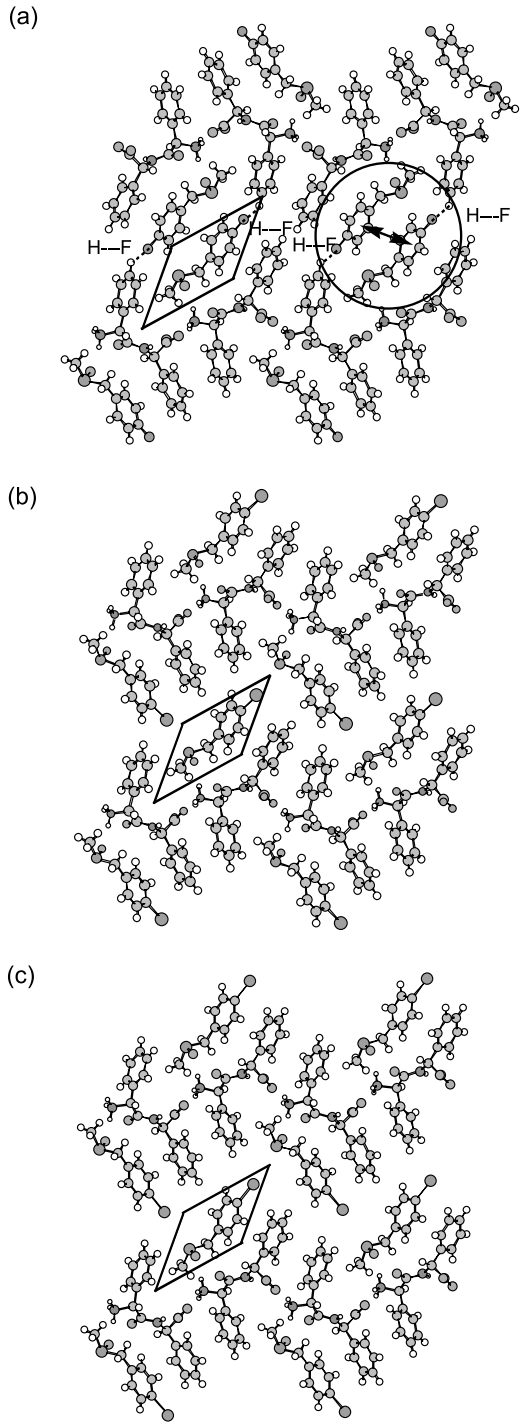


Figure 6. Layer structure of inclusion crystals. (a) *RR*-**1**·**G1**-F. (b) *RR*-**1**·**G1**-Br. (c) *RR*-**1**·**G1**-I.

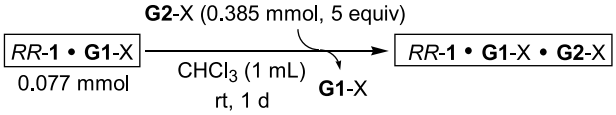
closest distances of H···halogen in these crystals are 3.10 and 3.28 Å, respectively, which are somewhat larger than the sum of van der Waals radii[7] of hydrogen and halogen (Br or I) [3.05 (1.20+1.85) Å, and 3.18 (1.20+1.98) Å]. The bromine atom and iodine atom in these inclusion crystals are surrounded not only by the phenyl group, but also by the sulfur atom of the adjacent guest sulfoxide. Since the closest distances of Br···S and I···S are 3.88 and 4.10 Å, respectively, it is likely that strong van der Waals interactions between these atoms do not work, comparing these values with the sum of their van der Waals radii,[7] 3.65 (1.85+1.80) Å and 3.78 (1.98+1.80) Å, respectively. Although the S–O bond of a sulfinyl group is so polar that the electron-deficient sulfinyl sulfur atom is known to serve as a counter for a halogen substituent,[14] this is not the case. Thus, these findings seem to show that the kind of halogen of the guest plays an important role in the formation of the inclusion crystals.

2.3. Guest exchange in inclusion crystals of *RR*-1

With several inclusion crystals of *RR*-**1** and *p*-halobenzyl methyl sulfoxides in hand, we were interested in the guest exchange in these crystals, which would reveal how strong the host framework interacts with the guest molecules. Fischer's lock-and-key model shows that polypeptides as enzymatic proteins catch their own substrate selectively and the product was easily exchanged with the upcoming substrate after the catalytic reaction in the cavity. Similarly, guest exchange processes of inclusion compounds are important for sensing and catalytic process of inclusion crystals in the solid phase. In such a crystal lattice inclusion by solid hosts, the guest exchange in solid inclusion compounds is usually examined using gaseous guest molecules, because reconstruction of dissolved host-guest complex in solution would be ruled out as a mechanistic problem.[15] However, there is a few reports which deal with guest exchange in inclusion crystals in solid-solution biphase.[16] Now, we discuss the guest exchange in these inclusion compounds from a standpoint of their crystal structures and host-guest interaction, using *RR*-**1**·**G1**-H and *RR*-**1**·**G1**-Br that have the parallel-arranged sheets therein.

At first, we performed a control experiment: when the inclusion crystals are suspended in chloroform and the resultant suspension was stirred for 1d, the guest was slowly released (the second column of Table 2). Next, solid *RR*-**1** was immersed in chloroform containing the guest molecules, but the inclusion phenomenon was not observed at all. This suggests that the once-dissolved *RR*-**1** molecules cannot form the inclusion crystals with the second guest (**G2**-**X**) in the solution phase. Hence, the exchange experiments were performed as follows: the inclusion compound (0.077 mmol, *RR*-**1**·**G1**-X) was stirred with 5 equiv of second *racemic* sulfoxide guest (0.385 mmol, **G2**-X) in $CHCl_3$ (1 mL) for 24 h. It should be noted that the

Table 2. Guest exchange experiment



RR-**1**·**G1**-X	Guest ratios[a] in *RR*-**1**·**G1**-X·**G2**-X		
	Blank	**G2**-H	**G2**-Br
RR-**1**·**G1**-H	**G1**-H 0.0	**G1**-H:**G2**-H-d_3 0.59:0.04 *Rac.*[b]	**G1**-H:**G2**-Br 0.0:0.0
RR-**1**·**G1**-Br	**G1**-Br 0.81	**G1**-Br:**G2**-H 0.02:0.80 *Rac.*[b]	**G1**-Br:**G2**-Br-d_3 0.93:0.03

[a] Guest ratios based on *RR*-**1** in the inclusion compound.
[b] *Rac.* means enantioselectivity within 10%.

inclusion compounds of *RR*-**1** are insoluble. The results are summarized in Table 2.

In order to examine the guest exchange between the same sulfoxides of *RR*-**1**·**G1**-X, we used (*p*-halo)benzyl d_3-methyl sulfoxides (**G2**-X-d_3) as the second guest. In the presence of **G2**-Br-d_3 (5 equiv), the release of the guest molecules from *RR*-**1**·**G1**-Br was retarded. Apparently, this result can be explained by considering that the high concentration of the guest (**G2**-Br-d_3) in solution phase slows down the release of the **G1**-Br, because adsorption of the guest (**G2**-Br-d_3) on the crystal of *RR*-**1**·**G1**-Br is promoted. However, the remained guest in crystals did not contain **G2**-Br-d_3. This means that the guest exchange was not induced so easily by the same guest. Recently, Aida and his coworkers reported the similar behavior for the guest exchange by porphyrinogen host molecules: the exchange of CH_3OH with CD_3OH, which should require no structural change of the crystal lattice, took place extremely slow.[15a] These cases have no driving force for guest exchange because these crystals are also isostructural. Next, we treated the inclusion crystal *RR*-**1**·**G1**-Br with benzyl methyl sulfoxide (**G2**-H). As soon as *RR*-**1**·**G1**-Br was stirred together with **G2**-H (5 equiv), the guest exchange started and occurred smoothly within 5 min, as shown in Figure 7. The stereochemistry of included **G2**-H was *racemic*, which agrees with the selectivity shown in Table 1. On the other hand, *RR*-**1**·**G1**-H did not allow the guest exchange with **G2**-Br. In this case, the release of **G1**-H occurred exclusively to give insoluble *RR*-**1** solid. Thus, we found that the guest exchange takes place in the case that the backbone sheets are extended from a pleated form into a flat form. Since these are preliminary results, we are going to continue our investigation on the guest exchange in the various inclusion crystals using various guest molecules.

3. Conclusion

Substitution by halogen atoms on *para*-position of benzyl methyl sulfoxide, a guest molecule, disrupted original benzene–benzene interaction of the host-guest inclusion system with *RR*-**1** as the host so as to generate a new structure of inclusion compounds. The guest molecules induced the conformational change of *RR*-**1** to give the novel rhombic cavity suitable for the shape of (*R*)-halobenzyl methyl sulfoxides, where *RR*-**1** aggregates in parallel and zigzags to construct a pleated sheet. We demonstrated the first example of guest exchange process in the layered dipeptide inclusion compounds. These findings show that dipeptides are able to capture their own substrate (guest) selectively and exchange easily with the upcoming substrate as if enzymatic polypeptides do.

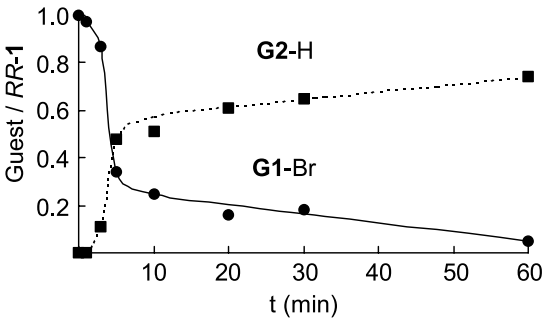


Figure 7. Time-course of guest exchange. Exposure of *RR*-**1**·**G1**-Br to **G2**-H in $CHCl_3$.

4. Experimental

4.1. General methods

^{1}H NMR spectra were recorded at 300 MHz using a Varian Gemini-2000NMR spectrometer and chemical shifts were referenced to TMS as an internal standard. Decomposition points (Dec.) were measured on a TG-DTA (MAC Science TG-DTA2000S). X-ray powder diffractions (PXRD) were obtained with a MAC Science MXP18 diffractometer using graphite-monochromated Cu $K\alpha$ radiation (30 kV, 200 mA). The spectra were measured at room temperature between 2 and 50° in the $2\theta/\theta$-scan mode with steps of 0.01° in 2θ and 4 deg min^{-1}. Elemental analyses were performed at the Chemical Analysis Center, Chiba University, Japan.

4.2. Materials

Chiral compounds, (*R*)-phenylglycine (99% ee) and (*S*)-α-methoxyphenyl acetic acid [(*S*)-MPAA] as a chiral shift reagent, were purchased from Tokyo Chemical Industry and Aldrich Chemical Co., respectively. Iodomethane-d_3 (99.5+atom%D) was purchased from C/D/N isotopes. (*R*)-phenylglycyl-(*R*)-phenylglycine (*RR*-**1**) was prepared according to our procedures previously reported.[6b] *p*-Halophenylmethanethiols were prepared from corresponding *p*-halobenzyl bromides and thiourea according to the literature.[17] By Williamson-type sulfide synthesis, sulfides were obtained from the *p*-halophenylmethanethiols and methyl iodide (or D-labeled methyl iodide).[18] Sulfides were oxidized to sulfoxides (**G1**-X, X=H, F, Cl, Br, or I) by hydrogen peroxide and acetic acid.[19]

4.3. Preparation of inclusion compounds of *RR*-1 and benzyl methyl sulfoxides

RR-**1** was dissolved in 0.1 M aqueous HCl, then the pH was adjusted to about 6.5 by addition of 0.1 M aqueous NaOH. After the addition of a *racemic* sulfoxide (**G1**-X, X=H, F, Cl, Br, or I, 2.2 equiv) to the aqueous solution of *RR*-**1**, the resulting mixture was allowed to stand at an ambient temperature. Then the deposited inclusion compound was collected by filtration and washed with chloroform and water.

4.4. Determination of efficiency, stereochemistry, and enantiomeric excess in the inclusion

After decomposition of the inclusion compound with diluted DCl in D_2O, inclusion efficiency was determined by NMR measurement. The included sulfoxide was isolated by dissolution of the inclusion compound in 0.1 M aqueous HCl and extraction with $CHCl_3$. Absolute stereochemistry of recognized sulfoxides was determined by a chiral shift reagent, (*S*)-α-methoxyphenyl acetic acid[20][(*S*)-α-MPAA:

3 equiv for the sulfoxide]. Enantiomeric excess of the sulfoxide was determined by a chiral HPLC (Daicel Chiralcel OB).

4.4.1. Compound *RR*-1·G1-F. Colorless crystals; dec. 190 °C; ^{1}H NMR (D_2O-DCl) δ 7.56–7.39 (m, 10H), 7.40 (dd, 2H, J=8.72, 5.43 Hz), 7.22 (dd, 2H, J=8.86 Hz), 5.50 (s, 1H), 5.22 (s, 1H), 4.28 (d, 1H, J=13.5 Hz), 4.07 (d, 1H, J=13.5 Hz), 2.64 (s, 3H); IR (KBr) 3242, 1674, 1626, 1593, 1523, 1009 cm^{-1}; powder X-ray diffraction [Å(I/I_0)] 13.4 (0.12), 12.1 (0.13), 4.44 (1.0), 4.15 (0.43), 3.88 (0.39). Anal. Calcd for $C_{16}H_{16}N_2O_3\cdot 1.00C_8H_9FOS$: C, 63.14; H, 5.52; N, 6.14. Found: C, 62.84; H, 5.44; N, 6.12. Included **G1**-F: $[\alpha]_D^{25}=-90.8$ (c=1.0, acetone); 99% ee *R* by HPLC; chiralcel OB, eluent, hexane/2-propanol=9/1, flow rate=0.7 mL/min, $t_R(R)$=33 min; ^{1}H NMR (with (*S*)-MPAA in $CDCl_3$, 98% ee *R*) δ 4.08 (d, 1.0H, J=13.1 Hz, *R* major and *S* minor), 3.98 (d, 0.01H, J=13.1 Hz, *S* minor), 3.97 (d, 0.99H, J=13.1 Hz, *R* major), 2.52 (s, 2.97H, *R* major), 2.49 (s, 0.03H, *S* minor). Aromatic 4H could be not identified because of (*S*)-MPAA.

4.4.2. Compound *RR*-1·G1-Cl. Colorless crystals; dec. 173 °C; ^{1}H NMR (300 MHz, D_2O-DCl) δ 7.56–7.39 (m, 10H), 7.48 (d, 2H, J=8.38 Hz), 7.35 (d, 2H, J=8.67 Hz), 5.50 (s, 1H), 5.22 (s, 1H), 4.27 (d, 1H, J=13.5 Hz), 4.05 (d, 1H, J=13.5 Hz), 2.63 (s, 3H); IR (KBr) 3240, 1674, 1624, 1591, 1523, 1009 cm^{-1}; powder X-ray diffraction [Å(I/I_0)] 14.2 (0.29), 12.1 (0.11), 4.49 (1.0), 4.20 (0.37), 3.93 (0.28). Anal. Calcd for $C_{16}H_{16}N_2O_3\cdot 1.00C_8H_9ClOS$: C, 60.94; H, 5.33; N, 5.92. Found: C, 60.77; H, 5.24; N, 5.94. Included **G1**-Cl: $[\alpha]_D^{25}=-73.5$ (c=1.0, acetone); 86% ee *R* by HPLC; chiralcel OB, HPLC eluent, hexane/2-propanol=9/1, flow rate=0.7 mL/min, $t_R(S)$=29 min, $t_R(R)$=44 min; ^{1}H NMR (with (*S*)-MPAA in $CDCl_3$, 83% ee *R*) δ 4.09 (d, 1.0H, J=12.9 Hz, *R* major and *S* minor), 3.98 (d, 0.08H, J=12.9 Hz, *S* minor), 3.97 (d, 0.92H, J=12.9 Hz, *R* major), 2.52 (s, 2.76H, *R* major), 2.50 (s, 0.24H, *S* minor). Aromatic 4H could be not identified because of (*S*)-MPAA.

4.4.3. Compound *RR*-1·G1-Br. Colorless crystals; dec. 179 °C; ^{1}H NMR (300 MHz, D_2O-DCl) δ 7.63 (d, 2H, J=8.52 Hz), 7.56–7.39 (m, 10H), 7.28 (d, 2H, J=8.38 Hz), 5.50 (s, 1H), 5.22 (s, 1H), 4.25 (d, 1H, J=13.3 Hz), 4.04 (d, 1H, J=13.3 Hz), 2.62 (s, 3H); IR (KBr) 3240, 1676, 1626, 1591, 1527, 1011 cm^{-1}; powder X-ray diffraction [Å(I/I_0)] 14.8 (0.053), 10.1 (0.052), 5.12 (0.61), 4.58 (0.83), 4.20 (0.82), 3.96 (1.0). Anal. Calcd for $C_{16}H_{16}N_2O_3\cdot 1.00C_8H_9BrOS$: C, 55.71; H, 4.87; N, 5.41. Found: C, 55.58; H, 4.87; N, 5.36. Included **G1**-Br: $[\alpha]_D^{25}=-77.4$ (c=0.99, acetone); 96% ee *R* by HPLC; chiralcel OB, HPLC eluent, hexane/2-propanol=9/1, flow rate=0.7 mL/min, $t_R(S)$=31 min, $t_R(R)$=46 min; ^{1}H NMR (with (*S*)-MPAA in $CDCl_3$, 96% ee *R*) δ 4.06 (d, 1.0H, J=12.9 Hz, *R* major and *S* minor), 3.96 (d, 0.02H, J=12.9 Hz, *S* minor), 3.95 (d, 0.98H, J=12.9 Hz, *R* major), 2.52 (s, 2.94H, *R* major), 2.50 (s, 0.06H, *S* minor). Aromatic 4H could be not identified because of (*S*)-MPAA.

4.4.4. Compound *RR*-1·G1-I. Colorless crystals; dec. 188 °C; ^{1}H NMR (300 MHz, D_2O-DCl) δ 7.84 (d, 2H, J=8.38 Hz), 7.56–7.39 (m, 10H), 7.15 (d, 2H, J=8.38 Hz), 5.50 (s, 1H), 5.22 (s, 1H), 4.24 (d, 1H, J=13.2 Hz), 4.02 (d, 1H, J=13.2 Hz), 2.62 (s, 3H); IR (KBr) 3377, 3238, 1676, 1626, 1591, 1527, 1012 cm^{-1}; powder X-ray diffraction [Å(I/I_0)] 6.71 (0.28), 6.21 (0.26), 5.10 (0.37), 4.53 (0.52), 4.15 (1.00), 3.95 (0.83). Anal. Calcd for $C_{16}H_{16}N_2O_3\cdot 1.00C_8H_9IOS$: C, 51.07; H, 4.46; N, 4.96. Found: C, 51.26; H, 4.48; N, 5.00. Included **G1**-I: $[\alpha]_D^{25}=-61.6$ (c=1.02, acetone); 95% ee *R* by HPLC; Chiralcel OB, HPLC eluent, hexane/2-propanol=9/1, flow rate=0.7 mL/min, $t_R(S)$=35 min, $t_R(R)$=55 min; ^{1}H NMR (with (*S*)-MPAA in $CDCl_3$, 93% ee *R*) δ 4.04 (d, 1.0H, J=12.9 Hz, *R* major and *S* minor), 3.94 (d, 0.03H, J=12.9 Hz, *S* minor), 3.93 (d, 0.97H, J=12.9 Hz, *R* major), 2.51 (s, 2.91H, *R* major), 2.50 (s, 0.09H, *S* minor). Aromatic 4H could be not identified because of (*S*)-MPAA.

4.5. Typical guest exchange experiment

Starting inclusion compounds (*RR*-**1**·**G1**-X) were prepared by crystallization of *RR*-**1** with first sulfoxide guest (**G1**-X) as mentioned above. The finely pulverized inclusion crystals (*RR*-**1**·**G1**-X, 0.077 mmol) was stirred with 5 equiv of second *racemic* sulfoxide guest (**G2**-X, 0.385 mmol) in $CHCl_3$ (1 mL) for 24 h at room temperature. Then resulting solid was collected by filtration and washed with chloroform (3 mL). The obtained crystal (*RR*-**1**·**G1**-X·**G2**-X) were analyzed by X-ray powder diffractions and infrared spectroscopy. The inclusion efficiency of the included **G1**-X and **G2**-X was determined by ^{1}H NMR. The absolute stereochemistry and enantiomeric excess of the included sulfoxide were determined by a chiral HPLC (Daicel Chiralcel OB-H).

RR-**1**·**G1**-Br·**G2**-H; Powder X-ray diffraction [Å(I/I_0)] 12.7 (0.89), 5.29 (0.11), 5.14 (0.19), 4.26 (1.00), 4.11 (0.23), 4.02 (0.34); IR (KBr) 3359, 1672, 1579, 1508, 1022 cm^{-1}. **G2**-H in *RR*-**1**·**G1**-Br·**G2**-H; *racemic* by HPLC; Chiralcel OB-H, HPLC eluent, hexane/2-propanol=5/1, flow rate=1.0 mL/min, $t_R(S)$=17 min, $t_R(R)$=21 min.

4.6. Crystallographic data for the inclusion compounds

To the solution of *RR*-**1** was added the sulfoxide guest directly in a vial, then a lid of the vial was loosely closed for evaporation of the solvent. The samples were allowed to stand for several days to form the desirable single crystals. Data collection was performed on a Mac Science MXC18 four-circle diffractometer with graphite-monochromated Cu $K\alpha$ (λ=1.54178) radiation using the $2\theta-\omega$ scan technique, and the X-ray intensities were measured up to 2θ=140° at 298 K. The structures were solved by a direct method *SIR97*[21] and *DIRDIF96*[22] and refined by a computer program package; maXus ver. 1.1 from MAC Science Co. Ltd. Hydrogen atoms were placed in calculated position with C–H=0.96 Å. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 249240–249242. 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].

4.6.1. Compound *RR*-1·G1-F. $C_{24}H_{25}FN_2O_4S$, M=

456.54, crystal dimensions 0.30×0.10×0.02 mm, orthorhombic, $P2_12_12_1$, a=14.037 (7) Å, b=27.903 (3) Å, c=5.513 (7) Å, V=2159 (2) Å^3, Z=4, ρ_{calcld}=1.404 g cm^{-3}, T=173 K, 2432 reflections measured, 2403 independent, refinement on F^2, R=0.057 (1476 reflections with $I>1.00\sigma(I)$), w$R(F^2)$=0.160, S=1.036, 289 parameters, with heavy atoms refined anisotropically, residual electron density 1.03/−1.93.

4.6.2. Compound *RR*-1·G1-Br. $C_{24}H_{25}BrN_2O_4S$, M=517.45, crystal dimensions 0.20×0.15×0.02 mm, monoclinic, $P2_1$, a=5.451 (4) Å, b=13.802 (8) Å, c=15.368 (7) Å, β=96.87 (5), V=1148.0 (12) Å^3, Z=2, ρ_{calcld}=1.497 g cm^{-3}, T=173 K, 4862 reflections measured, 2394 independent, refinement on F^2, R=0.078 (1848 reflections with $I>1.00\sigma(I)$), w$R(F^2)$=0.181, S=1.047, 289 parameters, with heavy atoms refined anisotropically, residual electron density 0.52/−1.14.

4.6.3. Compound *RR*-1·G1-I. $C_{24}H_{25}IN_2O_4S$, M=564.44, crystal dimensions 0.30×0.20×0.10 mm, monoclinic, $P2_1$ a=15.851 (6) Å, b=13.940 (9) Å, c=5.443 (2) Å, β=82.08 (3), V=1191.3 (10) Å^3, Z=2, ρ_{calcld}=1.574 g cm^{-3}, T=298 K, 2629 reflections measured, 2492 independent, refinement on F^2, R=0.069 (2063 reflections with $I>1.00\sigma(I)$), w$R(F^2)$=0.156, S=1.043, 290 parameters, with heavy atoms refined anisotropically, residual electron density 0.77/−1.45.

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The combination of 2-$NsNH_2$/NCS and MeCN as the nitrogen sources for the regio- and stereoselective formation of imidazolines from α,β-unsaturated ketones

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Dedicated to Professor Victor J. Hruby on the occasion of his 65th birthday

Abstract—A new nitrogen source combination was found for the regio- and stereoselective diamination of α,β-unsaturated ketones. This combination employs the readily available and inexpensive combination of NCS and 2-$NsNH_2$ as the electrophilic nitrogen source, and acetonitrile as the nucleophilic nitrogen source, respectively. The reaction is easily performed by mixing olefin, 2-$NsNH_2$, NCS and 4 Å molecular sieves in freshly distilled acetonitrile at room temperature. The reaction is chemoselective without the formation of any haloamine side products. A new aziridinium ion formed from enones and 2-NsNHCl is suggested to exist and to react with nitrile via a [2+3] cycloaddition mechanism, which is responsible for the excellent regio-, stereoselectivity of the resulting diamination products.

1. Introduction

1,2-Vicinal diamines play an important role in medicinal and pharmaceutical research.[1–3] For example, the ability of α,β-unsaturated carboxylate-derived diamines to mimic α and β amino acids is of great importance in peptide and protein studies. Meanwhile, these compounds have also been widely utilized as auxiliaries and ligands in asymmetric synthesis and catalysis.[4–7] Until now, most of the syntheses of these compounds have centered around the use of transition metal precusors.[2b,5] The development of efficient synthetic approaches to this functionality in regio- and stereoselective fashions is still a challenging topic, especially when functionalized olefins such as cinnamic esters and α,β-unsaturated ketones are employed as the substrates.

Recently, we and others have developed the regio- and stereoselective diamination of α,β-unsaturated ketones for the synthesis of 2-nitrobenzenesulfonyl-protected diamine derivatives.[3b,8–10] Our diamination reaction was carried out in a tandem manner by using *N*,*N*-dichloro-2-nitrobenzenesulfonamide (2-$NsNCl_2$) and acetonitrile as the nitrogen sources and stirring at room temperature for a period of 24 h without using any catalysts (Eq. (1), Scheme 1).[9b] 4 Å Molecular sieves were found to be crucial for controlling the diamine products at the stage of 1-*o*-nitrobenzenesulfonyl-3-dichloromethyl-4,5-imidazolines. Under slightly modified conditions, we were also able to control the reaction at the stage of 1-*o*-nitrobenzenesulfonyl-3-trichloromethyl-4,5-imidazolines (Eq. (2), Scheme 1). The latter reaction was achieved by performing the reaction at an elevated temperature in the absence of 4 Å molecular sieves. A similar synthesis has also been achieved for the *p*-tosyl-based diamination in which rhodium (II) acetate was utilized as the catalyst.[8e]

Very recently, we found that the diamination reaction can proceed with the combination of 4-$TsNH_2$ and NCS as the nitrogen source to replace 4-$TsNCl_2$ which is relatively unstable at room temperature.[9a] This method alleviates the need to prepare and store the relatively unstable 4-$TsNCl_2$ and demonstrates the first example for the aziridinium intermediate formation from the reaction of TsNHCl with olefins. We next attempted to use 2-$NsNH_2$ to replace 4-$TsNH_2$ with the anticipation of forming 1-*o*-nitrobenzenesulfonyl-3-dichloromethyl-4,5-imidazolines. In this paper, we would like to report the successful diamination of α,β-unsaturated ketones by using the new combination of 2-$NsNH_2$/NCS and MeCN as the electrophilic and

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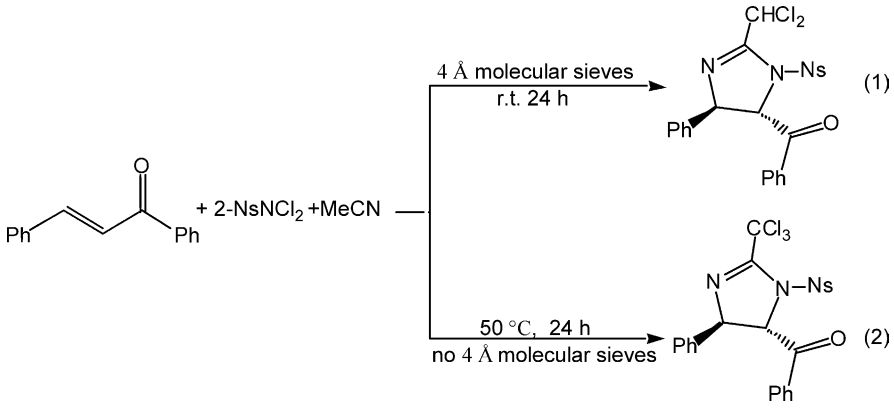
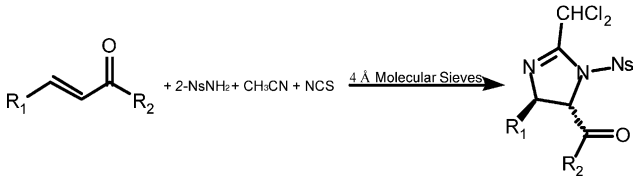


Scheme 1.

nucleophilic nitrogen sources. The reaction is described by Scheme 2, with the results summarized in Table 1.



Scheme 2.

Similar to our previous diamination, the present diamination is conveniently performed by simply by mixing olefin (1.0 mmol), 2-$NsNH_2$ (2.0 mmol), NCS (4.0 mmol), and 4 Å molecular sieves (0.40 g) in freshly distilled acetonitrile. The reaction vessel was capped, and the mixture was stirred at 50 °C for 1 day (without special protection of an inert gas atmosphere), at which time a second portion of NCS (2.0 mmol) was added. The reaction was again capped and stirred at 50 °C until the alkene starting materials were completely consumed, as determined by TLC or NMR spectroscopy.

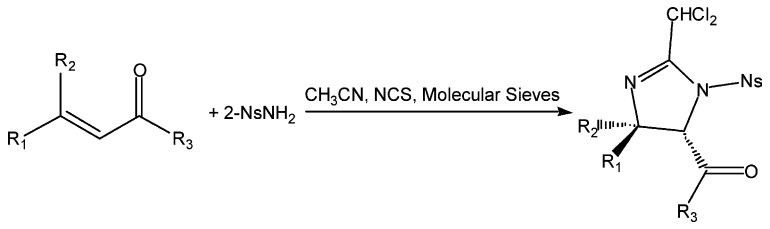
The initial optimization experiments employed *trans*-4-phenyl-3-buten-2-one as the substrate. 4 Å Molecular sieves were found to play a crucial role in the reaction, which is also similar to the previous system, which used the *p*-$TsNH_2$/NCS mixture. Although the reaction could proceed at room temperature, much longer reaction times were required for completion. Two-step of addition of NCS again proved to increase yields ~10–15%. Eight enone examples were examined under the present condition (Table 1).

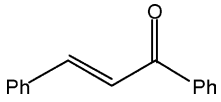
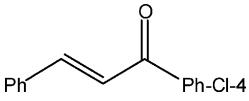
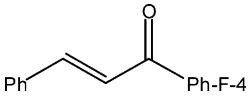
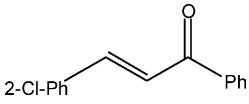
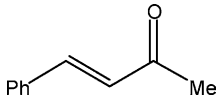
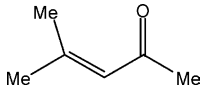
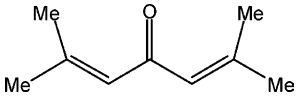
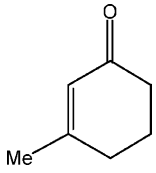
Interestingly, several obvious differences were found between the current 2-$NsNH_2$/NCS-based reaction and the previous 2-$NsNCl_2$-based imidazoline formation. First, in the absence of 4 Å molecular sieves, the nitrogen-source mixture of 2-$NsNH_2$ and NCS did not result in any trichlorinated products. This is in contrast to the previous diamination in which the resulting 3-dichloromethylimidazolines can proceed with a third chlorination on the 3-dichlomethyl group to give 1-*o*-nitrobenzenesulfonyl-3-dichloromethyl-4,5-imidazolines if 4 Å molecular sieves were not employed. Second, the current 2-$NsNH_2$/NCS-based diamination proceeded at a much slower speed. Also, for the present system, the use of less than 2 equiv of 2-$NsNH_2$ led to diminished chemical yields. Most substrates of the current diamination gave similar yields to those of the 2-$NsNCl_2$-based reaction. Unfortunately, the enone substrates with strong electron withdrawing groups (e.g., 3- and 4-nitrochalcone) failed to give any products at all. As can be seen from Table 1, both aromatic (entries 1–5) and aliphatic (entries 6–8) enone substrates worked well for this new process. Aliphatic enones typically gave faster reaction rates than aromatic ones, but no significant differences in chemical yields were observed. In no cases were the aminohalogenation side-product observed. Interestingly, the dienone substrate (entry 7) did not give any diamine product under the previous 2-$NsNCl_2$-based condition, but worked well in the present system and gave a chemical yield of 74%.

We believe that the mechanism of this reaction is similar to that of the $TsNH_2$/NCS-based diamination.[9a] The first step involves the formation of *N*-monochloro-2-nitrobenzenesulfonamide (2-NsNHCl) which reacts with olefin to form *N*-(2-nosyl)aziridinium intermediate (**A**, in Scheme 3). This intermediate joins the family of three other aziridinium ion intermediates, *N*-(*p*-tosyl),*N*-chloroaziridinium ion, *N*-(2-nosyl),*N*-chloroaziridinium ion and *N*-(*p*-tosyl), *N*-protonaziridinium ion, that have been found to serve for both electrophilic diamination and aminohalogenation reactions that we have established so far.[8–9] The next step of aziridinium ring opening proceeds through [2+3] cyclic addition by acetonitrile and **A** to form 1*N*-(2-nosyl)imidazolinium (**B**). This is the key step for explanation of regioselectivity and *anti* stereoselectivity (from *syn* addition). The following repeated deprotonation, chlorination and S_N2' type displacement result in the final products.

In summary, a new regio-, stereo- and chemoselective diamination of enones has been established without the observation of any haloamines. The reaction employs the readily available and inexpensive combination of NCS and 2-$NsNH_2$ as electrophilic nitrogen source, and acetonitrile as nucleophilic nitrogen source. A new aziridinium intermediate formed from enones and 2-NsNHCl has been proposed to exist during the reaction process, and to react with nitrile via a [2+3] cycloaddition mechanism. The concerted [2+3] cycloaddition for the aziridinium ring

Table 1. 2-$NsNH_2$/NCS/MeCN-based Diamination of enones



Entry	Substrate	Product no.	Mp/°C	Selectivity[a]	Time/h	Yield[b]
1		**1**	161–163	>95%	72	80
2		**2**	150–152	>95%	72	66
3		**3**	124–126	>95%	72	79
4		**4**	142–144	>95%	72	60
5		**5**	134–136	>95%	72	65
6		**6**	170–172	N/A	60	83
7		**7**	148–150	N/A	60	74
8		**8**	144 (dec)	>95%	60	67

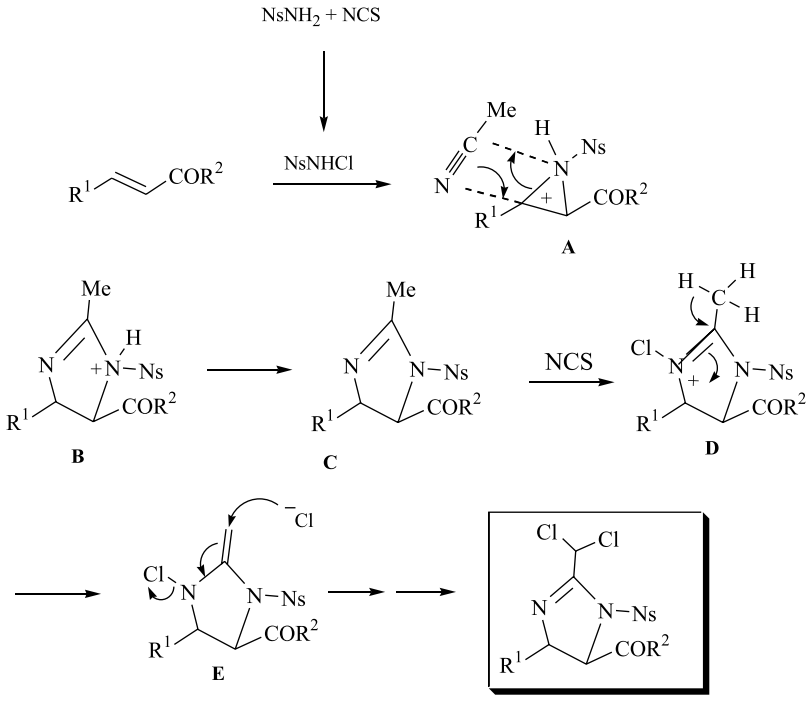
[a] Determined by crude ^1H NMR analysis. >95% Means that no minor isomer was detected.
[b] Yield analytically pure sample after column chromatography.

opening determines the regio- and stereoselectivity of the resulting diamination products.

2. Experimental

2.1. General

The representative procedure is demonstrated by the reaction of phorone with 2-nitrobenzenesulfonamide and NCS (entry 7, Table 1). Into a dry vial was added phorone (1.0 mmol), 2-$NsNH_2$ (2.0 mmol), NCS (4.0 mmol) and acetonitrile (6.0 mL). The vial was capped and stirred at 50 °C for 24 h. A second portion of NCS (2.0 mmol) was added, and the reaction vial was again capped and stirred at 50 °C until phorone was completely consumed. The resulting slurry was filtered, concentrated under reduced pressure, and purified via column chromatography to afford the pure product **7**, 1-(2-Dichloromethyl-3-(2-nitrobenzenesulfonyl)-5,5-dimethyl-4,5-dihydro-3*H*-imidazol-4-yl)-3-methyl-but-2-en-1-one, as a white solid (332 mg, 74% yield). Mp 148–150 °C. ^1H NMR (500 MHz, $CDCl_3$): 8.21–8.18 (m, 1H), 7.90–7.28 (m, 3H), 6.94 (s, 1H), 6.37 (m, 1H), 3.93 (s, 1H), 2.18 (d, $J=1$ Hz, 3H), 1.96 (d, $J=1$ Hz, 3H), 1.29 (s, 3H), 1.02 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 195.0, 161.3, 153.3, 147.8, 135.4, 133.2, 132.8,



Scheme 3.

125.5, 120.1, 70.5, 63.3, 30.2, 28.2, 23.1, 21.4. HRMS (ESI-TOF high-acc) m/z ($M^+ + 1$) found 448.0493, expected 448.0495. FTIR (cm^{-1}): 2980.8, 2937.0, 1686.9, 1617.6.

2.1.1. (2-Dichloromethyl-3-(2-nitrobenzenesulfonyl)-5-phenyl-4,5-dihydro-3*H*-imidazol-4-yl)-phenyl-methanone (1). Isolated as a white solid (414 mg, 80% yield). Mp 161–163 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 8.24–8.30 (m, 1H), 7.80–7.86 (m, 2H), 7.70–7.80 (m, 3H), 7.62–7.70 (m, 1H), 7.46–7.53 (m, 2H), 7.28–7.35 (m, 3H), 7.20 (s, 1H), 7.05–7.14 (m, 2H), 5.73 (d, J=4.0 Hz, 1H), 5.10 (d, J=4.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 192.7, 156.4, 147.9, 137.9, 135.2, 134.5, 133.0, 132.6, 132.0, 131.2, 129.2, 129.1, 129.0, 128.9, 126.5, 125.1, 72.3, 72.2, 62.4. HRMS (MALDI-FTMS) m/z ($M^+ + 1$) found 518.0348, calcd for $C_{23}H_{17}N_3O_5SCl_2$ 518.0339.

2.1.2. (4-Chloro-phenyl)-(2-dichloromethyl-3-(2-nitrobenzenesulfonyl)-5-phenyl-4,5-dihydro-3*H*-imidazol-4-yl)-methanone (2). Isolated as a white solid (364 mg, 66% yield). Mp 150–152 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 8.22–8.30 (m, 1H), 7.70–7.86 (m, 5H), 7.43–7.51 (m, 2H), 7.29–7.38 (m, 3H), 7.19 (s, 1H), 7.03–7.12 (m, 2H), 5.67 (d, J=4.0 Hz, 1H), 5.07 (d, J=4.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 191.6, 156.3, 147.8, 141.1, 137.7, 135.3, 132.6, 131.9, 131.2, 131.0, 130.2, 129.5, 129.2, 129.0, 126.4, 125.1, 77.2, 72.2, 62.3. Spectroscopic data are identical with previously reported literature values.[9b]

2.1.3. (2-Dichloromethyl-3-(2-nitrobenzenesulfonyl)-5-phenyl-4,5-dihydro-3*H*-imidazol-4-yl)-(4-fluoro-phenyl)-methanone (3). Isolated as a white solid (421 mg, 79% yield). Mp 124–126 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 8.25–8.30 (m, 1H), 7.83–7.90 (m, 2H), 7.73–7.83 (m, 3H), 7.28–7.36 (m, 3H), 7.13–7.21 (m, 3H), 7.06–7.12 (m, 2H), 5.69 (d, J=4.0 Hz, 1H), 5.08 (d, J=4.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 191.3, 167.5, 165.5, 156.4, 147.9, 137.8, 135.3, 132.6, 132.0, 131.8, 131.7, 131.2, 129.3, 129.1, 126.5, 125.1, 116.6, 116.4, 72.4, 72.3, 62.4. Spectroscopic data are identical with previously reported literature values.[9b]

2.1.4. [5-(2-Chloro-phenyl)-2-dichloromethyl-3-(2-nitrobenzenesulfonyl)-4,5-dihydro-3*H*-imidazol-4-yl]-phenyl-methanone (4). Isolated as a white solid (329 mg, 60% yield). Mp 142–144 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 8.16–8.20 (m, 1H), 7.80–7.86 (m, 2H), 7.74–7.80 (m, 2H), 7.68–7.74 (m, 1H), 7.58–7.66 (m, 1H), 7.42–7.49 (m, 2H), 7.29–7.35 (dd, J=7.5, 1.5 Hz, 1H), 7.17–7.28 (m, 2H), 7.08–7.17 (m, 2H), 5.71 (d, J=5.0 Hz, 1H), 5.55 (d, J=5.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 193.7, 157.2, 147.7, 135.9, 135.3, 134.3, 133.6, 132.6, 132.5, 132.2, 130.9, 129.9, 129.8, 129.0, 128.9, 128.2, 127.6, 125.2, 70.7, 69.1, 62.4. Spectroscopic data are identical with previously reported literature values.[9b]

2.1.5. 1-(2-Dichloromethyl-3-(2-nitrobenzenesulfonyl)-5-phenyl-4,5-dihydro-3*H*-imidazol-4-yl)-ethanone (5). Isolated as a white solid (295 mg, 65% yield). Mp 134–136 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 7.93–8.00 (dd, J=8.0, 1.5 Hz, 1H), 7.66–7.77 (m, 2H), 7.55–7.65 (m, 1H), 7.13 (s, 1H), 7.00–7.10 (m, 3H), 6.94–7.00 (m, 2H), 5.22 (d, J=3.5 Hz, 1H), 4.23 (d, J=3.5 Hz, 1H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 205.4, 156.8, 147.5, 139.0, 135.5, 132.8, 132.6, 129.2, 128.7, 128.0, 125.6, 125.5, 75.7, 71.7, 63.5, 26.3. Spectroscopic data are identical with previously reported literature values.[9b]

2.1.6. 1-(2-Dichloromethyl-3-(2-nitrobenzenesulfonyl)-5,5-dimethyl-4,5-dihydro-3*H*-imidazol-4-yl)-ethanone (6). Isolated as a white solid (338 mg, 83% yield). Mp 170–172 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 8.13–8.21 (m, 1H),

7.80–7.96 (m, 3H), 6.92 (s, 1H), 3.89 (s, 1H), 2.37 (s, 3H), 1.29 (s, 3H), 0.98 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 205.4, 153.5, 147.8, 135.7, 133.2, 132.9, 129.6, 125.7, 77.2, 70.4, 63.2, 30.0, 27.8, 23.0. HRMS (MALDI-FTMS) *m/z* ($M^+ + 1$) found 408.0177, calcd for $C_{14}H_{15}N_3O_5SCl_2$ 408.0182.

2.1.7. 2-Dichloromethyl-3-(2-nitrobenzenesulfonyl)-7a-methyl-3,3a,5,6,7,7a-hexahydro-benzoimidazol-4-one (8). Isolated as a white solid (238 mg, 67% yield). Mp: decomposed at 144 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 8.18–8.30 (m, 1H), 7.82–7.90 (m, 3H), 6.92 (s, 1H), 3.87 (s, 1H), 2.69–2.81 (m, 1H), 2.31–2.45 (m, 1H), 2.05–2.15 (m, 1H), 1.84–2.05 (m, 2H), 1.60–1.70 (m, 1H), 1.30 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 206.1, 154.9, 148.1, 135.4, 133.6, 132.7, 129.8, 125.2, 74.2, 73.3, 62.7, 36.4, 34.0, 27.4, 18.6. Spectroscopic data are identical with previously reported literature values.[9b]

Acknowledgements

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Synthesis of isotopically labeled puromycin derivatives for kinetic isotope effect analysis of ribosome catalyzed peptide bond formation

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Abstract—The mechanism by which the ribosome catalyze peptide bond formation remains controversial. Here we describe the synthesis of dinucleotides that can be used in kinetic isotope effect experiments to assess the transition state of ribosome catalyzed peptide bond formation. These substrates are the isotopically labeled dinucleotide cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-L-phenylalanyl-N^6,N^6-dimethyladenosine (Cm^6A_NPhe-NH_2) and cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-(L-2-hydroxy-3-phenylpropionyl)-N^6,N^6-dimethyl-adenosine (Cm^6A_NPhe-OH). These substrates are active in peptide bond formation and can be used to measure kinetic isotope effects in ribosome catalyzed protein synthesis.

1. Introduction

The ribosome is the ribonucleoprotein-complex responsible for protein synthesis in all cells. The structure of the 50 S ribosomal subunit was determined recently by X-ray crystallography.[1,2] The structure revealed that the peptidyl transferase center of the ribosome resides solely within the 23 S ribosomal RNA (rRNA), that is, the ribosome is an RNA enzyme or ribozyme.[1] The ribosome catalyses peptide bond formation between two substrates, the aminoacyl tRNA bound in the ribosomal A site, and the peptidyl tRNA bound in the ribosomal P site. The reaction involves nucleophilic attack of the A-site tRNA α-amino group on the carbonyl-ester linking the nascent peptide to the P-site tRNA. The products of this reaction are a deacylated P-site tRNA and an A-site tRNA linked to the nascent peptide, which has been extended by one amino acid.

The ribosome enhances the rate of peptide bond formation by more than 100,000 fold.[3] The pH dependence of the reaction suggests that an ionizable functional group in the ribosome is catalytically important.[3–5] This group could act as a general base to deprotonate the nucleophile, or deprotonation may cause a conformation change in the ribosome that enhances the reaction rate. Several chemical pathways have been proposed for the reaction, all of which invoke a tetrahedral transition state.[6–8] They differ with regard to the point along the reaction coordinate at which the nucleophile is deprotonated and the leaving group is protonated.

Transition state stabilization is a fundamental strategy employed by enzymes to promote chemical reactions. Characterization of the transition state would provide information essential to understanding how the ribosome enhances the peptidyl transferase reaction; but the transient nature of the free energy maxima between products and reactants makes such investigations extremely challenging. One approach to transition state analysis is the measurement of kinetic isotope effects.[9–12] Reactive functional groups in the substrates are isotopically labeled and the relative reaction rates of the heavy and light substrates determined by enzyme kinetics. Kinetic isotope effects arise from changes in vibrational states between the ground state and the transition state in a chemical reaction.[13] The magnitude and direction of these effects provides the information needed to successfully predict the reaction transition state, including reactions catalyzed by enzymes such as the ribosome.[13–18]

Keywords: Ribosome; Puromycin; Kinetic isotope effect; Solid-phase synthesis.
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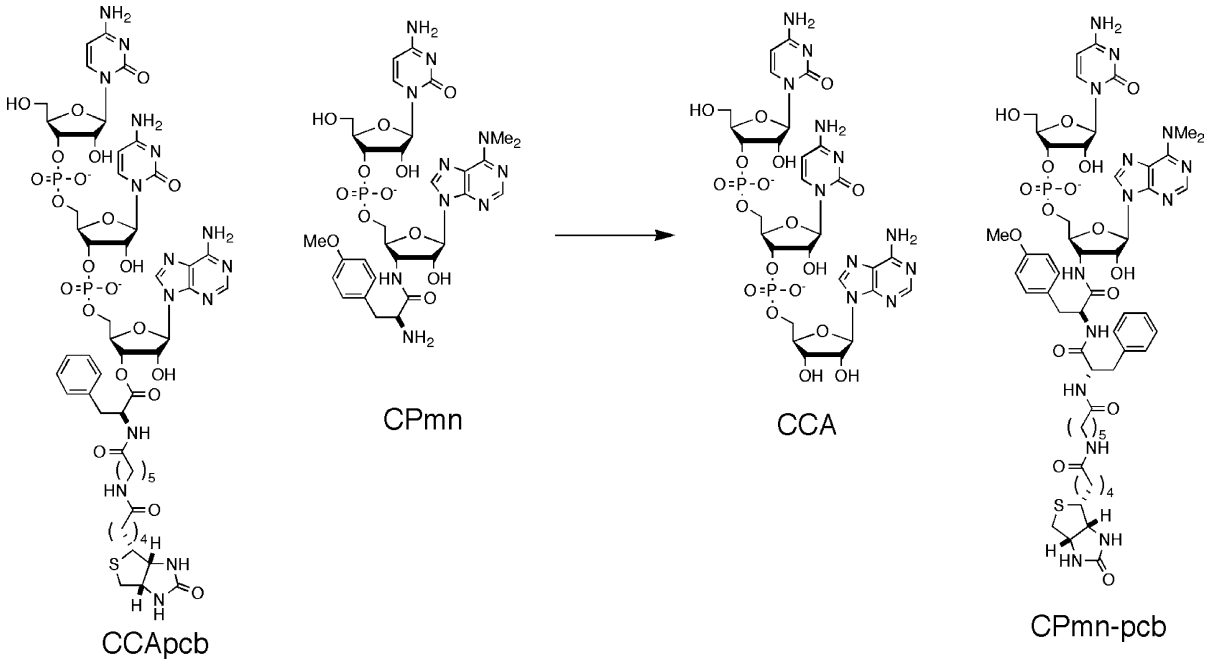


Scheme 1. Schematic of the modified fragment assay.

In order to measure a kinetic isotope effect, the isotope sensitive step must be rate-limiting. Ribosomal protein synthesis involves a multi-step process aided by GTP dependent protein factors. It is known from kinetic studies that accommodation of the amino-acyl tRNA is rate-limiting during the elongation step of protein synthesis.[19] Therefore, an assay that does not include accommodation is necessary for kinetic isotope effect measurements. The 50 S ribosomal subunit alone can catalyze peptide bond formation using two small synthetic substrates that mimic the A-site and P-site tRNAs (Scheme 1).[20] In this modified fragment assay, cytidylyl-(3′-5′)-puromycin (CPmn) functions in place of the A-site tRNA, while cytidylyl-(3′-5′)-cytidylyl-(3′-5′)-3′-(biotinyl-ε-aminocaproyl-L-phenylalanyl)adenosine (CCApcb) serves in place of the P-site tRNA. Like the standard peptidyl transferase reaction, the α-amino group of the A-site substrate attacks the ester bond in the P-site substrate, to produce a new peptide bond.[21] This simplified reaction is ideal for measuring kinetic isotope effects for two reasons: (i) the small substrates can be chemically synthesized to include specific isotopic substitutions, and (ii) the reaction is mechanistically simplified to the reversible binding of two small substrates, a chemical reaction, and product release.

In order to measure kinetic isotope effects on the ribosome, it was necessary to synthesize substrates with heavy atom substitutions on the reactive functional groups. Here we describe the synthesis of CPmn derivatives with ^{15}N substitution at the α-amino group and remote positions (**1**, **1*** and **1*****), and derivatives in which the α-amino is substituted with an ^{18}O α-hydroxyl group (**2** and **2***) (Fig. 1). We also demonstrate that these molecules serve as ribosome substrates.

2. Results and discussion

2.1. Synthesis of $Cm^6A_NPhe\text{-}NH_2$

The CPmn analogs $Cm^6A_NPhe\text{-}NH_2$ (**1** and **1***),[22] where **N** is either ^{14}N or ^{15}N, were prepared by solid phase synthesis.

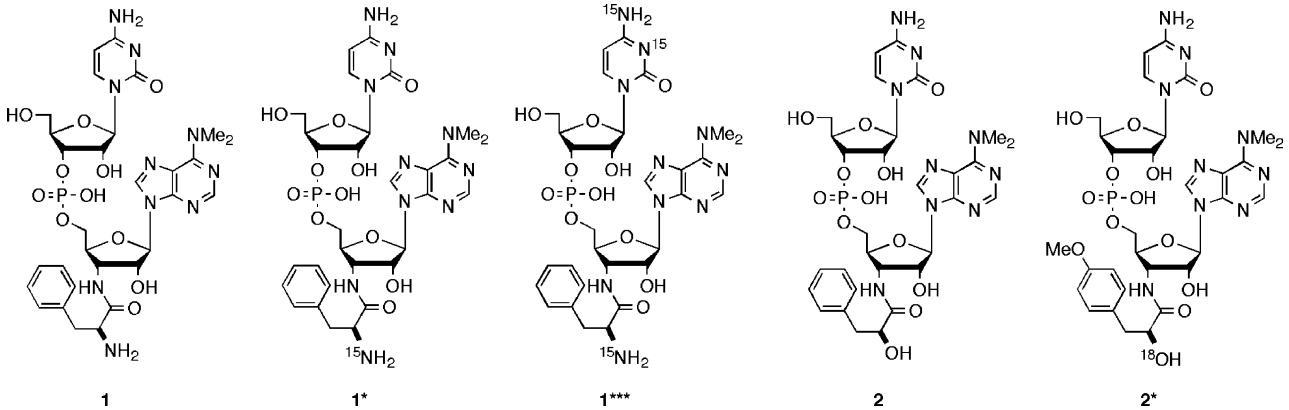


Figure 1. Synthetic targets $Cm^6A_NPhe\text{-}NH_2$ and $Cm^6A_NPhe\text{-}OH$ and their isotopic derivatives.

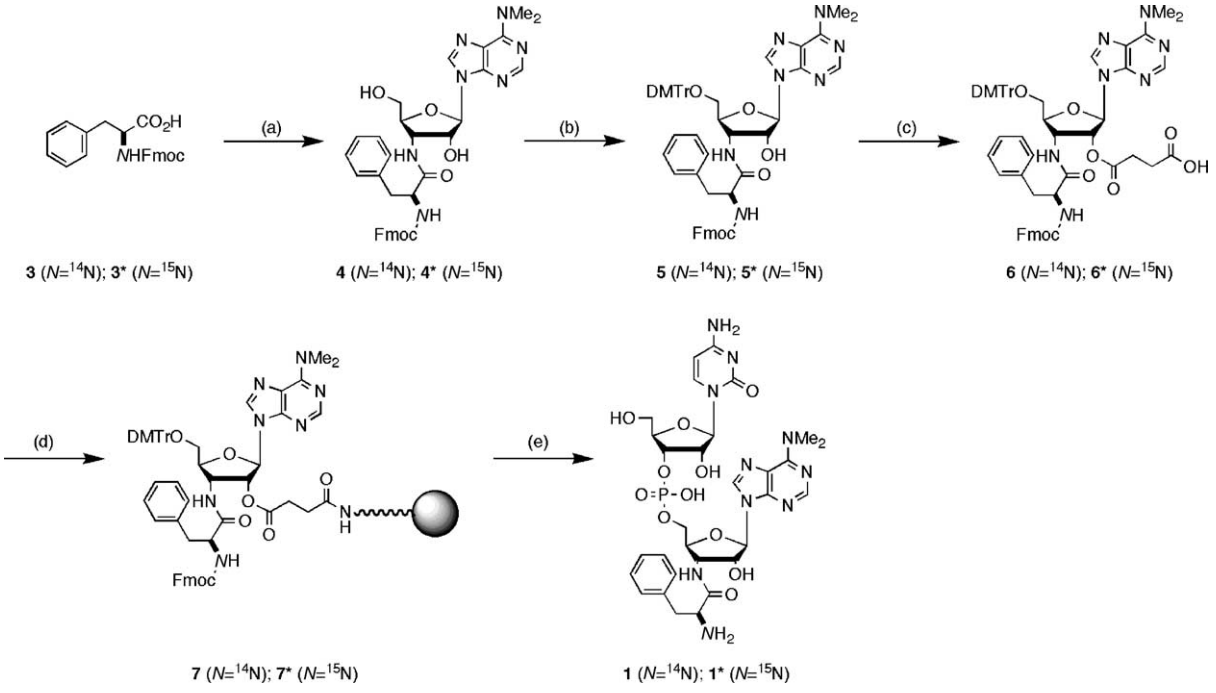
For this purpose, the appropriately protected puromycin analogs were attached to solid support and coupled to cytidine as shown in Scheme 2.

The 3′-amino group of puromycin aminonucleoside was selectively derivatized with commercially available *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanine (**3** and **3***) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI). Further derivatization of the 5′ and 2′ hydroxyl groups was performed using *p*,*p*′-dimethoxytrityl chloride (DMTrCl) and succinic anhydride, respectively. This transformation was accomplished using methods analogous to those reported in the literature[23,24] to yield suitably protected compounds **6** and **6***. These were attached to LCAA-polystyrene support and the loading quantified spectroscopically by the DMTr cation method.[25] The solid support (**7** and **7***) was coupled to the protected cytidine phosphoramidite, 4-acetyl-5′-*O*-[benzhydroxybis-(trimethylsiloxy)silyl]-2′-*O*-[bis(2-acetoxyethoxy)methyl]-cytidine-3′-(methyl-*N*,*N*-diisopropyl)phosphoramidite (**8**). The dinucleotide was cleaved from the solid support and deprotected as described previously.[26] Subsequent purification by reversed-phase HPLC yielded **1** and **1***, respectively.

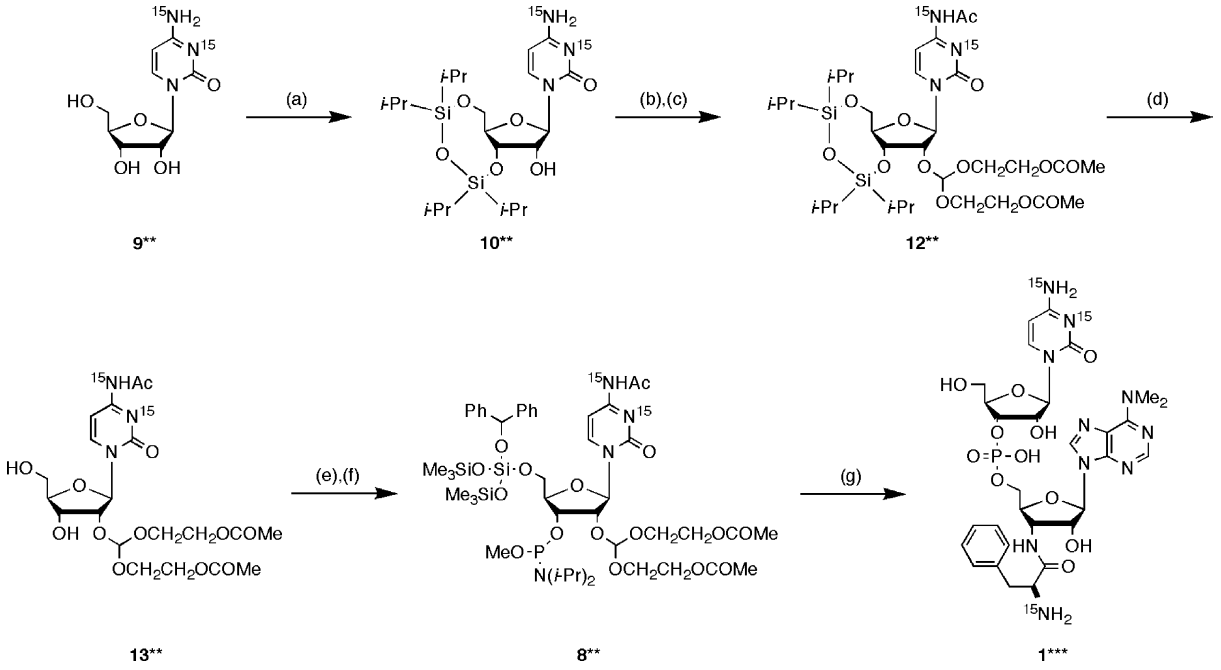
2.2. Synthesis of [3-^{15}N,4-$^{15}NH_2$]Cm^6A_NPhe-$^{15}NH_2$

The mass difference between Cm^6A_NPhe-$^{14}NH_2$ (**1**) and Cm^6A_NPhe-$^{15}NH_2$ (**1***) is only 1 Da. Whole molecule mass spectroscopic analysis of the two isotopes would be complicated by the significant size of the M+1 peaks that arises from natural ^{13}C abundance. To increase the mass difference between the two isotopes, we incorporated two additional remote substitutions in the ^{15}N labeled substrate. This is reminiscent of the remote labeling method that used radioactive isotopes as markers for heavy atom substitutions in kinetic isotope effect studies.[27] Because the cytidine moiety does not participate directly in the chemical reaction, heavy isotope substitution should not affect the reaction, nor should it show an isotope effect. Any affects can be controlled for by characterization of a dinucleotide containing only the remote labels. We selected [3-^{15}N,4-$^{15}NH_2$]cytidine[28] (**9****) for this purpose. The preparation of the [3-^{15}N,4-$^{15}NH_2$]cytidine phosphoramidite (**8****) was accomplished as follows (Scheme 3).[29]

Compound **9**** was prepared from uridine in five steps.[28] The ^{15}N at position N3 was introduced by the rearrangement reaction caused by the attack of $^{15}NH_3$ at the C4 of 2′,3′,5′-tri-*O*-acetyl-3-nitrouridine. The $^{15}NH_2$ substitution at the N4 position was introduced from the 4-(tetrazol-1-yl) intermediate by the $^{15}NH_3$ replacement reaction. Compound **9**** was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDSCl$_2$) in pyridine to simultaneously protect the 3′ and 5′ hydroxyl groups to produce **10****. The N4 amino group was acetyl protected (**11****), and the 2′ hydroxyl group protected with tris(2-acetoxyethoxy)orthoformate in the presence of 4-*tert*-butyldimethylsiloxy-3-penten-2-one in CH_2Cl_2 under reflux to give **12****. The 5′-3′ silyl protecting group was removed by fluoride (**13****), and the 5′ hydroxyl group was again protected by benzhydroxybis(trimethylsiloxy)silyl chloride (BzhCl) and diisopropylamine (**14****). Finally the 3′ hydroxyl group was derivatized with methyl tetraisopropyl phosphorodiamidite and 1*H*-tetrazole to yield target phosphoramidite (**8****). Coupling of **8**** to **7*** and deprotection was performed by standard



Scheme 2. Synthesis of Cm^6A_NPhe-NH_2 (**1**, **1***). Reagents and conditions: (a) puromycin aminonucleoside, EDCI, *N*-hydroxysuccinimide, DMF, 0 °C to room temperature, 74% (**4**), 77% (**4***); (b) DMTrCl, triethylamine, pyridine, room temperature, 86% (**5**), 89% (**5***); (c) succinic anhydride, DMAP, pyridine, room temperature, 65% (**6**), 60% (**6***); (d) LCAA-polystyrene, EDCI, DMAP, triethylamine, pyridine, room temperature, 109 μmol/g (**7**), 110 μmol/g (**7***); (e) as described in Ref. 26.



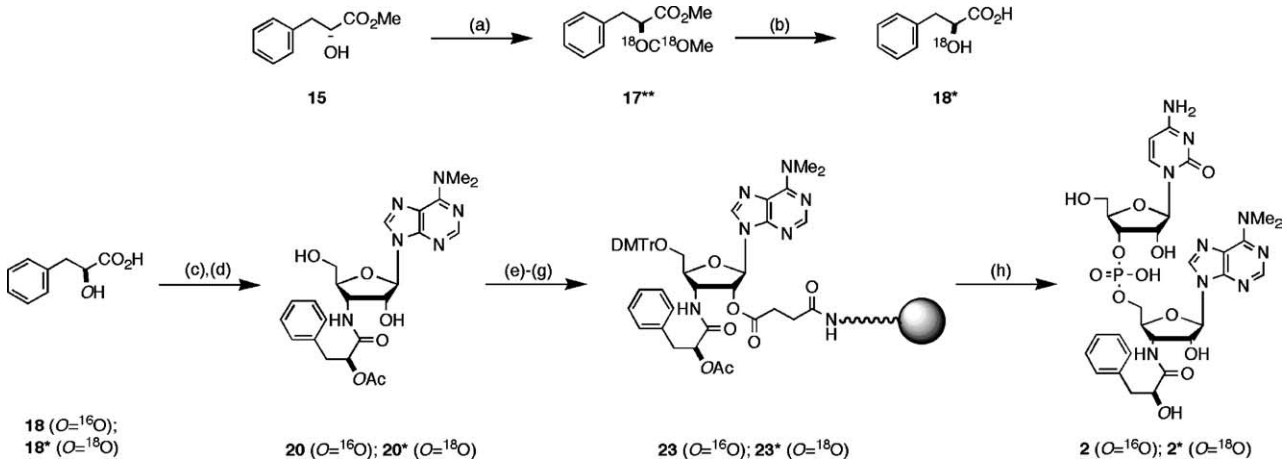
Scheme 3. Synthesis of [3-^{15}N,4-^{15}NH$_2$]Cm^{6}A$_N$Phe-^{15}NH$_2$ (**1*****). Reagents and conditions: (a) TIPDSCl$_2$, pyridine, 0 °C to room temperature, 78%; (b) acetic anhydride, DMF, room temperature, 100%; (c) tris(2-acetoxyethoxy)orthoformate, pyridnium *p*-toluenesulfonate, 4-*tert*-butyldimethylsiloxy-3-penten-2-one, CH_2Cl_2, reflux, 85%; (d) *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, 48% HF aq, acetonitrile, room temperature, 99%; (e) BzhCl, diisopropylamine, CH_2Cl_2, 0 °C, 96%; (f) methyl tetraisopropyl phosphorodiamidite, 1*H*-tetrazole, CH_2Cl_2, 0 °C, 89%; (g) as described in Ref. 26.

methods of solid phase oligoribonucleotide synthesis.[26] Purification by reverse-phase HPLC yielded [3-^{15}N,4-^{15}NH$_2$]-Cm^{6}A$_N$Phe-^{15}NH$_2$ (**1*****), which has three ^{15}N labels.

2.3. Synthesis of Cm^{6}A$_N$Phe-OH

The A-site substrate hydroxy-puromycin (Pmn-OH) has proven useful for investigating the peptidyl transferase reaction, because it is a substrate that does not have a neutral pK_a.[5] Pmn-OH participates in a transesterification reaction in which the peptide in the P-site is transferred to the A-site substrate via a new ester linkage. Pmn-OH has been used to deconvolute the contribution of the subtrate pK_a from that of the ribosome.[3]

In order to explore kinetic isotope effects on this transesterification reaction we set out to prepare the CPmn-OH derivatives (**2** and **2***) with ^{18}O substitution at the nucleophilic hydroxyl. Preparation of Cm^{6}A$_N$Phe-OH followed a synthetic scheme analogous to that described above (Scheme 4). The 2 hydroxyl group of D-methyl 2-hydroxy-3-phenylpropionate[30] (**15**) was converted to the triflate by trifluoromethanesulfonic anhydride in the



Scheme 4. Synthesis of Cm^{6}A$_N$Phe-OH (**2** and **2***); Reagents and conditions: (a) trifluoromethanesulfonic anhydride, pyridine, CH_2Cl_2, 0 °C to room temperature, then acetic $^{18}O_2$-acid, K_2CO_3, acetonitrile, room temperature, 83%; (b) 5 N KOH aq, MeOH, room temperature, 54%; (c) acetic anhydride, pyridine, room temperature, 100% (**19**), 95% (**19***); (d) puromycin aminonucleoside, EDCI, *N*-hydroxysuccinimide, DMF, 0 °C to room temperature, 73% (**20**), 71% (**20***); (e) DMTrCl, triethylamine, pyridine, room temperature, 89% (**21**), 89% (**21***); (f) succinic anhydride, DMAP, pyridine, room temperature, 57% (**22**), 62% (**22***); (g) LCAA-polystyrene, EDCI, DMAP, triethylamine, pyridine, room temperature, 70 μmol/g (**23**), 103 μmol/g (**23***); (h) as described in Ref. 26.

presence of pyridine and CH_2Cl_2.[31] The crude intermediate (**16**) was directly transformed to the ^{18}O-labeled acetoxy compound (**17****) with inverted stereochemistry using acetic $^{18}O_2$-acid and K_2CO_3 in acetonitrile. The L-[2-^{18}OH]-3-phenyllactic acid (**18***) was obtained by alkaline hydrolysis. L-3-Phenyllactic acid (**18** and **18***) was converted to acetate by acetic anhydride in pyridine to give **19** and **19***, respectively. As above, the 3′-amino group of puromycin aminonucleoside was selectively derivatized with **19** or **19*** using EDCI, the 5′ and 2′ hydroxyl groups protected with DMTrCl and succinic anhydride, respectively, and the resulting compounds attached to solid support. Solid-phase coupling of the cytidine phosphoramidite (**8**), followed by deprotection and HPLC purification yielded substrates, **2** and **2***. Unlike the ^{15}N containing compound **1***, the difference of two mass units between ^{16}O and ^{18}O is sufficient for kinetic isotope effect measurements by whole molecule mass spectrometry.

2.4. Ribosome 50 S subunit reaction assay

We tested if **1** and **2** serve as acceptors in the ribosomal peptidyl transferase reaction. Each substrate was incubated with 50 S ribosomal subunits in the presence of the P-site substrate CCApcb (Dharmacon Inc.)[21] that had been 5′-^{32}P radiolabeled with the enzyme polynucleotide kinase. Production of the P-site product CCA was monitored by gel electrophoresis (Fig. 2a). A new band of increased mobility increased as a function of time. The aminolysis and alcoholysis reactions proceed at a rate >100-fold and >10-fold above the background rate of hydrolysis, respectively. The A-site product was visualized by a peak of increased retention on the HPLC and the assignment confirmed by mass spectroscopic analysis (Fig. 2b, data shown for **1**). Similar results were obtained for compounds **1***, **1*****. These data indicate that **1**, **1***, **1*****, **2** and **2*** serve as acceptors in the peptidyl transferase reaction. These substrates will make it possible to perform kinetic isotope experiments on the ribosome.

3. Experimental

3.1. General

All reactions were monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Chromatography was performed with the indicated solvent system using Silicycle 0.040–0.060 mm silica gel. NMR spectra were measured on Brucker Avance

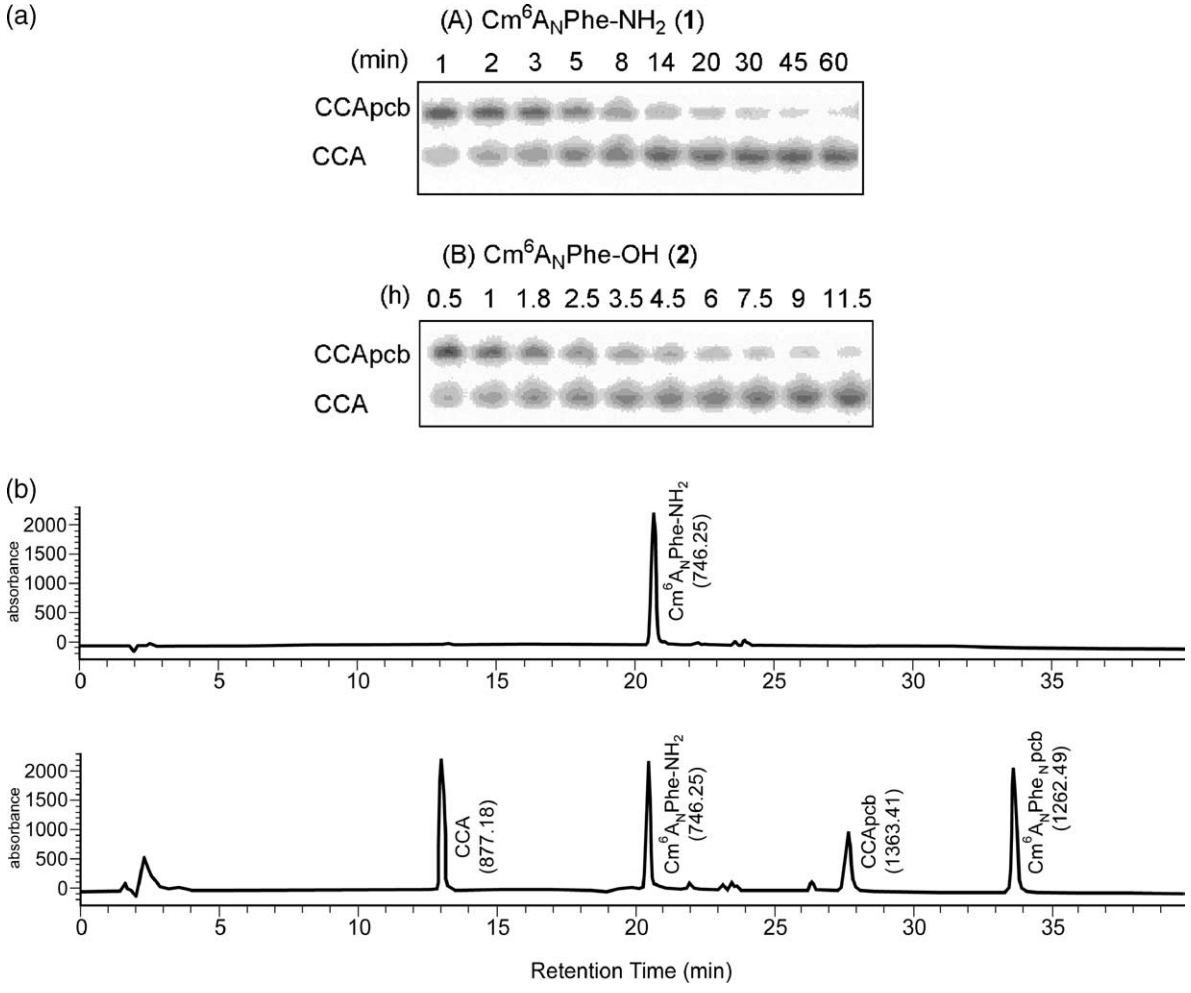


Figure 2. a. Demonstration of the peptidyl transferase reaction of **1** (A) and **2** (B) with ^{32}pCCApcb catalyzed by the 50 S ribosome subunit. The top bands are ^{32}pCCApcb and the bottom bands are the deacylated product ^{32}pCCA. b. HPLC trace of the reaction of **1** with CCApcb at an intermediate time point. Peaks for each of the reactants and nucleotide containing products are visible. Their observed molecule weights are indicated.

DPX-400 and Brucker Avance DPX-500 spectrometers. ^{1}H and ^{13}C NMR chemical shifts were recorded relative to the standard of tetramethylsilane, ^{15}N NMR chemical shifts were recorded relative to nitromethane as an external standard, and ^{31}P NMR chemical shifts were recorded relative to 85% phosphoric acid as an external standard. Mass spectra were collected on Waters Micromass LCT and Waters Micromass ZQ mass spectrometers. Optical rotation was performed on Perkin–Elmer Polarimeter 341.

Amino-derivatized polystyrene beads (Primer Support 30 HL, Amino-derivatized with a loading level of 161 μmol/g) was purchased from Amersham Biosciences. Pyridine was dried using Molecular Sieves. All other chemicals were used as received from commercial suppliers. [3-^{15}N,4-$^{15}NH_2$]cytidine[28] (**9****) and D-methyl 3-phenyllactate[30] (**15**) were synthesized according to the literature procedure.

3.1.1. 3′-Amino-3′-deoxy-3′-[*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-N^6,N^6-dimethyladenosine (4). To the solution of puromycin aminonucleoside (Sigma) (200.3 mg, 0.681 mmol), *N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanine (**3**, 290.4 mg, 0.750 mmol), and *N*-hydroxysuccinimide (290.4 mg, 0.748 mmol) in DMF (9.0 ml), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (145.0 mg, 0.756 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then stirred at room temperature for 24 h. After evaporation, the oily residue was crystallized with ethyl acetate (15 ml). This crude product was washed with ethyl acetate (30 ml), water (15 ml), ethyl acetate (10 ml), successively to give the pure product **4** as a colorless powder (333.9 mg, 74%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.44 (s, 1H, H8), 8.23 (s, 1H, H2), 8.17 (d, 1H, J=7.2 Hz, 3′-NH), 7.87 (d, 2H, J=8.0 Hz, Fmoc-aromatic), 7.64 (m, 3H, Fmoc-aromatic, Phe-NH), 7.42–7.18 (m, 9H, Fmoc-aromatic, Phe-aromatic), 6.10 (d, 1H, J=4.4 Hz, 2′-OH), 6.00 (s, 1H, H1′), 5.19 (t, 1H, J=4.0 Hz, 5′-OH), 4.52–4.47 (m, 2H, H2′, H3′), 4.42–4.37 (m, 1H, Phe-CH), 4.17–4.08 (m, 3H, Fmoc-CH, Fmoc-CH_2), 3.94 (m, 1H, H4′), 3.72–3.65 (m, 1H, H5′), 3.49 (br, 7H, H5′, NCH_3), 3.04–2.97 (m, 1H, Phe-CH_2), 2.85–2.72 (m, 1H, Phe-CH_2); ^{13}C NMR (125.8 MHz, DMSO-d_6/$CDCl_3$=3:1): 171.8, 155.7, 154.3, 151.6, 149.6, 143.7, 140.7, 140.6, 137.9, 137.7, 129.3, 127.9, 127.5, 126.9, 126.9, 126.1, 125.2, 125.1, 119.8, 119.8, 89.4, 83.5, 73.2, 65.7, 60.9, 56.1, 50.4, 46.6, 37.9 (N^6,N^6-dimethyl carbon was overlapped by DMSO-d_6, confirmed by DEPT.); ESI-MS (ES^+): *m*/*z* calcd for $C_{36}H_{37}N_7O_6$ 663.3, found 664.5 (MH^+); HRMS *m*/*z* calcd for $C_{36}H_{37}N_7O_6$ 664.2883 (MH^+), found 664.2871.

3.1.2. 3′-Amino-3′-deoxy-3′-[[2-^{15}NH]-*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-N^6,N^6-dimethyladenosine (4*). The product (**4***) was obtained from the coupling of puromycin aminonucleoside (250.7 mg, 0.852 mmol) and [2-^{15}NH]-*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanine (**3***, 398.9 mg, 0.938 mmol) as described in Section 3.1.1. **4*** was obtained as a colorless powder (435.0 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.44 (s, 1H, H8), 8.23 (s, 1H, H2), 8.20 (d, 1H, J=7.6 Hz, 3′-NH), 7.87 (d, 2H, J=7.6 Hz, Fmoc-aromatic), 7.64 (t, 2H, J=7.8 Hz, Fmoc-aromatic), 7.63 (dd, 1H, J_{HN}=92.4 Hz, J_{HH}=8.8 Hz, Phe-NH), 7.42–7.16 (m, 9H, Fmoc-aromatic, Phe-aromatic), 6.10 (d, 1H, J=4.0 Hz, 2′-OH), 5.98 (d, 1H, J=0.8 Hz, H1′), 5.18 (t, 1H, J=4.2 Hz, 5′-OH), 4.50–4.46 (m, 2H, H2′, H3′), 4.42–4.37 (m, 1H, Phe-CH), 4.18–4.08 (m, 3H, Fmoc-CH, Fmoc-CH_2), 3.96 (m, 1H, H4′), 3.67 (m, 1H, H5′), 3.44 (br, 7H, H5′, NCH_3), 3.04–2.97 (m, 1H, Phe-CH_2), 2.84–2.75 (m, 1H, Phe-CH_2); ^{13}C NMR (125.8 MHz, DMSO-d_6/$CDCl_3$=3:1): 171.8, 155.6 (d, J_{CN}=27.5 Hz), 154.3, 151.6, 149.6, 143.7, 140.6, 140.6, 137.9, 137.7, 129.3, 127.9, 127.5, 126.9, 126.9, 126.1, 125.3, 125.1, 119.9, 119.8, 89.4, 83.4, 73.2, 65.7, 60.9, 56.0 (d, J_{CN}=11.3 Hz), 50.3, 46.6, 37.9 (N^6,N^6-dimethyl carbon was overlapped by DMSO-d_6, confirmed by DEPT.); ^{15}N NMR (50.7 MHz, DMSO-d_6): −292.4 (d, J_{HN}=94.7 Hz); ESI-MS ($ES+$): *m*/*z* calcd for $C_{36}H_{37}N_6{}^{15}NO_6$ 664.3, found 665.3 (MH^+), 687.3 ($M+Na^+$); HRMS *m*/*z* calcd for $C_{36}H_{37}N_6{}^{15}NO_6$ 665.2854 (MH^+), found 665.2865.

3.1.3. 3′-Amino-3′-deoxy-3′-[*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine (5). 4 (175.0 mg, 0.264 mmol) was dried by repeated co-evaporation with pyridine, then dissolved in pyridine (8.0 ml). Triethylamine (0.12 ml, 0.861 mmol) and *p*,*p*′-dimethoxytrityl chloride (DMTrCl) (283.6 mg, 0.795 mmol) were added to the solution, then stirred at room temperature for 3 h. After addition of MeOH (2 ml) to quench the reaction, the mixture was evaporated. Further co-evaporation with toluene twice was followed by column chromatography (gradient from 1% MeOH in CH_2Cl_2 to 2% MeOH in CH_2Cl_2) to give the pure product (**5**) as a white foam (219.7 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.22 (s, 1H, H8), 7.99 (s, 1H, H2), 7.75 (d, 2H, J=7.2 Hz, Fmoc-aromatic), 7.54 (t, 2H, J=7.0 Hz, Fmoc-aromatic), 7.40–7.11 (m, 18H, Fmoc-aromatic, DMTr-aromatic, Phe-aromatic), 6.76 (d, 4H, J=8.4 Hz, DMTr-aromatic), 6.40 (br, 1H, 3′-NH), 5.58 (br, 1H, H1′), 5.45 (br, 1H, J=6.4 Hz, Phe-NH), 4.67 (br, 1H, J=4.4 Hz, H2′), 4.41(br, 1H, H3′), 4.36 (br.d, 3H, J=6.4 Hz, Phe-CH, Fmoc-CH_2), 4.19 (m, 2H, H4′, Fmoc-CH), 3.76 (s, 6H, DMTr-OCH_3), 3.54 (br, 6H, NCH_3), 3.44 (d, 1H, J=8.8 Hz, H5′), 3.32 (dd, 1H, J=10.6, 3.4 Hz, H5′), 3.10 (br, 1H, Phe-CH_2), 2.91 (br, 1H, Phe-CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$): 171.2, 158.5, 155.9, 154.9, 151.7, 149.1, 144.4, 143.8, 143.6, 141.3, 141.3, 136.4, 136.0, 135.7, 135.6, 130.1, 130.0, 129.2, 128.8, 128.2, 127.8, 127.8, 127.7, 127.1, 127.0, 126.8, 125.1, 120.6, 120.0, 119.9, 113.1, 91.3, 86.5, 84.1, 74.5, 67.1, 63.6, 56.4, 55.2, 52.6, 47.1, 39.2, 38.7 (br); ESI-MS (ES^+): *m*/*z* calcd for $C_{57}H_{55}N_7O_8$ 965.4, found 988.6 ($M+Na^+$); HRMS *m*/*z* calcd for $C_{57}H_{55}N_7O_8$ 966.4190 (MH^+), found 966.4185.

3.1.4. 3′-Amino-3′-deoxy-3′-[[2-^{15}NH]-*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine (5*). The product (**5***) was obtained as a white powder from **4*** (199.4 mg, 0.300 mmol) using the method described in Section 3.1.3 (257.1 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.22 (s, 1H, H8), 7.98 (s, 1H, H2), 7.74 (d, 2H, J=7.6 Hz, Fmoc-aromatic), 7.53 (t, 2H, J=7.6 Hz, Fmoc-aromatic), 7.40–7.10 (m, 18H, Fmoc-aromatic, DMTr-aromatic, Phe-aromatic), 6.76 (d, 4H, J=8.4 Hz, DMTr-aromatic), 6.42 (br, 1H, 3′-NH), 5.59–5.35 (m, 2H, H1′, Phe-NH), 4.66 (t, 1H, J=5.0 Hz, H2′), 4.41 (m., 1H, H3′), 4.35 (br.d, 3H, J=6.0 Hz, Phe-CH, Fmoc-CH_2), 4.18 (m, 2H, H4′, Fmoc-CH),

3.76 (s, 6H, DMTr-OCH_3), 3.54 (br, 6H, NCH_3), 3.43 (dd, 1H, J=10.8, 2.0 Hz, H5′), 3.29 (dd, 1H, J=11.0, 3.4 Hz, H5′), 3.08 (br, 1H, Phe-CH_2), 2.89 (br, 1H, Phe-CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$): 171.2, 158.5, 154.9, 151.7, 149.2, 144.4, 143.7 (d, J_{CN}=13.1 Hz), 141.3, 136.4, 136.0, 135.7, 135.6, 130.1, 129.2, 128.8, 128.2, 127.8, 127.7, 127.1, 126.8, 125.1, 120.6, 120.0, 120.0, 113.1, 91.3, 86.5, 84.2, 74.5, 67.2, 63.7, 56.4 (d, J_{CN}=12.7 Hz), 55.2, 52.7, 47.1, 39.2, 38.7 (br); ^{15}N NMR (50.7 MHz, $CDCl_3$): −294.1 (d, J_{HN}=91.6 Hz); ESI-MS (ES^+): m/z calcd for $C_{57}H_{55}N_6{}^{15}NO_8$ 966.4, found 989.5 ($M+Na^+$); HRMS m/z calcd for $C_{57}H_{55}N_6{}^{15}NO_8$ 967.4160 (MH^+), found 967.4136.

3.1.5. 3′-Amino-3′-deoxy-3′-[*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-5′-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-succinate (6). **5** (167.7 mg, 0.173 mmol) was dried by repeated co-evaporation with pyridine and dissolved in pyridine (1.4 ml). Succinic anhydride (52.1 mg, 0.521 mmol) and 4-(dimethylamino)pyridine (DMAP) (11.0 mg, 0.090 mmol) was added to the solution and stirred at room temperature for 24 h. The mixture was evaporated, followed by addition of 0.1 M $NaHCO_3$ aq (25 ml) and extraction with CH_2Cl_2 (25 ml×7). The combined organic phase was evaporated. Further co-evaporation with toluene twice was followed by column chromatography (gradient from 2% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to give the pure product (**6**) as a white foam (120.7 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.30 (s, 1H, H8), 7.94 (s, 1H, H2), 7.75 (d, 2H, J=7.6 Hz, Fmoc-aromatic), 7.50 (t, 2H, J=7.8 Hz, Fmoc-aromatic), 7.45–7.00 (m, 18H, Fmoc-aromatic, DMTr-aromatic, Phe-aromatic), 6.80 (d, 4H, J=7.6 Hz, DMTr-aromatic), 6.46 (d, 1H, J=9.2 Hz, 3′-NH), 6.10 (d, 1H, J=1.2 Hz, H1′), 5.79 (dd, 1H, J=5.4, 1.8 Hz, H2′), 5.72 (d, 1H, J=9.6 Hz, Phe-NH), 5.19 (m, 1H, H3′), 4.90 (m, 1H, Phe-CH), 4.33–4.20 (m, 2H, Fmoc-CH_2), 4.11 (t, 1H, J=7.0 Hz, Fmoc-CH), 3.83 (br, 1H, H4′), 3.74 (s, 3H, DMTr-OCH_3), 3.73 (s, 3H, DMTr-OCH_3), 3.51 (br, 6H, NCH_3), 3.40–3.28 (m, 2H, H5′), 2.85–2.58 (m, 6H, Phe-CH_2, succinic ester-CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$): 175.4 (br), 171.2, 171.0, 158.5, 156.5, 154.9, 152.5, 149.8, 144.4, 143.5, 141.2, 141.2, 136.2, 136.1, 135.6, 130.2, 129.3, 128.5, 128.4, 127.8, 127.8, 127.1, 126.9, 126.9, 125.1, 125.0, 120.4, 120.0, 113.1, 87.6, 86.4, 82.3, 75.7, 67.6, 62.6, 55.2, 49.5, 46.9, 40.2, 38.6 (br), 29.8; ESI-MS (ES^+): m/z calcd for $C_{61}H_{59}N_7O_{11}$ 1,065.4, found 1,066.6 (MH^+); HRMS m/z calcd for $C_{61}H_{59}N_7O_{11}$ 1066.4350 (MH^+), found 1066.4330.

3.1.6. 3′-Amino-3′-deoxy-3′-[[2-^{15}NH]-*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-succinate (6*). The product (**6***) was obtained as a white foam from **5*** (242.2 mg, 0.250 mmol) using the method described in Section 3.1.5 (161.2 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.30 (s, 1H, H8), 7.94 (s, 1H, H2), 7.75 (d, 2H, J=7.6 Hz, Fmoc-aromatic), 7.50 (t, 2H, J=7.6 Hz, Fmoc-aromatic), 7.44–7.01 (m, 18H, Fmoc-aromatic, DMTr-aromatic, Phe-aromatic), 6.80 (d, 4H, J=8.4 Hz, DMTr-aromatic), 6.47 (d, 1H, J=7.2 Hz, 3′-NH), 6.09 (s, 1H, H1′), 5.78 (d, 1H, J=5.6 Hz, H2′), 5.73 (dd, 1H, J_{HN}=91.6 Hz, J_{HH}=8.4 Hz, Phe-NH), 5.18 (m, 1H, H3′), 4.90 (m, 1H, Phe-CH), 4.34–4.20 (m, 2H, Fmoc-CH_2), 4.11 (t, 1H, J=7.2 Hz, Fmoc-CH), 3.83 (br, 1H, H4′), 3.74 (s, 3H, DMTr-OCH_3), 3.73 (s, 3H, DMTr-OCH_3), 3.51 (br, 6H, NCH_3), 3.40–3.29 (m, 2H, H5′), 2.85–2.58 (m, 6H, Phe-CH_2, succinic ester-CH_2); ^{13}C NMR (125.8 MHz, $CDCl_3$): 175.2, 171.3, 170.9, 158.5, 156.5 (d, J_{CN}=27.8 Hz), 154.9, 152.6, 149.8, 144.4, 143.5, 141.2, 141.2, 136.3, 136.1, 135.6, 135.6, 130.2, 129.3, 128.5, 128.4, 127.8, 127.8, 127.2, 126.9, 126.9, 125.1, 125.0, 120.4, 120.0, 113.1, 87.5, 86.5, 82.3, 75.8, 67.5, 62.6, 55.1 (2C), 49.4, 46.9, 40.2, 38.6 (br), 29.8, 29.7; ^{15}N NMR (50.7 MHz, $CDCl_3$): −291.5 (d, J_{HN}=91.4 Hz); ESI-MS (ES^+): m/z calcd for $C_{61}H_{59}N_6{}^{15}NO_{11}$ 1,066.4, found 1,067.6 (MH^+); HRMS m/z calcd for $C_{61}H_{59}N_6{}^{15}NO_{11}$ 1067.4320 (MH^+), found 1067.4310.

3.1.7. 3′-Amino-3′-deoxy-3′-[*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-(LCAA-polystyrene)succinate (7). Amino-derivatized polystyrene support (850.0 mg) was suspended in pyridine (8.5 ml) with **6** (180.0 mg, 0.169 mmol), DMAP (10.7 mg, 0.0876 mmol), EDCI (327.2 mg, 1.71 mmol), and triethylamine (70 μl, 0.498 mmol). The mixture was rocked gently for 26 h. The support was filtered and washed successively with pyridine (8.5 ml), MeOH (17 ml), and CH_2Cl_2 (25.5 ml). The support was capped to acetylate unreacted amino residues by suspension in 0.5 M acetic anhydride, 0.5 M pyridine, 1 M *N*-methyl imidazole solution in THF (8.5 ml) and rocked for 2.5 h. The support was filtered and successively washed with MeOH (17 ml) and CH_2Cl_2 (25.5 ml) to yield the product (**7**). The nucleoside loading was 109 μmol/g.

3.1.8. 3′-Amino-3′-deoxy-3′-[[2-^{15}NH]-*N*-(9-fluorenylmethoxycarbonyl)-L-phenylalanyl]-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-(LCAA-polystyrene)succinate (7*). The product (**7***) was obtained from **6*** (180.0 mg, 0.169 mmol) using the method described in Section 3.1.7. The nucleoside loading was 110 μmol/g.

3.1.9. Cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-L-phenylalanyl-N^6,N^6-dimethyladenosine (1). The coupling of 4-acetyl-5′-*O*-[benzhydroxybis(trimethylsiloxy)silyl]-2′-*O*-[bis(2-acetoxyethoxy)methyl]cytidine-3′-(methyl-*N*,*N*-diisopropyl)phosphoramidite (**8**) to 5′ hydroxyl group of **7** and successive deprotection was performed as described previously.[26] After lyophilization, the deprotected product was purified over a C-18 column with 0.1 M triethylammonium acetate buffer (pH 6.5) and acetonitrile (from 10:0 to 6:4). Lyophilization resulted in the purified product **1**. ^{1}H NMR (500 MHz, D_2O): 8.19 (s, 1H, H8-puromycin), 8.04 (s, 1H, H2-puromycin), 7.54 (d, 1H, J=7.5 Hz, H6-cytosine), 7.35–7.15 (m, 5H, Phe-aromatic), 5.93 (br, 1H, H1′-puromycin), 5.60 (d, 1H, J=7.5 Hz, H5-cytosine), 5.42 (br, 1H, H1′-cytosine), 4.32 (m, 1H, Phe-CH), 4.22 (dd, 1H, J=13.0, 8.1 Hz, H5′-cytosine), 4.09 (m, 1H), 4.03 (m, 2H), 3.85 (m, 1H), 3.71 (dd, 1H, J=12.8, 2.1 Hz, H5′-puromycin), 3.63 (dd, 1H, J=13.2, 4.2 Hz, H5′-puromycin), 3.61 (m, 1H), 3.25 (br, 6H, NCH_3), 3.12–2.93 (m, 4H); ESI-MS (ES^+): m/z calcd for $C_{30}H_{39}N_{10}O_{11}P$ 746.3, found 747.0 (MH^+); HRMS m/z calcd for $C_{30}H_{39}N_{10}O_{11}P$ 769.2437 ($M+Na^+$), found 769.2433.

3.1.10. Cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-([2-$^{15}NH_2$]-L-phenylalanyl)-N^6,N^6-dimethyladenosine (1*). The product (**1***) was obtained from **7*** using the method described in Section 3.1.9; ESI-MS (ES^+): *m/z* calcd for $C_{30}H_{39}N_9{}^{15}NO_{11}P$ 747.3, found 748.0 (MH^+); HRMS *m/z* calcd for $C_{30}H_{39}N_9{}^{15}NO_{11}P$ 748.2586 (MH^+), found 748.2585.

3.1.11. [3-^{15}N,4-$^{15}NH_2$]-3′,5′-*O*-(1,1,3,3-Tetraisopropyl-1,3-disiloxanediyl)cytidine (10).** [3-^{15}N,4-$^{15}NH_2$]-cytidine[28] (**9****, 1.63 g, 6.65 mmol) was dried by co-evaporation twice with pyridine (25 ml) and then dissolved in pyridine (55 ml). 1,3-Dichloro-1,1,3,3-tetraisopropyl-disiloxane (2.34 ml, 7.31 mmol) was added to the mixture dropwise at 0 °C over 1 min. The reaction mixture was stirred for 1 h at 0 °C, then stirred at room temperature for 13 h. After evaporation, addition of water (100 ml) was followed by extraction using CH_2Cl_2 (100 ml×3). The organic phases were combined, dried over $MgSO_4$, then evaporated followed by co-evaporation twice with toluene (40 ml). The pure product **10**** was obtained by column chromatography (gradient from 4% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2) as a white solid (2.53 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$=3:1): 7.96 (d, 1H, *J*=7.2 Hz, H6), 5.81 (d, 1H, *J*=7.2 Hz, H5), 5.68 (s, 1H, H1′), 4.29–4.17 (m, 3H, H3′, H4′, H5′), 4.09 (d, 1H, *J*=3.6 Hz, H2′), 4.02 (dd, 1H, *J*=13.2, 2.0 Hz, H5′), 1.13–0.96 (m, 28H, 4×$CH(CH_3)_2$); ^{13}C NMR (125.8 MHz, $CDCl_3/CD_3OD$=3:1): 166.3 (dd, J_{CN}=21.4, 4.2 Hz), 156.5 (d, J_{CN}=5.0 Hz), 141.0, 94.7, 91.8, 81.8, 75.3, 68.4, 60.2, 17.6, 17.6, 17.4, 17.4, 17.1, 17.1, 17.0, 16.9, 13.7, 13.3, 13.2, 12.7; ^{15}N NMR (50.7 MHz, $CDCl_3/CD_3OD$=3:1): −176.9 (d, J_{HN}=4.5 Hz), −292.5 (br); ESI-MS (ES^+): *m/z* calcd for $C_{21}H_{39}N^{15}N_2O_6Si_2$ 487.2, found 488.5 (MH^+); HRMS *m/z* calcd for $C_{21}H_{39}N^{15}N_2O_6Si_2$ 488.2396 (MH^+), found 488.2414.

3.1.12. [3-^{15}N,4-$^{15}NH_2$]-4-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)cytidine (11).** To a mixture of **10**** (2.47 g, 5.06 mmol) and DMF (50 ml) was added acetic anhydride (2.39 ml, 25.3 mmol). The reaction mixture became clear soon after acetic anhydride addition. The solution was stirred for additional 6 h, evaporated, and co-evaporated twice with MeOH (30 ml). The pure product **11**** was obtained by column chromatography (3% MeOH in CH_2Cl_2) as a white foamy powder (2.72 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): 10.11 (d, 1H, J_{HN}=90.4 Hz, NH), 8.21 (d, 1H, *J*=7.2 Hz, H6), 7.44 (d, 1H, *J*=7.2 Hz, H5), 5.82 (s, 1H, H1′), 4.29–4.20 (m, 4H, H2′, H3′, H4′, H5′), 4.01 (dd, 1H, *J*=13.6, 2.8 Hz, H5′), 2.30 (d, 3H, J_{HN}=1.6 Hz, N4-$COCH_3$), 1.11–0.90 (m, 28H, 4×$CH(CH_3)_2$); ^{13}C NMR (125.8 MHz, $CDCl_3$): 171.2 (d, J_{CN}=11.2 Hz), 163.2 (dd, J_{CN}=18.1, 6.9 Hz), 155.0 (dd, J_{CN}=6.9, 4.8 Hz), 144.4, 96.6, 91.5, 82.0, 75.2, 68.6, 60.0, 24.9 (d, J_{CN}=9.1 Hz), 17.5, 17.4, 17.3, 17.3, 17.0, 17.0, 16.9, 16.8, 13.4, 13.0, 12.9, 12.5; ^{15}N NMR (50.7 MHz, $CDCl_3$): −152.0, −233.1 (d, J_{HN}=86.0 Hz); ESI-MS (ES^+): *m/z* calcd for $C_{23}H_{41}N^{15}N_2O_7Si_2$ 529.2, found 530.5 (MH^+); HRMS *m/z* calcd for $C_{23}H_{41}N^{15}N_2O_7Si_2$ 530.2502 (MH^+), found 530.2511.

3.1.13. [3-^{15}N,4-$^{15}NH_2$]-4-Acetyl-2′-*O*-[bis(2-acetoxyethoxy)methyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)cytidine (12).** To a stirred solution of **11**** (2.65 g, 5.00 mmol) in CH_2Cl_2 were added tris(2-acetoxyethoxy)orthoformate tris (ACE)orthoformate (4.51 g, 14.0 mmol), pyridinium *p*-toluenesulfonate (251 mg, 1.00 mmol) and 4-*tert*-butyldimethylsiloxy)-3-penten-2-one (2.13 ml, 9.01 mmol). The reaction mixture was refluxed under Ar atmosphere for 10 h, cooled to the room temperature, and quenched by the addition of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (0.38 ml, 2.50 mmol). The mixture was subjected directly to a chromatography column (gradient from 50% ethyl acetate in *n*-hexane to 100% ethyl acetate) to afford the pure product **12**** as a white foamy powder (3.19 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): 9.94 (d, 1H, J_{HN}=89.6 Hz, NH), 8.30 (d, 1H, *J*=7.2 Hz, H6), 7.44 (d, 1H, *J*=7.2 Hz, H5), 5.85 (s, 1H, H1′), 5.84 (s, 1H, ACE-CH), 4.32–4.17 (m, 8H, 2×ACE-CH_2, H2′, H3′, H4′, H5′), 4.02–3.84 (m, 5H, 2×ACE-CH_2, H5′), 2.28 (d, 3H, J_{HN}=1.2 Hz, N4-$COCH_3$), 2.07 (s, 3H, ACE-$COCH_3$), 2.06 (s, 3H, ACE-$COCH_3$), 1.11–0.91 (m, 28H, 4×$CH(CH_3)_2$); ^{13}C NMR (125.8 MHz, $CDCl_3$): 170.9, 170.9, 170.8 (d, J_{CN}=12.3 Hz), 163.1 (dd, J_{CN}=18.3, 7.1 Hz), 154.7 (dd, J_{CN}=6.5, 5.2 Hz), 144.2, 111.9, 96.4, 89.9, 82.0, 77.3, 67.5, 63.5, 63.3, 61.7, 61.2, 59.3, 24.9 (d, J_{CN}=9.1 Hz), 20.9, 20.8, 17.5, 17.4, 17.3, 17.3, 17.1, 16.9, 16.9, 16.8, 13.4, 13.1, 12.9, 12.6; ^{15}N NMR (50.7 MHz, $CDCl_3$): −151.0 (d, J_{HN}=5.9 Hz), −233.3 (dd, J_{HN}=89.7, 6.1 Hz); ESI-MS (ES^+): *m/z* calcd for $C_{32}H_{55}N^{15}N_2O_{13}Si_2$ 747.3, found 770.5 ($M+Na^+$); HRMS *m/z* calcd for $C_{32}H_{55}N^{15}N_2O_{13}Si_2$ 770.3112 ($M+Na^+$), found 770.3100.

3.1.14. [3-^{15}N,4-$^{15}NH_2$]-4-Acetyl-2′-*O*-[bis(2-acetoxyethoxy)methyl]cytidine (13).** To the mixture of acetonitrile (42 ml) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (3.13 ml, 4.14 mmol) 48% HF aq (0.53 ml, 14.6 mmol) was added dropwise over 1 min at 0 °C. **12**** (3.10 g, 4.14 mmol) was added to the solution, then stirred at room temperature for 4 h. The reaction mixture was evaporated. Pure product **13**** was obtained by column chromatography (gradient from 5% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2) as a white foamy powder (2.08 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): 9.67 (d, 1H, J_{HN}=89.6 Hz, NH), 8.30 (d, 1H, *J*=7.6 Hz, H6), 7.43 (d, 1H, *J*=7.2 Hz, H5), 5.80 (d, 1H, *J*=2.4 Hz, H1′), 5.62 (s, 1H, ACE-CH), 4.63 (dd, 1H, *J*=5.4, 3.0 Hz, H2′), 4.39 (q, 1H, *J*=5.7 Hz, H3′), 4.26–4.02 (m, 7H, 2×ACE-CH_2, H4′, H5′, 5′-OH), 3.89–3.78 (m, 5H, 2×ACE-CH_2, H5′), 3.54 (d, 1H, *J*=6.0 Hz, 3′-OH), 2.26 (d, 3H, J_{HN}=0.8 Hz, N4-$COCH_3$), 2.07 (s, 3H, ACE-$COCH_3$), 2.06 (s, 3H, ACE-$COCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): 171.1 (2C), 171.0 (d, J_{CN}=13.3 Hz), 162.9 (dd, J_{CN}=18.4, 6.9 Hz), 155.5 (dd, J_{CN}=5.1, 4.5 Hz), 146.6, 112.7, 96.9, 92.1, 85.1, 76.8, 68.4, 63.1, 63.0, 62.9, 62.8, 60.6, 24.9 (d, J_{CN}=9.2 Hz), 20.9, 20.9; ^{15}N NMR (50.7 MHz, $CDCl_3$): −149.5 (d, J_{HN}=4.7 Hz), −233.2 (dd, J_{HN}=90.2, 6.6 Hz); ESI-MS (ES^+): *m/z* calcd for $C_{20}H_{29}N^{15}N_2O_{12}$ 505.2, found 506.4 (MH^+); HRMS *m/z* calcd for $C_{20}H_{29}N^{15}N_2O_{12}$ 506.1770 (MH^+), found 506.1793.

3.1.15. [3-^{15}N,4-$^{15}NH_2$]-4-Acetyl-5′-*O*-[benzhydroxybis(trimethylsiloxy)silyl]-2′-*O*-[bis(2-acetoxyethoxy)methyl]cytidine (14).** A solution of **13**** (2.02 g, 4.00 mmol) and diisopropylamine (0.56 ml, 4.00 mmol)

in CH_2Cl_2 (22 ml) was cooled to 0 °C. Benzhydroxybis(trimethylsiloxy)silyl chloride (BzhCl) (3.47 g, 8.17 mmol) was dissolved in CH_2Cl_2 (13.5 ml), then diisopropylamine (0.56 ml, 4.00 mmol) was added dropwise to this mixture at 0 °C. This mixture was added dropwise to the previous solution over 2 h at 0 °C. Upon completion, the addition of 5% $NaHCO_3$ aq (50 ml) quenched the reaction. The mixture was separated, then the aqueous phase was extracted with CH_2Cl_2 (30 ml×3). The organic layers were combined, washed with brine, dried over $MgSO_4$, and evaporated. The pure product **14**** was obtained by column chromatography (gradient from 50% ethyl acetate in *n*-hexane to 10% MeOH in CH_2Cl_2) as a colorless oil (3.43 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.98 (d, 1H, J_{HN}=89.6 Hz, NH), 8.33 (d, 1H, J=7.6 Hz, H6), 7.36–7.25 (m, 9H, H5, phenyl), 7.22–7.17 (m, 2H, phenyl), 5.94 (s, 1H, Bzh-CH), 5.93 (s, 1H, H1′), 5.71 (s, 1H, ACE-CH), 4.28–4.21 (m, 5H, 2×ACE-CH_2, H2′), 4.11–3.98 (m, 3H, H3′, H4′, H5′), 3.93–3.83(m, 5H, 2×ACE-CH_2, H5′), 2.85 (d, 1H, J=8.0 Hz, 3′-OH), 2.25 (d, 3H, J_{HN}=1.6 Hz, N4-$COCH_3$), 2.08 (s, 3H, ACE-$COCH_3$), 2.05 (s, 3H, ACE-$COCH_3$), 0.09 (s, 9H, $Si(CH_3)_3$), 0.08 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125.8 MHz, $CDCl_3$): 170.9, 170.9, 169.9 (d, J_{CN}=11.2 Hz), 162.5 (dd, J_{CN}=19.1, 7.5 Hz), 155.0 (dd, J_{CN}=6.9, 5.3 Hz), 144.7, 143.9 (d, J_{CN}=5.4 Hz), 128.3, 127.3, 126.4, 126.3, 113.0, 96.2, 89.6, 83.9, 78.6, 67.1, 63.1, 63.1, 63.0, 60.5, 25.0 (d, J_{CN}=9.4 Hz), 20.9, 20.8, 1.5 (6C); ^{15}N NMR (50.7 MHz, $CDCl_3$): −149.8 (d, J_{HN}=6.9 Hz), −233.8 (dd, J_{HN}=88.6, 7.2 Hz); ESI-MS (ES^+): *m/z* calcd for $C_{39}H_{57}N^{15}N_2O_{15}Si_3$ 893.3, found 894.6 (MH^+); HRMS *m/z* calcd for $C_{39}H_{57}N^{15}N_2O_{15}Si_3$ 916.2936 ($M+Na^+$), found 916.2926.

3.1.16. [3-^{15}N,4-$^{15}NH_2$]-4-Acetyl-5′-*O*-[benzhydroxybis(trimethylsiloxy)silyl]-2′-*O*-[bis(2-acetoxyethoxy)methyl]cytidine-3′-(methyl-*N*,*N*-diisopropyl)phosphoramidite (8).** To a stirred solution of **14**** (3.38 g, 3.78 mmol) in CH_2Cl_2 (25 ml) were added methyl tetraisopropyl phosphorodiamidite (3.05 ml, 10.6 mmol) and 1*H*-tetrazole (291 mg, 4.16 mmol). The resulting solution was stirred for 19 h. The reaction was quenched by the addition of 5% $NaHCO_3$ aq (50 ml). The organic phase was separated, and the aqueous phase was extracted by CH_2Cl_2 (30 ml×4). The organic fractions were combined, dried over $MgSO_4$, and evaporated. The pure product **8**** was obtained by column chromatography (gradient from 30% CH_2Cl_2: 60% *n*-hexane: 10% triethylamine to 50% CH_2Cl_2: 40% *n*-hexane: 10% triethylamine) as a colorless oil (3.55 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): (mixture of diastereomers) 10.05 (d, 1H, J_{HN}=88.8 Hz, NH), 8.37 (d, 0.4H, J=7.6 Hz, H6), 8.35 (d, 0.6H, J=7.6 Hz, H6), 7.38–7.18 (m, 11H, H5, phenyl), 6.02 (s, 0.4H, H1′), 6.00 (s, 0.6H, H1′), 5.97 (s, 0.4H, Bzh-CH), 5.96 (s, 0.6H, Bzh-CH), 5.81 (s, 0.6H, ACE-CH), 5.75 (s, 0.4H, ACE-CH), 4.31–4.16 (m, 8H), 4.11–4.03 (m, 1H), 3.95–3.80 (m, 4H), 3.61–3.53 (m, 2H, $CH(CH_3)_2$), 3.35 (d, 1.6H, J=13.6 Hz, OCH_3), 3.32 (d, 1.4H, J=13.6 Hz, OCH_3), 2.28 (s, 3H, N4-$COCH_3$), 2.05 (s, 1.5H, ACE-$COCH_3$), 2.05 (s, 1.5H, ACE-$COCH_3$), 2.04 (s, 1.5H, ACE-$COCH_3$), 2.04 (s, 1.5H, ACE-$COCH_3$), 1.17–1.14 (m, 12H, $CH(CH_3)_2$), 0.09 (s, 4.5H, $Si(CH_3)_3$), 0.09 (s, 4.5H, $Si(CH_3)_3$), 0.07 (s, 4.5H, $Si(CH_3)_3$), 0.06 (s, 4.5H, $Si(CH_3)_3$); ^{15}N NMR (50.7 MHz, $CDCl_3$): −151.8, −233.2 (d, J_{HN}=88.4 Hz); ^{31}P NMR (161.9 MHz, $CDCl_3$): 151.8, 151.0; ESI-MS (ES^+): *m/z* calcd for $C_{46}H_{73}N_2{}^{15}N_2O_{16}PSi_3$ 1054.4, found 1055.6 (MH^+).

3.1.17. [3-^{15}N, 4-$^{15}NH_2$]Cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-([2-^{15}NH]-phenylalanyl)-N^6,N^6-dimethyladenosine (1*).** The product (**1*****) was obtained from **9*** and **8**** using the method described in Section 3.1.9; ESI-MS (ES^+): *m/z* calcd for $C_{30}H_{39}N_7{}^{15}N_3O_{11}P$ 749.2, found 750.3 (MH^+); HRMS *m/z* calcd for $C_{30}H_{39}N_7{}^{15}N_3O_{11}P$ 750.2526 (MH^+), found 750.2537.

3.1.18. D-Methyl 2-trifluoromethanesulfonyl-3-phenyllactate (16). Trifluoromethanesulfonic anhydride (2.4 ml, 14.3 mmol) was added dropwise over 2 min to a solution of D-methyl 3-phenyllactate[30] (**15**, 500.0 mg, 2.77 mmol) in CH_2Cl_2 (25 ml) and pyridine (1.2 ml) at 0 °C. After addition of CH_2Cl_2 (20 ml), the mixture was stirred at room temperature for 2 h. The mixture was further diluted by addition of CH_2Cl_2 (60 ml), and successively washed with 0.8 N $NaHCO_3$ aq (120 ml), 1 N HCl aq (120 ml), and brine (120 ml). The organic layer was dried over $MgSO_4$, and evaporated to give crude product (**16**) as an oil (0.70 g). ^{1}H NMR (400 MHz, $CDCl_3$): 7.37–7.20 (m, 5H, phenyl), 5.25 (dd, 1H, J=8.8, 4.4 Hz, CH), 3.84 (s, 3H, CH_3), 3.35 (dd, 1H, J=14.8, 4.4 Hz, CH_2), 3.21 (dd, 1H, J=14.8, 8.4 Hz, CH_2).

3.1.19. L-Methyl [2-^{18}O,1′-^{18}O]-2-acetoxy-3-phenyllactate (17).** Crude **16** (0.70 g) was dissolved in an acetic $^{18}O_2$-acid (200 mg, 3.12 mmol) solution in acetonitrile (15 ml). K_2CO_3 (402.6 mg, 2.914 mmol) was added and the mixture was stirred at room temperature for 29 h. CH_2Cl_2 (100 ml) was added and the organic phase was washed successively with 0.8 N $NaHCO_3$ aq (100 ml), 1 N HCl aq (100 ml), and brine (120 ml). The organic layer was dried over $MgSO_4$, and evaporated to give product (**17****) as an oil (521.6 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.33–7.21 (m, 5H, phenyl), 5.22 (dd, 1H, J=8.8, 4.4 Hz, CH), 3.73 (s, 3H, CO_2CH_3), 3.17 (dd, 1H, J=14.2, 4.6 Hz, CH_2), 3.09 (dd, 1H, J=14.0, 8.8 Hz, CH_2), 2.08 (s, 3H, acetoxy-CH_3); ESI-MS (ES^+): *m/z* calcd for $C_{12}H_{14}O_2{}^{18}O_2$ 226.1, found 249.2 ($M+Na^+$).

3.1.20. L-[2-^{18}OH]-3-Phenyllactic acid (18*). 17** (496.1 mg, 2.19 mmol) was dissolved in MeOH (10 ml) and 5 N KOH aq (10 ml), and stirred at room temperature for 5 h. After addition of conc. HCl (10 ml), the mixture was evaporated at 60 °C to give a white semi-solid. The crude product was washed with ethyl acetate (40 ml×4). The combined organic phase was filtered and evaporated. Recrystallization from $CHCl_3$/*n*-hexane gave the product (**18***) as needles (200.6 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.35–7.26 (m, 5H, phenyl), 4.53 (br.s, 1H, CH), 3.23 (d, 1H, J=11.6 Hz, CH_2), 3.01 (dd, 1H, J=13.4, 7.0 Hz, CH_2); ESI-MS (ES^+): *m/z* calcd for $C_9H_{10}O_2{}^{18}O$ 168.1, found 190.9 ($M+Na^+$); $[\alpha]_D^{22}$: −27.8 (*c*0.18, MeOH).

3.1.21. L-2-Acetoxy-3-phenyllactic acid.[32] (19) Acetic anhydride (0.3 ml, 3.15 mmol) was added to a L-3-phenyllactic acid (**18**) (250.4 mg, 1.48 mmol) solution in pyridine (3.0 ml), and the mixture was stirred at room

temperature for 1 day. The reaction was quenched by addition of MeOH (5 ml), and the mixture was evaporated. The resulting oil was dissolved in 0.1 N HCl aq (20 ml), and extracted with CH_2Cl_2 (20 ml×3). The combined organic phase was washed with brine (30 ml), dried over $MgSO_4$, and evaporated to give product (**19**) as a colorless oil (313.2 mg, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.34–7.24 (m, 5H, phenyl), 5.25 (dd, 1H, J=9.0, 4.2 Hz, CH), 3.23 (dd, 1H, J=14.2, 3.8 Hz, CH_2), 3.12 (dd, 1H, J=14.4, 8.8 Hz, CH_2), 2.08 (s, 3H, CH_3); ESI-MS (ES^+): *m/z* calcd for $C_{11}H_{12}O_4$ 208.1, found 231.1 ($M+Na^+$).

3.1.22. L-[2-^{18}O]-Acetoxy-3-phenyllactic acid (19*). The product (**19***) was obtained as a colorless oil from **18*** (154.6 mg, 0.919 mmol) using method described in Section 3.1.21 (184.0 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.34–7.24 (m, 5H, phenyl), 4.53 (dd, 1H, J=9.0, 4.2 Hz, CH), 3.24 (dd, 1H, J=14.4, 4.0 Hz, CH_2), 3.12 (dd, 1H, J=14.2, 8.6 Hz, CH_2), 2.08 (s, 3H, CH_3); ESI-MS (ES^+): *m/z* calcd for $C_{11}H_{12}O_3{}^{18}O$ 210.1, found 232.9 ($M+Na^+$).

3.1.23. 3′-Amino-3′-deoxy-3′-(L-2-acetoxy-3-phenylpropionyl)-*N*^{6},*N*^{6}-dimethyladenosine (20). EDCI (176.0 mg, 0.900 mmol) was added at 0 °C to a solution of puromycin aminonucleoside (240.4 mg, 0.817 mmol), **19** (172.5 mg, 0.828 mmol), and *N*-hydroxysuccinimide (107.1 mg, 0.903 mmol) in DMF (10 ml). The reaction mixture was stirred at 0 °C for 1 h, then stirred at room temperature for 24 h. After evaporation, the oily residue was subjected to column chromatography (gradient from 2% MeOH in CH_2Cl_2 to 4% MeOH in CH_2Cl_2) to give the crude product as a white powder. This material was washed with ethyl acetate (10 ml) to give the pure product **20** (288.9 mg, 73%) as a colorless fine powder. ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD=1:1): 8.29 (s, 1H, H8), 8.24 (s, 1H, H2), 7.32–7.21 (m, 5H, phenyl), 5.85 (d, 1H, J=2.8 Hz, H1′), 5.30 (dd, 1H, J=7.4, 5.8 Hz, Phe-CH), 4.49–4.41 (m, 2H, H2′, H3′), 4.05 (dt, 1H, J=7.2, 2.0 Hz, H4′), 3.95 (dd, 1H, J=12.8, 2.0 Hz, H5′), 3.69 (dd, 1H, J=12.8, 2.4 Hz, H5′), 3.53 (br, 6H, NCH_3), 3.36 (m, 1H, 3′-NH), 3.18 (dd, 1H, J=14.2, 5.4 Hz, Phe-CH_2), 3.11 (dd, 1H, J=13.8, 7.4 Hz, Phe-CH_2), 2.12 (s, 3H, acetyl-CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$/CD_3OD=1:1): 171.4, 171.1, 155.5, 152.2, 149.4, 138.2, 136.2, 129.9, 129.0, 127.5, 121.3, 91.5, 84.6, 75.1, 74.3, 61.8, 50.8, 39.0 (br), 38.3, 20.7; ESI-MS (ES^+): *m/z* calcd for $C_{23}H_{28}N_6O_6$ 484.2, found 485.2 (MH^+), 507.2 ($M+Na^+$); HRMS *m/z* calcd for $C_{23}H_{28}N_6O_6$ 485.2148 (MH^+), found 485.2154.

3.1.24. 3′-Amino-3′-deoxy-3′-(L-[2-^{18}O]-2-acetoxy-3-phenylpropionyl)-*N*^{6},*N*^{6}-dimethyladenosine (20*). The product (**20***) was obtained as a colorless powder from **19*** (173.1 mg, 0.823 mmol) using the method described in Section 3.1.23 (306.1 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD=3:1): 8.28 (s, 1H, H8), 8.24 (s, 1H, H2), 7.32–7.21 (m, 5H, phenyl), 5.85 (d, 1H, J=2.8 Hz, H1′), 5.30 (dd, 1H, J=7.2, 5.6 Hz, Phe-CH), 4.49–4.42 (m, 2H, H2′, H3′), 4.06 (dt, 1H, J=6.9, 2.1 Hz, H4′), 3.95 (dd, 1H, J=13.0, 2.2 Hz, H5′), 3.70 (dd, 1H, J=12.8, 2.4 Hz, H5′), 3.54 (br, 6H, NCH_3), 3.36 (m, 1H, 3′-NH), 3.18 (dd, 1H, J=13.8, 5.8 Hz, Phe-CH_2), 3.11 (dd, 1H, J=14.2, 7.4 Hz, Phe-CH_2), 2.12 (s, 3H, acetyl-CH_3); ESI-MS (ES^+): *m/z* calcd for $C_{23}H_{28}N_6O_5{}^{18}O$ 486.2, found 487.2 (MH^+); HRMS *m/z* calcd for $C_{23}H_{28}N_6O_5{}^{18}O$ 487.2191 (MH^+), found 487.2209.

3.1.25. 3′-Amino-3′-deoxy-3′-(L-2-acetoxy-3-phenylpropionyl)-5′-(*p*,*p*′-dimethoxytrityl)-*N*^{6},*N*^{6}-dimethyladenosine (21). The product (**21**) was obtained as a white foam from **20** (242.0 mg, 0.499 mmol) using the method described in Section 3.1.3 (350.9 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.26 (s, 1H, H8), 7.97 (s, 1H, H2), 7.35–7.14 (m, 14H, DMTr-aromatic, Phe-aromatic), 6.77 (d, 4H, J=9.2 Hz, DMTr-aromatic), 6.65 (d, 1H, J=5.2 Hz, 3′-NH), 6.01 (s, 1H, 2′-OH), 5.77 (d, 1H, J=4.0 Hz, H1′), 5.32 (dd, 1H, J=7.2, 5.6 Hz, Phe-CH), 4.65 (t, 1H, J=5.4 Hz, H2′), 4.44 (dd, 1H, J=11.6, 5.6 Hz, H3′), 4.33 (m, 1H, H4′), 3.77 (s, 6H, DMTr-OCH_3), 3.54 (br, 6H, NCH_3), 3.47 (dd, 1H, J=10.4, 2.4 Hz, H5′), 3.37 (dd, 1H, J=10.8, 3.6 Hz, H5′), 3.17 (dd, 1H, J=14.2, 5.4 Hz, Phe-CH_2), 3.09 (dd, 1H, J=14.2, 7.4 Hz, Phe-CH_2), 2.07 (s, 3H, acetyl-CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$): 169.7, 169.6, 158.5, 155.0, 151.7, 149.2, 144.4, 136.1, 135.9, 135.7, 135.6, 130.1, 129.5, 128.5, 128.2, 127.8, 127.0, 126.8, 120.7, 113.1, 91.5, 86.5, 84.4, 74.6, 74.4, 63.7, 55.2, 52.6, 38.6 (br), 37.7, 20.8; ESI-MS (ES^+): *m/z* calcd for $C_{44}H_{46}N_6O_8$ 786.4, found 787.5 (MH^+), 809.5 ($M+Na^+$); HRMS *m/z* calcd for $C_{44}H_{46}N_6O_8$ 787.3435 (MH^+), found 787.3447.

3.1.26. 3′-Amino-3′-deoxy-3′-(L-[2-^{18}O]-2-acetoxy-3-phenylpropionyl)-5′-(*p*,*p*′-dimethoxytrityl)-*N*^{6},*N*^{6}-dimethyladenosine (21*). The product (**21***) was obtained as a white foam from **20*** (249.4 mg, 0.513 mmol) using the method described in Section 3.1.3 (359.9 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.27 (s, 1H, H8), 7.96 (s, 1H, H2), 7.32–7.16 (m, 14H, DMTr-aromatic, Phe-aromatic), 6.75 (d, 4H, J=9.2 Hz, DMTr-aromatic), 6.69 (d, 1H, J=4.8 Hz, 3′-NH), 6.02 (s, 1H, 2′-OH), 5.73 (d, 1H, J=4.4 Hz, H1′), 5.34 (dd, 1H, J=7.0, 5.4 Hz, Phe-CH), 4.71 (t, 1H, J=5.6 Hz, H2′), 4.41 (dd, 1H, J=11.4, 5.0 Hz, H3′), 4.37 (m, 1H, H4′), 3.78 (s, 3H, DMTr-OCH_3), 3.77 (s, 3H, DMTr-OCH_3), 3.54 (br, 6H, NCH_3), 3.46 (dd, 1H, J=10.8, 2.8 Hz, H5′), 3.37 (dd, 1H, J=10.6, 3.4 Hz, H5′), 3.18 (dd, 1H, J=14.0, 5.2 Hz, Phe-CH_2), 3.10 (dd, 1H, J=14.2, 7.4 Hz, Phe-CH_2), 2.08 (s, 3H, acetyl-CH_3); ESI-MS (ES^+): *m/z* calcd for $C_{44}H_{46}N_6O_7{}^{18}O$ 788.3, found 811.4 ($M+Na^+$); HRMS *m/z* calcd for $C_{44}H_{46}N_6O_7{}^{18}O$ 789.3487 (MH^+), found 789.3477.

3.1.27. 3′-Amino-3′-deoxy-3′-(L-2-acetoxy-3-phenylpropionyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-*N*^{6},*N*^{6}-dimethyladenosine 2′-*O*-succinate (22). The product (**22**) was obtained as a white foam from **21** (299.7 mg, 0.380 mmol) using the method described in Section 3.1.5 (192.7 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.29 (s, 1H, H8), 7.94 (s, 1H, H2), 7.32–7.16 (m, 14H, DMTr-aromatic, Phe-aromatic), 6.77 (d, 4H, J=8.8 Hz, DMTr-aromatic), 6.43 (br, 1H, 3′-NH), 6.10 (s, 1H, H1′), 5.61 (d, 1H, J=3.2 Hz, H2′), 5.41 (t, 1H, J=5.2 Hz, Phe-CH), 5.18 (dd, 1H, J=15.0, 8.2 Hz, H3′), 3.91 (br, 1H, H4′), 3.73 (s, 3H, DMTr-OCH_3), 3.72 (s, 3H, DMTr-OCH_3), 3.50 (br, 6H, NCH_3), 3.38–3.31 (m, 2H, H5′), 3.03 (dd, 1H, J=14.0, 7.6 Hz, Phe-CH_2), 2.92 (dd, 1H, J=14.2, 5.4 Hz, Phe-CH_2), 2.65 (br, 2H, succinic ester-CH_2), 2.60 (br, 2H, succinic ester-CH_2), 2.08 (s, 3H, acetyl-CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): 170.7, 170.2, 169.1, 158.4, 154.9, 152.3, 149.4, 144.3,

139.4, 136.3, 135.7, 135.6, 135.5, 130.1, 129.7, 129.5, 129.1, 128.5, 128.2, 127.9, 127.8, 127.1, 127.0, 126.8, 120.3, 113.1, 113.0, 87.2, 86.6, 77.2, 75.9, 74.1, 62.5, 55.3, 55.2, 49.6, 38.6 (br), 37.8, 29.9, 29.7 (br), 20.9; ESI-MS (ES^+): *m/z* calcd for $C_{48}H_{50}N_6O_{11}$ 886.4, found 887.6 (MH^+); HRMS *m/z* calcd for $C_{48}H_{50}N_6O_{11}$ 887.3538 (MH^+), found 887.3550.

3.1.28. 3′-Amino-3′-deoxy-3′-(L-[2-^{18}O]-2-acetoxy-3-phenylpropionyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-succinate (22*). The product (**22***) was obtained as a white foam from **21*** (312.5 mg, 0.396 mmol) using the method described in Section 3.1.5 (217.0 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): 8.29 (s, 1H, H8), 7.94 (s, 1H, H2), 7.32–7.16 (m, 14H, DMTr-aromatic, Phe-aromatic), 6.77 (d, 4H, J=8.8 Hz, DMTr-aromatic), 6.43 (br, 1H, 3′-NH), 6.10 (d, 1H, J=2.0 Hz, H1′), 5.62 (d, 1H, J=4.4 Hz, H2′), 5.41 (t, 1H, J=6.4 Hz, Phe-CH), 5.18 (dd, 1H, J=14.2, 8.6 Hz, H3′), 3.91 (br, 1H, H4′), 3.74 (s, 3H, DMTr-OCH_3), 3.73 (s, 3H, DMTr-OCH_3), 3.50 (br, 6H, NCH_3), 3.39–3.32 (m, 2H, H5′), 3.03 (dd, 1H, J=14.4, 7.2 Hz, Phe-CH_2), 2.93 (dd, 1H, J=13.6, 5.2 Hz, Phe-CH_2), 2.65 (br, 2H, succinic ester-CH_2), 2.61 (br, 2H, succinic ester-CH_2), 2.08 (s, 3H, acetyl-CH_3); ESI-MS (ES^+): *m/z* calcd for $C_{48}H_{50}N_6O_{10}{}^{18}O$ 888.4, found 889. 6 (MH+), 911.7 ($M+Na^+$); HRMS *m/z* calcd for $C_{48}H_{50}N_6O_{10}{}^{18}O$ 889.3658 (MH^+), found 889.3656.

3.1.29. 3′-Amino-3′-deoxy-3′-(L-2-acetoxy-3-phenylpropionyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-(LCAA-polystyrene)succinate (23). The product (**23**) was obtained from **22** (180.0 mg, 0.169 mmol) using the solid support derivatization method described in Section 3.1.7. The nucleoside loading was 70 μmol/g.

3.1.30. 3′-Amino-3′-deoxy-3′-(L-[2-^{18}O]-2-acetoxy-3-phenylpropionyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-N^6,N^6-dimethyladenosine 2′-*O*-(LCAA-polystyrene)succinate (23*). The product (**23***) was obtained from **22*** (180.0 mg, 0.169 mmol) using the solid support derivatization method described in Section 3.1.7. The nucleoside loading was 103 μmol/g.

3.1.31. Cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-(L-2-hydroxy-3-phenylpropionyl)-N^6,N^6-dimethyladenosine (2). The product (**2**) was obtained by coupling the cytidine phosphoramidite (**8**) to **23** using the method described in Section 3.1.9. ESI-MS (ES^+): *m/z* calcd for $C_{30}H_{38}N_9O_{12}P$ 747.2, found 748.3 (MH^+); HRMS *m/z* calcd for $C_{30}H_{38}N_9O_{12}P$ 770.2275 ($M+Na^+$), found 770.2284.

3.1.32. Cytidylyl-(3′-5′)-3′-amino-3′-deoxy-3′-(L-[2-^{18}OH]-2-hydroxy-3-phenylpropionyl)-N^6,N^6-dimethyladenosine (2*). The product (**2***) was obtained by coupling the cytidine phosphoramidite (**8**) to **23*** using the method described in Section 3.1.9. ESI-MS (ES^+): *m/z* calcd for $C_{30}H_{38}N_9O_{11}{}^{18}OP$ 749.2, found 750.4 (MH^+); HRMS *m/z* calcd for $C_{30}H_{38}N_9O_{11}{}^{18}OP$ 750.2498 (MH^+), found 750.2509.

3.2. 50 S subunit reaction assay

Large ribosomal subunits were isolated from *E. coli*MRE600 cells by a procedure modified from the literature.[33] CCApcb was 5′-^{32}P end labeled by phosphorylation with T4 polynucleotide kinase and [γ-^{32}P]ATP. The reaction of 1.0 mM **1** or **2** with ^{32}pCCApcb and 9 μM 50 S ribosome was performed in 7 mM Mg^{2+}, 7 mM K^+, 166 mM $NH_4{}^+$, 0.1 mM EDTA, 0.2 mM DTT, 25 mM MES, 25 mM MOPS, 50 mM Tris-HCl buffer (pH 7.0) at 25 °C. The ribosomes were incubated for 2 min at 37 °C before beginning the reaction. The samples were analyzed by polyacrylamide gel electrophoresis (7 M urea/50 mM Tris-sodium phosphate (pH 6.5)/12% polyacrylamide gel with 50 mM Tris-sodium phosphate buffer (pH 6.5) at 30 W).

For HPLC analysis, 20 nmol of A-site substrate was added to 30 nmol of CCApcb (Fig. 2b). The buffer conditions were the same as above. 4.5 μM 50 S ribosomal subunits were added to begin the reaction. Once the reaction had proceeded to greater than 50% reacted, it was quenched by addition of ~50 mM EDTA. The reaction was purified on an Agilent Technologies XBD-C_{18} reverse phase HPLC column using 10 mM triethylamine acetate (TEAA), pH 6.5 as the mobile phase. Substrates and products were separated by HPLC using a gradient of 0–30% acetonitrile over 30 min followed by an isocratic run for 10 min at 30% acetonitrile. Mass spectrometry was used to determine the identity of each HPLC peak. All HPLC fractions were frozen, lyophilized to dryness, and desalted by multiple rounds of lyophilization. The samples were analyzed on an Applied Biosystems PE SciEX API 3000 triple quadrupole mass spectrometer with an electrospray ion source (ESI-MS). For ESI-MS analysis, samples were resuspended in 1:1 10 mM TEAA:acetonitrile and injected by direct infusion at a rate of 10 μl/min. The same procedure was used for reactions with **1**, **1***, and **1***** each serving as the A-site substrate. As expected, the ^{15}N substitutions did not effect the HPLC retention time. The exact mass for each of the A-site substrates **1**, **1***, and **1***** and their products $Cm^6A_NPhe_N$-pcb, $Cm^6A_NPhe_{15N}$pcb, and [3-^{15}N,4-$^{15}NH_2$]-$Cm^6A_NPhe_{15N}$pcb are 746.25, 747.25, 749.25, 1262.49, 1263.49, and 1265.49, respectively.

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Synthesis and purity assessment of tetra- and pentaacyl lipid A of *Chlamydia* containing (*R*)-3-hydroxyicosanoic acid

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Abstract—Based on structural data of lipid A from *Chlamydia trachomatis* strains, chemically pure tetra- and pentaacyl 1,4′-bisphosphoryl as well as the related 4′-monophosphoryl derivatives of lipid A were synthesized. (*R*)-3-Hydroxyicosanoic acid as a chiral constituent was prepared via Noyori-reduction of methyl-3-oxoicosanoic acid. Synthetic intermediates were *O*-acylated with myristoic acid residues at positions 3 and 3′ and *N*-acylated with (*R*)-3-hydroxyicosanoic acid at both glucosamine units. Efficient purification methods for highly hydrophobic long-chain tri-, tetra- and pentaacyl progenitors of lipid A have been developed. Purity and homogeneity of the synthetic target compounds were confirmed by NMR and MS-data as well as a sensitive immunostaining approach. The tetra- and pentaacyl species serve as biomedical probes to investigate the endotoxic potential of chlamydial lipid A and to clarify its role in *Chlamydia* associated infections.

1. Introduction

Chlamydiae are obligatory intracellular Gram-negative pathogens with a biphasic lifecycle that cause acute and chronic diseases in animals and humans.[1] The species *Chlamydia trachomatis* is implicated in eye trachoma and chronic urogenital infections leading to infertility in women.[2] The common opportunistic pathogen *Chlamydophila pneumoniae* is suspected to contribute to the pathogenesis of human atherosclerosis, myocardial infarction and stroke.[3,4] Macrophages infected by *Chl. pneumoniae* are thought to be responsible for the mediation of inflammatory and autoimmune processes leading to atherosclerosis.[5] The role of chlamydial lipopolysaccharide (LPS) in these chronic infections and the underlying pathophysiological mechanisms have not yet been clarified.[6] The structure of lipid A—as the endotoxically active component of LPS—has recently been established for the lipid A from *C. trachomatis* serotypes L_2, E and F as well as *Chl. psittaci* 6BC, showing unique tetra- and pentaacylated species with only minor variations in the lipid A acylation pattern (Fig. 1).[7–9] Chlamydial LPS has been shown to be at least 10-fold less stimulatory than enterobacterial LPS, and the structural basis for this low potency was attributed to the higher hydrophobicity of chlamydial lipid A containing fatty acyl groups of longer chain length (up to C-21) and only non-hydroxylated fatty acids ester-linked to the sugar backbone.[10,11] Chlamydial LPS, due to the obligatory intracellular growth of the bacteria, is only available in minor quantities. Moreover, chlamydial lipid A comprises a complex mixture of structural homologs having four or five long-chain acyl groups of different chain lengths. Thus, isolation of homogeneous chlamydial lipid A is not possible, and the study of potential agonistic or antagonistic properties of differently acylated lipid A variants is not viable. On the basis of the reported structures for lipid A from *C. trachomatis* we have chemically synthesized two representative forms, the chlamydial tetra- and pentaacyl lipid A derivatives **1** and **2** as well as the corresponding 4′-monophosphoryl analogues **3** and **4** (Fig. 2). The compounds serve as model compounds for authentic chlamydial lipid A, for immunobiological studies and as substrates for chlamydial CMP-Kdo-transferases.

2. Results and discussion

The basic synthetic approach to a large variety of lipid A and analogues has been elaborated in the groups of Shiba and Kusumoto.[12] A crucial issue in the context of evaluating the bioactivity of lipid A relates to the purity of lipid A samples. Controls of synthetically prepared lipid A mostly

Keywords: Lipid A; *Chlamydia*; Lipopolysaccharide; Sugar phosphates; Glycolipid.
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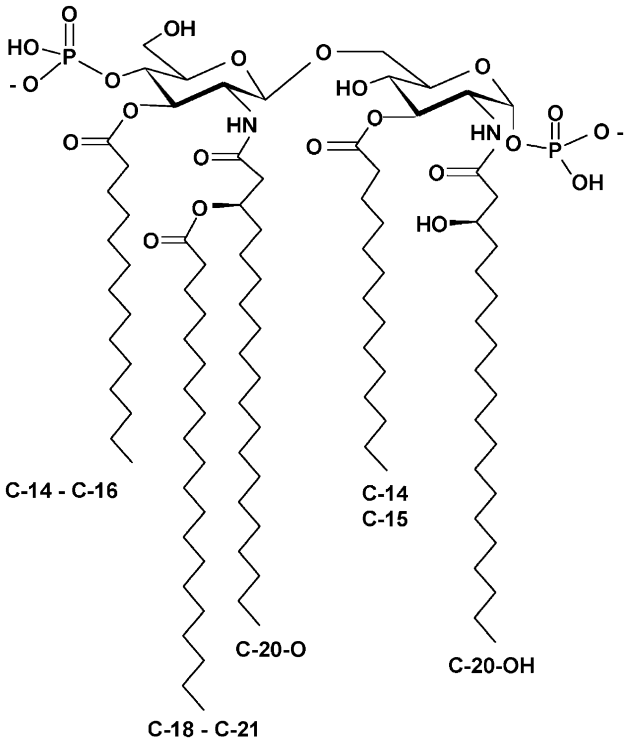
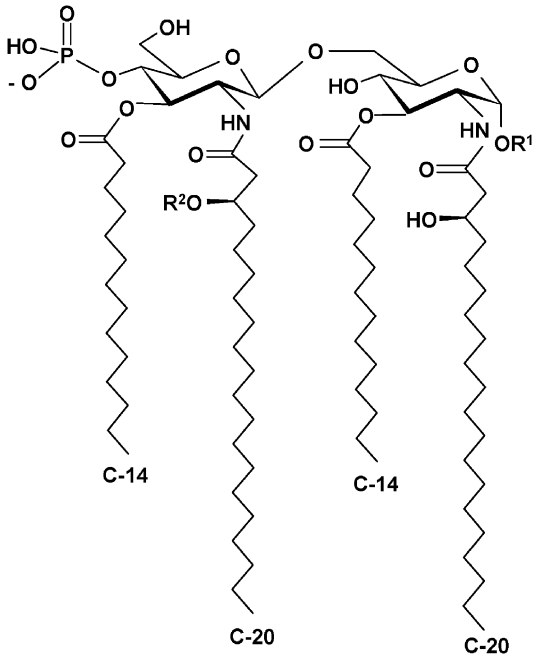


Figure 1. Structure of major lipid A species from *C. trachomatis* serotypes L_2, E and F.



1 $R^1 = PO_3H^-$, $R^2 = H$
2 $R^1 = PO_3H^-$, $R^2 = -CO(CH_2)_{16}CH_3$
3 $R^1 = R^2 = H$,
4 $R^1 = H$, $R^2 = -CO(CH_2)_{16}CH_3$

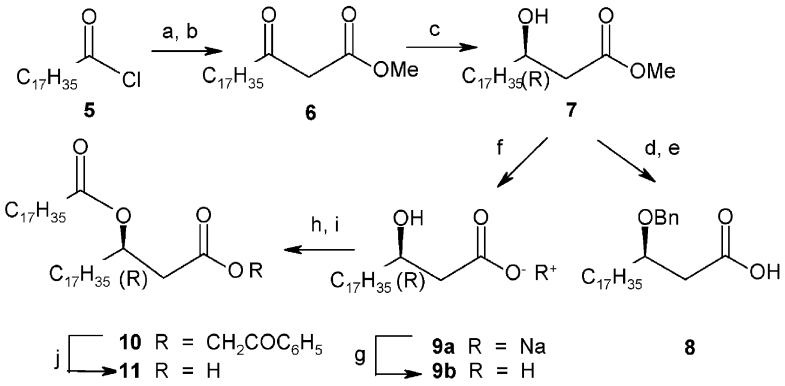
Figure 2. Synthetic chlamydial tetraacyl lipid A **1**, pentaacyl lipid A **2** and monophosphoryl lipid A analogues **3** and **4**.

rely upon spectral analysis of the final products by MS- and NMR data. Highly amphiphilic, poorly soluble lipid A samples display weakly resoluted signals in the NMR spectra making impossible the identification of potential lipid-derived impurities. Yet minor contaminants may be detected by highly sensitive immunostaining using a monoclonal antibody recognizing the bisphosphorylated backbone of lipid A.[13] Therefore, the synthetic strategy towards divergently acylated chlamydial lipid A **1** and **2** was also focused on the purity assessment of hydrophobic synthetic intermediates and the development of efficient purification protocols. The target lipid A derivatives **1** and **2** and their monophosphoryl analogues (MLA) **3** and **4** were prepared in a highly convergent manner from the following major constituents: optically pure (*R*)-3-hydroxyalkanoic acids **8** and **11** (Scheme 1), the trichloroacetimidate glycosyl donor **20** (Scheme 2) and the bisacylated 4-*O*-benzyl protected acceptor **36** (see Scheme 3). The synthesis of β-(1→6)-linked disaccharides **28** and **37** followed by final *N*-acylation and phosphorylation (Schemes 4 and 5, respectively), and approaches towards isolation of fully acylated intermediates will be successively presented.

2.1. Preparation of optically pure 3-hydroxyalkanoic acids

Whereas the preparation of a variety of (*R*)-3-hydroxyalkanoic acids and their derivatives has been previously reported,[14–16] 3-hydroxyicosanoic acid was only prepared as a racemic mixture.[17] Herein we present the first enantioselective synthesis of (*R*)-3-hydroxyicosanoic acid **8** and (*R*)-3-(octadecanoyloxy)-icosanoic acid **11**—the long chain acyl constituents of chlamydial lipid A. To this end, methyl 3-oxoicosanoate **6** was prepared from octadecanoyl chloride **5** via chain lengthening with 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) and subsequent decarboxylation in 46% yield.[18] Enantioselective Noyori hydrogenation[16,19] of the prochiral 3-oxo-ester **6** in methanol using $RuCl_2$[(*R*)-Binap] at 60 °C and 85 kg cm^{-2} hydrogen pressure afforded methyl (*R*)-hydroxyicosanoate **7** in 77% yield with excellent optical purity (ee≥99%). Comparison of its specific optical rotation value with those reported for shorter methyl (*R*)-3-hydroxyalkanoates[14] allowed to assign the absolute configuration of **7** as (*R*). The enantiomeric purity was determined by ^1H NMR analysis using a chiral shift reagent europium tris[3-(heptafluoropropylhydroxy-methylene)-(+)-camphorate] $Eu(hfc)_3$.[14,20] The 3-hydroxy group in **7** was protected by one-pot reductive benzylation according to improved[21] Nishizawa method[22] using benzaldehyde, hexamethyldisiloxane, trimethylsilyl trifluoromethanesulfonate (TMSOTf) and triethylsilane (Et_3SiH), which afforded methyl (*R*)-3-(benzyloxy)icosanoate in 90% yield. Subsequent hydrolysis of the methyl ester group with $LiOH \cdot H_2O$ gave (*R*)-3-(benzyloxy)icosanoic acid **8** in 90% yield. (*R*)-3-(Octadecanoyloxy)icosanoic acid **11** was prepared from 3-hydroxyalkanoic ester **7** in four steps (Scheme 1).

Alkaline cleavage of the methyl ester group of **7** afforded **9a**, and subsequent treatment with phenacyl bromide followed by 3-*O*-acylation with octadecanoyl chloride in CH_2Cl_2/DMAP afforded ester **10**. Cleavage of the phenacyl

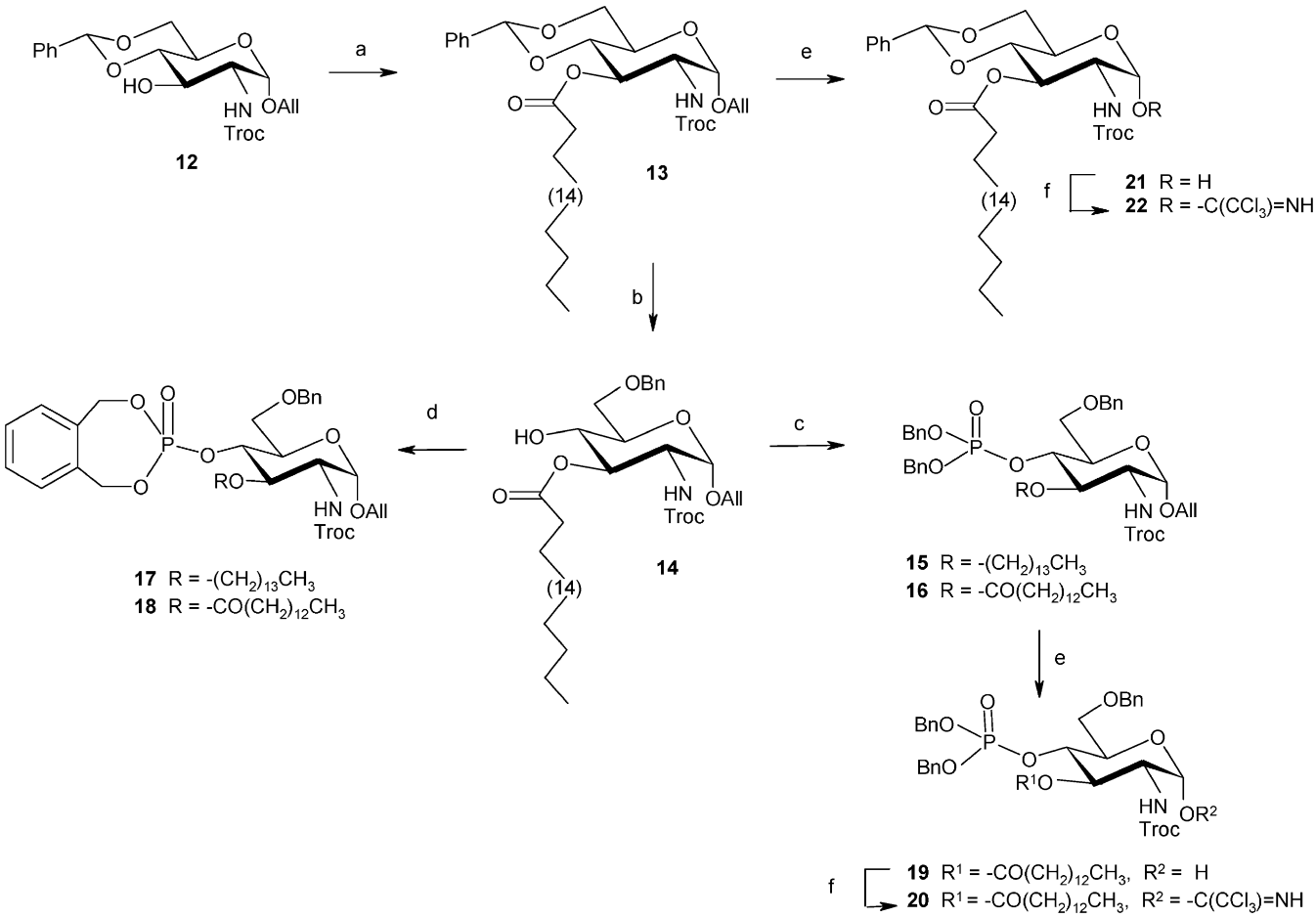


Scheme 1. Synthesis of icosanoic acid derivatives: (a) 2,2-dimethyl-1,3-dioxane-4,6-dione, pyridine/CH_2Cl_2; (b) MeOH, reflux, 46% (two steps); (c) $RuCl_2$[(*R*)-Binap], 60 °C, 85 kg/cm^{-2} H_2, 77%; (d) C_6H_5CHO, $(TMS)_2O$, TMSOTf, Et_3SiH, 90%; (e) $LiOH\cdot H_2O$, 90%; (f) NaOH; (g) Dowex H^+; (h) phenacyl bromide, Et_3N, EtOAc, 45 °C; (i) $C_{17}H_{35}COCl$, 4-DMAP, CH_2Cl_2, 88% for steps (h)+(i); (j) Zn–Cu couple, AcOH/toluene, then aq HCl, 85%.

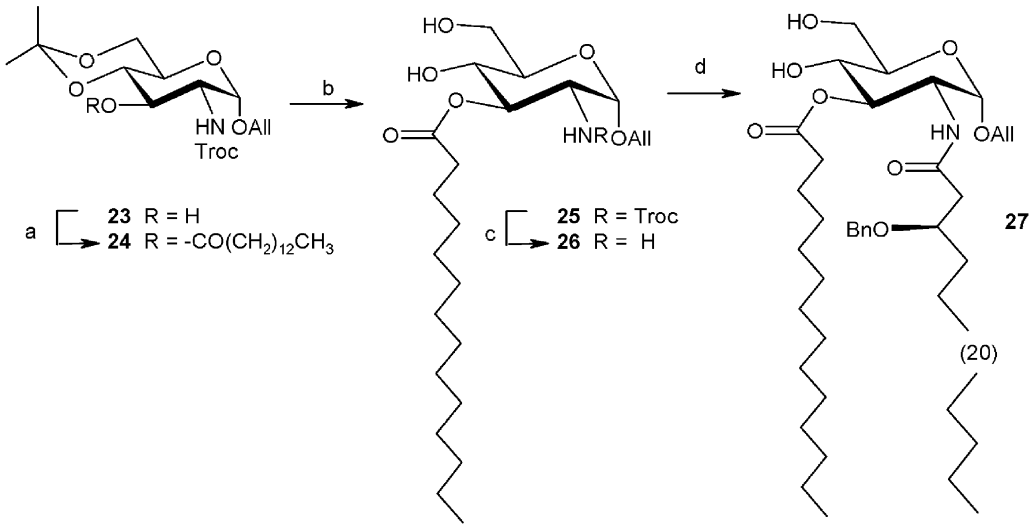
group by Zn–Cu couple in AcOH–toluene gave free acid **11**. The sodium salt **9a** was converted into the free acid **9b** by treatment with Dowex® AG 50 (H^+-form).

2.2. Glycosyl donor and acceptor synthesis

A common glycosyl donor for the synthesis of **1**–**4** was prepared from the known intermediate **12**[23] (Scheme 2). The 3-OH group in **12** was acylated with myristoyl chloride/pyridine in 95% yield to give fully protected **13**, which was further employed for the preparation of both donor and acceptor moieties. Reductive cleavage of the benzylidene acetal in **13** to yield the 6-*O*-benzyl-protected derivative **14** was achieved first by application of a frequently used

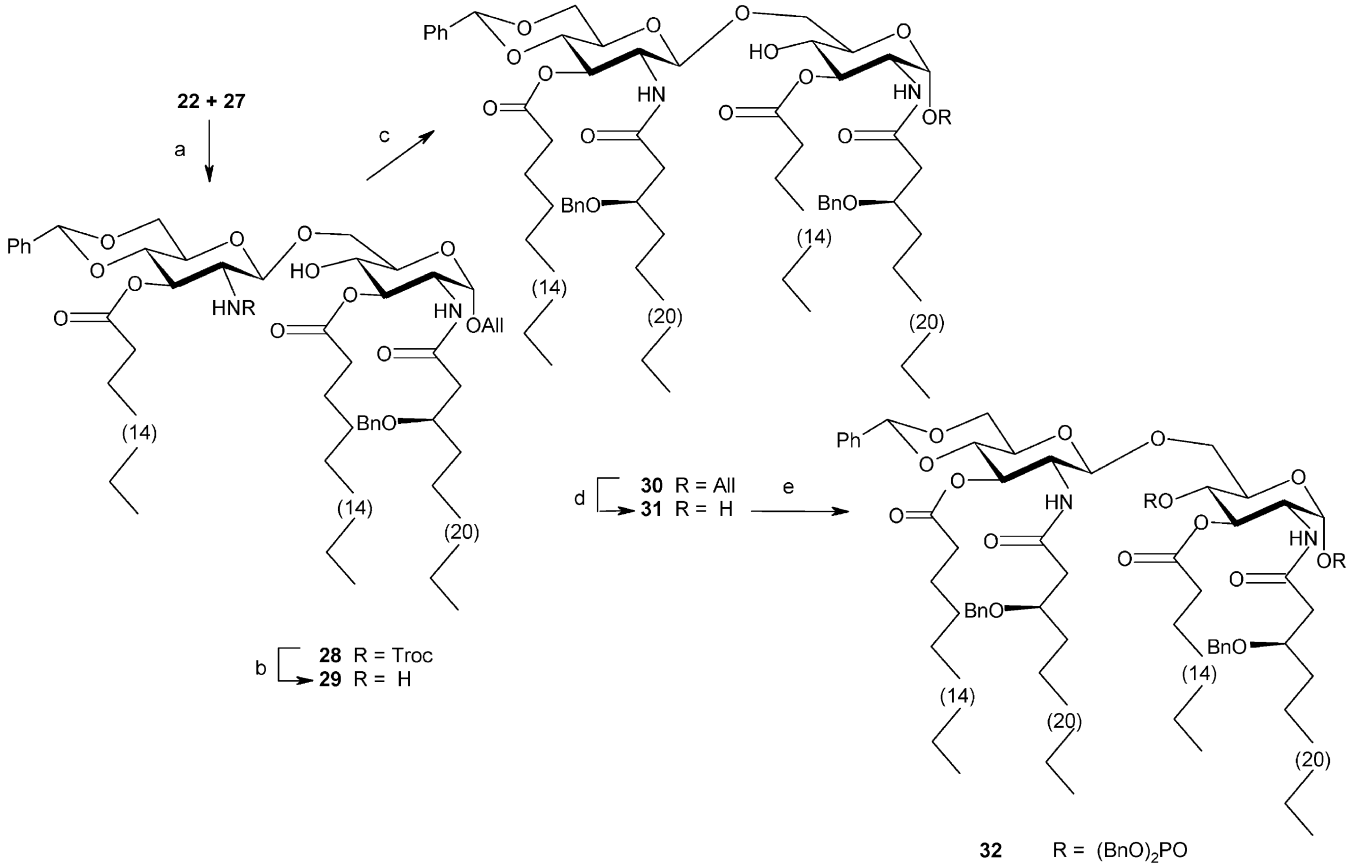


Scheme 2. Synthesis of glycosyl donor and acceptor: (a) MyrCl, pyridine, THF, 95%; (b) method A: $BH_3\cdot Me_2NH$, $BF_3\cdot OEt_2$, CH_2Cl_2, 0 °C→ rt; 62%; method B: Et_3SiH, TfOH, CH_2Cl_2, mol. sieves 0.4 nm, −78 °C, 92%; (c) di-*O*-benzyloxy(*N*,*N*-diisopropylamino)phosphine, 1*H*-tetrazole, CH_2Cl_2, then *tert*-BuOOH, (method A: **16** 44% **15** 6%; method B: **16** 78%); (d) *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2, then 3-chloroperbenzoic acid (**18** 47%, **17** 8%); (e) {[bis(methyldiphenyl)phosphine](1,5-cyclooctadiene)iridium(I)} hexafluorophosphate, THF, then aq I_2, 91% for **19**, 83% for **21**; (f) trichloroacetonitrile, Cs_2CO_3, CH_2Cl_2.

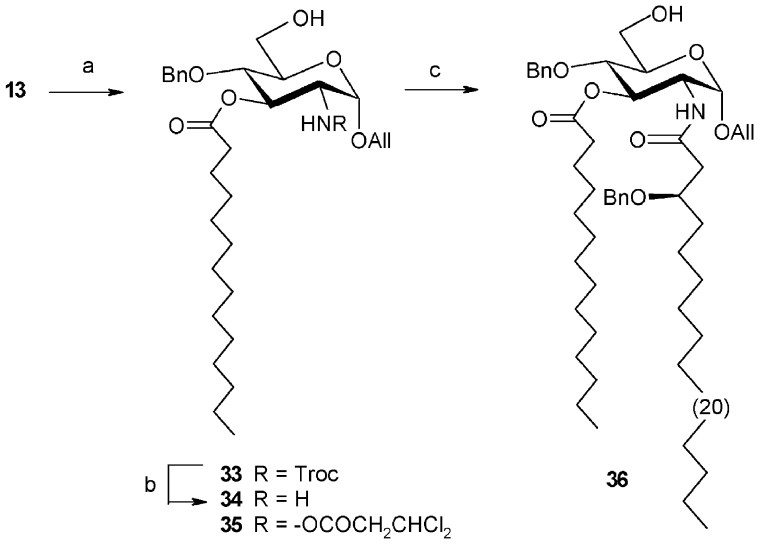


Scheme 3. Synthesis of glycosyl acceptor **27**: (a) $CH_3(CH_2)_{12}COOH$, DCC, 4-DMAP, CH_2Cl_2, 86%; (b) 90% aq AcOH, 90 °C, 96%; (c) Zn–Cu couple, AcOH, 70%; (d) **8**, DCC, CH_2Cl_2, 86%.

method in lipid A chemistry employing dimethylamine–borane complex $BH_3 \cdot Me_2NH$ as reagent with $BF_3 \cdot OEt_2$ as a promoter in acetonitrile.[21,24,25] This approach proved to be disadvantageous in our case with respect to moderate yields of 6-*O*-benzyl derivative **14** (60%) and persistent contamination of the latter with dimethylamine–borane, which is partially soluble in organic solvents. In spite of multiple treatments of the reaction mixture with aqueous 1 M HCl and thorough chromatographic purification of the product **14**, the presence of minor amounts of $BH_3 \cdot Me_2NH$ was detrimental in the next phosphorylation step. Thus, phosphitylation of contaminated **14** with di-*O*-benzyloxy(*N*,*N*-diisopropylamino)phosphine in the presence of 1*H*-tetrazole as an acid catalyst and subsequent oxidation



Scheme 4. Phosphorylation of 4-unprotected precursor **31**: (a) TMSOTf, CH_2Cl_2, molecular sieves 0.4 nm, −25 °C, 76%; (b) Zn–Cu couple, AcOH, 66%; (c) **8**, IIDQ, $CH_2Cl_2/CHCl_3$, 76%; (d) {[bis(methyldiphenyl)phosphine](1,5-cyclooctadiene)iridium(I)}hexafluorophosphate, THF, then aq I_2, 94%; (e) $[(BnO)_2P(O)]_2O$, $(TMS)_2NLi$, THF, −78 °C, 19%.



Scheme 5. Synthesis of 4-*O*-protected glycosyl acceptor: (a) Et_3SiH, $PhBCl_2$, CH_2Cl_2, mol. sieves 0.4 nm, −78 °C, 96%; (b) method A: Zn–Cu couple, AcOH, (**34** 60%, **35** 5%), method B: Zn, AcOH, 89% for **34**; (c) **8**, WSCD·HCl, HOBt, $CH_2Cl_2/CHCl_3$, 85%.

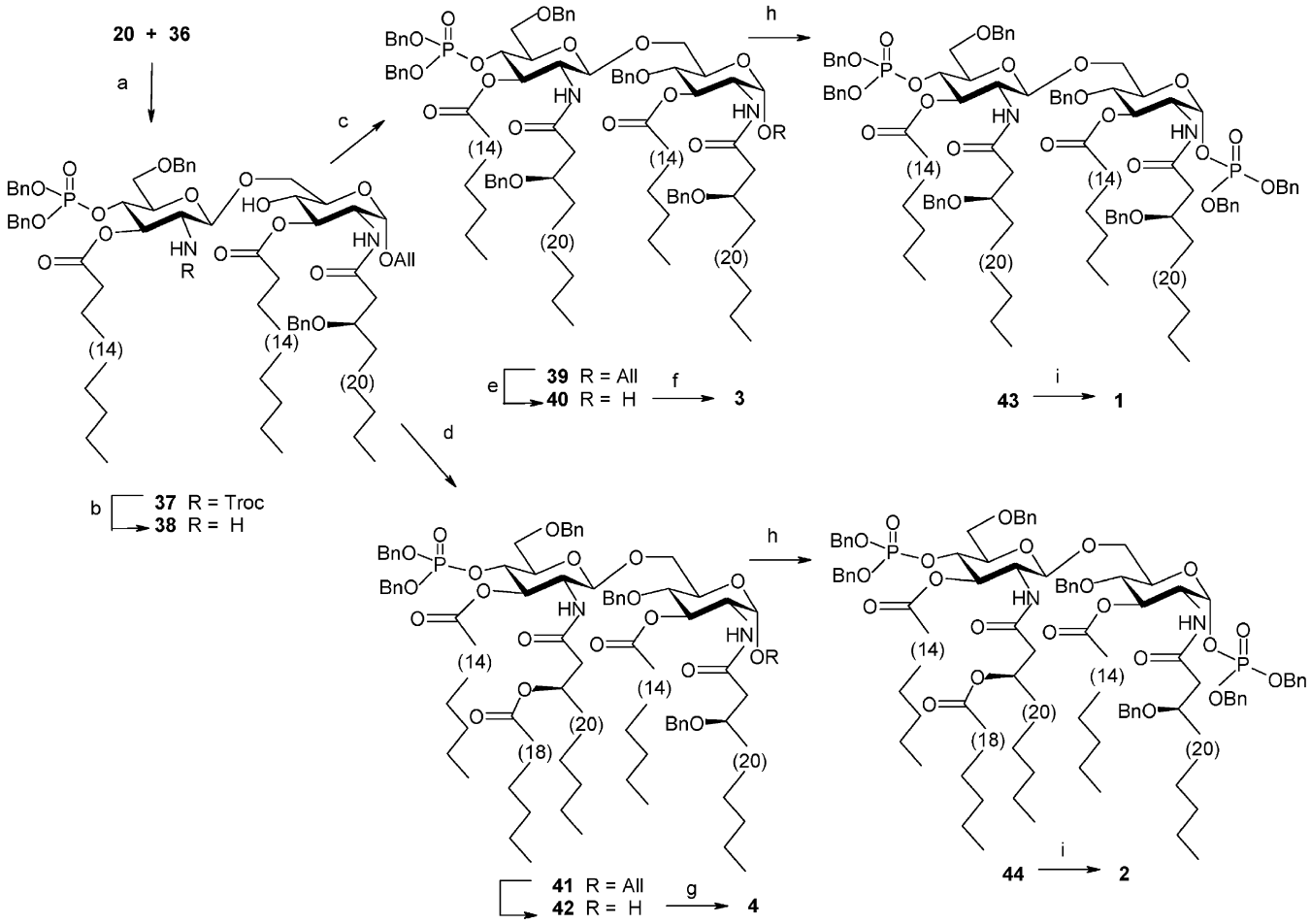
with *t*BuOOH gave rise to the desired phosphate **16** (44%) along with the co-migrating 3-*O*-alkyl analogue **15** (15% crude yield, 6% isolated yield). Partial reduction of the 3-*O*-acyl group took place independently of the phosphitylating reagent used. Employment of the Watanabe reagent[26] (*N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine) and subsequent oxidation furnished the expected 3-*O*-acyl-4-*O*-phosphate **18**, but also the undesired 3-*O*-alkyl derivative **17**.[27] Complete separation of 3-*O*-acyl-4-*O*-phosphotriesters **16** and **18** from their co-migrating 3-*O*-alkyl counterparts **15** and **17**, respectively, required repeated preparative HPLC purifications which resulted in low yields (44 and 47%, respectively) of chemically pure phosphates **16** and **18**.

These shortcomings could be avoided by using Et_3SiH as a reductive reagent at −78 °C in CH_2Cl_2 in the presence of activated molecular sieves and trifluoromethanesulfonic acid (TfOH)[28,29] for the regioselective opening of the benzylidene acetal.[28] In this way, benzylidene acetal **13** was efficiently converted in 92% yield into the corresponding 6-*O*-benzyl derivative **14** (Scheme 2). Furthermore, phosphitylation and subsequent oxidation of the alcohol **14** proceeded without by-product formation and the dibenzyl-phosphotriester **16** was readily isolated in 78% yield. The allyl group in **16** was isomerized by treatment with H_2-activated iridium complex {[bis(methyldiphenyl)-phosphine](1,5-cyclooctadiene) iridium(I)}hexafluoro-phosphate.[30] The propenyl group was cleaved with aq iodine to furnish **19** which was transformed into glycosyl donor **20** by treatment with trichloroacetonitrile/Cs_2CO_3. The 4,6-*O*-benzylidene acetal derivative **13** was also employed for the preparation of the non-phosphorylated donor **22** by successive cleavage of the allyl group to afford **21** and treatment of **21** with trichloroacetonitrile/Cs_2CO_3.

2.3. Disaccharide synthesis

4,6-Diol glucosamine acceptors have previously been effectively used in the synthetic preparation of different lipid A derivatives.[20,23] Formation of a 1→6-glycosidic linkage with this type of acceptor is known to proceed regioselectively, and the 4-OH group was regarded to remain unaffected during final BuLi-assisted 1-*O*-phosphorylation of the fully acylated β(1→6)-linked disaccharide 4′-phosphate progenitor of lipid A.[20,23,31] In a first approach, the diol acceptor **27** was prepared according to a literature procedure.[32] The known acetonide **23** was *O*-acylated with tetradecanoic acid in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine which furnished **24**. Subsequent acidic hydrolysis of the isopropylidene group afforded diol **25**. Removal of trichloroethoxycarbonyl (Troc) protection with Zn–Cu couple and final *N*-acylation of the free amine **26** with (*R*)-3-(benzyloxy)icosanoic acid **8** under assistance of DCC furnished 4,6-diol derivative **27** (Scheme 3) in 57% yield (for three steps).[33]

To test the regioselectivity of 1-*O*-phosphorylation in the last step of the synthesis, the diol-acceptor **27** was first utilized for the synthesis of 4′,6′-*O*-benzylidene-protected 1-*O*-monophosphoryl lipid A progenitor **31**. Glycosylation of **27** with 4,6-*O*-benzylidene protected trichloroacetimidate **22** afforded disaccharide **28** (76%). Reductive cleavage of *N*-Troc protecting group with Zn–Cu couple in AcOH–toluene and subsequent *N*-acylation of the free amine **29** with (*R*)-3-(benzyloxy)icosanoic acid **8** in the presence of 1-isopropyloxycarbonyl-2-isopropyloxy-1,2-dihydroquinoline (IIDQ) furnished the tetraacyl precursor **30**. After the removal of the anomeric allyl group, the reducing diol **31** was subjected to phosphorylation with tetrabenzyl diphosphate in the presence of lithium bis(trimethylsilyl)amide at −78 °C.[25] The high hydrophobicity and low reactivity of **31** required the use of four-times excess of the reagents to complete the reaction, which led to an undesired phosphorylation at the unprotected 4-position. The major product isolated in 19% yield by chromatography was identified as 1,4-*O*-bisphosphate **32**, while the more polar



Scheme 6. *N*-Acylation, 1-*O*-phosphorylation and final deprotection: (a) TMSOTf, CH_2Cl_2, mol. sieves 0.4 nm, −25 °C, 93%; (b) Zn, AcOH, 84%; (c) **8**, HATU, DMF, DIPEA, 35 °C, 92%; (d) **11**, HBTU, DIPEA, DMF/THF, 58 °C, 86%; (e) {[bis(methyldiphenyl)phosphine](1,5-cyclooctadiene)iridium(I)}hexafluorophosphate, THF, then aq I_2, 85% for **40**, 88% for **42**; (f) Pd/C, H_2, then DEAE-cellulose, 60% for **3**; (g) Pd/C, H_2, 5:1 toluene/MeOH, then silica gel and DEAE-cellulose chromatography, 49%; (h) $[(BnO)_2OP]_2O$, $(TMS)_2NLi$, THF, −78 °C, 85% for **43**, 86% for **44**; (i) Pd/C, H_2, 5:1 toluene/MeOH, then DEAE-cellulose, 51% for **1**, 45% for **2**.

1-*O*-monophosphoryl component of the mixture was destroyed due to the prolonged contact with silica gel.

Taking into consideration the lability of reducing phosphotriesters and the difficulty of their isolation in chemically pure form, the importance of a full-protection strategy and, therefore, the option of selective 1-*O*-monophosphorylation using excess of reagents became evident.[34] To this end the 4-*O*-benzyl glycosyl acceptor **33** was obtained in an excellent yield (96%) by regioselective reductive opening of benzylidene acetal **13** using Et_3SiH as reductive reagent and phenyldichloroborane $PhBCl_2$ as Lewis-acid catalyst (Scheme 5).[28] Reductive cleavage of Troc protection with Zn–Cu couple in aqueous AcOH[23] led unexpectedly to partial reduction of trichloroethoxycarbonyl group with the formation of *N*-2,2-dichloroethoxycarbonyl derivative **35**, thereby reducing the yield of the amine **34** to 66%. The employment of Zn in AcOH, however, afforded free amine **34** in 89% isolated yield without any detectable partial reduction of the trichloroethoxycarbonyl group.[35] Subsequent *N*-acylation with (*R*)-3-(benzyloxy)icosanoic acid **8** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (water soluble carbodiimide, WSCD·HCl) and 1-hydroxy-benzotriazole (HOBt)[21] furnished amphiphilic acceptor **36**, which was extensively purified by partition-adsorption chromatography on silica gel using toluene as stationary phase and aqueous MeOH as mobile phase.

For the assembly of synthetic chlamydial lipid A the protected 6-OH acceptor **36** and trichloroacetimidate donor **20** were employed (Scheme 6). Glycosylation of **36** with *N*-alkoxycarbonyl-protected trichloroacetimidate **20** gave rise exclusively to β-disaccharide **37**.[36] The β(1→6)-linkage was supported by ^1H NMR experiments which revealed a coupling constant $^3J_{1',2'}$ of the disaccharide of 8.3 Hz. The next step, reductive cleavage of Troc-protection with Zn in AcOH at 50 °C, and thorough purification of the generated free amine by chromatography afforded **38** in 84% yield.[37,38] The isolation of **38** in chemically pure form was crucial, since its Troc-protected precursor **37**, when not fully deprotected or being partially reduced to a dichloroethoxycarbonyl derivative, co-migrates with tetra- and pentaacyl lipid A progenitors **39** and **41** prepared in the subsequent acylation step. A series of conditions towards divergent *N*-acylation of the disaccharide **38** with either (*R*)-3-(benzyloxy)icosanoic acid **8** or (*R*)-3-(octadecanoyloxy)-icosanoic acid **11** was explored. Acylation of **38**

Table 1. Conditions for the *N*-acylation of lipid A precursor **38** with acids **8** or **11** to yield tetraacyl- (**39**) or pentaacyl (**41**) derivatives

Entry	Acid	Conditions	Coupling reagent, auxiliary nucleophile, base	Yield (%)
1	**8**	THF, 20 °C, 24 h	WSCD·HCl, HOBt	20
2	**8**	DMF, 40 °C, 40 h	WSCD·HCl, HOAt, DIPEA	20
3	**8**	THF, 20 °C, 40 h	EEDQ	40
4	**8**	THF, 20 °C, 30 h	IIDQ	50
5	**8**	DMF/THF, 20 °C, 3 h	HATU, DIPEA	50
6	**8**	DMF, 35 °C, 5 h	HATU, DIPEA	92
7	**11**	DMF, 50 °C, 8 h	HATU, DIPEA	75
8	**8**	DMF, 35 °C, 6 h	HBTU, DIPEA	80
9	**11**	DMF, 58 °C, 5 h	HBTU, DIPEA	86

with less bulky and less hydrophobic acid **8** in the presence of water-soluble carbodiimide (WSCD·HCl)[21] provided the tetraacyl derivative **39** in only 20% yield independently of the nature of the auxiliary nucleophile, base and solvent used (Table 1, entries 1 and 2). Employment of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[35] or IIDQ as coupling reagents in THF required prolonged reaction times and only slightly improved the isolated yields (40–50%). The optimal reaction conditions were established by employing the uronium salts *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis-tetramethyluronium hexafluorophosphate (HBTU)[39] in the presence of diisopropylethylamine (DIPEA), which provided tetraacyl disaccharide **39** and sterically hindered pentaacyl derivative **41** in 92 and 86% yield, respectively (Table 1, entries 6 and 9). Subsequent isomerisation of the allyl protection of the fully acylated disaccharides **38** and **41** with H_2-activated iridium complex and hydrolysis of the propenyl group by treatment with aq iodine furnished reducing disaccharides **40** and **42**, respectively.

2.4. Purification of disaccharide intermediates

While a variety of synthetic approaches towards divergently acylated lipid A structures is adequately presented in the literature, the issues of isolation of protected synthetic intermediates of lipid A containing three or more long chain fatty acids received little attention. In the commonly used solvent mixtures (CH_2Cl_2–acetone or $CHCl_3$–MeOH) for chromatographic purification of such synthetic intermediates of lipid A[21,23,25,32] the potential by-products stemming from cleavage, migration, or reduction of acyl groups display similar chromatographic behaviour to those of the products, thus making the detection of the former on TLC and the isolation of the latter rather intricate. Consequently, minor co-migrating by-products accumulate throughout the multistep synthesis, leading to unseparable and hardly detectable contaminations in the final amphiphilic lipid A preparations. Therefore, development of an efficient purification protocol for highly hydrophobic tri-, tetra- and pentaacyl synthetic intermediates of lipid A has been strongly demanded. Herein a highly efficient, mild and simple isolation procedure for synthetic precursors of chlamydial lipid A is presented. The method comprises sequential precipitation of the desired reaction product with successively polar (EtOH or MeOH or acetone) and unpolar (*n*-hexane) solvents from CH_2Cl_2 or CH_2Cl_2/toluene, such that the lipid-derived by-products and excess of the reagents are withheld in the filtrates (Fig. 3). This purification scheme provides a high-yield alternative to affinity-separation which eliminates an excess of reagents and non-lipid impurities only.[40] To facilitate the detection of co-migrating by-products by TLC a four-component one-phase mixture composed of toluene/dichloromethane/methanol/water (150:100:15:1), where toluene/CH_2Cl_2 served as unpolar stationary phase and MeOH/H_2O as polar mobile phase, was elaborated. Apart from perfect resolution on TLC, an application of this solvent mixture for preparative adsorption–partition chromatography on silica gel allowed efficient isolation of protected tetra- (**39**, **40**) and pentaacyl (**41**, **42**) intermediates in chemically pure form.

2.5. Phosphorylation and deprotection

For the introduction of α-phosphate at the reducing end of the tetraacyl lipid A progenitor **40**, the phosphoramidite procedure was first examined. Phosphitylation of **40** with di-*O*-benzyloxy-(*N*,*N*-diisopropylamino)phosphine in the presence of 1*H*-tetrazole and in situ oxidation with *t*BuOOH afforded a 3:2 α/β mixture of phosphotriesters. Although α-selectivity of 1-OH phosphitylation using the phosphoramidite approach has been reported in the literature,[41,42] and was rationalized on the basis of the extreme instability of the anomeric β-phosphate,[42] exclusively α-linked phosphates could not be prepared using this approach. Applying the very efficient α-selective phosphorylation of reducing disaccharides **40** and **42** via 1-*O*-lithiation using lithium bis(trimethylsilyl)amide followed by phosphorylation with tetrabenzyl diphosphate at −78 °C[25] afforded α-configured anomeric bisphosphotriesters **43** and **44**, respectively. The propensity of these compounds to undergo fast acidic

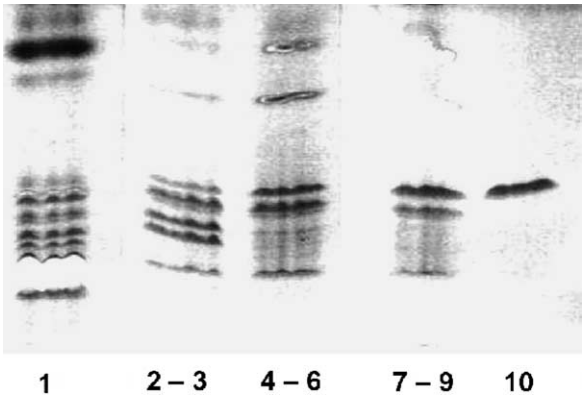


Figure 3. Thin layer chromatography of final precipitate and filtrates arising from purification of protected pentaacyl lipid A precursor **41** by sequential precipitation: TLC of filtrates from sequential precipitations from CH_2Cl_2 with EtOH (lanes 1–3), *n*-hexane (lanes 4–6), EtOH (lanes 7–9) and TLC of final precipitate (lane 10).

hydrolysis on silica gel with the loss of reducing phosphate stipulated the waste of more than 50% of synthesized diphosphates during chromatography. As reported in the literature, 1-*O*-phosphotriesters of fully protected lipid A are either not isolated and used as crude mixtures in the final deprotection step[25] or just roughly purified by flash chromatography.[20,21,23,34] Again, the precipitation method proved to be superior to chromatography in the purification step of reducing phosphotriesters **43** and **44**, which were isolated in 90% yield by sequential precipitation (compared to 30–40% yield by conventional chromatography for the same degree of purity). Thus, the purification conditions set forth herein show a broad scope, as they proved equally useful for both stable and labile acylated hydrophobic intermediates of lipid A. Hydrogenolytic cleavage of benzyl protecting groups in monophosphates **40** and **42** with Pd/C in THF or toluene/methanol, respectively, afforded a complex mixture of monophosphoryl lipid A (MLA) and their partially benzylated intermediates. Purification by chromatography on silica gel in 50:20:20:3:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/AcOH and subsequent ion-exchange chromatography on DEAE-cellulose (CH_3COO^--form) afforded monophosphoryl analogues **3** and **4** in 60 and 49% yield, respectively.

For the hydrogenolytic deprotection of the labile bisphosphotriesters **43** and **44**, the employment of Pd/C in 5:1 toluene/methanol was found to be the best option; in spite of concomitant formation of methyl glycosides, partial hydrolysis of reducing 1-phosphates and adsorption of the amphiphilic products on charcoal, the yields were considerably higher than those obtained by application of Pd-black in THF.[34] Tetraacyl lipid A **1** and pentaacyl lipid A **2** were finally purified by anion-exchange chromatography on DEAE-cellulose[43] (CH_3COO^--form) in 2:3:1 $CHCl_3$/MeOH/aq $CH_3COO^-HNEt_3^+$ using a stepwise gradient of triethylammonium acetate and by subsequent desalting with Bligh–Dyer solvent system.[44]

Tetra- and pentaacyl lipid A **1** and **2** and their monophosphoryl analogs **3** and **4** were characterized and their structures and purity were confirmed by NMR spectroscopy (Fig. 4), positive MALDI-TOF and ESI- mass spectrometry and immunostaining.

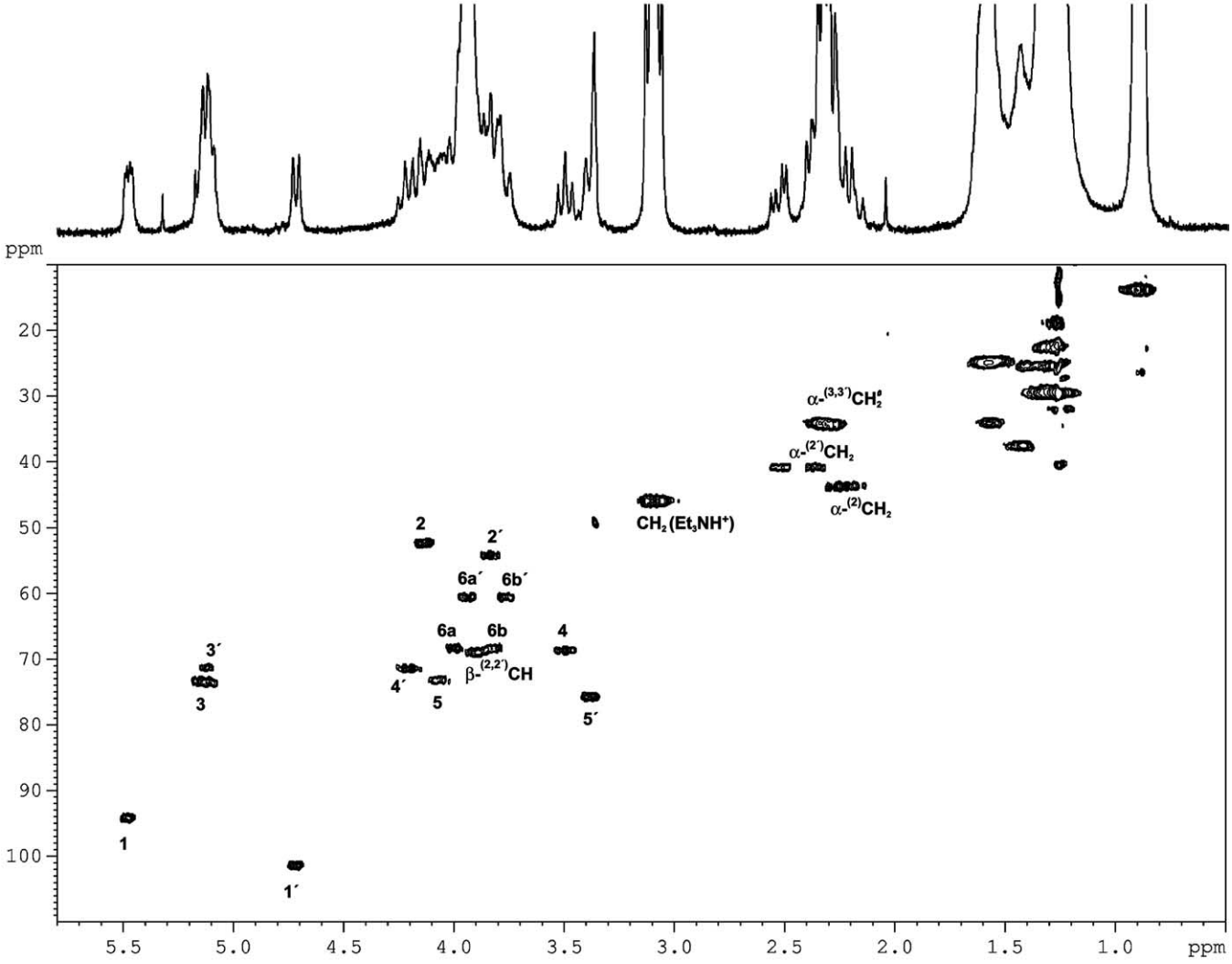


Figure 4. ^1H–^{13}C HMQC spectrum of synthetic pentaacyl lipid A **2**.

In the NMR spectra of compounds **3** and **4** the anomeric protons were seen at 5.10–5.13 ppm with a coupling constant $^{3}J_{1,2}=3.3$ Hz confirming α-anomeric configuration of the reducing end, whereas the coupling constant ($^{3}J_{1',2'}=8.5$ Hz) displayed by the anomeric protons of the distal glucosamine unit proved the presence of a β-glycosidic linkage. The sites of esterification of fatty acids at the 3 and 3′ positions were consistent with the observed proton downfield shifts to 5.10–5.18 ppm, whereas the downfield shifts of the proton at 4′-position (4.28 and 4.24 ppm, respectively), confirmed the presence of the phosphate group (Table 2). NMR experiments of the monotriethylammonium salts of lipid A derivatives **1** and **2** were attempted in different non-destructive solvent mixtures (2:1 $CDCl_3$/DMF-*d*7, 2:3:1 $CDCl_3/CD_3OD/D_2O$, 4:1 $CDCl_3$/2-PrOD-*d*7, 4:1 $CDCl_3/CD_3OD$) in the temperature range 27–37 °C and in concentrations of 5–8 mg/mL. Limited solubility and time-dependent aggregation led generally to broad unresolved lines. Only in 4:1 $CDCl_3/CD_3OD$[45] the ^{1}H NMR signals were sharp and sufficiently resolved and significant dephosphorylation at the anomeric position was not detected upon storage over several days at room temperature. The latter fact might be attributed to the actual presence of large amount of aggregated water in the samples (which is observed as broad intensive signal of HDO at approx. 3.9 ppm) in connection with the reported stability of purified intact lipid A in $CDCl_3/CD_3OD/D_2O$.[46] The well-resolved double–doublet resonances at 5.44 and 5.48 ppm were assigned to the anomeric protons, the doublets at 4.78 and 4.72 were assigned to the H-1′ signal of the distal sugar (for **1** and **2**, respectively). The respective small and large coupling constants ($^{3}J_{1,2}=3.2$–3.5 Hz, $^{3}J_{1',2'}=8.3$–8.5 Hz) proved α- and β-pyranosyl forms for the proximal and distal glucosamine rings, respectively. Two phosphorous resonances confirmed the bisphosphorylated structure. The downfield positions of H-1 (doublet of doublets) and H-4′(pseudo triplet) at 5.44–5.48 and 4.26–4.23 ppm, respectively, and phosphorus–proton coupling $^{3}J_{1,P}=7.0$–7.4 Hz supported the sites of phosphorylation (the coupling value $^{3}J_{4',P}=10.0$–10.5 Hz was very close to that of vicinal protons $^{3}J_{3',4'}=^{3}J_{4',5'}=9.0$ Hz). Two anomeric carbon resonances for **1** and **2**, respectively, were observed at 93–94 and 100.4–101.4 ppm and represented the phosphorylated anomeric position C-1 of the proximal sugar unit and C-1′ of the distal glucosamine residue, respectively. H-2 and H-2′ signals correlated with the upfield-shifted carbon resonances at 52.3–52.4 and 53.8–54.3 ppm (C-2 and C-2′, respectively) reflecting the presence of acylamido groups (Table 3). Downfield-shifted signals of H-3 and H-3′ displayed overlapped cross-peaks to downfield-shifted C-3 and C-3′ carbon resonances at 72.7–73.6 and at 73.4–71.4 ppm, respectively, thus confirming the presence of acyl groups at both positions. The broad CD_3OH and HOD signals at 3.95–3.80 ppm obscured the resonances from H-5, H 6a, H-6a′, β-2CH and β-2′CH; the chemical shifts of these protons could be determined from two-dimensional ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC-experiments (Fig. 4).

Purity and molecular masses of the tetra- and pentacyl lipid A derivatives **1** and **2** were confirmed by MALDI TOF-MS and high-resolution ESI FT-MS. Furthermore, compounds **1** and **2** were subjected to separation by TLC and subsequent immunostaining with a monoclonal antibody against lipid

Table 2. ^{1}H NMR data (ppm)[a] of tetraacyl lipid A (**1**), pentaacyl lipid A (**2**) and monophosphoryl analogues **3** and **4**

→	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	β-$^{(2)}$CH	α-$^{(2)}$CH$_2$	α-$^{(3)}$CH$_2$
6GlcN										
1[b]	5.44, $J_{1,P}=7.0$	4.12, $J_{1,2}=3.2$	5.13, $J_{2,3}=10.7$	3.44, $J_{3,4}=9.2$	4.08, $J_{4,5}=9.5$	4.03 n.d.[c]	3.80 n.d.[c]	3.88	2.33–2.18	2.38–2.30
2[b]	5.48, $J_{1,P}=7.4$	4.14, $J_{1,2}=3.5$	5.15, $J_{2,3}=10.4$	3.49, $J_{3,4}=9.5$	4.07, $J_{4,5}=9.5$	4.01 n.d.[c]	3.80 n.d.[c]	3.91	2.30–2.15	2.40–2.23
3[d]	5.10, $J_{1,2}=3.3$	4.12, $J_{2,3}=10.5$	5.17, $J_{3,4}=9.4$	3.46, $J_{4,5}=9.7$	4.03 n.d.[c]	4.17 n.d.[c]	3.65 n.d.[c]	3.90	2.35–2.15	2.40–2.20
4[e]	5.13 n.d.[c]	4.10 n.d.[c]	5.11 n.d.[c]	3.48, $J_{4,5}=9.5$	4.03 n.d.[c]	4.02 n.d.[c]	3.76 n.d.[c]	3.86	2.38–2.20	2.45–2.30
GlcN1→										
1[b]	4.78, $J_{1',2'}=8.3$	3.95, $J_{2',3'}=10.5$	5.09, $J_{3',4'}=9.0$	4.26, $J_{4',P}=10.5$	3.37, $J_{4',5'}=9.0$	3.97 n.d.[c]	3.74 n.d.[c]	3.98	2.18–2.33	2.38–2.28
2[b]	4.72, $J_{1',2'}=8.5$	3.83, $J_{2',3'}=10.5$	5.12, $J_{3',4'}=9.0$	4.23, $J_{4',P}=10.0$	3.38, $J_{4',5'}=9.0$	3.94, $J_{5',6'b}=2.5$	3.77, $J_{6'a,6'b}=12.7$	3.90	2.52/2.36	2.40–2.23
3[d]	4.62, $J_{1',2'}=8.7$	4.00, $J_{2',3'}=10.3$	5.12, $J_{3',4'}=9.0$	4.28, $J_{4',P}=10.5$	3.38, $J_{4',5'}=9.0$	3.97 n.d.[c]	3.70 n.d.[c]	3.92 n.d.[c]	n.d.[c]	n.d.[c]
4[e]	4.76, $J_{1',2'}=8.5$	3.88 n.d.[c]	5.18 n.d.[c]	4.24, $J_{4',P}=10.5$	3.38, $J_{4',5'}=9.0$	3.94 n.d.[c]	3.79 n.d.[c]	3.86	2.55/2.38	2.45–2.30

[a] Coupling constants (Hz) are first order values.
[b] Chemical shifts at 300 K in $CDCl_3/CD_3OD$ (4:1, v/v).
[c] Not determined.
[d] Chemical shifts at 300 K in $CDCl_3$/2-PrOD-*d*7 (4:1, v/v).
[e] Chemical shifts at 318 K in $CDCl_3$/2-PrOD-*d*7 (4:1, v/v).

Table 3. ^{13}C NMR data[a] (ppm) of tetraacyl lipid A (**1**), pentaacyl lipid A (**2**) and the monophosphoryl analogs **3** and **4**

GlcN	C-1	C-2	C-3	C-4	C-5	C-6	βCH	αCH$_2$
1[b]	93.0	52.3	72.7	68.5	73.1	67.3	68.9	43.9
2[b]	94.3	52.4	73.6	68.7	73.3	68.4	69.2	44.0
3[c]	91.4	52.4	73.6	68.7	70.5	68.9	68.7	43.4
4[d]	91.9	52.8	71.9	69.6	71.6	68.9	69.0	43.5
GlcN′								
1[b]	100.4	53.8	73.4	70.8	75.3	60.3	68.9	43.9
2[b]	101.4	54.3	71.4	71.5	75.8	60.7	69.2	41.1
3[c]	101.7	53.8	73.4	70.7	75.8	60.4	68.7	43.6
4[d]	102.0	54.6	74.2	72.0	76.7	61.3	69.0	41.9

[a] Recorded at 75.47 MHz.
[b] Chemical shifts at 300 K in $CDCl_3/CD_3OD$ (4:1, v/v).
[c] Chemical shifts at 300 K in $CDCl_3$/2-PrOD-*d*7 (4:1, v/v).
[d] Chemical shifts at 318 K in $CDCl_3$/2-PrOD-*d*7 (4:1, v/v).

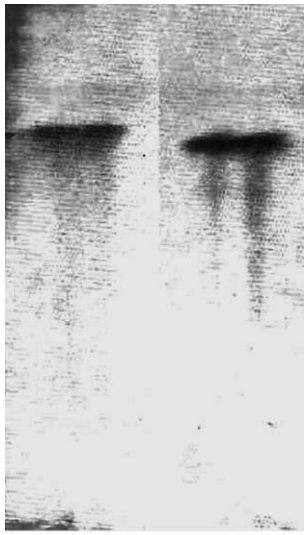


Figure 5. Immunostaining of compounds **1** and **2**. Compounds **1** and **2** were separated by TLC (500 ng) each, transferred to PVDF membrane and stained with a monoclonal antibody against the 1,4′-bisphosphorylated glucosamine disaccharide of lipid A.

A.[47] This method was shown to detect lipid A in amounts as low as 10–50 ng. As depicted in Figure 5, the pentaacyl lipid A derivative **2** (left lane) was free of any visible contaminants, whereas the tetraacyl lipid A derivative **1** showed a very minor trace contaminant which migrated slightly lower than the main compound (right lane).

In summary, the synthesis of pure tetra- and pentaacyl lipid A species from Chlamydiae has been achieved starting from the readily available precursor **12** in 11 steps in excellent overall yields. Studies of the endotoxic activities of synthetic chlamydial lipid A and as acceptors for chlamydial Kdo-transferase are currently in progress and will be reported elsewhere.

3. Experimental

3.1. General

Column chromatography was performed on silica gel 60 (230–400 mesh, Merck), HPLC was performed on silica gel 60 (10 μm). Anion-exchange chromatography was performed on BioRad DEAE-Cellulose (Pharmacia). Reactions were monitored by TLC on (A): Silica gel 60 F_{254} precoated glass plates (Merck) or on (B): Silica gel 60 F_{254} HPTLC precoated glass plates with 2.5 cm concentration zone (Merck), unless stated otherwise; spots were visualized by spraying with anisaldehyde–H_2SO_4; phosphorus-containing compounds were additionally detected with a molybdate solution [0.02 M solution of ammonium cerium(IV)sulfate dihydrate and ammonium molybdate(VI)tetrahydrate in aq H_2SO_4]. Concentration of solutions was performed at reduced pressure at temperatures <25 °C. Diisopropylethylamine, dry tetrahydrofuran, dry $CHCl_3$ and dry MeOH were purchased from Merck. Triethylamine and dichloromethane were dried by refluxing with CaH_2 (5 g/L) for 16 h, then distilled and stored under argon. Toluene was distilled from phosphorus pentaoxide and redistilled from CaH_2. The liquids were stored over molecular sieves 0.4 nm. DMF was stirred with CaH_2 (5 g/L) for 16 h at 20 °C, distilled under reduced pressure and stored over activated molecular sieves 0.3 nm. Triethylammonium acetate (TEAA) (1 M) buffer was purchased from Fluka. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. $[\alpha]_D^{20}$-Values are given in units of 10^{-1} deg cm^3 g^{-1}. NMR-spectra were recorded at 297 K in $CDCl_3$ (unless stated otherwise) with a Bruker DPX 300 spectrometer (^1H at 300.13 MHz, ^{13}C at 75.47 MHz and ^{31}P at 121.50 MHz) using standard Bruker NMR software. ^1H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to chloroform (δ 77.00). ^{31}P NMR spectra were referenced externally to 85% aq H_3PO_4 (δ 0.0). Elemental analyses were provided by Dr. J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien. MALDI-TOF-MS spectra were recorded on a Dynamo (Thermo BioAnalysis) instrument in the positive ion mode with 2% 2,5-dihydroxybenzoic acid as matrix. Laser-desorption-MS spectra were recorded on a laser microprobe mass analyzer (LAMMA 500, Leybold AG). ESI-MS spectra were recorded on a 7-Tesla Apex II, Bruker Daltonics instrument. Melting points were determined with a Kofler hot stage microscope and are uncorrected. Thin layer chromatography and immunostaining: compounds (500 ng) were separated by TLC on precoated silica 60 aluminum plates (Merck) using 30:70:16:10 $CHCl_3$/pyridine/88% aq HCOOH/H_2O. Dried plates were transferred to polyvinylidene difluoride (PVDF)

membranes as described[13] and stained with monoclonal antibody A6 recognizing the bisphosphorylated glucosamine disaccharide of lipid A.[47]

3.1.1. Methyl 3-oxoicosanoate (6). Octadecanoyl chloride **5** (106.0 g, 0.35 mol) was added dropwise during 20 min at 0 °C to a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (50.4 g, 0.35 mol) and pyridine (55.4 g, 0.70 mol) in CH_2Cl_2 (150 mL). The mixture was allowed to warm to rt over a period of 20 min and stirred for 3 h, then concentrated. The residue was repeatedly evaporated with toluene (2×300 mL), dissolved in $CHCl_3$ (500 mL) and extracted in turn with 1 M aq HCl (2×500 mL) and brine (500 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was dissolved in methanol (500 mL) and the solution was stirred under reflux for 6.5 h. During this period methanol was added twice (after 3.5 and 5 h, 100 mL each). The solution was cooled to 0 °C and kept at 4 °C for 12 h. The precipitated solid was collected by filtration and crystallized from ethanol (400 mL, 4 °C) to afford **6** as yellowish crystals (55.0 g, 46%), mp=53–55 °C. ^{1}H NMR: δ 3.71 (s, 3H, CO_2CH_3), 3.42 (s, 2H, $COCH_2CO$), 2.50 (t, 2H, ^3J=7.4 Hz, CH_2CH_2-CO), 1.56 (t, 2H, CH_2CH_2CO), 1.35–1.20 (m, 28H, $14CH_2$), 0.85 (t, 3H, ^3J=6.9 Hz, CH_3). Anal. Calcd for $C_{21}H_{40}O_3$: C, 74.07; H, 11.84. Found: C, 73.85; H, 11.55.

3.1.2. Methyl (*R*)-3-hydroxyicosanoate (7). A suspension of **6** (30.0 g, 88.0 mmol) and (*R*)-(+)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl-$RuCl_2$ {prepared from (*R*)-Binap (100 mg, 0.16 mmol) and [$RuCl_2$(cyclooctadiene)] (41 mg)}[19] in MeOH (40 mL) was stirred under 85 kg cm^{-2} H_2 at 60 °C for 44 h. The mixture was cooled to 0 °C, H_2 was evacuated and the solids were collected by filtration. The residue was crystallized from hexane (100 mL), light-yellow crystals were collected on the filter and washed with cold (0 °C) MeOH. Yield 23.3 g (77%, ee>99%), mp=62–63 °C. $[\alpha]_D^{20}=-10.4$ (*c* 1.0, $CHCl_3$). LAMMA *m/z*: 365.0 $[M+Na]^+$. ^{1}H NMR: δ 3.99 (m, 1H, βCH), 3.70 (s, 3H, CO_2CH_3), 2.78 (br s, 1H, OH), 2.49 (dd, 1H, $^2J_{\alpha CHH}$= 16.5 Hz, $^3J_{\alpha CH\mathit{H},\beta CH}$=3.1 Hz, αC*H*H), 2.40 (dd, 1H, $^3J_{\alpha CH\mathit{H},\beta CH}$=9.0 Hz, αCH*H*), 1.53–1.40 (m, 2H, CH_2), 1.35–1.20 (m, 30H, $15CH_2$), 0.86 (t, 3H, ^3J=6.9 Hz, CH_3). Anal. Calcd for $C_{21}H_{42}O_3$: C, 73.63; H, 12.36. Found: C, 73.60; H, 12.33.

3.1.3. (*R*)-3-(Benzyloxy)icosanoic acid (8). To a solution of **7** (3.0 g, 8.8 mmol) and benzaldehyde (2.67 mL, 26.3 mmol) in THF (30 mL) were added hexamethyldisiloxane (11.2 mL, 52.6 mmol) and trimethylsilyl trifluoromethanesulfonate (1.3 mL, 7 mmol) at 0 °C. After the mixture was stirred for 15 min at 0 °C, triethylsilane (4.9 mL, 30.7 mmol) was added and the mixture was stirred at 0 °C for 3 h, then allowed to warm to rt. The reaction was quenched by addition of satd aq $NaHCO_3$ (30 mL), diluted with EtOAc (400 mL) and extracted in turn with satd aq $NaHCO_3$ (200 mL) and brine (200 mL). The organic phase was dried (Na_2SO_4) and concentrated. Chromatography on silica gel (toluene→100:3 toluene/EtOAc) afforded methyl (*R*)-3-(benzyloxy)icosanoate (3.4 g, 90%), R_f 0.6 (9:1 toluene/EtOAc). A solution of methyl (*R*)-3-(benzyloxy)-icosanoate (0.8 g, 1.86 mmol) in THF–H_2O (5:1, 30 mL) was vigorously stirred with an aqueous solution of $LiOH\cdot H_2O$ (0.44 g, 10.9 mmol, 10 mL) under reflux for 3 h. The mixture was cooled to rt and the pH was adjusted to 7.0 by slow addition of 1.5 M aq HCl. The mixture was diluted with CH_2Cl_2 (300 mL), washed with aq $NaHCO_3$ (100 mL), dried (Na_2SO_4) and concentrated. Purification of the residue on silica gel ($CHCl_3$→10:1 $CHCl_3$/MeOH) afforded **8** as white solid (0.7 g, 90%), mp=42–44 °C; $[\alpha]_D^{20}=-2.4$ (*c* 0.25, $CHCl_3$); ^{1}H NMR: δ 7.35–7.25 (m, 5H, $CH_2\mathit{Ph}$), 4.56 (s, 2H, $C\mathit{H}_2$Ph), 3.87 (m, 1H, βCH), 2.62 (dd, 1H, $^2J_{\alpha CHH}$=15.5 Hz, $^3J_{\alpha CH\mathit{H},\beta CH}$=6.8 Hz, αC*H*H), 2.57 (dd, 1H, $^3J_{\alpha CH\mathit{H},\beta CH}$=5.0 Hz, αCH*H*), 1.69–1.53 (m, 2H, CH_2), 1.39–1.25 (m, 30H, $15CH_2$), 0.87 (t, 3H, ^3J= 6.8 Hz, CH_3); LAMMA *m/z* 417.6 $[M-H]^-$. Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07. Found: C, 77.23; H, 11.17.

3.1.4. Sodium (*R*)-3-hydroxyicosanoate (9a). To a solution of **7** (1 g, 2.3 mmol) in THF (20 mL) a solution of NaOH (0.6 g, 15 mmol) in H_2O (3 mL) was added and the mixture was vigorously stirred at reflux for 4 h, then cooled to 25 °C. The pH was adjusted to 7.0 by addition of 1.5 M aq HCl. The mixture was taken up in EtOAc (200 mL) and washed with aq $NaHCO_3$ (200 mL), which afforded three phases. The middle phase was collected and its volume was reduced to 10 mL. *n*-Hexane (100 mL) was added, the suspension was heated to 60 °C until the solids dissolved, then cooled to 0 °C, and the precipitate was collected on a filter. Yield 916 mg (95%), mp=176–180 °C; LAMMA *m/z* 327.5 $[M-H]^-$; ^{1}H NMR (DMSO, 65 °C): δ 3.80 (m, 1H, βCH), 2.24–2.20 (m, 2H, αCH_2), 1.34–1.24 (m, 32H, $16CH_2$), 0.86 (t, 3H, ^3J=6.9 Hz, CH_3). Anal. Calcd for $C_{20}H_{39}NaO_3\times H_2O$: C, 66.82; H, 11.21. Found: C, 66.60; H, 11.31.

3.1.5. (*R*)-3-Hydroxyicosanoic acid (9b). To a stirred suspension of **9a** (1 g, 2.27 mmol) in MeOH (300 mL) Dowex® AG 50 W-X8 resin (H^+-form) was gradually added until pH 5 was reached and a clear solution was obtained. The resin was filtered off and the filtrate was concentrated to give 0.93 g, (98%) of **9b** as a white solid. Mp=88–90 °C; $[\alpha]_D^{23}=-11.5$ (*c* 0.3, $CHCl_3$); ^{1}H NMR: δ 4.02 (m, 1H, βCH), 2.59 (dd, 1H, $^2J_{\alpha CHH}$=16.6 Hz, $^3J_{\alpha CH\mathit{H},\beta CH}$=3.3 Hz, αC*H*H), 2.47 (dd, 1H, $^3J_{\alpha CH\mathit{H},\beta CH}$= 8.8 Hz, αCH*H*), 1.60–1.44 (m, 2H, CH_2), 1.40–1.20 (m, 30H, $15CH_2$), 0.88 (t, 3H, ^3J=6.5 Hz, CH_3). Anal. Calcd for $C_{20}H_{40}O_3\times H_2O$: C, 71.17; H, 12.24. Found: C, 71.84; H, 12.60.

3.1.6. Phenacyl (*R*)-3-(octadecanoyloxy)icosanoate (10). To a stirred suspension of **9a** (886 mg, 2.12 mmol) and phenacyl bromide (517 mg, 2.6 mmol) in EtOAc (100 mL) triethylamine (0.6 mL, 4.3 mmol) was added at 0 °C and the mixture was stirred at 45 °C for 20 h. The precipitate was removed by filtration and washed with EtOAc (200 mL). The combined filtrates were extracted with aq $NaHCO_3$ (30 mL), brine (80 mL), dried (Na_2SO_4) and concentrated. Purification on silica gel (100:1→100:25 CH_2Cl_2/MeOH) afforded 1.1 g (1.97 mmol, 93%) of phenacyl (*R*)-3-hydroxyicosanoate as white solid. R_f 0.35 (9:1 toluene/EtOAc).

To a solution of phenacyl (*R*)-3-hydroxyicosanoate (400 mg, 0.75 mmol) in CH_2Cl_2 (10 mL) a solution of octadecanoyl chloride (300 mg, 0.99 mmol) in CH_2Cl_2

(10 mL) and DMAP (234 mg, 1.9 mmol) were added and the mixture was stirred for 3 h at rt. The reaction mixture was diluted with EtOAc (300 mL), washed with aq $NaHCO_3$ (50 mL), brine (50 mL), dried (Na_2SO_4) and concentrated. Purification by chromatography on silica gel (EtOAc→100:5 toluene/EtOAc) gave **10** as a white solid (475 mg, 88%). $[\alpha]_D^{20}=\pm 0.0$ (*c* 0.55, $CHCl_3$); ^1H NMR: δ 7.84–7.81 (m, 2H, *ortho*-Ph), 7.56–7.51 (m, 1H, *para*-Ph), 7.43–7.38 (m, 2H, *meta*-Ph), 5.26 (s, 2H, CH_2COPh), 5.23 (m, 1H, βCH), 2.74–2.61 (m, 2H, αCH_2), 2.38 (t, 2H, $^3J=7.5$ Hz, $\alpha CH_{2'}$), 2.24 (t, 2H, $^3J=7.8$ Hz, $\beta CH_{2'}$) 1.63–1.51 (m, 6H, $3CH_2$), 1.27–1.20 (m, 54H, $27CH_2$), 0.81 (t, 6H, $^3J=6.9$ Hz, $2CH_3$); MALDI-TOF-MS: *m/z*: 735.9 $[M+Na]^+$. Anal. Calcd for $C_{46}H_{80}O_5$: C, 77.48; H, 11.31. Found: C, 77.22; H, 11.44.

3.1.7. (*R*)-3-(Octadecanoyloxy)icosanoic acid (11). A solution of **10** (200 mg, 0.27 mmol) in 1:2 AcOH–toluene (20 mL) was stirred with Zn–Cu couple [1.3 g, made from Zn (1.0 g) and 5% aq $CuSO_4$ (7.0 mL)] at rt for 2 h. The solids were removed by filtration through a pad of Celite, the filtrate was concentrated, redissolved in toluene and concentrated (3×60 mL). The residue was redissolved in EtOAc (200 mL) and extracted with satd aq $NaHCO_3$ (50 mL), brine (2×70 mL) and acidified water (2×50 mL, pH adjusted to 4.5 with HCl). The organic phase was dried (Na_2SO_4), concentrated and purified on silica gel ($CHCl_3$→100:5 $CHCl_3$/MeOH) to afford **11** as a white solid (142 mg, 85%), mp=49–50 °C; $[\alpha]_D^{23}=-1.3$ (*c* 0.3, $CHCl_3$); ^1H NMR: δ 5.21 (m, 1H, βCH), 2.62 (dd, 1H, $^2J_{\alpha CHH}=15.4$ Hz, $^3J_{\alpha CH\mathit{H},\beta CH}=7.3$ Hz, αC*H*H), 2.57 (dd, 1H, $^3J_{\alpha CH\mathit{H},\beta CH}=5.1$ Hz, αCH*H*), 2.26 (t, 2H, $^3J=7.5$ Hz, $\alpha CH_2'$), 1.61–1.58 (m, 6H, $3CH_2$), 1.29–1.15 (m, 56H, $28CH_2$), 0.88 (t, 6H, $^3J=6.9$ Hz, $2CH_3$); MALDI-TOF-MS: *m/z*: 617.7 $[M+Na]^+$. Anal. Calcd for $C_{38}H_{74}O_4\times H_2O$: C, 75.56; H, 12.51. Found: C, 75.80; H, 12.6.

3.1.8. Allyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (13). To a stirred solution of allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside **12**[23] (3 g, 6.21 mmol) in dry pyridine (10 mL) a solution of myristoyl chloride (2.22 g, 2.45 mL, 9 mmol) in dry THF (5 mL) was added during 15 min under N_2. The mixture was stirred for 1 h, diluted with EtOAc (200 mL), washed with satd aq $NaHCO_3$ (50 mL), brine (50 mL), dried (Na_2SO_4) and concentrated. Purification of the residue on silica gel (toluene→50:1 toluene/EtOAc) afforded **13** as a white solid (4.2 g, 95%); mp=85–88 °C; $[\alpha]_D^{20}=+36.3$ (*c* 0.3, $CHCl_3$); ^1H NMR: δ 7.45–7.43 (m, 2H, CH*Ph*), 7.36–7.34 (m, 3H, CH*Ph*), 5.90 (m, 1H, CH=), 5.52 (s, 1H, C*H*Ph), 5.40 (t, 1H, $^3J_{3,2}=^3J_{3,4}=10.0$ Hz, H-3), 5.36 (d, 1H, $^3J_{NH,2}=10.0$ Hz, NH), 5.31 (dq, 1H, $=CH_{2trans}$), 5.24 (dq, 1H, $=CH_{2cis}$), 4.93 (d, 1H, $^3J_{1,2}=3.5$ Hz, H-1), 4.71 (s, 2H, CH_2CCl_3), 4.29 (dd, 1H, $^2J_{6a,6b}=10.3$ Hz, $^3J_{5,6a}=4.9$ Hz, H-6a), 4.22 (dd, 1H, OC*H*H, All), 4.07–4.01 (m, 2H, H-2, OCH*H*, All), 3.94 (dd, 1H, H-5), 3.78 (t, 1H, $^3J_{5,6b}=10.3$ Hz, H-6b), 3.71 (t, 1H, $J_{4,5}=10.0$ Hz, H-4), 2.34–2.24 (m, 2H, αCH_2), 1.59–1.55 (m, 2H, CH_2), 1.30–1.17 (m, 20H, $10CH_2$), 0.87 (t, 3H, CH_3); MALDI-TOF-MS: *m/z*: 714.0, 716.0 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{33}H_{48}Cl_3NO_8$: C, 57.19; H, 6.98; N, 2.02. Found: C, 57.18; H, 7.46; N, 2.28.

3.1.9. Allyl 6-*O*-benzyl-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (14). *Method A*. Boron trifluoride etherate $BF_3\cdot OEt_2$ (0.33 mL, 2.80 mmol) was added to a stirred solution of **13** (400 mg, 0.56 mmol) and borane–dimethylamine complex $BH_3\cdot Me_2NH$ (166 mg, 2.82 mmol) in acetonitrile (10 mL) at 0 °C under N_2. The mixture was stirred for 30 min at 0 °C and for 1 h at rt. The solution was cooled to 4 °C and neutralized with satd aq $NaHCO_3$ (60 mL). The mixture was diluted with EtOAc (200 mL) and washed with satd aq $NaHCO_3$ (80 mL), 1 M aq HCl (2×80 mL), H_2O (100 mL), $NaHCO_3$ (30 mL). The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified on silica gel (toluene→100:15 toluene/EtOAc) which afforded **14** as a syrup (247 mg, 62%). $[\alpha]_D^{20}=+49.3$ (*c* 0.15, $CHCl_3$); ^1H NMR: δ 7.34–7.29 (m, 5H, CH_2Ph), 5.89 (m, 1H, CH=), 5.29 (dq, 1H, $=CH_{2trans}$), 5.40 (d, 1H, $^3J_{NH,2}=9.7$ Hz, NH), 5.22 (dq, 1H, $=CH_{2cis}$), 5.14 (dd, 1H, $^3J_{3,2}=10.3$ Hz, $^3J_{3,4}=8.7$ Hz, H-3), 4.91 (d, 1H, $^3J_{1,2}=3.4$ Hz, H-1), 4.73 and 4.67 (AB, 2H, $^2J=12.1$ Hz, CH_2CCl_3), 4.65 and 4.58 (AB, 2H, $^2J=12.1$ Hz, CH_2Ph), 4.22 (m, 1H, OC*H*H, All), 4.03 (m, 1H, OCH*H*, All), 4.0 (ddd, 1H, H-2), 3.88–3.77 (m, 3H, H-4, H-5, H-6a), 3.74 (dd, 1H, $^2J_{6a,6b}=10.3$ Hz, $^3J_{6b,5}=3.9$ Hz, H-6b), 2.68 (d, 1H, OH), 2.35 (m, 2H, αCH_2), 1.59–1.55 (m, 2H, CH_2), 1.30–1.17 (m, 20H, $10CH_2$), 0.88 (t, 3H, CH_3); MALDI-TOF-MS: *m/z*: 716.5, 718.7 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{33}H_{50}Cl_3NO_8$: C, 57.02; H, 7.25; N, 2.02. Found: C, 56.75; H, 7.14; N, 1.94.

Method B. A solution of **13** (1.3 g, 1.83 mmol) in CH_2Cl_2 (20 mL) and powdered activated molecular sieves 0.4 nm were stirred for 3 h under N_2 at ambient temperature. The suspension was cooled to −78 °C and Et_3SiH (320 mg, 440 μL, 2.75 mmol) and a solution of TfOH (275 μL, 3.11 mmol) in CH_2Cl_2 (2 mL) were added successively at −78 °C under N_2. The mixture was stirred for 40 min at −78 °C. Et_3N (2 mL) and MeOH (2 mL) were added successively. The mixture was stirred for 15 min, then warmed up to rt, diluted with EtOAc (30 mL) and filtered over a pad of Celite. The filtrate was diluted with EtOAc (300 mL) and washed successively with satd aq $NaHCO_3$ (50 mL), H_2O (100 mL), and brine (100 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by flash chromatography on silica gel (5:1→4:1 toluene/EtOAc) which afforded **14** as a syrup (1.2 g, 92%).

3.1.10. Allyl 6-*O*-benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (15) and allyl 6-*O*-benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (16). *Variant A*. To a solution of **14** (prepared according to method B) (330 mg, 0.47 mmol) and di-*O*-benzyloxy-(*N*,*N*-diisopropylamino) phosphine (228 mg, 222 μL, 0.66 mmol) in CH_2Cl_2 (10 mL) a solution of 1*H*-tetrazole (46 mg, 0.66 mmol) in CH_3CN (3 mL) was added. The mixture was stirred for 30 min under N_2, then cooled to 0 °C and a solution of *tert*-butylhydroperoxide (300 mL) in CH_3CN (3 mL) was slowly added. The stirring was continued for 6 h at 0 °C, the mixture was diluted with EtOAc (200 mL) and washed with satd aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic

phase was dried (Na_2SO_4), concentrated and the residue was purified by chromatography on silica gel (100:1→100:15 toluene/EtOAc) which afforded 350 mg (0.37 mmol, 78%) of **16**, R_f 0.50 (A, 3:1 toluene/EtOAc). $[\alpha]_D^{20} = +39.5$ (*c* 1, $CHCl_3$); ^{1}H NMR: δ 7.40–7.25 (m, 15H, Ph), 5.91 (m, 1H, CH=), 5.39 (dd, 1H, $^3J_{3,2}=10.7$ Hz, $^3J_{3,4}=9.3$ Hz, H-3), 5.34 (dq, 1H, $=CH_{2trans}$), 5.28 (d, 1H, $^3J_{2,NH}=10.0$ Hz, NH), 5.27 (dq, 1H, $=CH_{2cis}$), 5.0–4.87 (m, 4H, C*H*$_2$Ph), 4.92 (d, 1H, $^3J_{1,2}=5.3$ Hz, H-1), 4.70 (br s, 2H, C*H*$_2$Ph), 4.60 (t, 1H, $^3J_{4,5}=9.3$ Hz, H-4), 4.57 and 4.48 (2AB, 2H, $^2J=12.0$ Hz, CH_2CCl_3), 4.23 (m, 1H, OC*H*H, All), 4.07–3.92 (m, 3H, H-2, H-5, OCH*H*, All), 3.77 (dd, 1H, $^2J_{6a,6b}=11.0$ Hz, $^3J_{6a,5}=2.1$ Hz, H-6a), 3.71 (dd, 1H, $^3J_{6b,5}=4.6$ Hz, H-6b), 2.17 (t, 2H, $^3J=7.4$ Hz, αCH_2), 1.35–1.15 (m, 2H, βCH_2), 1.35–1.25 (m, 20H, $10CH_2$), 0.90 (t, 3H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.45 (1C, CO), 154.52 (1C, CONH), 138.45, 136.37 (2C, C_6H_5), 133.51 (=CH), 128.98, 128.86, 128.72, 128.53, 128.35, 128.0 (16C, Ph), 118.83 ($=CH_2$), 96.53 (C-1), 95.75 (CCl_3), 75.06 (*C*H$_2$Ph), 74.10 (C-4, $^2J_{4,P}=6.0$ Hz), 73.83 (CH_2, Troc), 71.43 (C-3, $^3J_{3,P}=2.3$ Hz), 70.36 (C-5, $^3J_{5,P}=6.0$ Hz), 69.98 and 69.91 (2C, *C*H$_2$Ph, $^2J_{C,P}=5.3$ Hz), 69.49 (OCH_2, All), 68.60 (C-6), 54.54 (C-2), 34.69 (αCH_2), 32.56, 30.10, 30.06, 30.04, 29.86, 29.76, 29.71, 29.49 ($10CH_2$), 24.98 (β CH_2), 14.55 (CH_3); ^{31}P NMR ($CDCl_3$): δ −0.9; MALDI-TOF-MS: *m*/*z*: 975.83, 977.92 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{47}H_{63}Cl_3NO_{11}P$: C, 59.09; H, 6.65; N, 1.47. Found: C, 59.25; H, 6.70; N, 1.42.

Variant B. The reaction was carried out from **14** (prepared according to method A) (330 mg, 0.47 mmol) in the same manner as described above. Purification, first by column chromatography on silica gel (100:1→100:15 toluene/EtOAc), then in three portions by HPLC (linear gradient toluene→100:15 toluene/EtOAc) afforded **16** as the main product (195 mg, 0.21 mmol, 44%) and **15** (28 mg, 0.03 mmol, 6%) as a by-product; R_f 0.46 (A, 3:1 toluene/EtOAc); ^{1}H NMR: δ 7.25–7.35 (m, 15H, Ph), 5.91 (m, 1H, CH=), 5.30 (dq, 1H, $=CH_{2trans}$), 5.24 (dq, 1H, $=CH_{2cis}$), 5.20 (d, 1H, $^3J_{2,NH}=9.7$ Hz, NH), 5.0 (m, 4H, C*H*$_2$Ph), 4.90 (d, 1H, $^3J_{1,2}=3.5$ Hz, H-1), 4.80 and 4.71 (2AB, 2H, $^2J=12.0$ Hz, C*H*$_2$CCl$_3$), 4.57 and 4.45 (2AB, 2H, $^2J=12.0$ Hz, C*H*$_2$Ph), 4.48 (t, 1H, $^3J_{4,5}=9.5$ Hz, H-4), 4.21 (m, 1H, OC*H*H, All), 4.05–3.97 (m, 2H, H-2, OCH*H*, All), 3.88 (m, 1H, H-5), 3.80–3.69 (m, 3H, H-6a, H-6b, αCH*H*), 3.63 (dd, 1H, $^3J_{3,2}=10.5$ Hz, $^3J_{3,4}=9.2$ Hz, H-3), 3.54 (m, 1H, αCH*H*), 1.48 (m, 2H, βCH_2), 1.30–1.18 (m, 20H, $10CH_2$), 0.90 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 154.43 (CONH), 138.45, 136.37 (2C, Ph), 138.60 (1C, Ph), 136.30, 136.21 (2C, Ph, $^3J_{C,P}=4.0$ Hz), 133.72 (=CH), 128.94–127.86 (15C, C_6H_5, Bn), 118.62 ($=CH_2$), 96.90 (C-1), 95.82 (CCl_3), 79.39 (C-3), 75.96 (C-4, $^2J_{4,P}=6.8$ Hz), 75.17 (CH_2, Troc), 73.71 (*C*H$_2$Ph), 72.78 (αCH_2), 70.68 (C-5, $^3J_{5,P}=4.5$ Hz), 69.87 and 69.79 (2C, *C*H$_2$Ph, $^2J_{C,P}=2.0$ Hz), 68.96 and 68.86 (2C, OCH_2, All, C-6), 54.73 (C-2), 32.32 (βCH_2), 30.59, 30.09, 30.02, 29.76, 26.36, 23.09 ($10CH_2$), 14.51 (CH_3); ^{31}P NMR ($CDCl_3$): δ 0.1; MALDI-TOF-MS: *m*/*z*: 962.34, 964.20 (24.47% ^{37}Cl) $[M+Na]^+$.

3.1.11. Allyl 6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-tetradecyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (17) and allyl 6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (18). A solution of **14** (prepared according to method A) (250 mg, 0.36 mmol), *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (260 mg, 1.08 mmol) and 1*H*-tetrazole (126 mg, 1.8 mmol) in CH_2Cl_2 (10 mL) was stirred for 20 min under N_2. The mixture was brought to −20 °C and the solution was stirred with 3-chloroperoxobenzoic acid (70%, 222 mg, 0.9 mmol) for 10 min. The reaction was quenched with satd aq $NaHCO_3$ (30 mL) at 0 °C. The mixture was diluted with $CHCl_3$ (100 mL) and washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on silica gel (3:1 hexane/Et_2O→Et_2O, then 4:1→3:2 toluene/EtOAc) and, in three portions, by HPLC (3:2 toluene/EtOAc) to afford **18** as a main product (148 mg, 0.17 mmol, 47%); R_f 0.53 (B, 2:1 hexane/EtOAc), R_f 0.62 (B, 2:1 toluene/EtOAc); $[\alpha]_D^{20} = +30.5$ (*c* 0.2, $CHCl_3$); ^{1}H NMR: δ 7.37–7.17 (m, 9H, Ph), 5.90 (m, 1H, CH=), 5.40 (dd, 1H, $^3J_{3,2}=10.3$ Hz, $^3J_{3,4}=9.5$ Hz, H-3), 5.32 (dq, 1H, $=CH_{2trans}$), 5.30–5.20 (m, 2H, NH, $=CH_{2cis}$), 5.17–5.02 [m, 4H, $C_6H_4(CH_2O)_2P$], 4.96 (d, 1H, $^3J_{1,2}=3.7$ Hz, H-1), 4.77 (t, 1H, $^3J_{4,5}=9.5$ Hz, H-4), 4.73 and 4.69 (2AB, 2H, $^2J=12.0$ Hz, CH_2CCl_3), 4.67 and 4.59 (2AB, 2H, $^2J=12.1$ Hz, C*H*$_2$Ph), 4.24 (m, 1H, OC*H*H, All), 4.10–3.97 (m, 3H, H-2, H-5, OCH*H*, All), 3.82 (dd, 1H, $^2J_{6a,6b}=11.1$ Hz, $^3J_{6a,5}=2.3$ Hz, H-6a), 3.75 (dd, 1H, $^3J_{5,6b}=4.9$ Hz, H-6b), 2.38 (t, 2H, $^3J=7.4$ Hz, αCH_2), 1.65–1.55 (m, 2H, βCH_2), 1.35–1.25 (m, 20H, $10CH_2$), 0.90 (t, 3H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.49 (1C, CO), 154.47 (1C, CONH), 138.33 (=CH), 135.12, 135.09 (2C, Ph), 129.37, 128.94, 128.84, 128.75, 128.25, 128.01 (10C, Ph), 118.92 ($=CH_2$), 96.58 (C-1), 95.73 (CCl_3), 75.06 (CH_2, Troc), 74.76 (C-4, $^2J_{4,P}=5.9$ Hz), 74.05 (*C*H$_2$Ph), 71.43 (C-3), 70.25 (C-5, $^3J_{5,P}=6.0$ Hz), 69.15 (OCH_2, All), 68.97 (C-6, $J_{6,P}=6.0$ Hz), 68.70 [2C, (*C*H$_2$O)$_2$P], 54.45 (C-2), 34.55 (αCH_2), 32.31, 30.08, 30.07, 30.04, 29.90, 29.74, 29.57, 23.08 ($10CH_2$), 25.07 (βCH_2), 14.51 (CH_3); ^{31}P NMR ($CDCl_3$): δ 0.0; MALDI-TOF-MS: *m*/*z*: 898.6, 900.4 (24.47% ^{37}Cl) $[M+Na]^+$.

Further elution gave **17** (25 mg, 0.03 mmol, 8%) as a minor product; R_f 0.50 (B, 2:1 hexane/EtOAc), 0.55 (B, 2:1 toluene/EtOAc). ^{1}H NMR: δ 7.30–7.10 [m, 9H, CH_2*Ph*, $C_6H_4(CH_2O)_2P$], 5.83 (m, 1H, CH=), 5.27–5.10 [m, 3H, $=CH_{2trans}$, $=CH_{2cis}$, $C_6H_4(CHHO)_2P$], 5.07 (d, 1H, $^3J_{2,NH}=6.8$ Hz, NH), 4.95–5.20 [m, 1H, $C_6H_4(CHHO)_2P$], 4.83 (d, 1H, $^3J_{1,2}=3.7$ Hz, H-1), 4.72 and 4.60 (2AB, 2H, $^2J=12.0$ Hz, CH_2CCl_3), 4.52 (t, 1H, $^3J_{4,5}=9.8$ Hz, H-4), 4.53 and 4.48 (2AB, 2H, $^2J=12.0$ Hz, C*H*$_2$Ph), 4.13 (m, 1H, OC*H*H, All), 3.97–3.82 (m, 3H, H-2, H-5, OCH*H*, All), 3.75 (dd, 1H, $^2J_{6a,6b}=11.0$ Hz, $^3J_{6a,5}=2.0$ Hz, H-6a), 3.70 (dd, 1H, $^3J_{6b,5}=5.1$ Hz, H-6b), 3.63 (ddd, 1H, αC*H*H), 3.58 (t, 1H, $^3J_{3,2}=^3J_{3,4}=9.8$ Hz, H-3), 3.53 (ddd, 1H, αCH*H*), 1.47 (d, 2H, βCH_2), 1.20–1.15 (m, 20H, $10CH_2$), 0.81 (t, 3H, CH_3); ^{13}C NMR($CDCl_3$): δ 154.37 (CONH), 138.57 (=CH), 135.53, 133.66 (2C, Ph), 129.34, 129.02, 128.68, 128.11, 127.88 (10C, Ph), 118.67 ($=CH_2$), 96.91 (C-1), 95.83 (CCl_3), 79.20 (C-3), 76.48 (C-4, $^2J_{4,P}=4.0$ Hz), 75.14 (CH_2, Troc), 73.83 (*C*H$_2$Ph), 72.62 (αCH_2), 70.51 (C-5, $^3J_{5,P}=2.0$ Hz), 68.90, 68.82, 68.71, 68.68 [4C, OCH_2, All, $(CH_2O)_2P$, C-6], 54.69 (C-2), 32.32 (β CH_2), 30.57, 30.10,

29.76, 26.45, 23.08 (10CH_2), 14.51 (CH_3); ^{31}P NMR ($CDCl_3$): δ 0.4; MALDI-TOF-MS: *m/z*: 884.02, 885.85 (24.47% ^{37}Cl) $[M+Na]^+$.

3.1.12. 6-*O*-Benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (19). To a degassed solution of **16** (600 mg, 0.63) in THF (20 mL) {[bis(methyldiphenyl)phosphine](1,5-cyclooctadiene) iridium(I)}hexafluorophosphate (25 mg, 0.03 mmol) was added. The mixture was degassed four times and filled with He, then three times degassed and filled with H_2 (which was kept for 10 s each time), and then four times degassed and filled with He. The mixture was stirred under He for 30 min, cooled to 0 °C and a solution of I_2 (304 mg, 1.2 mmol) in 2:1 THF/H_2O (3 mL) was added dropwise. The mixture was stirred at 0 °C for 6 h, then for 2 h at 25 °C, diluted with EtOAc (200 mL), washed with 5% aq $Na_2S_2O_3$ (20 mL), satd aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried (cotton), concentrated and the residue was purified by chromatography on silica gel (85:15→75:25 toluene/EtOAc) to afford **19** (527 mg, 91%) as a solid. R_f 0.45 (2:1 toluene/EtOAc); $[\alpha]_D^{20}=+16.0$ (*c* 1, $CHCl_3$); ^{1}H NMR (for α-anomer): δ 7.35–7.25 (m, 15H, Ph), 5.40 (dd, 1H, $^3J_{3,2}=10.9$ Hz, $^3J_{3,4}=9.3$ Hz, H-3), 5.32 (d, 1H, $^3J_{2,NH}=9.8$ Hz, NH), 5.0–4.87 (m, 4H, 2CH_2Ph), 4.92 (t, 1H, $^3J_{1,2}=^3J_{1,OH}=4.0$ Hz, H-1), 4.94, 4.93, 4.92, 4.91 (2AB, 4H, $^2J=11.5$ Hz, 2CH_2Ph), 4.72 and 4.66 (2AB, 2H, $^2J=11.8$ Hz, CH_2Ph), 4.54 and 4.45 (2AB, 2H, $^2J=12.0$ Hz, CH_2CCl$_3$), 4.44 (t, 1H, $^3J_{4,5}=9.3$ Hz, H-4), 4.19 (ddd, 1H, $^3J_{6a,5}=1.8$ Hz, $^3J_{6b,5}=7.1$ Hz, H-5), 3.95 (ddd, 1H, H-2), 3.77 (dd, 1H, $^2J_{6a,6b}=10.7$ Hz, H-6a), 3.66 (d, 1H, OH-1), 3.64 (dd, 1H, H-6b), 2.16 (t, 2H, $^3J=7.4$ Hz, αCH_2), 1.50–1.40 (m, 2H, βCH_2), 1.35–1.25 (m, 20H, 10CH_2), 0.90 (t, 3H, CH_3); ^{31}P NMR ($CDCl_3$): δ −1.5; MALDI-TOF-MS: *m/z*: 936.35, 938.35 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{44}H_{59}Cl_3NO_{11}P$: C, 57.74; H, 6.50; N, 1.53. Found: C, 57.52; H, 6.24; N, 1.58.

3.1.13. 6-*O*-Benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose trichloroacetimidate (20). A solution of **19** (526 mg, 0.575 mmol) in CH_2Cl_2 (10 mL) was stirred with trichloroacetonitrile (0.5 mL, 5 mmol) and Cs_2CO_3 (98 mg, 0.3 mmol) for 1 h. The reaction was stopped by addition of satd aq $NaHCO_3$ (5 mL). The mixture was diluted with CH_2Cl_2 (200 mL) and washed with satd aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried (cotton) and concentrated, the residue was purified by flash chromatography on silica gel (4:1:0.5 toluene/EtOAc/Et_3N) to give **20** as a syrup (360 mg, 0.34 mmol, 59%). R_f 0.58 (3:1 toluene/EtOAc); $[\alpha]_D^{20}=+37.0$ (*c* 1, $CHCl_3$); ^{1}H NMR: δ 8.68 (s, 1H, NH), 7.26–7.15 (m, 15H, Ph), 6.34 (d, 1H, $^3J_{1,2}=3.7$ Hz, H-1), 5.37 (dd, 1H, $^3J_{3,2}=11.2$ Hz, $^3J_{3,4}=9.3$ Hz, H-3), 5.16 (d, 1H, Troc, $^3J_{2,NH}=9.4$ Hz, NH), 4.93–4.80 (m, 4H, 2CH_2Ph), 4.70 (dd, 1H, $^3J_{4,5}=9.3$ Hz, H-4), 4.63 and 4.56 (2AB, 2H, $^2J=11.8$ Hz, CH_2Ph), 4.45 and 4.38 (2AB, 2H, $^2J=12.0$ Hz, CH_2CCl_3), 4.10 (ddd, 1H, H-2), 3.97 (m, 1H, H-5), 3.68 (m, 2H, H-6a, H-6b), 2.15 (t, 2H, $^3J=7.4$ Hz, αCH_2), 1.40–1.30 (m, 2H, βCH_2), 1.25–1.15 (m, 20H, 10CH_2), 0.90 (t, 3H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.44 (1C, CO), 160.39 (1C, O–C=N), 154.0 (1C, CONH), 137.82, 135.38 (2C, C_6H_5), 128.58, 128.46, 128.42, 127.86, 127.53, 127.32 (16C, Ph), 95.13 (C-1), 94.50 (CCl_3), 90.69 [C(NH)CCl_3], 74.59 (*C*H_2Ph), 73.37 (CH_2, Troc), 72.64 (C-4, $^2J_{4,P}=6.0$ Hz), 72.44 (C-5, $^3J_{5,P}=6.0$ Hz), 70.20 (C-3, $^3J_{3,P}=2.3$ Hz), 69.63 and 69.55 (2C, *C*H_2Ph, $^2J_{C,P}=5.3$ Hz), 67.46 (C-6), 54.04 (C-2), 34.69 (αCH_2), 32.56, 30.10, 30.06, 30.04, 29.86, 29.76, 29.71, 29.49 (10CH_2), 24.98 (βCH_2), 14.55 (CH_3); ^{31}P NMR ($CDCl_3$): δ 0.8.

3.1.14. Allyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (24). A solution of allyl 2-deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside **23**[32] (2.2 g, 5.06 mmol), tetradecanoic acid (1.44 g, 6.3 mmol), 1,3-dicyclohexylcarbodiimide (1.41 g, 6.83 mmol) and 4-*N*,*N*-dimethylaminopyridine (5 mg, 0.04 mmol) in CH_2Cl_2 (25 mL) was stirred for 30 min at rt. Methanol (1.5 mL) and acetic acid (0.6 mL) were added and the mixture was stirred for further 30 min. Insoluble materials were filtered off and the filtrate was concentrated to 10 mL. The suspension was filtered, the filtrate was diluted with EtOAc (150 mL) and extracted with satd aq $NaHCO_3$ (2×100 mL), H_2O (100 mL), dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (toluene→100:5 toluene/EtOAc) to afford **24** (2.8 g, 86%). $[\alpha]_D^{20}=+56.0$ (*c* 0.3, $CHCl_3$); ^{1}H NMR: δ 5.88 (m, 1H, CH=), 5.36 (d, 1H, $^3J_{2,NH}=9.8$ Hz, NH), 5.32 (dq, 1H, $=CH_{2trans}$), 5.29–5.18 (m, 2H, $=CH_{2cis}$, H-3), 4.89 (d, 1H, $^3J_{1,2}=3.7$ Hz, H-1), 4.73 and 4.68 (AB, 2H, $^2J=12.0$ Hz, CH_2CCl_3), 4.44–3.96 (m, 2H, H-2, OCH*H*, All), 4.20 (m, 1H, OC*H*H, All), 3.90 (m, 1H, H-6a), 3.82–3.68 (m, 3H, H-4, H-5, H-6b), 2.30 (m, 2H, αCH_2), 1.63 (m, 2H, βCH_2), 1.49 (s, 3H) and 1.40 (s, 3H, CMe_2), 1.34–1.24 (m, 20H, 8CH_2), 0.88 (t, 3H, CH_3); MALDI-TOF-MS: *m/z*: 666.4, 668.4 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{29}H_{48}Cl_3NO_8$: C, 54.00; H, 7.50; N, 2.17. Found: C, 53.91; H, 7.62; N, 2.15.

3.1.15. Allyl 2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (27). A suspension of **24** (307 mg, 0.48 mmol) in 90% aq AcOH (3 mL) was stirred at 90 °C for 20 min. The solution was concentrated and the residue was purified by chromatography (2:1→1:1 toluene/EtOAc) to give 270 mg (0.45 mmol, 95%) of **25** [allyl 2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside] as a syrup. R_f 0.55 (1:2 toluene/EtOAc); ^{1}H NMR: δ 5.90 (m, 1H, CH=), 5.34 (d, 1H, $^3J_{NH,2}=9.7$ Hz, NH), 5.32 (dq, 1H, $=CH_{2trans}$), 5.25 (dq, 1H, $=CH_{2cis}$), 5.15 (m, 1H, H-3), 4.92 (d, 1H, $^3J_{1,2}=3.6$ Hz, H-1), 4.76 and 4.68 (AB, 2H, $^2J=12.0$ Hz, CH_2CCl_3), 4.22 (m, 1H, OC*H*H, All), 4.03 (m, 1H, OC*H*H, All), 3.98 (m, 1H, H-2), 3.88 (AB, 2H, H-6a, H-6b), 3.80–3.72 (ABX, 2H, H-4, H-5), 2.72 (br s, 1H, OH), 2.35 (t, 2H, αCH_2), 2.03 (br s, 1H, OH), 1.65–1.55 (m, 4H, 2CH_2), 1.20–1.35 (m, 50H, 25CH_2), 0.88 (t, 6H, CH_3). A solution of **25** (270 mg, 0.45 mmol) in acetic acid (5 mL) was stirred with Zn–Cu couple [1.0 g, made from Zn (0.8 g) and 5% aq $CuSO_4$ (4 mL)] at rt for 2 h. The solids were removed by filtration, the filtrate was concentrated and was repeatedly evaporated with toluene (3×30 mL). The residue was dissolved in EtOAc (100 mL) and extracted with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The organic phase was dried (Na_2SO_4) and concentrated. The

residue was purified by flash chromatography on silica gel (100:5 EtOAc/MeOH) to give 135 mg (0.31 mmol, 70%) of **26** (allyl 2-amino-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside). R_f 0.3 (15:1 EtOAc/MeOH); ^{1}H NMR: δ 5.90 (m, 1H, CH=), 5.30 (dq, 1H, =CH_{2trans}), 5.20 (dq, 1H, =CH_{2cis}), 4.96 (t, 1H, $^3J_{3,4}$=9.8 Hz, H-3), 4.87 (d, 1H, $^3J_{1,2}$=3.5 Hz, H-1), 4.19 (m, 1H, OC*H*H, All), 4.0 (m, 1H, OC*H*H, All), 3.81 (AB, 2H, H-6a, H-6b), 3.69 (ABX, 1H, H-5), 3.55 (t, 1H, H-4), 2.83 (dd, 1H, $^3J_{2,3}$=9.8 Hz, H-2), 2.23 (br s, 3H, OH, NH_2), 2.40 (t, 2H, αCH_2), 1.68–1.59 (m, 4H, 2CH_2), 1.35–1.20 (m, 50H, 25CH_2), 0.88 (t, 6H, 2CH_3); ^{13}C NMR ($CDCl_3$): δ 175.93 (1C, CO), 134.06 (=CH), 118.04 (=CH_2), 99.03 (C-1), 95.73 (CCl_3), 78.41 (C-3), 72.46 (C-5), 70.31 (C-4), 68.91 (OCH_2), 62.46 (C-6), 54.79 (C-2), 34.83 (αCH_2), 32.28, 30.04–29.54 (10CH_2), 25.41 (βCH_2), 14.48 (CH_3).

The free amine **26** (135 mg, 0.31 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was stirred with **8** (200 mg, 0.48 mmol) and 1,3-dicyclohexylcarbodiimide (110 mg, 0.54 mmol) at rt for 2 h. Methanol (0.5 mL) and acetic acid (0.2 mL) were added and the mixture was stirred for further 30 min. Insoluble materials were separated and the filtrate was concentrated to 5 mL volume. The precipitate was filtered off, the filtrate was diluted with EtOAc (100 mL) and washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified on silica gel (1:1→1:3 cyclohexane/Et_2O) to give 221 mg (0.27 mmol, 86%) of **27** as a solid. R_f 0.4 (1:1 EtOAc/toluene); mp=84–86 °C; $[\alpha]_D^{23}$=+42.3 (*c* 0.3, $CHCl_3$); ^{1}H NMR: δ 7.34–7.26 (m, 5H, Ph), 6.31 (d, 1H, $^3J_{NH,2}$=9.5 Hz, NH), 5.73 (m, 1H, CH=), 5.19 (dq, 1H, =CH_{2trans}), 5.12–5.07 (m, 2H, =CH_{2cis}, H-3), 4.77 (d, 1H, $^3J_{1,2}$=3.5 Hz, H-1), 4.52 (AB, 2H, $^2J_{A,B}$=11.5 Hz, C*H*$_2$Ph), 4.29 (ddd, 1H, $^3J_{2,3}$=9.8 Hz, H-2), 4.03 (dd, 1H, OC*H*H, All), 3.87–3.82 (m, 2H, OCH*H*, All), 3.80–3.69 (m, 4H, H-4, H-5, H-6a, H-6b), 3.20 (d, 1H, $^3J_{4,OH}$=4.9 Hz, 4-OH), 2.60 (t, 1H, $^3J_{6,OH}$=6.0 Hz, 6-OH), 2.40–2.27 (m, 4H, 2αCH_2), 1.59–1.44 (m, 4H, 2CH_2), 1.30–1.25 (m, 50H, 25CH_2), 0.88 (t, 6H, 2CH_3); MALDI-TOF-MS: *m/z*: 852.3 $[M+Na]^+$. Anal. Calcd for $C_{50}H_{87}NO_8$: C, 72.33; H, 10.56; N, 1.69. Found: C, 72.31; H, 10.54; N, 1.69.

3.1.16. Allyl 6-*O*-[4,6-*O*-benzylidene-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (28). To a degassed solution of **13** (200 mg, 0.29 mmol) in THF (20 mL) iridium catalyst (25 mg, 0.03 mmol) was added and activated with H_2 as described for **19**. The mixture was stirred under He for 30 min, then cooled to 0 °C and a solution of I_2 (100 mg, 0.4 mmol) in 2:1 THF/H_2O (3 mL) was added dropwise. The solution was stirred at 0 °C for 4 h, then for 2 h at 25 °C. The mixture was diluted with EtOAc (200 mL), washed with 5% aq $Na_2S_2O_3$ (20 mL), satd aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried (Na_2SO_4), concentrated and the residue was purified by chromatography on silica gel (2:1 *n*-hexane/EtOAc) to afford **21** [allyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranose] (157 mg, 0.24 mmol, 83%) as a solid. The residue was taken up in CH_2Cl_2 (10 mL) and stirred with trichloroacetonitrile (0.5 mL, 5 mmol) and Cs_2CO_3 (98 mg, 0.3 mmol) for 1 h. The reaction was stopped by addition of satd aq $NaHCO_3$ (2 mL), the mixture was diluted with CH_2Cl_2 (150 mL) and washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was dried (cotton), concentrated and dried by repeated evaporations with dry toluene (2×15 mL). The crude **22** [4,6-*O*-benzylidene-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate] (0.24 mmol, crude) and **27** (150 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (5 mL) and stirred with powdered activated molecular sieves (0.4 nm) under N_2 for 2 h. The suspension was cooled to −25 °C and a solution of trimethylsilyl trifluoromethanesulfonate (3.6 μL, 0.02 mmol) in CH_2Cl_2 (1 mL) was added. The stirring was continued for 20 min and the reaction was stopped by addition of dry Na_2CO_3. The solids were removed by filtration over a pad of Celite, the filtrate was diluted with CH_2Cl_2 (150 mL) and washed with satd aq $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by chromatography on silica gel (30:1 $CHCl_3$/acetone). Appropriate fractions were collected, concentrated to dryness and purified by repeated precipitations. The residue was dissolved in CH_2Cl_2 (3 mL), then acetone (30 mL) was added. The volume was reduced to 15 mL by evaporation, the suspension was cooled to 4 °C, the white fluffy precipitate was separated on a glass-filter and washed with acetone (5 mL). The precipitate was redisolved in CH_2Cl_2 (10 mL) and the solution was concentrated to dryness which afforded **28** (200 mg, 0.14 mmol, 76% based on the acceptor **27**; 57% based on **22**) as a white solid. R_f 0.4 (B, 3:1 toluene/EtOAc), R_f 0.6 (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}$=+4.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.45–7.25 (m, 10H, Ph), 6.34 (d, 1H, $^3J_{2,NH}$=9.4 Hz, NH), 5.87 (d, 1H, $^3J_{2',NH'}$=9.3 Hz, NH′), 5.75 (m, 1H, CH=), 5.50 (s, 1H, C*H*Ph), 5.35 (dd, 1H, $^3J_{3',2'}$=10.2 Hz, $^3J_{3',4'}$=9.4 Hz, H-3′), 5.20 (dq, 1H, =CH_{2trans}), 5.12 (dq, 1H, =CH_{2cis}), 5.10 (dd, 1H, $^3J_{3,2}$=10.2 Hz, $^3J_{3,4}$=9.7 Hz, H-3), 4.79 (d, 1H, $^3J_{1,2}$=3.6 Hz, H-1), 4.78 and 4.68 (2AB, 2H, ^2J=11.8 Hz, CH_2, Troc), 4.56 and 4.52 (2AB, 2H, ^2J=12.0 Hz, C*H*$_2$Ph), 4.46 (d, 1H, $^3J_{1',2'}$=8.3 Hz, H-1′), 4.34 (dd, 1H, $^2J_{6a',6b'}$=10.2 Hz, $^3J_{5',6a'}$=4.9 Hz, H-6a′), 4.29 (ddd, 1H, H-2), 4.04 (m, 1H, OCH*H*, All), 3.99 (m, 1H, H-5), 3.89–3.70 (m, 6H, H-4, H-6a, H-6b, H-6b′, βC*H*OBn, OCH*H* All), 3.68 (t, 1H, $^3J_{4',5'}$=9.4 Hz), 3.50 (dt, 1H, Hz, $^3J_{5',6b'}$=10.5 Hz, H-5′), 3.20 (br s, 1H, OH-4), 2.38–2.32 (m, 6H, ^{Ico}αCH_2, 2^{Myr}αCH_2), 1.60 (m, 6H, 2^{Myr}βCH_2, ^{Ico}γCH_2), 1.35–1.15 (m, 74H, 37CH_2), 0.90 (t, 9H, 3CH_3); ^{13}C NMR ($CDCl_3$): δ 175.25 (CO), 174.31 (CO), 171.44 (CONH), 154.91 (CONH, Troc), 138.77, 137.30, 137.26 (2C, Ph), 133.75 (=CH), 129.41, 128.70, 128.59, 127.95, 127.92, 126.30 (10C, Ph), 118.22 (=CH_2), 102.62 (C-1′), 101.51 (*C*HPh), 96.86 (C-1), 95.83 (CCl_3), 79.19 (C-4′), 76.62 (^{Ico}*C*HOBn), 75.0 (CH_2, Troc), 74.14 (C-3), 71.76 (*C*H$_2$Ph), 71.45 (C-3′), 70.90 (C-4), 69.50 (C-5), 69.29 (C-6), 68.84 (C-6′), 68.64 (OCH_2, All), 66.60 (C-5′), 56.84 (C-2′), 51.78 (C-2), 42.0 (^{Ico}αCH_2), 34.73, 34.57, 32.26 (2^{Myr}αCH_2, ^{Ico}γCH_2), 30.05, 29.99, 29.91, 29.79, 29.70, 29.58, 29.56, 29.35, 25.69, 25.35, 25.28, 23.03 (37CH_2), 14.44 (3CH_3); MALDI-TOF-MS: *m/z*: 1485.95 $[M+Na]^+$, 1501.90 $[M+K]^+$.

3.1.17. Allyl 6-*O*-[2-amino-4,6-*O*-benzylidene-2-deoxy-3-*O*-tetradecanoyl-β-D-glucopyranosyl]-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (29). A solution of **28** (136 mg, 0.093 mmol) in 3:2 acetic acid/toluene (5 mL) was stirred with Zn–Cu couple [1.0 g, made from Zn (0.8 g) and 5% aq $CuSO_4$ (4 mL)] at rt for 1 h and at 40 °C for 30 min. The solids were removed by filtration over a pad of Celite and washed with 1:1 toluene/acetic acid (15 mL). The filtrate was concentrated, the residue was redissolved in toluene and concentrated (3×20 mL). The residue was dissolved in CH_2Cl_2 (150 mL) and extracted with satd aq $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL). The organic phase was dried (cotton), concentrated and purified by chromatography on silica gel (2×20 cm, 100:0→100:2 CH_2Cl_2/MeOH) to afford **29** (80 mg, 0.06 mmol, 66%), R_f 0.2 (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}=+1.0$ (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.45–7.25 (m, 10H, Ph), 6.29 (d, 1H, $^3J_{2,NH}$= 9.5 Hz, NH), 5.73 (m, 1H, CH═), 5.50 (s, 1H, C*H*Ph), 5.20 (dq, 1H, $=CH_{2trans}$), 5.14 (dd, 1H, $^3J_{3,2}=10.5$ Hz, $^3J_{3,4}=$ 8.9 Hz, H-3), 5.13 (dd, 1H, $^3J_{3',2'}=10.0$ Hz, $^3J_{3',4'}=9.3$ Hz, H-3′), 5.12 (dq, 1H, $=CH_{2cis}$), 4.80 (d, 1H, $^3J_{1,2}=3.7$ Hz, H-1), 4.56 and 4.52 (2AB, 2H, $^2J=11.7$ Hz, CH_2Ph), 4.40 (d, 1H, $^3J_{1',2'}=8.0$ Hz, H-1′), 4.36 (dd, 1H, $^2J_{6a',6b'}=$ 10.7 Hz, $^3J_{5',6a'}=5.2$ Hz, H-6a′), 4.33 (ddd, 1H, H-2), 4.16 (dd, 1H, $^2J_{6a,6b}=9.9$ Hz, $^3J_{5,6a}=1.5$ Hz, H-6a), 4.05 (m, 1H, OCH*H*, All), 3.90–3.76 (m, 6H, H-4, H-5, H-6b, H-6b′, βC*H*OBn, OCH*H*, All), 3.64 (t, 1H, $^3J_{4',5'}=9.5$ Hz, H-4′), 3.53 (ddd, 1H, $^3J_{5',6b'}=10.0$ Hz, H-5′), 2.95 (dd, 1H, H-2′), 2.35–2.15 (m, 7H, $^{Ico}CH_2$, $2^{Myr}\alpha CH_2$, OH-4), 1.70–1.55 (m, 6H, $2^{Myr}\beta CH_2$, $^{Ico}\gamma CH_2$), 1.35–1.15 (m, 74H, $37CH_2$), 0.90 (t, 9H, $3CH_3$); ^{13}C NMR($CDCl_3$): δ 175.19 (CO), 174.09 (CO), 171.42 (CONH), 138.81, 137.40 (2C, $2C_6H_5$), 133.76 (═CH), 129.38, 128.77, 128.57, 128.0, 126.45 (10C, Ph), 118.26 (═CH_2), 105.85 (C-1′), 101.76 (*C*HPh), 97.14 (C-1), 79.38 (C-4′), 76.71 (^{Ico}*C*HOBn), 74.06 (2C, C-3, C-3′), 71.84 (*C*H_2Ph), 71.25 (C-4), 70.13 (C-6), 69.30 (C-6′), 69.05 (C-5), 68.81 (OCH_2, All), 67.10 (C-5′), 57.36 (C-2′), 51.79 (C-2), 42.10 ($^{Ico}\alpha CH_2$), 34.78, 34.76, 34.42 ($2^{Myr}\alpha CH_2$, $^{Ico}\gamma CH_2$), 32.32, 30.11, 30.07, 29.95, 29.80, 29.75, 29.66, 29.58, 29.42, 25.76, 25.53, 25.34, 23.08 ($37CH_2$), 14.51 ($3CH_3$).

3.1.18. Allyl 6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-(octadecanoyloxy)icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (30). To a stirred solution of **29** (90 mg, 0.07 mmol) and **8** (50 mg, 0.12 mmol) in CH_2Cl_2 (3 mL) under N_2 a solution of 1-isopropyloxycarbonyl-2-isopropyloxy-1,2-dihydroquinoline (36 mg, 35 μL, 0.12 mmol) in CH_2Cl_2 (1 mL) was added. After stirring for 3 h, a white precipitate was formed. The mixture was diluted with $CHCl_3$ (2 mL) and purged with N_2 until the volume of the mixture was reduced to 2 mL. Stirring was continued for 10 h, the mixture was diluted with CH_2Cl_2 (150 mL) and extracted with satd aq $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL). The organic phase was dried (cotton), concentrated and purified by repeated precipitations with acetone from CH_2Cl_2 (×2) (see purification of **28**) and with acetone from $CHCl_3$ (×3) as follows. The residue was dissolved in $CHCl_3$ (2 mL), then acetone (30 mL) was added. The volume was reduced to 10 mL by evaporation, the suspension was cooled to 4 °C, the white fluffy precipitate was separated on the glass-filter and washed with acetone (5 mL). The precipitate was redissolved in CH_2Cl_2 (10 mL), the solution was concentrated to dryness which afforded **30** (90 mg, 0.07 mmol, 76%). R_f 0.65 (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}=\pm 0$ (*c* 0.5, $CHCl_3$); ^{1}H NMR: δ 7.35–7.20 (m, 15H, Ph), 6.40 (d, 1H, $^3J_{2',NH'}=9.0$ Hz, NH′), 6.29 (d, 1H, $^3J_{2,NH}=9.5$ Hz, NH), 5.65 (m, 1H, CH═), 5.40 (s, 1H, C*H*Ph), 5.12 (dd, 1H, $^3J_{3',2'}=10.2$ Hz, $^3J_{3',4'}=9.5$ Hz, H-3′), 5.11 (dq, 1H, $=CH_{2trans}$), 5.02 (dd, 1H, $^3J_{3,2}=10.5$ Hz, $^3J_{3,4}=8.9$ Hz, H-3), 5.01 (dq, 1H, $=CH_{2cis}$), 4.67 (d, 1H, $^3J_{1,2}=3.8$ Hz, H-1), 4.56 and 4.41 (2AB, 2H, $^2J=12.1$ Hz, CH_2Ph), 4.46 and 4.42 (2AB, 2H, $^2J=12.0$ Hz, CH_2Ph), 4.25 (dd, 1H, $^2J_{6a',6b'}=10.6$ Hz, $^3J_{5',6a'}=5.0$ Hz, H-6a′), 4.19 (ddd, 1H, H-2), 4.17 (d, 1H, $^3J_{1',2'}=8.1$ Hz, H-1′), 3.93 (m, 1H, OCH*H*, All), 3.90 (ddd, 1H, H-2′), 3.81 (dd, 1H, $^2J_{6a,6b}=10.0$ Hz, $^3J_{6a,5}=1.8$ Hz, H-6a), 3.75–3.55 (m, 7H, H-4, H-5, H-6b, H-6b′, 2βC*H*OBn, OCH*H*, All), 3.57 (t, 1H, $^3J_{4',5'}=9.5$ Hz, H-4′), 3.33 (ddd, 1H, $^3J_{5',6b'}=10.0$ Hz, H-5′), 3.20 (d, 1H, $^2J=4.0$ Hz, OH-4), 2.30–2.18 (m, 8H, $2^{Ico}\alpha CH_2$, $2^{Myr}\alpha CH_2$), 1.55–1.45 (m, 8H, $2^{Myr}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.3–1.10 (m, 110H, $55CH_2$), 0.80 (t, 12H, $4CH_3$); ^{13}C NMR($CDCl_3$): δ 175.04 (CO), 173.92 (CO), 172.03 (CONH), 171.27 (CONH), 138.80, 138.47, 137.26 (3C, Ph), 133.78 (═CH), 129.38, 129.05, 128.68, 128.53, 128.28, 128.16, 127.95, 127.88, 126.39 (15C, Ph), 117.97 (═CH_2), 102.31 (C-1′), 101.63 (*C*HPh), 96.92 (C-1), 79.06 (C-4′), 76.63 (^{Ico}*C*HOBn), 76.45 (^{Ico}*C*HOBn), 73.82 (C-3), 71.77 (*C*H_2Ph), 71.35 (C-3′), 70.87 (*C*H_2Ph), 70.58 (C-4), 69.48 (C-5), 69.21 (C-6), 68.90 (C-6′), 68.60 (OCH_2, All), 66.88 (C-5′), 54.85 (C-2′), 51.85 (C-2), 42.10 and 41.47 ($2^{Ico}\alpha CH_2$), 34.70, 34.61, 34.40, 33.98 ($2^{Myr}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.27, 30.05, 29.91, 29.80, 29.79, 29.70, 29.62, 29.54, 29.36, 25.68, 25.45, 25.41, 25.28, 23.03 ($55CH_2$), 14.45 ($4CH_3$); MALDI-TOF-MS: *m*/*z*: 1712.37 $[M+Na]^+$, 1728.34 $[M+K]^+$; calcd 1712.28 $[M+Na]^+$, 1728.37 $[M+K]^+$.

3.1.19. 6-*O*-{4,6-*O*-Benzylidene-2-deoxy-2-[(*R*)-3-(octadecanoyloxy)icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-D-glucopyranose (31). To a degassed solution of **30** (46 mg, 0.27 mmol) in THF (10 mL) iridium catalyst (15 mg, 0.02 mmol) was added and activated with H_2 as described for **19** and the mixture was stirred under He for 30 min, then cooled to 0 °C. A solution of I_2 (20 mg, 0.08 mmol) in 2:1 THF/H_2O (1 mL) was added dropwise. The mixture was stirred at 0 °C for 4 h, then for 2 h at 25 °C. The solution was diluted with EtOAc (100 mL), washed with 5% aq $Na_2S_2O_3$ (20 mL), satd aq $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL). The organic phase was dried (cotton), concentrated and the residue was purified by precipitation with acetone from $CHCl_3$ (2×) (as described for **30**) and by chromatography on silica gel (10:1 $CHCl_3$/acetone) to afford **31** (42 mg, 0.025 mmol, 94%) as a solid. R_f 0.3 (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}=-10.0$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (for α-anomer): δ 7.45–7.30 (m, 15H, Ph), 6.45 (d, 1H, $^3J_{2',NH'}=8.7$ Hz, NH′), 6.34 (d, 1H, $^3J_{2,NH}=9.4$ Hz, NH), 5.50 (s, 1H, C*H*Ph), 5.23 (t, 1H, $^3J_{2',3'}=^3J_{3',4'}=9.9$ Hz, H-3′), 5.0 (dd, 1H, $^3J_{2,3}=10.4$ Hz, $^3J_{3,4}=9.7$ Hz, H-3), 4.95 (dd, 1H, $^3J_{1,2}=3.7$ Hz, $^2J_{1,OH}=2.5$ Hz, H-1), 4.62 and 4.48

(2AB, 2H, $^{2}J=11.5$ Hz, CH_2Ph), 4.56 and 4.53 (2AB, 2H, $^{2}J=12.0$ Hz, CH_2Ph), 4.52 (d, 1H, $^{3}J_{1',2'}=8.5$ Hz, H-1′), 4.36 (d, 1H, OH-1), 4.35 (dd, 1H, $^{2}J_{6a',6b'}=10.0$ Hz, $^{3}J_{5',6a'}=5.1$ Hz, H-6a′), 4.16 (ddd, 1H, H-2), 4.06 (dd, 1H, $^{2}J_{6a,6b}=11.5$ Hz, $^{3}J_{5,6a}=2.2$ Hz, H-6a), 3.98 (ddd, 1H, H-2′), 3.90–3.82 (m, 2H, 2βC*H*OBn), 3.80 (t, 1H, $^{3}J_{5',6b'}=10.0$ Hz, H-6b′), 3.72 (ddd, 1H, $^{3}J_{4,5}=9.7$ Hz, $^{3}J_{5,6b}=7.2$ Hz, H-5), 3.68 (t, 1H, $^{3}J_{4',5'}=9.5$ Hz, H-4′), 3.55 (dd, 1H, $^{3}J_{5,6b}=7.2$ Hz, H-6b), 3.45 (ddd, 1H, H-5′, $^{3}J_{5',6b'}=10.0$ Hz), 3.37 (dt, 1H, $^{2}J_{4,OH}=6.5$ Hz, H-4), 2.65 (d, 1H, OH-4), 2.45–2.20 (m, 8H, $2^{Ico}\alpha CH_2$, $2^{Myr}\alpha CH_2$), 1.65–1.40 (m, 8H, $2^{Myr}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.35–1.20 (m, 110H, 55CH_2), 0.90 (t, 12H, 4CH_3); ^{13}C NMR($CDCl_3$): δ 175.53 (CO), 173.93 (CO), 172.24 (CONH), 171.57 (CONH), 138.86, 138.11, 137.34 (3C, 3C_6H_5), 129.46, 129.06, 128.78, 128.59, 128.47, 128.18, 128.07, 126.47 (15C, Ph), 102.81 (C-1′), 101.72 (*C*HPh), 91.92 (C-1), 79.09 (C-4′), 76.89 (2C, 2^{Ico}*C*HOBn), 73.99 (C-3), 71.85 (CH_2, Bn), 71.71 (C-3′), 71.48 (*C*H_2Ph), 71.30 (C-5), 70.59 (C-4), 70.03 (C-6), 68.93 (C-6′), 66.93 (C-5′), 55.10 (C-2′), 52.01 (C-2), 42.11 and 41.85 ($2^{Ico}\alpha CH_2$), 34.68, 34.61, 34.45, 33.03 ($2^{Myr}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.26, 30.06, 30.0, 29.88, 29.80, 29.70, 29.64, 29.49, 29.37, 25.61, 25.38, 25.27, 23.02 (55CH_2), 14.45 (4CH_3); MALDI-TOF-MS: *m*/*z*: 1672.34 $[M+Na]^+$, 1688.31 $[M+K]^+$; calcd 1672.23 $[M+Na]^+$, 1688.34 $[M+K]^+$.

3.1.20. 6-*O*-{4,6-*O*-Benzylidene-2-deoxy-2-[(*R*)-3-(octadecanoyloxy)icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-1,4-*O*,*O*-bis[(dibenzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-1-α-D-glucopyranose (32). A 1.0 M solution of lithium bis(trimethylsilyl)amide in *n*-hexane (60 μL, 0.06 mmol) was added to a stirred solution of **31** (32 mg, 0.019 mmol) and tetrabenzyl diphosphate (43 mg, 0.08 mmol) in anhydrous THF (5 mL) at −78 °C under N_2. The mixture was stirred for 30 min, then allowed to warm up to 0 °C within 5 min and the reaction was quenched with satd aq $NaHCO_3$ (0.5 mL). The mixture was diluted with $CHCl_3$ (100 mL) and washed with satd aq $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The organic phase was dried (cotton), concentrated and purified by chromatography on silica gel (100:0.2:0→100:0.2:15 CH_2Cl_2/acetone). Appropriate fractions were collected and purified by a second chromatography (1.5×30 cm, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O) which afforded **32** (8 mg, 0.004 mmol, 19%) as a solid. R_f 0.7 (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}=+1.0$ (*c* 0.4, $CHCl_3$); ^{1}H NMR: δ 7.38–7.15 (m, 36H, 7Ph, NH′), 6.05 (d, 1H, $^{3}J_{2,NH}=8.8$ Hz, NH), 5.49 (dd, 1H, $^{3}J_{1,2}=3.3$ Hz, $^{3}J_{1,P}=5.3$ Hz, H-1), 5.80 (s, 1H, C*H*Ph), 5.19 (dd, 1H, $^{3}J_{3,2}=10.9$ Hz, $^{3}J_{3,4}=9.2$ Hz, H-3), 5.07 (t, 1H, $^{3}J_{3',2'}=^{3}J_{3',4'}=9.9$ Hz, H-3′), 4.94–4.82 (m, 8H, 4CH_2Ph), 4.56 and 4.45 (2AB, 2H, $^{2}J=11.9$ Hz, CH_2Ph), 4.50 (t, 1H, $^{3}J_{4,5}=9.2$ Hz, H-4), 4.42 and 4.31 (2AB, 2H, $^{2}J=11.8$ Hz, CH_2Ph), 4.38 (d, 1H, $^{3}J_{1',2'}=8.5$ Hz, H-1′), 4.24 (ddd, 1H, H-2), 4.17 (dd, 1H, $^{2}J_{6a',6b'}=10.7$ Hz, $^{3}J_{5',6a'}=5.2$ Hz, H-6a′), 4.09 (ddd, 1H, H-2′), 3.85 (m, 1H, H-6a), 3.81 (m, 1H, H-5), 3.72–3.56 (m, 3H, H-6b′, 2βC*H*OBn), 3.54 (t, 1H, $^{3}J_{4',5'}=9.5$ Hz, H-4′), 3.37 (dd, 1H, $^{2}J_{6a,6b}=8.7$ Hz, $^{3}J_{5,6b}=3.7$ Hz, H-6a), 3.12 (ddd, 1H, $^{3}J_{5',6b'}=10.0$ Hz, H-5′), 2.25–2.19 (m, 8H, $2^{Ico}\alpha CH_2$, $2^{Myr}\alpha CH_2$), 1.50–1.40 (m, 8H, $2^{Myr}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.30–1.10 (m, 110H, 55CH_2), 0.90 (t, 12H, 4CH_3); ^{1}H–^{13}C HMQC ($CDCl_3$): 102.4 (*C*HPh), 101.5 (C-1′), 96.4 (C-1), 79.3 (C-4′), 77.5 (C-5), 76.5 ($^{Ico}\beta$C*H*), 75.7 ($^{Ico}\beta$C*H*), 72.2 (C-4), 71.3 (*C*H_2Ph), 70.2 (CH_2, Bn), 68.9 (C-6′), 66.9 (C-5′), 66.8 (C-6), 54.5 (C-2′), 52.3 (C-2), 41.7 ($^{Ico}\alpha CH_2$), 41.40 ($^{Ico}\alpha CH_2$), 34.7–34.3 ($2^{Myr}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.0–23.0 (55CH_2), 14.4 (4CH_3); ^{31}P NMR ($CDCl_3$): δ −1.63 (attached to C-4), −2.26 (attached to C-1); MALDI-TOF-MS: *m*/*z*: 2192.50 $[M+Na]^+$, 2208.47 $[M+K]^+$; calcd 2192.36 $[M+Na]^+$, 2208.46 $[M+K]^+$.

3.1.21. Allyl 4-*O*-benzyl-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (33). A solution of **13** (820 mg, 1.16 mmol) in CH_2Cl_2 (20 mL) and powdered molecular sieves 0.4 nm (500 mg) were stirred for 3 h under N_2 at ambient temperature. The suspension was cooled to −78 °C and Et_3SiH (202 mg, 278 μL, 1.74 mmol) and a solution of $PhBCl_2$ (313 mg, 256 μL, 1.97 mmol) in CH_2Cl_2 (2 mL) were added successively under N_2. The mixture was stirred for 1 h. Et_3N (1 mL) and MeOH (1 mL) were added and the mixture was stirred for 15 min, then warmed up to rt, diluted with EtOAc (30 mL) and filtered over a pad of Celite. The filtrate was diluted with EtOAc (300 mL) and washed successively with satd aq $NaHCO_3$ (50 mL), H_2O (50 mL) and brine (50 mL). The organic phase was dried (cotton) and concentrated. The residue was purified on silica gel (4:1→3:1 toluene/EtOAc) which afforded **33** as a syrup (790 mg, 96%). R_f 0.53 (A, 2:1 toluene/EtOAc); $[\alpha]_D^{20}=+56.8$ (*c* 1, $CHCl_3$); ^{1}H NMR: δ 7.40–7.25 (m, 5H, Ph), 5.89 (m, 1H, CH=), 5.40 (dd, 1H, $^{3}J_{2,3}=10.7$ Hz, $^{3}J_{3,4}=8.3$ Hz, H-3), 5.35 (d, 1H, $^{3}J_{NH,2}=9.7$ Hz, NH), 5.30 (dq, 1H, $=CH_{2trans}$), 5.23 (dq, 1H, $=CH_{2cis}$), 4.92 (d, 1H, $^{3}J_{1,2}=3.6$ Hz, H-1), 4.73 and 4.69 (AB, 2H, $^{2}J=12.1$ Hz, CH_2CCl_3), 4.70 and 4.65 (AB, 2H, $^{2}J=12.0$ Hz, CH_2Ph), 4.19 (m, 1H, OC*H*H, All), 3.96 (m, 1H, OC*H*H, All), 3.94 (ddd, 1H, H-2), 3.88–3.72 (m, 4H, H-4, H-5, H-6a, H-6b), 2.23 (m, 2H, αCH_2), 1.82 (t, 1H, OH), 1.58–1.52 (m, 2H, βCH_2), 1.33–1.22 (m, 20H, 10CH_2), 0.89 (t, 3H, CH_3); ^{13}C NMR (HMQC, in $CDCl_3$): δ 138.5 (=CH), 135.0–128.0 (Ph), 119.0 (=CH_2), 96.5 (C-1), 76.0 (C-4), 75.3 (*C*H_2Ph), 74.8 (CH_2, Troc), 72.7 (C-3), 71.5 (C-5), 69.1 (OCH_2, All), 61.8 (C-4), 55.0 (C-2), 34.5 (αCH_2), 32.0–28.0 (9CH_2), 25.0 (β CH_2), 23.0 (CH_2), 14.51 (CH_3); MALDI-TOF-MS: *m*/*z*: 716.32, 718.31 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{33}H_{50}Cl_3NO_8$: C, 57.02; H, 7.25; N, 2.02. Found: C, 56.78; H, 7.26; N, 1.97.

3.1.22. Allyl 2-amino-4-*O*-benzyl-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (34). *Method A*. A solution of **33** (723 mg, 1.04 mmol) in AcOH (20 mL) was stirred with Zn–Cu couple [made from Zn (1.2 g) and 5% aq $CuSO_4$ (5 mL)] at ambient temperature for 1 h. The solids were removed by filtration over a pad of Celite, the filtrate was concentrated and repeatedly evaporated with toluene (3× 30 mL). The residue was dissolved in EtOAc (250 mL) and washed with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by chromatography on silica gel (100:1→100:5 EtOAc/MeOH) to give **35** [allyl 4-*O*-benzyl-2-deoxy-2-(2,2-dichloroethoxycarbonylamino)-3-*O*-tetradecanoyl-α-D-glucopyranoside] as faster eluted product (34 mg, 0.05 mmol, 5%); R_f 0.52 (A, 2:1 toluene/EtOAc); ^{1}H NMR: δ 7.38–7.26 (m, 5H, Ph), 5.88 (m, 1H,

CH=), 5.82 (t, 1H, $^3J=6.1$ Hz, CH_2CHCl_2), 3.37 (dd, 1H, $^3J_{2,3}=10.8$ Hz, $^3J_{3,4}=8.7$ Hz, H-3), 5.29 (dq, 1H, $=CH_{2trans}$), 5.23 (d, 1H, $^3J_{NH,2}=9.7$ Hz, NH), 5.23 (dq, 1H, $=CH_{2cis}$), 4.89 (d, 1H, $^3J_{1,2}=3.6$ Hz, H-1), 4.70 and 4.64 (AB, 2H, $^2J=12.1$ Hz, CH_2Ph), 4.39 (dd, 2H, $^3J=6.1$, 7.7 Hz, CH_2CHCl_2), 4.18 (m, 1H, OC*H*H, All), 3.96 (m, 1H, OC*H*H, All), 3.91 (ddd, 1H, H-2), 3.86–3.71 (m, 4H, H-4, H-5, H-6a, H-6b), 2.24 (m, 2H, αCH_2), 1.77 (t, 1H, OH), 1.58–1.52 (m, 2H, βCH_2), 1.33–1.22 (m, 20H, $10CH_2$), 0.89 (t, 3H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.29 (1C, CO), 155.23 (1C, CONH), 138.04 (=CH), 133.55, 128.94, 128.42, 128.25 (6C, Ph), 118.63 ($=CH_2$), 96.86 (C-1), 75.78 (C-4), 75.26 (CH_2Ph), 73.30 (C-3), 71.61 (C-5), 69.26 (1C, CH_2CHCl_2), 69.10 (OCH_2, All), 68.94 (1C, CH_2CHCl_2), 61.84 (C-6), 54.88 (C-2), 34.75 (αCH_2), 32.36, 30.09, 30.07, 29.86, 29.72, 29.55, 23.11 ($10CH_2$), 25.27 (β CH_2), 14.56 (CH_3). Further elution gave **34** (320 mg, 0.62 mmol, 60%) as a syrup; R_f 0.5 (A, 15:1 EtOAc/MeOH); $[\alpha]_D^{20}=+92.0$ (*c* 1, $CHCl_3$); ^{1}H NMR: δ 7.37–7.26 (m, 5H, Ph), 5.97 (m, 1H, CH=), 5.33 (dq, 1H, $=CH_{2trans}$), 5.31 (dd, 1H, $^3J_{2,3}=10.5$ Hz, $^3J_{3,4}=9.3$ Hz, H-3), 5.22 (dq, 1H, $=CH_{2trans}$), 5.18 (d, 1H, $^3J_{1,2}=3.5$ Hz, H-1), 4.68 and 4.64 (AB, 2H, $^2J=11.5$ Hz, CH_2, 4-O-Bn), 4.20 (m, 1H, OC*H*H, All), 4.12 (m, 1H, OC*H*H, All), 3.85–3.75 (m, 3H, H-5, H-6a, H-6b), 3.68 (t, 1H, $^3J_{4,5}=9.4$ Hz, H-4), 3.11 (dd, 1H, H-2), 2.50 (br s, 1H, OH), 2.31 (m, 2H, αCH_2), 1.62–1.52 (m, 2H, βCH_2), 1.33–1.22 (m, 20H, $10CH_2$), 0.89 (t, 3H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.82 (CO), 138.15 (1C, Ph), 134.07 (=CH), 128.86, 128.29, 128.05 (5C, Ph), 118.16 ($=CH_2$), 99.14 (C-1), 76.63 (C-4), 76.16 (C-3), 74.59 (CH_2Ph), 71.49 (C-5), 68.70 (OCH_2, All), 61.66 (C-6), 55.51 (C-2), 34.69 (αCH_2), 32.09, 29.84, 29.81, 29.77, 29.61, 29.52, 29.46, 29.41 (9C, CH_2), 25.12 (β CH_2), 22.86 (CH_2), 14.29 (CH_3); MALDI *m*/*z* 542.40 $[M+Na]^+$. Anal. Calcd for $C_{30}H_{49}NO_6$: C, 69.33; H, 9.50; N, 2.70. Found: C, 69.10; H, 9.26; N, 2.70.

Method B. A solution of **33** (218 mg, 0.314 mmol) in AcOH (5 mL) was stirred with Zn powder at rt for 4 h, then diluted with AcOH (20 mL). The solids were removed by filtration over a pad of Celite, the filtrate was concentrated, redissolved in toluene and concentrated (3×30 mL). The residue was dissolved in EtOAc (100 mL) and washed with satd aq $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by chromatography on silica gel (1.5×60 cm, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O) to give **34** (145 mg, 0.28 mmol, 89%); R_f 0.2 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O).

3.1.23. Allyl 4-*O*-benzyl-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (36). To a stirred solution of **8** (437 mg, 1.04 mmol) in CH_2Cl_2 (3 mL) HOBt (140 mg, 0.91 mmol) and WSCD·HCl (175 mg, 0.91 mmol) were added successively under N_2. The suspension was sonicated to afford a clear solution, which was stirred for 1 h. A solution of **34** (467 mg, 0.90 mmol) in $CHCl_3$ (3 mL) was added to the mixture and stirring was continued for 1.5 h under N_2. The mixture was taken up in EtOAc (200 mL), washed with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by chromatography on silica gel (3×40 cm, 80:10:0.4 toluene/MeOH/H_2O) to give **36** (703 mg, 0.76 mmol, 85%) as a solid R_f 0.3 (B, 2:1 toluene/EtOAc) or R_f 0.4 (A, 80:10:0.4 toluene/MeOH/H_2O). $[\alpha]_D^{20}=+42.0$ (*c* 1, $CHCl_3$); ^{1}H NMR: δ 7.38–7.25 (m, 5H, Ph), 6.25 (d, 1H, $^3J_{2,NH}=9.5$ Hz, NH), 5.73 (m, 1H, CH=), 5.38 (ddd, 1H, $^3J_{2,3}=10.7$ Hz, $^3J_{3,4}=9.2$ Hz, $^5J_{3,CH}=3.8$ Hz, H-3), 5.18 (dq, 1H, $=CH_{2trans}$), 5.12 (dq, 1H, $=CH_{2cis}$), 4.78 (d, 1H, $^3J_{1,2}=3.5$ Hz, H-1), 4.70 and 4.64 (AB, 2H, $^2J=11.5$ Hz, CH_2, 4-O-Bn), 4.56 and 4.52 (AB, 2H, $^2J=12.0$ Hz, CH_2Ph), 4.27 (ddd, 1H, H-2), 4.02 (m, 1H, OC*H*H, All), 3.88–3.70 (m, 6H, H-4, H-5, H-6a, H-6b, βCH, OC*H*H, All), 2.35 (d, 2H, $^2J<1$ Hz, $^3J=7.0$ Hz, $\alpha CH_{2'}$), 2.23 (m, 2H, αCH_2), 1.76 (m, 1H, OH), 1.65–1.45 (m, 2H, βCH_2, $\gamma CH_{2'}$), 1.33–1.22 (m, 50H, $25CH_2$), 0.89 (t, 6H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.24 (CO), 171.58 (CONH), 138.92 and 138.18 (2C, $2C_6H_5$, Bn), 133.74 (=CH), 128.87, 128.74, 128.31, 128.19, 128.02, 127.94 (10C, $2C_6H_5$, Bn), 118.24 ($=CH_2$), 97.05 (C-1), 76.70 (β'CH), 76.08 (C-4), 75.21 (CH_2, 4-*O*-Bn), 73.50 (C-3), 71.83 (CH_2, Bn), 71.61 (C-5), 68.87 (OCH_2, All), 61.88 (C-6), 52.58 (C-2), 42.23 ($\alpha'CH_2$), 34.79, 34.47 (2C, αCH_2, $\gamma'CH_2$), 32.33, 30.11, 30.08, 30.06, 29.91, 29.76, 29.74, 29.62 ($23CH_2$), 25.72, 25.26 ($2CH_2$), 23.09 (CH_2), 14.29 (CH_3). Anal. Calcd for $C_{57}H_{93}NO_8$: C, 74.39; H, 10.19; N, 1.52. Found: C, 74.16; H, 9.90; N, 1.79; MALDI *m*/*z* 942.75 $[M+Na]^+$.

3.1.24. Allyl 4-*O*-benzyl-6-*O*-{6-*O*-benzyl-4-*O*-[bis-(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (37). A solution of **20** (445 mg, 0.42 mmol) and **36** (385 mg, 0.42 mmol) in CH_2Cl_2 (5 mL) was stirred with powdered activated molecular sieves (0.4 nm) under N_2 for 2 h. The suspension was cooled to −25 °C and a solution of trimethylsilyl trifluoromethanesulfonate (7.5 μL, 0.04 mmol) in CH_2Cl_2 (1 mL) was added. The stirring was continued for 20 min and the reaction was stopped by addition of dry Na_2CO_3. The mixture was let to warm up to rt and the solids were removed by filtration through a pad of Celite. The filtrate was diluted with CH_2Cl_2 (200 mL) and washed with satd aq $NaHCO_3$ (50 mL), H_2O (50 mL) and brine (50 mL). The organic phase was dried (cotton) and concentrated. The residue was purified in three equal portions by chromatography on silica gel (1.5×60 cm, 30:40:15:0.3 toluene/CH_2Cl_2/acetone/H_2O) which afforded **37** as a solid (710 mg, 0.39 mmol, 93%). R_f 0.5 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O); R_f 0.7 (B, 2:1 toluene/EtOAc). $[\alpha]_D^{20}=+20.7$ (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.38–7.24 (m, 25H, 5Ph), 6.21 (d, 1H, $^3J_{2,NH}=9.4$ Hz, NH), 5.73 (m, 1H, CH=), 5.36 (dd, 1H, $^3J_{3,2}=10.8$ Hz, $^3J_{3,4}=9.1$ Hz, H-3), 5.32 (dd, 1H, $^3J_{3',2'}=10.7$ Hz, $^3J_{3',4'}=9.1$ Hz, H-3′), 5.14 (dq, 1H, $=CH_{2trans}$), 5.05 (dq, 1H, $=CH_{2cis}$), 4.85 (d, 1H, $^3J_{2,NH}=7.8$ Hz, NH), 4.93–4.82 [m, 4H, CH_2, $(BnO)_2P(O)$–], 4.70 (d, 1H, $^3J_{1,2}=3.6$ Hz, H-1), 4.54 and 4.48 (2AB, 2H, $^2J=12.2$ Hz, CH_2Ph), 4.56 and 4.52 (2AB, 2H, $^2J=11.8$ Hz, CH_2Ph), 4.66 and 4.57 (2AB, 2H, $^2J=11.8$ Hz, CH_2CCl_3), 4.67 and 4.61 (2AB, 2H, $^2J=11.6$ Hz, CH_2Ph), 4.47 (d, 1H, $^3J_{1',2'}=8.3$ Hz, H-1′), 4.44 (t, 1H, $^3J_{4',5'}=9.1$ Hz, H-4′), 4.29 (ddd, 1H, H-2), 4.11 (dd, 1H, $^2J_{6a,6b}=10.8$ Hz, $^3J_{6a,5}=1.6$ Hz, H-6a), 4.03 (m, 1H, OCH*H*, All), 3.88–3.82 (m, 2H, H-5, βC*H*OBn), 3.84–3.60 (m, 7H, OCH*H*, All, H-6b, H-6′a, H-6′b, H-2′,

H-4, H-5′), 2.35 (m, 2H, $^{Ico}\alpha CH_2$), 2.28–2.17 (m, 4H, $2^{Myr}\alpha CH_2$), 1.65–1.40 (m, 6H, $2^{Myr}\beta CH_2$, $^{Ico}\gamma CH_2$), 1.35–1.20 (m, 74H, $37CH_2$), 0.90 (t, 9H, CH_3); ^{13}C NMR($CDCl_3$): δ 174.06 (CO), 173.96 (CO), 171.62 (CONH), 154.11 (CONH, Troc), 138.81, 138.39 (2C, Ph), 135.80, 135.70 (2C, Ph), 133.64 (=CH), 128.91, 128.88, 128.87, 128.63, 128.61, 128.24, 127.90, 127.86, 127.82 (25C, Ph), 118.17 ($=CH_2$), 101.01 (C-1′), 96.87 (C-1), 76.58 (^{Ico}*C*HOBn), 76.04 (C-4), 74.91 (*C*H_2Ph), 74.82 (C-5′), 74.65 (*C*H_2Ph), 74.31 (C-4′, $^2J_{4',P}$=6.0 Hz), 73.78 (C-3), 73.73 (CH_2, Troc), 72.19 (C-3′, $^3J_{3',P}$=1.5 Hz), 71.73 (CH_2, ^{Ico}Bn), 70.27 (C-5), 69.93 and 69.86 [$2CH_2$, $^2J_{C,P}$=3.0 Hz, $(BnO)_2P(O)$–], 68.98 (C-6′), 68.61 (OCH_2, All), 67.95 (C-6), 56.52 (C-2′), 52.20 (C-2), 42.17 ($^{Ico}\alpha CH_2$), 34.71, 34.41, 34.24 ($2^{Myr}\alpha CH_2$ $^{Ico}\gamma CH_2$), 32.22, 30.0, 29.95, 29.82, 29.75, 29.65, 29.59, 29.53, 29.38, 25.62, 25.17, 24.89, 22.98 ($37CH_2$), 14.60 ($3CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.52; MALDI-TOF-MS: *m/z*: 1838.0, 1853.97 (24.47% ^{37}Cl) $[M+Na]^+$. Anal. Calcd for $C_{101}H_{150}Cl_3N_2O_{18}P$: C, 66.74; H, 8.32; N, 1.54. Found: C, 66.17; H, 8.20; N, 1.44.

3.1.25. Allyl 6-*O*-{2-amino-6-*O*-benzyl-2-deoxy-4-*O*-[bis(benzyloxy)phosphoryl]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-4-*O*-benzyl-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (38). A solution of **37** (176 mg, 0.097 mmol) in acetic acid (10 mL) was stirred with Zn powder (300 mg) at 50 °C for 2 h under N_2. The solids were removed by filtration over a pad of Celite, the filtrate was concentrated and repeatedly evaporated with toluene (3×30 mL). The residue was dissolved in CH_2Cl_2 (200 mL) and extracted with satd aq $NaHCO_3$ (30 mL), H_2O (50 mL) and brine (50 mL). The organic phase was dried (cotton), concentrated and purified in two equal portions by chromatography on silica gel (1.5×60 cm, 30:40:15:0.3 toluene/CH_2Cl_2/acetone/H_2O) to afford **38** (134 mg, 0.081 mmol, 84%) as a transparent foam. Compound **38** was further purified by chromatography on silica gel (100:1→100:10 CH_2Cl_2/acetone) which afforded 105 mg of **38** (0.064 mmol, 66%). R_f 0.5 (B, 30:40:15:0.3 toluene/CH_2Cl_2/acetone/H_2O); $[\alpha]_D^{20}=+20.6$ (*c* 1.0, $CHCl_3$); ^1H NMR: δ 7.33–7.22 (m, 25H, 5Ph), 6.20 (d, 1H, $^3J_{2,NH}$=9.5 Hz, NH), 5.70 (m, 1H, CH=), 5.36 (dd, 1H, $^3J_{3',2'}$=10.7 Hz, $^3J_{3',4'}$=9.2 Hz, H-3′), 5.15 (dq, 1H, $=CH_{2trans}$), 5.07 (dq, 1H, $=CH_{2cis}$), 5.05 (dd, 1H, $^3J_{3,2}$=10.8 Hz, $^3J_{3,4}$=9.2 Hz, H-3), 4.93–4.87 [m, 4H, CH_2, $(BnO)_2P(O)$–], 4.77 (d, 1H, $^3J_{1,2}$=3.5 Hz, H-1), 4.65 and 4.60 (2AB, 2H, ^2J=12.2 Hz, CH_2Ph), 4.54 and 4.49 (2AB, 2H, ^2J=11.8 Hz, CH_2Ph), 4.51 and 4.44 (2AB, 2H, ^2J=11.6 Hz, CH_2Ph), 4.37 (t, 1H, $^3J_{4',5'}$=9.2 Hz, H-4′), 4.30 (ddd, 1H, H-2), 4.18 (d, 1H, $^3J_{1',2'}$=8.0 Hz, H-1′), 4.11 (dd, 1H, $^2J_{6a,6b}$=10.9 Hz, $^3J_{6a,5}$=1.5 Hz, H-6a), 4.01 (m, 1H, OCH*H*, All), 3.90–3.55 (m, 8H, H-5, βC*H*OBn, OCH*H* All, H-6b, H-6′a, H-6′b, H-4, H-5′), 2.90 (dd, 1H, H-2′), 2.33 (m, 2H, $^{Ico}\alpha CH_2$), 2.30–2.10 (m, 4H, $2^{Myr}\alpha CH_2$), 1.60–1.40 (m, 6H, $2^{Myr}\beta CH_2$, $^{Ico}\gamma CH_2$), 1.35–1.20 (m, 74H, $37CH_2$), 0.90 (t, 9H, $3CH_3$); ^{13}C NMR($CDCl_3$): δ 174.0 (CO), 173.87 (CO), 171.20 (CONH), 138.63, 138.35, 137.95 (3C, $3C_6H_5$), 135.79, 135.69 [2C, Ph, $^3J_{C,P}$=5 Hz, $(BnO)_2P(O)$–], 133.45 (=CH), 128.68, 128.61, 128.48, 128.42, 128.07, 127.99, 127.74, 127.69, 127.63 (25C, Ph), 118.0 ($=CH_2$), 104.53 (C-1′), 96.69 (C-1), 76.41 (^{Ico}*C*HOBn), 76.36 (C-4), 75.22 (C-3′, $^3J_{3',P}$=1.6 Hz), 74.78 (CH_2Ph), 74.70 (C-5′), 74.40 (C-4′, $^2J_{4',P}$=6.3 Hz), 73.53 (CH_2Ph), 73.47 (C-3), 71.57 ($^{Ico}CH_2$, Bn), 70.38 (C-5), 69.69 and 69.62 [$2CH_2$, $^2J_{C,P}$=3.2 Hz, $(BnO)_2P(O)$–], 69.06 (C-6′), 68.52 (OCH_2, All), 68.39 (C-6), 56.25 (C-2′), 52.08 (C-2), 42.0 ($^{Ico}\alpha CH_2$), 34.52, 34.31, 34.23 ($2^{Myr}\alpha CH_2$, $^{Ico}\gamma CH_2$), 32.06, 29.85, 29.79, 29.64, 29.63, 29.50, 29.36, 29.30 ($37CH_2$), 14.25 ($3CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.12. Anal. Calcd for $C_{98}H_{149}N_2O_{16}P$: C, 71.67; H, 9.15; N, 1.71. Found: C, 71.44; H, 8.94; N, 1.71; MALDI-TOF-MS: *m/z*: 1642.14 $[M+H]^+$, 1664.12 $[M+Na]^+$, 1680.06 $[M+K]^+$.

3.1.26. Allyl 4-*O*-benzyl-6-*O*-{6-*O*-benzyl-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-4-*O*-[bis(benzyloxy)-phosphoryl]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (39). To a solution of **8** (100 mg, 0.24 mmol) and DIPEA (42 μL, 0.24 mmol) in DMF (2 mL) a solution of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 110 mg, 0.29 mmol) in DMF (1 mL) was added and the mixture was stirred under N_2 for 30 min. The solution containing activated acid **8** was added to a stirred solution of **38** (137 mg, 0.083 mmol) in DMF (5 mL) and the mixture was stirred under N_2 for 20 min at rt and for 5 h at 35 °C. The mixture was diluted with CH_2Cl_2 (200 mL) and washed with satd aq $NaHCO_3$ (20 mL), H_2O (50 mL) and brine (40 mL). The organic phase was dried (cotton), concentrated and purified by precipitation with EtOH from CH_2Cl_2 (3×) and by precipitation with hexane from CH_2Cl_2 (2×) as follows: precipitation with EtOH from CH_2Cl_2. The residue was dissolved in CH_2Cl_2 (3 mL), then acetone (0.5 mL) and EtOH (25 mL) were successively added. The volume was reduced to 10 mL by concentration, the suspension was diluted with EtOH (10 mL), the white fluffy precipitate was separated on the glass-filter and washed with EtOH (10 mL). The precipitate was redissolved in CH_2Cl_2 (10 mL) and the solution was concentrated to dryness. Precipitation with hexane from CH_2Cl_2. The residue was dissolved in CH_2Cl_2 (2 mL), then hexane (30 mL) was added and the volume was reduced to 10 mL by concentration under diminished pressure. The suspension was diluted with hexane (10 mL) and cooled to 4 °C, the transparent gel-like precipitate was separated on the glass-filter and washed with cold (4 °C) hexane (10 mL). The precipitate was recovered from the filter by dissolution in CH_2Cl_2 (10 mL), the solution was concentrated to dryness. Yield 155 mg (0.076 mmol, 92%) of **39** as a white solid; R_f 0.55 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O); R_f 0.7 (B, 2:1 toluene/EtOAc); $[\alpha]_D^{20}=+11.6$ (*c* 1.0, $CHCl_3$); ^1H NMR: δ 7.40–7.22 (m, 30H, 6Ph), 6.46 (d, 1H, $^3J_{2',NH'}$=8.8 Hz, NH′), 6.22 (d, 1H, $^3J_{2,NH}$=9.4 Hz NH), 5.70 (m, 1H, CH=), 5.34 (dd, 1H, $^3J_{3',2'}$=0.7 Hz, $^3J_{3',4'}$=9.2 Hz, H-3′), 5.33 (dd, 1H, $^3J_{3,2}$=10.8 Hz, $^3J_{3,4}$=9.1 Hz, H-3), 5.18 (dq, 1H, $=CH_{2trans}$), 5.07 (dq, 1H, $=CH_{2cis}$), 4.97–4.86 [m, 4H, $2CH_2$, $(BnO)_2P(O)$–], 4.75 (d, 1H, $^3J_{1,2}$=3.4 Hz, H-1), 4.59–4.42 (m, 8H, 4CH_2Ph), 4.47 (d, 1H, $^3J_{1',2'}$=8.3 Hz, H-1′), 4.42 (t, 1H, $^3J_{4',5'}$=9.2 Hz, H-4′), 4.28 (ddd, 1H, H-2), 4.01 (m, 1H, OCH*H*, All), 3.99 (dd, 1H, $^2J_{6a,6b}$=10.5 Hz, $^3J_{6a,5}$=1.9 Hz, H-6a), 3.88–3.57 (m, 10H, H-5, H-2′, 2βC*H*OBn, OCH*H* All, H-6b, H-6′a, H-6′b, H-4, H-5′), 2.33 (m, 4H, $2^{Ico}\alpha CH_2$), 2.25–2.0 (m, 4H, $2^{Myr}\alpha CH_2$, Myr), 1.60–1.40 (m, 8H,

$2^{Myr}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.35–1.10 (m, 110H, $55CH_2$), 0.90 (t, 12H, $4CH_3$); ^{13}C NMR($CDCl_3$): δ 174.10 (CO), 173.85 (CO), 171.55 (CONH), 171.34 (CONH), 138.85, 138.51, 138.24 (4C, $4\times C_6H_5$), 135.90, 135.81 [2C, $2C_6H_5$, $^3J_{C,P}=5.0$ Hz, $(BnO)_2P(O)$–], 133.69 (=CH), 128.95, 128.89, 128.75, 128.66, 128.63, 128.26, 128.19, 128.11, 127.98, 127.94, 127.89 (30C, Ph), 118.09 ($=CH_2$), 100.91 (C-1′), 96.68 (C-1), 76.59 (C-4), 76.49 and 76.45 (2C, $2^{Ico}\beta CH$), 74.83 (C-5′), 74.73 (CH_2Ph), 74.61 (C-4′, $^2J_{4',P}=6.0$ Hz), 73.70 (CH_2Ph), 73.55 (C-3), 72.66 (C-3′, $^3J_{3',P}=1.5$ Hz), 71.73 and 71.09 ($2CH_2$, 2^{Ico}Bn), 70.42 (C-5), 69.91 and 69.85 [$2CH_2$, $^2J_{C,P}=3.2$ Hz, $(BnO)_2P(O)$–], 69.10 (C-6′), 68.47 (OCH_2, All), 68.94 (C-6), 54.53 (C-2′), 52.41 (C-2), 42.18 ($^{Ico}\alpha CH_2$), 41.44 ($^{Ico}\alpha CH_2$), 34.68, 34.46, 34.36, 33.98 ($2^{Myr}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.27, 30.06, 30.02, 29.90, 29.71, 29.57, 25.68, 25.36, 25.19, 24.93, 23.04 ($55CH_2$), 14.46 ($4CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.57; MALDI-TOF-MS: *m/z*: 2064.41 $[M+Na]^+$, 2080.40 $[M+K]^+$. Alternatively, **39** [prepared from the same amount of **38** (137 mg, 0.083 mmol)] was purified in three equal portions by adsorption-partition chromatography on silica gel (1.5×60 cm, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O), to yield 118 mg (0.058 mmol, 76%) of **39**.

3.1.27. 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-4-*O*-[bis(benzyloxy)phosphoryl]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-D-glucopyranose (40). To a degassed solution of **39** (138 mg, 0.068 mmol) in THF (20 mL) iridium catalyst (25 mg, 0.03 mmol) was added and activated with H_2 as described for **19**. The mixture was stirred under He for 30 min and cooled to 0 °C. A solution of I_2 (50 mg, 0.2 mmol) in 2:1 THF/H_2O (2 mL) was added dropwise. The mixture was stirred at 0 °C for 6 h, then for 2 h at 25 °C, diluted with CH_2Cl_2 (200 mL), washed with 5% aq $Na_2S_2O_3$ (20 mL), satd aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by repeated precipitations (see purification of **39**) with EtOH from CH_2Cl_2 (3×), then with hexane from CH_2Cl_2 (3×) to afford **40** (120 mg, 0.06 mmol, 89%) as a solid. Alternatively, **40** (prepared from 127 mg, 0.062 mmol of **39**) was purified by sequential precipitations with EtOH from CH_2Cl_2 (1×), with hexane from CH_2Cl_2 (1×) and subsequent column chromatography on silica gel (1.5×60 cm, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O) which afforded **40** (106 mg, 0.053 mmol, 85%) as a solid. R_f 0.47 (α-anomer) and 0.38 (β-anomer) (B, 30:40:15:0.3 toluene/CH_2Cl_2/acetone/H_2O); $[\alpha]_D^{23}=+2.5$ (*c* 1.0, $CHCl_3$); ^1H NMR: δ 7.35–7.18 (m, 30H, 6Ph), 6.43 (d, 1H, $^3J_{2',NH'}=8.1$ Hz, NH′), 6.20 (d, 1H, $^3J_{2,NH}=9.4$ Hz, NH), 5.34 (dd, 1H, $^3J_{3',2'}=10.4$ Hz, $^3J_{3',4'}=9.0$ Hz, H-3′), 5.28 (dd, 1H, $^3J_{3,2}=10.5$ Hz, $^3J_{3,4}=9.2$ Hz, H-3), 4.93–4.85 [m, 4H, CH_2, $(BnO)_2P(O)$–], 4.95 (d, 1H, $^3J_{1,2}=3.5$ Hz, H-1), 4.86 (d, 1H, $^3J_{1',2'}=8.3$ Hz, H-1′), 4.71 (br s, 1H, 1-OH), 4.55 and 4.51 (2AB, 2H, $^2J=11.8$ Hz, CH_2Ph), 4.53 and 4.48 (2AB, 2H, $^2J=12.0$ Hz, CH_2Ph), 4.52 and 4.51 (2AB, 2H, $^2J=11.7$ Hz, CH_2Ph), 4.44 and 4.39 (2AB, 2H, $^2J=12.1$ Hz, CH_2Ph), 4.42 (t, 1H, $^3J_{4',5'}=9.2$ Hz, H-4′), 4.13 (ddd, 1H, H-2), 4.02–3.90 (m, 2H, H-5, H-6a), 3.80 (dd, 1H, $^2J_{6a',6b'}=10.3$ Hz, $^3J_{6a',5'}=2.0$ Hz, H-6a′), 3.79 (m, 2H, 2βC*H*OBn), 3.67–3.54 (m, 4H, H-2′, H-6b, H-6b′, H-5′), 3.28 (t, 1H, H-4), 2.40–2.30 (m, 4H, $2^{Ico}\alpha CH_2$), 2.25–2.10 (m, 4H, $2^{Myr}\alpha CH_2$), 1.62–1.40 (m, 8H, $2^{Myr}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.32–1.13 (m, 110H, $55CH_2$), 0.90 (t, 12H, $4CH_3$); ^{13}C NMR($CDCl_3$): δ 174.23 (CO), 173.66 (CO), 171.23 (CONH), 171.20 (CONH), 139.33, 139.01, 138.74 (4C, Ph), 136.40, 136.11 [2C, Ph, $^3J_{C,P}=5.0$ Hz, $(BnO)_2P(O)$–], 129.01, 128.82, 128.58, 128.33, 128.30, 128.25, 128.17, 128.13, 128.0, 127.96 (30C, Ph), 101.16 (C-1′), 91.74 (C-1), 77.55 (C-4), 76.78 and 76.64 (2C, $2^{Ico}\beta CH$), 74.74 (CH_2Ph), 74.63 (C-5′), 74.55 (C-4′, $^2J_{4',P}=5.8$ Hz), 73.84 (CH_2Ph), 73.47 (C-3), 72.99 (C-3′, $^3J_{3',P}=1.4$ Hz), 71.89 and 71.48 ($2CH_2$, 2^{Ico}Bn), 71.05 (C-5), 69.96 and 69.89 [$2CH_2$, $^2J_{C,P}=3.4$ Hz, $(BnO)_2P(O)$–], 69.36 (C-6′), 69.13 (C-6), 55.57 (C-2′), 52.84 (C-2), 42.29 ($^{Ico}\alpha CH_2$), 41.81 ($^{Ico}\alpha CH_2$), 34.79, 34.63, 34.37, 34.02 ($2^{Myr}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.33, 30.12, 30.07, 29.96, 29.91, 29.77, 29.62, 29.59, 25.68, 25.22, 24.96, 23.09 ($55CH_2$), 14.51 ($4CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.61; MALDI-TOF-MS: *m/z*: 2024.46 $[M+Na]^+$, 2040.40 $[M+K]^+$.

3.1.28. Allyl 4-*O*-benzyl-6-*O*-{6-*O*-benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-2-[(*R*)-3-(octadecanoyloxy)icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranoside (41). To a solution of **11** (62 mg, 0.104 mmol) in 1:1 DMF/THF (4 mL) a solution of *O*-(benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-bistetramethyluronium hexafluorophosphate (HBTU) (86 mg, 0.20 mmol) in DMF (1 mL) was added and the mixture was stirred under N_2 for 30 min. The solution containing activated fatty acid **11** was added to a stirred solution of **38** (98 mg, 0.06 mmol) and DIPEA (21 μL, 0.12 mmol) in DMF (5 mL) under N_2. The mixture was heated to 50 °C and the reaction vessel was purged with N_2 for 10 min (to blow off the THF). The reaction mixture was stirred for 5 h at 58 °C under N_2, cooled to rt, diluted with CH_2Cl_2 (150 mL) and washed with satd aq $NaHCO_3$ (20 mL), H_2O (50 mL) and brine (40 mL). The organic phase was dried (cotton), concentrated and purified by sequential precipitations with EtOH from CH_2Cl_2 (3×) (see purification of **39**), with hexane from CH_2Cl_2 (3×) and with EtOH from CH_2Cl_2 (2 times) to give **41** (114 mg, 0.052 mmol, 86%) as a white solid; R_f 0.56 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O) or R_f 0.7 (B, 2:1 toluene/EtOAc); $[\alpha]_D^{20}=+15.7$ (*c* 1.0, $CHCl_3$); ^1H NMR: δ 7.35–7.20 (m, 25H, 5Ph), 6.22 (d, 1H, $^3J_{2,NH}=9.5$ Hz NH), 5.94 (d, 1H, $^3J_{2',NH'}=8.4$ Hz, NH′), 5.70 (m, 1H, CH=), 5.45 (dd, 1H, $^3J_{3',2'}=10.6$ Hz, $^3J_{3',4'}=9.0$ Hz, H-3′), 5.34 (dd, 1H, $^3J_{3,2}=11.0$ Hz, $^3J_{3,4}=9.1$ Hz, H-3), 5.18 (dq, 1H, $=CH_{2trans}$), 5.08 (dq, 1H, $=CH_{2cis}$), 4.96 [βCH, (octadecanoyloxy)icosanoyl], 4.96–4.87 [m, 4H, $2CH_2$, $(BnO)_2P(O)$–], 4.86 (d, 1H, $^3J_{1',2'}=8.5$ Hz, H-1′), 4.77 (d, 1H, $^3J_{1,2}=3.5$ Hz, H-1), 4.58–4.44 (m, 6H, 3CH_2Ph), 4.43 (t, 1H, $^3J_{4',5'}=9.0$ Hz, H-4′), 4.30 (ddd, 1H, H-2), 4.07 (dd, 1H, $^2J_{6a,6b}=11.0$ Hz, $^3J_{6a,5}=1.6$ Hz, H-6a), 4.01 (m, 1H, OCH*H*, All), 3.87–3.62 (m, 9H, H-5, H-2′, βC*H*OBn, OCH*H* All, H-6b, H-6a′, H-6b′, H-4, H-5′), 2.43–2.10 (m, 10H, $2^{Ico}\alpha CH_2$, $2^{Myr}\alpha CH_2$, $^{Ste}\alpha CH_2$), 1.65–1.40 (m, 10H, $2^{Myr}\beta CH_2$, $^{Ste}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.35–1.10 (m, 134H, $67CH_2$), 0.90 (t, 15H, $5CH_3$); ^{13}C NMR($CDCl_3$): δ 174.16 (CO), 174.10 (CO), 173.92 (CO), 171.34 (CONH), 169.98 (CONH), 138.81, 138.46, 138.17 (3C, Ph), 135.94,

135.81 [2C, Ph, $^{3}J_{C,P}=5.0$ Hz, $(BnO)_2P(O)$–], 133.68 (=CH), 128.92, 128.89, 128.81, 128.69, 128.43, 128.29, 128.19, 128.04, 127.97, 127.88 (25C, Ph), 118.18 ($=CH_2$), 100.58 (C-1′), 96.82 (C-1), 76.58 ($^{Ico}\beta$CH), 76.43 (C-4), 74.85 (C-5′), 74.79 (CH_2Ph), 74.60 (C-4′, $^{2}J_{4',P}=6.2$ Hz), 73.68 (CH_2Ph), 73.60 (C-3), 72.46 (C-3′, $^{3}J_{3',P}=1.5$ Hz), 71.75 (CH_2Ph), 71.31 [βCH, (octadecanoyloxy)icosanoyl], 70.43 (C-5), 69.92 and 69.83 [$2CH_2$, $^{2}J_{C,P}=5.3$ Hz, $(BnO)_2P(O)$–], 69.08 (C-6′), 68.57 (OCH_2, All), 67.94 (C-6), 55.29 (C-2′), 52.32 (C-2), 42.19 ($2^{Ico}\alpha CH_2$), 34.81, 34.66, 34.50, 34.46, 34.29 ($2^{Myr}\alpha CH_2$, $^{Ste}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.26, 30.05, 30.0, 29.92, 29.88, 29.78, 29.70, 29.58, 29.54, 25.67, 25.61, 25.32, 25.18, 24.92, 23.02 ($67CH_2$), 14.44 ($5CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.59; MALDI-TOF-MS: *m/z*: 2240.70 $[M+Na]^+$, 2256.69 $[M+K]^+$.

3.1.29. 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-2-[(*R*)-3-(octadecanoyloxy)-icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-2-deoxy-3-*O*-tetradecanoyl-D-glucopyranose (42). To a degassed solution of **41** (95 mg, 0.043 mmol) in THF (20 mL) iridium catalyst (25 mg, 0.03 mmol) was added and activated with H_2 as described for the preparation of **19**. The mixture was stirred under He for 30 min and cooled to 0 °C. A solution of I_2 (20 mg, 0.08 mmol) in 2:1 THF/H_2O (2 mL) was added dropwise and the solution was stirred at 0 °C for 3 h, diluted with CH_2Cl_2 (200 mL), washed with 5% aq $Na_2S_2O_3$ (20 mL), satd aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic phase was dried (cotton) and concentrated. The residue was purified by precipitation with EtOH from CH_2Cl_2 (2×) (see preparation of **39**), then with MeOH from 1:1 toluene/hexane (2×) (see below) and, finally, by chromatography on silica gel (1.5×60 cm, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O) which afforded **42** (82 mg, 0.038 mmol, 88%) as amorphous solid. *Precipitation with MeOH from 1:1 toluene/hexane*. The residue was dissolved in CH_2Cl_2 (2 mL), then toluene (1 mL) and hexane (3 mL) were added and the volume was reduced to 2 mL by concentration. MeOH (20 mL) was added and the suspension was kept at 4 °C for 30 min. The white fluffy precipitate was separated on a glass-filter and washed with MeOH (10 mL). The precipitate was collected from the filter by dissolution in CH_2Cl_2 (10 mL), the solution was concentrated to dryness. R_f 0.45 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O), R_f 0.36 (α-anomer) and R_f 0.32 (β-anomer) (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}=+4.5$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (for α-anomer): δ 7.38–7.22 (m, 25H, 5Ph), 6.26 (d, 1H, $^{3}J_{2,NH}=9.5$ Hz, NH), 6.02 (d, 1H, $^{3}J_{2',NH'}=8.0$ Hz, NH′), 5.42 (dd, 1H, $^{3}J_{3',2'}=10.9$ Hz, $^{3}J_{3',4'}=9.4$ Hz, H-3′), 5.39 (dd, 1H, $^{3}J_{3,2}=10.7$ Hz, $^{3}J_{3,4}=9.1$ Hz, H-3), 5.17 (d, 1H, $^{3}J_{1',2'}=8.2$ Hz, H-1′), 5.11 (d, 1H, $^{3}J_{1,2}=3.2$ Hz, H-1), 4.96 [βCH, (octadecanoyloxy)icosanoyl], 4.95–4.85 [m, 4H, $2CH_2$, $(BnO)_2P(O)$–], 4.62 and 4.54 (AB, 2H, $^{2}J=11.8$ Hz, CH_2Ph), 4.58 and 4.51 (AB, 2H, $^{2}J=11.5$ Hz, CH_2Ph), 4.53 and 4.46 (AB, 2H, $^{2}J=12.0$ Hz, CH_2Ph), 4.52 (t, 1H, $^{3}J_{4',5'}=9.4$ Hz, H-4′), 4.22 (ddd, 1H, H-2), 4.12 (ddd, 1H, $^{3}J_{6a,5}=1.6$ Hz, $^{3}J_{6b,5}=8.0$ Hz, H-5), 3.98 (dd, 1H, $^{2}J_{6a,6b}=12.0$ Hz, H-6a), 3.84 (m, 1H, βC*H*OBn), 3.82 (dd, 1H, $^{2}J_{6a',6b'}=10.5$ Hz, $^{3}J_{6a',5'}=2.0$ Hz, H-6a′), 3.74 (dd, 1H, H-6b), 3.70–3.50 (m, 3H, H-2′, H-6b′, H-5′), 3.36 (t, 1H, H-4), 2.40–2.15 (m, 10H, $2^{Ico}\alpha CH_2$, $2^{Myr}\alpha CH_2$, $^{Ste}\alpha CH_2$), 1.65–1.40 (m, 10H, $2^{Myr}\beta CH_2$, $^{Ste}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.35–1.15 (m, 134H, $67CH_2$), 0.90 (t, 15H, $5CH_3$); ^{13}C NMR($CDCl_3$): δ 174.97 (CO), 173.94 (CO), 173.86 (CO), 171.47 (CONH), 170.59 (CONH), 138.84, 138.30, 138.89 (3C, $3C_6H_5$), 135.90, 135.81 [2C, Ph, $^{3}J_{C,P}=5.0$ Hz, $(BnO)_2P(O)$–], 128.86, 128.83, 128.74, 128.62, 128.23, 128.21, 128.12, 128.03, 127.99, 127.86 (25C, Ph), 99.96 (C-1′), 91.77 (C-1), 76.23 (C-4), 76.70 ($^{Ico}\beta$CH), 74.81 (CH_2Ph), 74.65 (C-5′, $^{2}J_{5',P}=4.0$ Hz), 74.51 (C-4′, $^{2}J_{4',P}=6.9$ Hz), 73.74 (CH_2Ph), 73.48 (C-3), 72.34 (C-3′, $^{3}J_{3',P}=1.2$ Hz), 71.86 (CH_2Ph), 71.80 [βCH, (octadecanoyloxy)icosanoyl], 70.66 (C-5), 69.86 and 69.79 [$2CH_2$, $^{2}J_{C,P}=5.4$ Hz, $(BnO)_2P(O)$–], 68.96 (C-6′), 67.93 (C-6), 55.77 (C-2′), 52.69 (C-2), 42.50 and 42.26 ($2^{Ico}\alpha CH_2$), 34.81, 34.70, 34.59, 34.57, 34.26 ($2^{Myr}\alpha CH_2$, $^{Ste}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.24, 30.04, 29.98, 29.92, 29.88, 29.82, 29.68, 29.54, 29.50, 25.58, 25.25, 25.14, 24.94, 24.78, 23.0 ($67CH_2$), 14.45 ($5CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.65; MALDI-TOF-MS: *m/z*: 2200.67 $[M+Na]^+$.

3.1.30. 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-[(*R*)-3-(benzyloxy)icosanoylamino]-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-1-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranose (43). To a stirred solution of **40** (58 mg, 0.029 mmol) and tetrabenzyl diphosphate (76 mg, 0.14 mmol) in anhydrous THF (10 mL) a 1.0 M solution of lithium bis(trimethylsilyl)amide in *n*-hexane (60 μL, 0.058 mmol) was added at −78 °C under N_2. The mixture was stirred for 30 min, then allowed to warm up to 0 °C within 5 min and the reaction was quenched with satd aq $NaHCO_3$ (0.5 mL). The mixture was diluted with $CHCl_3$ (100 mL) and washed with satd aq $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The organic phase was dried (cotton), concentrated and purified by repeated precipitation with EtOH from CH_2Cl_2 (5×) (see preparation of **39**) to give **43** (56 mg, 0.025 mmol, 85%) as a solid. R_f 0.46 (B, 2:1 toluene/EtOAc), R_f 0.43 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O). $[\alpha]_D^{20}=+11.3$ (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.35–7.19 (m, 40H, 8Ph), 7.01 (d, 1H, $^{3}J_{2',NH'}=9.1$ Hz, NH′), 6.20 (d, 1H, $^{3}J_{2,NH}=8.7$ Hz, NH), 5.67 (d, 1H, $^{3}J_{1,2}=3.6$ Hz, $^{3}J_{1,P}=5.3$ Hz, H-1), 5.26 (dd, 1H, $^{3}J_{3',2'}=10.8$ Hz, $^{3}J_{3',4'}=9.2$ Hz, H-3′), 5.22 (dd, 1H, $^{3}J_{3,2}=10.8$ Hz, $^{3}J_{3,4}=9.3$ Hz, H-3), 5.05–4.85 [m, 8H, $4CH_2$, $2\times(BnO)_2P(O)$–], 4.72 (d, 1H, $^{3}J_{1',2'}=8.5$ Hz, H-1′), 4.56–4.38 (m, $4CH_2$, 4Bn), 4.44 (t, 1H, $^{3}J_{4',5'}=9.2$ Hz, H-4′), 4.26 (ddd, 1H, $^{4}J_{2,P}=1.5$ Hz, H-2), 4.03 (ddd, 1H, $^{3}J_{6a,5}=2.0$ Hz, $^{3}J_{6b,5}=5.6$ Hz, H-5), 3.93 (ddd, 1H, H-2′), 3.90 (dd, 1H, $^{2}J_{6a,6b}=11.5$ Hz, H-6a), 3.83 (dd, 1H, $^{2}J_{6a',6b'}=11.1$ Hz, $^{3}J_{6a',5'}=1.8$ Hz, H-6a′), 3.78 (dd, 1H, H-6b), 3.77 (m, 1H, βC*H*OBn), 3.70 (m, 1H, βC*H*OBn), 3.67 (dd, 1H, $^{3}J_{6b',5'}=6.2$ Hz, H-6b′), 3.55 (ddd, 1H, H-5′), 3.54 (t, 1H, $^{3}J_{5,4}=9.3$ Hz, H-4), 2.48–2.28 (m, 4H, $2^{Ico}\alpha CH_2$), 2.27–2.05 (m, 4H, $2^{Myr}\alpha CH_2$), 1.55–1.40 (m, 8H, $2^{Myr}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.34–1.15 (m, 110H, $55CH_2$), 0.90 (t, 12H, $4CH_3$); ^{13}C NMR($CDCl_3$): δ 174.04 (CO), 173.82 (CO), 171.85 (CONH), 171.53 (CONH), 138.96, 138.75, 138.60, 137.78 (4C, Ph), 136.01, 135.92, 135.78, 135.68 [4C, Ph, $^{3}J_{C,P}=4.5$ Hz, $2(BnO)_2P(O)$–], 129.08, 129.02, 128.87, 128.84, 128.72, 128.65, 128.63 (20C, Ph), 128.38, 128.33, 128.24, 128.14, 128.10, 128.0, 127.93, 127.88, 127.80 (20C, Ph), 101.39 (C-1′), 96.39 (C-1, $^{2}J_{1,P}=6.6$ Hz), 76.27 ($^{Ico}\beta$CH), 75.94 (C-4), 75.88 ($^{Ico}\beta$CH), 75.18 (CH_2Ph), 74.79 (C-5′, $^{3}J_{5',P}=3.2$ Hz), 74.70 (C-4′,

$^{2}J_{4',P}$=6.0 Hz), 74.13 (C-5), 73.64 (CH_2Ph), 73.38 (C-3′, $^{3}J_{3',P}$=2.0 Hz), 72.10 (C-3), 71.31 and 71.29 ($2CH_2$, 2^{Ico}Bn), 70.26 and 70.19 [$2CH_2$, $^{2}J_{C,P}$=1.7 Hz, $(BnO)_2P(O)$–], 69.87 and 69.79 [$2CH_2$, $^{2}J_{C,P}$=6.0 Hz, $(BnO)_2P(O)$–], 69.06 (C-6′), 66.61 (C-6), 55.57 (C-2′), 52.52 (C-2, $^{3}J_{C,P}$=8.8 Hz), 41.73 ($^{Ico}\alpha CH_2$), 41.40 ($^{Ico}\alpha CH_2$), 34.67, 34.57, 34.39, 34.32 ($2^{Myr}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.27, 30.07, 30.02, 29.93, 29.86, 29.76, 29.71, 29.58, 29.55, 25.69, 25.62, 25.46, 25.11, 24.91, 23.04 ($55CH_2$), 14.46 ($4CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.60 (attached to C-4′), −2.55 (attached to C-1); MALDI-TOF-MS: *m/z*: 2284.59 $[M+Na]^+$, 2300.58 $[M+K]^+$.

3.1.31. 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-4-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-2-[(*R*)-3-(octadecanoyloxy)-icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-(benzyloxy)icosanoylamino]-1-*O*-[bis(benzyloxy)phosphoryl]-2-deoxy-3-*O*-tetradecanoyl-α-D-glucopyranose (44). To a stirred solution of **42** (45 mg, 0.021 mmol) and tetrabenzyl diphosphate (54 mg, 0.1 mmol) in anhyd THF (10 mL) a 1.0 M solution of lithium bis(trimethylsilyl)amide in *n*-hexane (40 μL, 0.04 mmol) was added at −78 °C under N_2. The mixture was stirred for 30 min, then allowed to warm up to 0 °C within 5 min and the reaction was quenched with 10% aqueous $NaHCO_3$ (0.5 mL). The mixture was diluted with $CHCl_3$ (100 mL) and washed with satd aq $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The organic phase was dried (cotton) and concentrated. Purification by repeated precipitations with EtOH from CH_2Cl_2 (3×) (see preparation of **39**) and with MeOH from toluene (3×) (see below) gave **44** (43 mg, 0.018 mmol, 86%) as a solid. *Precipitation with MeOH from toluene*. The residue was dissolved in CH_2Cl_2 (2 mL), then toluene (1 mL) was added and the volume was reduced to 1 mL by concentration. MeOH (15 mL) was added and the suspension was kept at 4 °C for 30 min. The white fluffy precipitate was filtered off and washed with MeOH (10 mL). The precipitate was recovered from the filter by dissolution in CH_2Cl_2 (10 mL), the solution was concentrated to dryness. Alternatively, purification of the same amount of diphosphate **44** (starting from 45 mg of **42**) by subsequent precipitation with EtOH from CH_2Cl_2 (2×) and chromatography on silica gel (1.5× 10 cm, 100:7→100:10 CH_2Cl_2/acetone) afforded 21 mg (0.088 mmol, 41%) of **44**. R_f 0.64 (B, 150:100:15:1 toluene/CH_2Cl_2/MeOH/H_2O), R_f 0.45 (B, 10:1 CH_2Cl_2/acetone); $[\alpha]_D^{20}$=+15.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.38–7.22 (m, 35H, 7PH), 6.92 (d, 1H, $^{3}J_{2',NH'}$=9.2 Hz, NH′), 6.24 (d, 1H, $^{3}J_{2,NH}$=8.8 Hz, NH), 5.67 (dd, 1H, $^{3}J_{1,2}$=3.2 Hz, $^{3}J_{1,P}$= 5.3 Hz, H-1), 5.27 (dd, 1H, $^{3'}J_{3',2'}$=10.6 Hz, $^{3}J_{3',4'}$= 9.1 Hz, H-3′), 5.26 (dd, 1H, $^{3}J_{3,2}$=11.0 Hz, $^{3}J_{3,4}$=9.5 Hz, H-3), 5.10 [m, 1H, βCH, (octadecanoyloxy)icosanoyl], 5.09–4.88 [m, 8H, $4CH_2$, $2(BnO)_2P(O)$–], 4.90 (d, 1H, $^{3}J_{1',2'}$=8.3 Hz, H-1′), 4.50 and 4.39 (AB, 2H, ^{2}J=12.0 Hz, CH_2Ph), 4.61 and 4.55 (AB, 2H, ^{2}J=11.7 Hz, CH_2Ph), 4.52 and 4.49 (AB, 2H, ^{2}J=12.1 Hz, CH_2Ph), 4.43 (t, 1H, $^{3}J_{4',5'}$=9.1 Hz, H-4′), 4.28 (ddd, 1H, H-2), 4.09 (m, 1H, H-5), 3.95–3.80 (m, 4H, H-2′, H-6a, H-6b, H-6a′), 3.73 (m, 1H, βC*H*OBn), 3.68 (dd, 1H, $^{2}J_{6a',6b'}$=11.2 Hz, $^{3}J_{6b',5'}$= 5.6 Hz, H-6b′), 3.55 (m, 1H, H-5′), 3.51 (t, 1H, H-4), 2.50–2.15 (m, 10H, $2^{Ico}\alpha CH_2$, $2^{Myr}\alpha CH_2$, $^{Ste}\alpha CH_2$), 1.65–1.40 (m, 10H, $2^{Myr}\beta CH_2$, $^{Ste}\beta CH_2$, $2^{Ico}\gamma CH_2$), 1.35–1.15 (m, 134H, $67CH_2$), 0.90 (t, 15H, $5CH_3$); ^{13}C NMR($CDCl_3$): δ 174.12 (CO), 173.85 (CO), 173.82 (CO), 171.59 (CONH), 170.34 (CONH), 138.79, 138.70, 137.68 (3C, $3C_6H_5$), 136.09, 136.01, 135.78, 135.69 [4C, Ph, $^{3}J_{C,P}$=4.0 Hz, $2(BnO)_2P(O)$–], 129.17, 128.92, 128.88, 128.72, 128.68, 128.43, 128.36, 128.31, 128.29, 128.15, 128.08, 127.97, 127.56 (25C, Ph), 100.24 (C-1′), 96.39 (C-1, $^{2}J_{1',P}$= 6.6 Hz), 76.10 (C-4), 75.94 ($^{Ico}\beta CH$), 75.31 (CH_2Ph), 74.86 (C-5′), 74.74 (C-4′, $^{2}J_{4',P}$=5.3 Hz), 73.69 (CH_2Ph), 73.42 (C-5), 72.15 (C-3), 72.14 (C-3′), 71.34 (CH_2Ph), 71.31 [βCH, (octadecanoyl oxy)icosanoyl], 69.36 and 70.30 [$2CH_2$, $^{2}J_{C,P}$=1.8 Hz, $(BnO)_2P(O)$–], 70.04 and 69.85 [$2CH_2$, $^{2}J_{C,P}$=7.5 Hz, $(BnO)_2P(O)$–], 68.13 (C-6′), 66.69 (C-6), 54.50 (C-2′), 52.61 (C-2, $^{2}J_{2,P}$= 8.7 Hz), 41.58 and 41.47 ($2^{Ico}\alpha CH_2$), 34.85, 34.64, 34.42, 34.35, 34.30 ($2^{Myr}\alpha CH_2$, $^{Ste}\alpha CH_2$, $2^{Ico}\gamma CH_2$), 32.33, 30.13, 30.08, 29.99, 29.92, 29.87, 29.77, 29.63, 25.78, 25.75, 25.42, 25.17, 24.98, 23.10 ($67CH_2$), 14.51 ($5CH_3$); ^{31}P NMR ($CDCl_3$): δ −1.6 (attached to C-4′), −2.9 (attached to C-1); MALDI-TOF-MS: *m/z*: 2200.67 $[M+Na]^+$.

3.1.32. Monotriethylammonium 2-deoxy-6-*O*-{2-deoxy-2-[(*R*)-3-hydroxyicosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxyicosanoylamino]-3-*O*-tetradecanoyl-α-D-glucopyranose 1,4′-bisphosphate (1). A solution of **43** (27 mg, 0.012 mmol) in 5:1 toluene/MeOH (10 mL) was hydrogenated in the presence of Pd/C (10%, 80 mg) at rt and atmospheric pressure for 20 h. The reaction mixture was diluted with 5:1 toluene/MeOH (10 mL), sonicated (10 min) and filtered through a membrane filter (0.45 μm, regenerated cellulose). The catalyst was removed from the filter, resuspended in THF (20 mL) and the suspension was sonicated (10 min) and again membrane-filtered. The procedure was repeated twice. The combined filtrates were concentrated at diminished pressure under N_2, dissolved in 4:1 $CHCl_3$/MeOH (5 mL) and let slowly adsorb on a DEAE-cellulose column (CH_3COO^--form, 1×8 cm) equilibrated with 2:3:1 $CHCl_3$/MeOH/H_2O. The column was washed with 2:3:1 $CHCl_3$/MeOH/H_2O (50 mL) and then developed with the stepwise gradient of 2:3:1 $CHCl_3$/MeOH/aq $CH_3COO^-HNEt_3^+$ (30 mL of $CHCl_3$/MeOH/0.06 M aq $CH_3COO^-HNEt_3^+$, 60 mL of $CHCl_3$/MeOH/0.08 M aq $CH_3COO^-HNEt_3^+$, 30 mL of $CHCl_3$/MeOH/0.2 M aq $CH_3COO^-HNEt_3^+$ and 100 mL of $CHCl_3$/MeOH/1 M aq $CH_3COO^-HNEt_3^+$). Appropriate fractions (**1** was eluted at 0.1 M aq $CH_3COO^-HNEt_3^+$) were collected, the total volume was adjusted to 240 mL by addition of 2:3:1 $CHCl_3$/MeOH/H_2O. The solution was transferred to an extraction funnel and converted to a two-phase Bligh–Dyer system by changing the solvent proportions to 2:2:1.8 by addition of $CHCl_3$ (40 mL) and water (68 mL). The phases were resolved, the lower phase was concentrated, the residue was redissolved in 2:3:1 $CHCl_3$/MeOH/H_2O (180 mL) and rendered to a Bligh–Dyer mixture by addition of $CHCl_3$ (40 mL), methanol (10 mL) and water (60 mL). The phases were resolved in the extraction funnel, the lower phase was separated and concentrated to afford **1** (10 mg, 0.0061 mmol, 51%). R_f 0.3 (A, 100:75:15 $CHCl_3$/MeOH/H_2O) or R_f 0.3 (A, 50:20:30:5:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/CH_3COOH); $[\alpha]_D^{20}$=+12 (*c* 0.2, 4:1 $CHCl_3$/MeOH); NMR data see Table 2; MALDI-TOF-MS: *m/z*: 1466.07 $[M-H_3PO_4+Na-H]^+$, 1482.44 $[M-H_3PO_4+K-H]^+$,

1564.07 $[M+Na]^+$, 1586.04 $[M-H+2Na]^+$; calcd 1466.07 $[M-H_3PO_4+Na-H]^+$, 1482.17 $[M-H_3PO_4+K-H]^+$, 1564.04 $[M+Na]^+$, 1586.02 $[M-H+2Na]^+$; ESI-MS-neg.: *m/z*: 1541.05 [M], calcd 1541.05 [M].

3.1.33. Monotriethylammonium 2-deoxy-6-*O*-{2-deoxy-2-[(*R*)-3-(octadecanoyloxy)icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxyicosanoylamino]-3-*O*-tetradecanoyl-α-D-glucopyranose 1,4′-bisphosphate (2). A solution of **44** (25 mg, 0.01 mmol) in 5:1 toluene/MeOH (10 mL) was hydrogenated in the presence of Pd/C (10%, 80 mg) at rt and atmospheric pressure for 20 h. The reaction mixture was diluted with 5:1 toluene/MeOH (10 mL), sonicated (10 min) and filtered over membrane filter as described for **1**. The combined filtrates were concentrated under N_2, dissolved in 4:1 $CHCl_3$/MeOH (5 mL) and purified on a DEAE-cellulose column (CH_3COO^--form, 1×8 cm) equilibrated with 2:3:1 $CHCl_3$/MeOH/H_2O. The column was washed with 2:3:1 $CHCl_3$/MeOH/H_2O (70 mL) and then developed with a stepwise gradient as described for **1**. Appropriate fractions (**2** was eluted at 0.1 M aq $CH_3COO^-HNEt_3^+$) were collected and the total volume was adjusted to 240 mL by addition of 2:3:1 $CHCl_3$/MeOH/H_2O, the solution was transferred to an extraction funnel and subjected to the Bligh–Dyer extraction as described for **1**. The phases were resolved in the extraction funnel and the lower phase was separated and concentrated under reduced pressure to afford **2** (8.5 mg, 0.0045 mmol, 45%) as monotriethylammonium salt. R_f 0.37 (A, 100:75:15 $CHCl_3$/MeOH/H_2O) or R_f 0.4 (A, 50:20:30:5:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/CH_3COOH); $[\alpha]_D^{20}=+8$ (*c* 0.7, 4:1 $CHCl_3$/MeOH); MALDI-TOF-MS: *m/z*: 1733.16 $[M-H_3PO_4+Na-H]^+$, 1749.17 $[M-H_3PO_4+K-H]^+$, 1755.16 $[M-H_3PO_4+2Na-2H]^+$, 1853.13 $[M-H+2Na]^+$, 1875.09 $[M-2H+3Na]^+$; calcd 1733.33 $[M-H_3PO_4+Na-H]^+$, 1749.44 $[M-H_3PO_4+K-H]^+$, 1755.32 $[M-H_3PO_4+2Na-2H]^+$, 1852.28 $[M-H+2Na]^+$, 1874.26 $[M-2H+3Na]^+$; ESI-MS-neg.: *m/z*: 1541.05 $[M-C_{18}H_{35}O]$, 1564.04 [M−Ste+Na], 1807.32 [M], calcd 1541.05 $[M-C_{18}H_{35}O]$, 1564.05 [M−Ste+Na], 1807.31 [M].

3.1.34. Monotriethylammonium 2-deoxy-6-*O*-{2-deoxy-2-[(*R*)-3-hydroxyicosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxyicosanoylamino]-3-*O*-tetradecanoyl-D-glucopyranose 4′-phosphate (3). A solution of **40** (20 mg, 0.01 mmol) in THF (10 mL) was hydrogenated in the presence of Pd/C (10%, 100 mg), at rt and atmospheric pressure for 20 h. The reaction mixture was diluted with THF (20 mL), sonicated (10 min) and filtered through PTFE-membrane (0.45 μm) syringe filter. The catalyst was removed from the filter, suspended in THF (20 mL) and the suspension was sonicated (10 min) and filtered [PTFE-membrane]. The combined filtrates were concentrated, dissolved in 4:1 $CHCl_3$/MeOH (3 mL) and slowly adsorbed on a resin bed of DEAE-cellulose column (CH_3COO^--form, 1.5×10 cm) equilibrated with 2:3:1 $CHCl_3$/MeOH/H_2O. The column was washed with 2:3:1 $CHCl_3$/MeOH/H_2O (50 mL) and then developed with a stepwise gradient of 2:3:1 $CHCl_3$/MeOH/aq $CH_3COO^-NH_4^+$ (0.02 M, 0.03 M, 0.04 M, 0.06 M, 0.08 M aq $CH_3COO^-NH_4^+$, 30 mL each). Appropriate fractions (**3** was eluted at 0.04–0.06 M aq $CH_3COO^-NH_4^+$) were collected and the total volume was adjusted to 120 mL by addition of 2:3:1 $CHCl_3$/MeOH/H_2O. The solution was transferred to an extraction funnel and rendered to a Bligh–Dyer mixture by addition of $CHCl_3$ (20 mL) and water (34 mL). The lower phase was concentrated and purified by chromatography on silica gel (1.5×20 cm) 50:20:20:3:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/CH_3COOH. Appropriate fractions were collected and concentrated. The residue was suspended in 1% aq Et_3N in water (10 mL) and freeze-dried which afforded **3** as monotriethylammonium salt (9 mg, 0.006 mmol, 60%). R_f 0.2 (A, 50:20:20:3:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/CH_3COOH) or R_f 0.65 (A, 100:75:15 $CHCl_3$/MeOH/H_2O); $[\alpha]_D^{20}=-16.4$ (*c* 0.6, $CHCl_3$/MeOH); MALDI-TOF-MS: *m/z*: 1484.14 $[M+Na]^+$, 1506.14 $[M-H+2Na]^+$; calcd 1484.09 $[M+Na]^+$, 1506.06 $[M-H+2Na]^+$; MALDI-TOF: *m/z*: $[M-H]^-$ 1460.07, calcd 1460.09 $[M-H]^-$.

3.1.35. Monotriethylammonium 2-deoxy-6-*O*-{2-deoxy-2-[(*R*)-3-(octadecanoyloxy)icosanoylamino]-3-*O*-tetradecanoyl-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxyicosanoylamino]-3-*O*-tetradecanoyl-D-glucopyranose 4′-phosphate (4). Compound **42** (40 mg, 0.018 mmol) was dissolved in 5:1 toluene/MeOH (10 mL) under sonication and the solution was hydrogenated in the presence of Pd/C (10%, 80 mg) at rt and atmospheric pressure for 20 h (the reaction mixture was sonicated every 30 min during first 2 h). The reaction mixture was diluted with 5:1 toluene/MeOH (10 mL), sonicated (10 min) and filtered over membrane filter (0.45 μ, regenerated cellulose). The catalyst was removed from the filter and suspended in THF (20 mL). The suspension was sonicated (10 min), and filtered over membrane filter (0.45 μ, regenerated cellulose); the procedure was repeated twice. The combined filtrates were concentrated, the residue was dissolved under sonication and heating (30 °C) in 50:20:20:3:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/CH_3COOH (3 mL) and purified by chromatography on silica gel (2×30 cm, 50:20:20:3:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/AcOH). Appropriate fractions were collected, concentrated under diminished pressure, redissolved in 4:1 $CHCl_3$/MeOH (5 mL) and slowly adsorbed on a resin bed of DEAE-cellulose column (CH_3COO^--form, 1×10 cm) equilibrated with 2:3:1 $CHCl_3$/MeOH/H_2O. The column was washed with 2:3:1 $CHCl_3$/MeOH/H_2O (50 mL) and then developed with stepwise gradient of 2:3:1 $CHCl_3$/MeOH/aq $CH_3COO^-NH_4^+$ (0.02 M, 0.04 M, 0.06 M, 0.08 M, 0.1 M aq $CH_3COO^-NHEt_3^+$, 50 mL each). Appropriate fractions (**4** was eluted at 0.06–0.08 M aq $CH_3COO^-NHEt_3^+$) were collected and the total volume was adjusted to 120 mL by addition of 2:3:1 $CHCl_3$/MeOH/H_2O. The solution was transferred to an extraction funnel and converted to a Bligh–Dyer system by addition of $CHCl_3$ (20 mL) and water (34 mL). The phases were resolved, the lower phase was concentrated to dryness to give **4** (16 mg, 0.0087 mmol, 49%) (triethylammonium salt). R_f 0.3 (A, 50:20:20:3:2 $CHCl_3$/*n*-hexane/MeOH/H_2O/CH_3COOH) or R_f 0.75 (A, 100:75:15 $CHCl_3$/MeOH/H_2O); $[\alpha]_D^{20}=-38$ (*c* 0.4, 4:1 $CHCl_3$/MeOH); MALDI-TOF-MS: *m/z*: 1750.43 $[M+Na]^+$, 1772.41 $[M-H+2Na]^+$; calcd 1750.35 $[M+Na]^+$, 1772.35 $[M-H+2Na]^+$.

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Synthesis of new thiazole analogues of pyochelin, a siderophore of *Pseudomonas aeruginosa* and *Burkholderia cepacia*. A new conversion of thiazolines into thiazoles

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Abstract—Three pyochelin analogues and their methyl esters all containing a thiazole ring have been synthesised from the same Weinreb amide key intermediate. One of these analogues called HPTT-COOH, a molecule released in the course of pyochelin and yersiniabactin biosynthesis, was efficiently synthesised using a new base induced conversion of the key compound 2′-(2-hydroxyphenyl)-2′-thiazoline-4′-(*N*-methoxy,*N*-methyl) carboxamide into 2′-(2-hydroxyphenyl)-2′-thiazole-4′-(*N*-methoxy,*N*-methyl) carboxamide.

1. Introduction

Under iron deficient conditions, microorganisms synthesise and excrete small molecules called siderophores which strongly chelate iron (III) and transport it into the cell.[1] In Gram-negative bacteria, the ferrisiderophore is recognised by a specific receptor in the outer membrane and the metal ion is then transported into the cytoplasm by a proton-motive force energised multiproteic system.[2] Our interest is focused on iron uptake systems in *Pseudomonas aeruginosa* and *Burkholderia cepacia*.[3] These bacteria are nosocomial opportunistic pathogens, causing severe and often lethal lung infections especially in cystic fibrosis patients. Both *P. aeruginosa* and *B. cepacia* excrete pyochelin **1**,[4] a hydroxyphenylthiazolinyl-thiazolidine type of siderophore which chelates iron (III) with a 2:1 stoichiometry.[5] We have recently reported the synthesis and biological properties of several synthetic pyochelin analogues and shown that both the 4′*R* and 4′*S* enantiomers of pyochelin chelate and transport iron(III) at very similar rates, suggesting that the configuration at carbon C-4′ has no effect on the biological properties of pyochelin.[6] These results prompted us to synthesise the thiazole pyochelin analogues **2**, **3** and **4** and to explore furthermore the influence of the C-4′, C-2″ and C-4″ assymetric centers on iron chelation and transport.

In compounds **2** and **3**, the thiazoline moiety was replaced by a thiazole ring where C-4′ and C-5′ are both sp^2, in contrast to pyochelin. In analogue **4** by replacement of the thiazolidine moiety with a thiazoline ring, C-4′ and C-2″ become sp^2 and C-4″ remains the only assymetric center (Fig. 1).

In this paper we describe the synthesis of two thiazole analogues of pyochelin **2** and **3** using the route developed in our laboratory for the synthesis of natural pyochelin **1** via

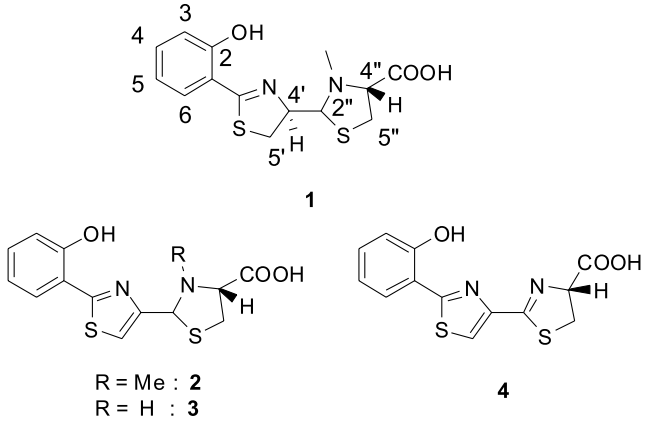

Figure 1.

Keywords: Pseudomonas; Siderophore; Pyochelin; Yersiniabactin; HPTT-COOH; Thiazole; Thiazoline; Weinreb amide.

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the thiazole hydroxamate **5** (Fig. 2).[7] We also report a new conversion procedure of thiazolines into thiazoles which we have applied to the synthesis of **4** a third thiazolic analogue of pyochelin.

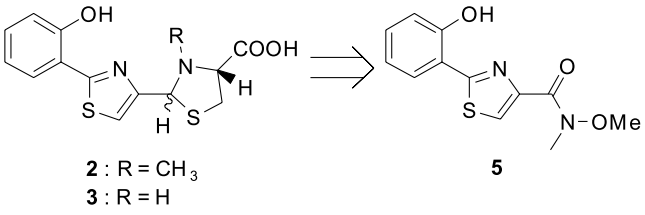
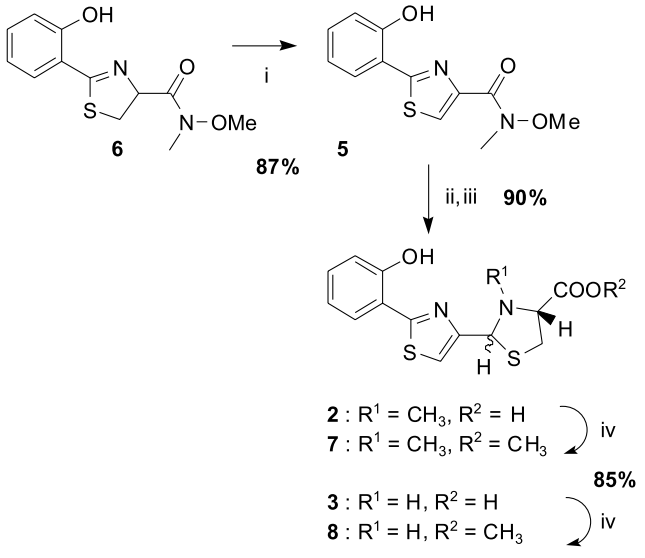


Figure 2.

2. Results and discussion

The thiazole intermediate **5**, was prepared from the known Weinreb amide **6**[7] which was used as starting material and reacted under several different conditions. First attempts performed using manganese dioxide under various conditions yielded the expected thiazole intermediate **5** but in poor to average yields. Better results were obtained when a mixture of $CBrCl_3$/DBU was used, giving compound **5** as a single product in 87% yield.[8] The Weinreb amide **5** was then reduced with lithium aluminum hydride,[9] into a very labile aldehyde. This latter was straightforwardly condensed with either (*R*)-cysteine or (*R*)-*N*-methylcysteine hydrochloride[10] in the presence of potassium acetate leading to the pyochelin analogues **3** and **2** respectively, both isolated in 90% yield over two steps. Compounds **2** and **3** were further converted into the corresponding methyl esters **7** and **8**, both isolated in 85% yield, using trimethylsilyldiazomethane (Scheme 1).



Scheme 1. (i) DBU, $CBrCl_3$, CH_2Cl_2, 20 °C. (ii) $LiAlH_4$, THF, −40 to −20 °C. (iii) (*R*)-cysteine or (*R*)-*N*-methylcysteine.HCl, AcOK, EtOH/H_2O, 20 °C. (iv) $TMSCHN_2$, MeOH/CH_2Cl_2, 20 °C.

Pyochelin is usually extracted from culture broth or synthesised as a mixture of diastereoisomers. Actually the very labile C-2″ position is readily epimerised in absence of metal.

Previous reports strongly suggest a template effect of both the metal ion and the configuration of the C-4″ asymmetric center, in the definition of the C-2″ stereocenter.[7c] In our hands, compounds **2**, **3**, **7** and **8** were isolated as mixtures of two diastereoisomers **a** (2″*R*, 4″*R*) and **b** (2″*S*, 4″*R*) in equimolar proportions. The relative configurations of the stereocenters were unambiguously assigned by NOESY experiments.[7,11] When proton H-2″ was saturated a marked Overhauser effect with H-4″ proton was observed for **a** (2″*R*, 4″*R*) isomer (i.e. *cis* isomer) whereas no NOE was observed for **b** (2″*S*, 4″*R*). In addition, using COSY and ^1H–^{13}C correlation it was then possible to assign the chemical shifts of both diastereoisomers for **2**, **3**, **7** and **8** (Fig. 3).

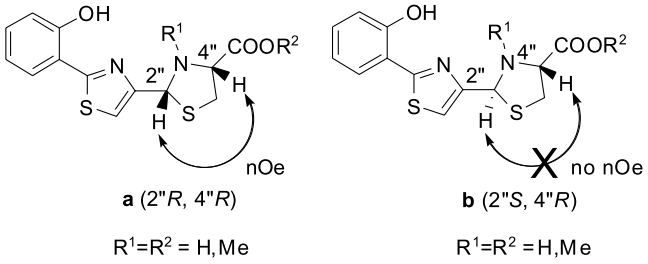
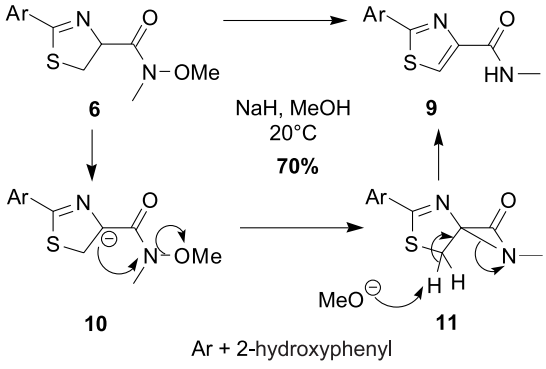


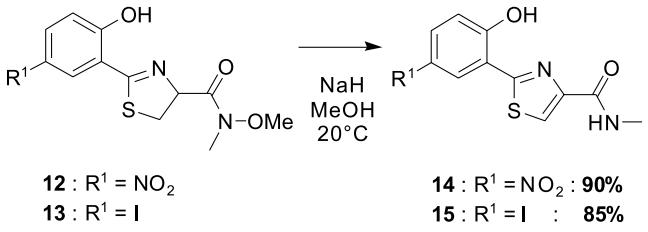
Figure 3.

In the course of the synthesis of hydroxamic ester **5** we have observed that if DBU was added a long time before $CBrCl_3$, the thiazole by-product **9** was isolated in significant amounts along with thiazole **5**. Moreover when $CBrCl_3$ was omitted, conversion of **6** into **9** proceeded sluggishly indicating that the basic nature of DBU promotes the conversion of **6** into **9**. Other base/solvent combinations were tested (TMSOK/THF, *t*BuOK/*t*BuOH, TBAF/THF, NaH/MeOH). The best yields were obtained when an excess of sodium hydride in dry methanol was used, where compound **6** was efficiently converted into compound **9** in 70% isolated yield. A plausible explanation of this result is illustrated in Scheme 2. First the base abstracts the acidic proton of the Schiff base **6** giving the intermediate **10**. Subsequent intramolecular attack of the carbanion on the methoxy amide and release of the methoxide anion affords the second intermediate **11**. Finally aromatisation and strain release favour the proton elimination and the cleavage of the aziridone moiety to yield compound **9** (Scheme 2).



Scheme 2.

In addition, when hydroxamic esters **12** and **13**, were treated in the same conditions, conversion into the corresponding 2-arylthiazole-4-methylcarboxamides **14** and **15** proceeded similarly, in very high yield. It is worthwhile pointing out that the best yield was obtained with the derivatives bearing the strongly electron withdrawing nitro group (Scheme 3).

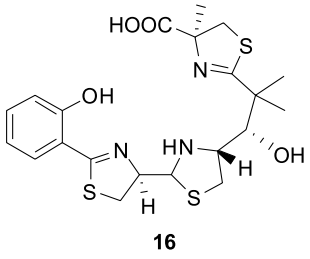


12 : $R^1 = NO_2$
13 : R^1 = I

14 : $R^1 = NO_2$: **90%**
15 : R^1 = I : **85%**

Scheme 3.

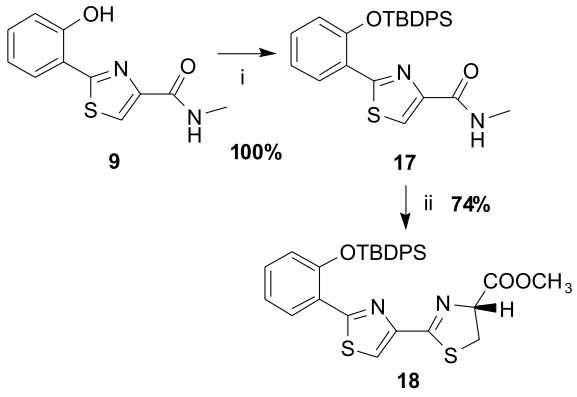
To the best of our knowledge this conversion of the Weinreb amide is unprecedented and we wished to apply it to the synthesis of other pyochelin thiazole analogues. In the literature, such thiazole-4-methylcarboxamide structurally related compounds were described recently as powerful synthons in an approach to natural thiazolylthiazoline compounds.[12] In connection to this observation, thiazole **9** should be a good substrate for a straightforward synthesis of molecules such as analogue **4**. This compound, called HPTT-COOH, was previously isolated and described as an oxidised form of an hydrolytic intermediate in the nonribosomal biosynthesis of pyochelin **1** and yersiniabactin **16** (Fig. 4).[13,14]



16

Figure 4.

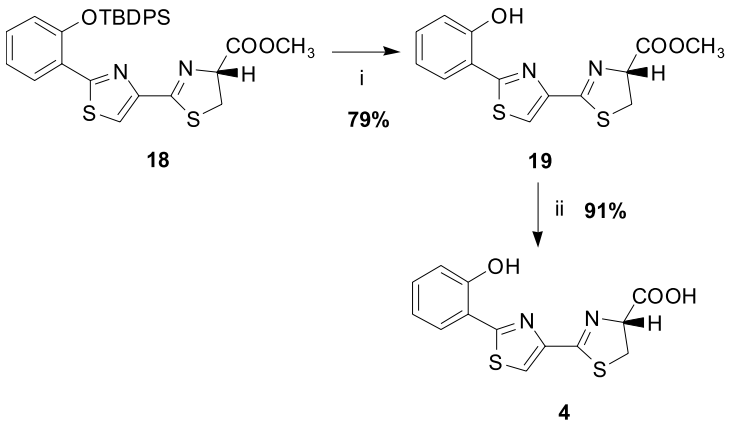
Initially, the phenol function of compound **9** was protected with a silyl group. Both *t*-butyldimethylsilyl and *t*-butyldiphenylsilyl groups were introduced in parallel studies but the latter proved to be more suitable due to its higher stability during the synthetic sequence.[7] Thus, compound **9** was treated with *t*-butyldiphenylsilyl chloride in presence of triethylamine, leading quantitatively to the corresponding silyl ether **17**. Using the conditions reported by Charette and co-workers,[12] compound **17** was successively treated with triflic anhydride and with *O*-methylcysteine hydrochloride, in the presence of pyridine, leading to the expected tricyclic compound **18**, isolated in 74% yield (Scheme 4).



18

Scheme 4. (i) TBDPSCl, NEt_3, CH_2Cl_2, 20 °C. (ii) (a) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −30 to 20 °C. (b) *O*-Methylcysteine.HCl, pyridine, −30 to 20 °C.

The silylated compound **18** appeared to be unstable and was therefore immediately deprotected with TBAF, leading to the methylester **19**, isolated in 79% yield. This ester was converted into the expected HPTT-COOH **4** after saponification with lithium hydroxide in wet tetrahydrofurane. This compound, which has been previously described in the literature,[13d] was actually prepared from a multienzymatic synthetic pathway and to the best of our knowledge the present report is the first which describes a straightforward efficient chemical synthetic access to HPTT-COOH (Scheme 5).



Scheme 5. (i) TBAF, THF, 20 °C. (ii) $LiOH \cdot H_2O$, THF/H_2O, 25 °C.

3. Conclusion

In conclusion we have synthesised in good overall yields, three pyochelin analogues **2**, **3** and **4** and their methyl esters **7**, **8** and **19**, bearing all a thiazole moiety. During the synthetic exploration, we have discovered a new base induced conversion of 2-aryl-4,5-dihydrothiazole-4-methoxymethylcarboxamide into the corresponding 2-arylthiazole-4-methylcarboxamide. This reaction was applied to the synthesis of HPTT-COOH **4**. The different pyochelin analogues described herein might be useful tools in order to investigate the pyochelin dependent iron uptake systems from siderophore biosynthesis to the ferripyochelin internalisation processes. Detailed analysis of pyochelin-dependent iron transport should help us to develop a new generation of antibiotics focused against emerging multi-resistant strains of *P. aeruginosa* and *B. cepacia*.

4. Experimental

4.1. General procedures

All reactions were carried out under argon. Solvents used were of analytical grade purity. Amines were distilled and stored on KOH before use. All reactions were monitored by thin-layer chromatography (TLC) using Merck precoated silica gel $60F^{254}$ (0.25 mm). Column chromatography purifications were performed using Merck kieselgel 60 (63–200 μm). Melting points were determined with a Stuart Scientific Bibby SMP3 apparatus. IR spectra were scanned neat using a Perkin–Elmer Spectrum one spectrophotometer. UV–visible spectra were measured on a Kontron Uvikon 930 spectrophotometer. NMR spectra were recorded either on a Bruker Avance 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) or a Bruker Avance 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C). Elemental analysis were performed at the Service d'Analyses de l'Institut de Chimie at Université Louis Pasteur of Strasbourg. Mass were performed on a Bruker Daltonic MicroTOF mass spectrometer.

4.1.1. 2′-(2-Hydroxyphenyl)-2′-thiazole-4′-(*N*-methoxy-*N*-methyl) carboxamide (5). To a solution of **6**[7] (521 mg, 1.96 mmol) and DBU (589 μL, 599 mg, 3.93 mmol, 2.00 equiv) in CH_2Cl_2 (20 mL), cooled to 0 °C was added dropwise $CBrCl_3$ (338 μL, 680 mg, 3.43 mmol, 1.75 equiv). After 20 h of gentle stirring at 20 °C, the mixture was adsorbed onto silica gel and filtered through a silica gel column (30 g SiO_2, hexane/acetone: 85/15). The expected thiazole **5** (452 mg, yield: 87%) was isolated as a white powder. Mp 111–114 °C, R_f 0.43 (hexane/acetone: 2/1), IR (neat) 3167, 2925, 2160, 1634, 1583, 1478, 1399, 1378, 1275, 1219, 1183, 1153, 1132, 1070, 1037, 1006, 976, 934, 889, 837, 818, 798, 751, 743, 706, 663 cm^{-1}. UV (MeOH) 214 (25240), 277 (10070), 323 (8320), ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.44 (s, 3H), 3.82 (s, 3H), 6.92–6.97 (m,1H), 7.08 (dd, J=8.2, 0.9 Hz, 1H), 7.36 (ddd, J=8.8, 7.3, 1.6 Hz, 1H), 7.65 (dd, J=7.9, 1.6 Hz, 1H), 8.03 (s, 1H), 11.96 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.89, 61.86, 116.50, 117.89, 119.58, 123.78, 127.34, 132.34, 147.44, 161.56, 163.75, 168.57. MS (ES+) *m/z* 265 ($M+H^+$, 100), 287 ($M+Na^+$, 49), 551 ($2M+Na^+$, 12), 582 (20). Anal. Calcd for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60. Found: C, 54.73; H, 4.64; N, 10.53.

4.1.2. ′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″,4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid (2) and 2′-(2-hydroxyphenyl)-4′-(2″,3″,4″,5″-tetrahydro)-[2″,4′]-bisthiazolyl-4″-carboxylic acid (3). To a solution of Weinreb amide **5** (150 mg, 0.57 mmol) in dry THF (8 mL), cooled down −60 °C, $LiAlH_4$ (0.74 mL of a 1 M solution in THF, 0.73 mmol, 1.30 equiv) was added dropwise by syringe. The reaction temperature was allowed to raise to −20 °C over 30 mn and then hydrolysed by successive additions of saturated aqueous solution of NH_4Cl (12 mL) and 1 M aqueous solution of $KHSO_4$ (5 mL). The mixture was allowed to warm to room temperature and vigorous stirring was applied until two phases were formed. After extraction with Et_2O (2×30 mL), the organic layers were combined, dried over Na_2SO_4 and filtered before being evaporated. The crude aldehyde, isolated as a yellow powder, was very sensitive to oxidation and was used directly for subsequent reactions. It was dissolved into a mixture of ethanol (20 mL) and water (6 mL) and to this solution were successively added, potassium acetate (650 mg, 3.79 mmol, 6.65 equiv) and either (*R*)-*N*-methyl-cysteine hydrochloride (207 mg, 1.21 mmol, 2.12 equiv) for the synthesis of **2** or (*R*)-cysteine (255 mg, 2.11 mmol, 3.70 equiv) for **3**. The mixture was then gently stirred in the dark during one hour before being successively washed with hexane (30 mL) and diluted with water (30 mL). This aqueous layer was then acidified to pH 2.0 by addition of solid citric acid before being extracted with CH_2Cl_2 (2× 35 mL). The organic layers were collected, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The expected pyochelin analogues **2** (165 mg, yield: 90%) and **3** (160 mg, yield: 90%) were respectively isolated as yellow powders.

4.1.3. 2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″,4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid (2). Mp 100–103 °C. IR (neat) 3432, 2956, 2526, 1977, 1716, 1619, 1579, 1456, 1380, 1307, 1248, 1207, 1156, 1017, 948, 856, 821, 751. SM (ES−) *m/z* 321 ($M-H^+$, 100), 643 ($2M-H^+$, 8).

4.1.4. (2″*R*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″, 4″, 5″-tetrahydro)-[2″,4′]bisthiazolyl-4″carboxylic carboxylic acid (2a). ^{1}H NMR (400 MHz, CD_3COCD_3) δ 2.63 (s, 3H), 3.34 (ABX, J_{AX}=6.6 Hz, J_{BX}=7.7 Hz, J_{AB}= 11.0 Hz, 2H), 3.80 (dd, J=7.7, 6.6 Hz, 1H), 5.35 (s, 1H), 6.94 (t, J=7.6 Hz, 1H), 6.99 (d, J=8.8 Hz, 1H), 7.33–7.39 (m, 1H), 7.69 (s, 1H), 7.72–7.77 (m, 1H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 34.33, 41.50, 72.07, 73.00, 115.92, 117.72, 118.19, 120.45, 128.09, 132.74, 156.51, 157.28, 169.69, 172.00.

4.1.5. (2″*S*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″, 4″,5″-tetrahydro-[2″,4′]bisthiazolyl-4″-carboxylic acid (2b). ^{1}H NMR (400 MHz, CD_3COCD_3) δ 2.52 (s, 3H), 3.24 (dd, J=10.4, 4.0 Hz, 1H), 3.46 (dd, J=10.4, 6.8 Hz, 1H), 4.30 (dd, J=6.8, 4.2 Hz, 1H), 5.62 (s, 1H), 6.94 (t, J= 7.6 Hz, 1H), 6.99 (d, J=8.8 Hz, 1H), 7.33–7.39 (m, 1H), 7.60 (s, 1H), 7.72–7.77 (m, 1H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 33.13, 36.71, 69.38, 69.96, 116.08, 117.83,

118.24, 120.42, 128.04, 132.74, 156.31, 157.41, 169.74, 171.52.

4.1.6. 2′-(2-Hydroxyphenyl)-4′-(2″,3″,4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid (3). Mp 165–168 °C. IR (neat) 3119, 3062, 3021, 2161, 1641, 1615, 1573, 1479, 1429, 1361, 1336, 1311, 1292, 1270, 1248, 1200, 1167, 1145, 1127, 1068, 1036, 1023, 1010, 950, 933, 906, 866, 843, 831, 819, 772, 729, 682. SM (ES−) *m/z* 307 ($M-H^+$, 100), 615 ($2M-H^+$, 51).

4.1.7. (2″*R*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(2″,3″,4″,5″-tetra hydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid (3a). ^{1}H NMR (400 MHz, CD_3SOCD_3) δ 3.01 (dd, $J=10.0$, 9.0 Hz, 1H), 3.39 (dd, $J=10.0$, 7.0 Hz, 1H), 3.93 (dd, $J=9.0$, 7.0 Hz, 1H), 5.73 (s, 1H), 6.92–7.02 (m, 2H), 7.28–7.33 (m, 1H), 7.72 (s, 1H), 8.06 (dd, $J=7.9$, 1.6 Hz, 1H), 11.14 (bs, 1H). ^{13}C NMR (100 MHz, CD_3SOCD_3) δ 38.33, 65.33, 66.99, 116.45, 116.56, 118.82, 119.45, 127.34, 131.08, 155.42, 163.66, 172.64, 174.52.

4.1.8. (2″*S*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(2″,3″,4″,5″-tetra hydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid (3b). ^{1}H NMR (400 MHz, CD_3SOCD_3) δ 3.06 (dd, $J=10.2$, 5.8 Hz, 1H), 3.31 (dd, $J=10.1$, 6.8 Hz, 1H), 4.31 (dd, $J=6.4$, 5.9 Hz, 1H), 5.89 (s, 1H), 6.92–7.02 (m, 2H), 7.28–7.33 (m, 1H), 7.56 (s, 1H), 8.01 (dd, $J=7.9$, 1.2 Hz, 1H), 11.22 (bs, 1H). ^{13}C NMR (100 MHz, CD_3SOCD_3) δ 38.00, 64.94, 66.90, 114.75, 116.56, 118.60, 119.43, 127.39, 131.08, 154.92, 164.13, 171.19, 172.21.

4.1.9. 2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″,4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid methyl ester (7) and 2′-(2-hydroxyphenyl)-4′-(2″,3″,4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid methylester (8). To a solution of **2** (77 mg, 0.24 mmol) or **3** (70 mg, 0.23 mmol) in a mixture of CH_2Cl_2 (8 mL) and MeOH (3 mL), trimethylsilyldiazomethane (480 μL of an approx. 2 M solution in hexane, 0.91 mmol, 4.00 equiv) was added dropwise in four successive injections (every 20 mn). After 16 h stirring at 20 °C, the mixture was evaporated and chromatographed on a silica gel column (5 g SiO_2, hexane/Et_2O: 1/1) leading respectively to methyl esters **7** (71 mg, yield: 85%) or **8** (63 mg, yield: 84%) isolated respectively as an orange oil and an yellow solid. Before NMR measurements, these compounds were purified again on preparative thin layer chromatography (eluent: Et_2O).

4.1.10. 2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″, 4″,5″-tetra hydro)-[2″,4′]bisthiazolyl-4″carboxylic acid methyl ester (7). IR (neat) 3108, 3043, 2992, 2950, 2850, 2790, 1737, 1619, 1580, 1475, 1456, 1436, 1400, 1346, 1269, 1250, 1216, 1201, 1155, 1057, 1018, 947, 909, 857, 821, 739. (ES+) *m/z* 337 ($M+H^+$, 100).

4.1.11. (2″*R*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″, 4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″carboxylic acid methyl ester (7a). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (s, 3H), 3.20 (dd, $J=11.0$, 6.2 Hz, 1H), 3.33 (dd, $J=10.8$, 9.2 Hz, 1H), 3.69 (dd, $J=9.2$, 6.1 Hz, 1H), 3.76 (s, 3H), 5.20 (s, 1H), 6.90 (m, 1H), 7.05 (m, 1H), 7.32 (m, 1H), 7.48 (s, 1H), 7.60 (d, $J=10.5$ Hz, 1H), 12.00 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.76, 41.49, 52.46, 71.11, 72.43, 114.26, 116.90, 117.77, 119.36, 127.01, 131.80, 155.27, 156.78, 169.46, 171.16.

4.1.12. (2″*S*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(3″-methyl-2″,3″, 4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid methyl ester (7b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 3.20 (dd, $J=10.8$, 4.6 Hz, 1H), 3.46 (dd, $J=10.5$, 6.7 Hz, 1H), 3.80 (s, 3H), 4.20 (dd, $J=6.6$, 4.1 Hz, 1H), 5.54 (s, 1H), 6.90 (m, 1H), 7.05 (m, 1H), 7.27 (s, 1H), 7.32 (m, 1H), 7.61 (d, $J=10.5$ Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.71, 36.61, 52.13, 68.36, 69.26, 113.95, 117.74, 119.37, 127.01, 131.80, 156.13, 156.70, 169.57, 171.69.

4.1.13. 2′-(2-Hydroxyphenyl)-4′-(2″,3″,4″,5″-tetrahydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid methylester (8). Mp 80–83 °C. IR (neat) 3482, 3272, 3101, 3000, 2949, 1732, 1619, 1582, 1475, 1431, 1402, 1377, 1332, 1304, 1265, 1203, 1175, 1157, 1137, 1037, 1005, 974, 948, 925, 880, 845, 821, 790, 743, 721, 700. (ES+) *m/z* 323 ($M+H^+$, 100).

4.1.14. (2″*R*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(2″,3″,4″,5″-tetra hydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid methyl ester (8a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.11–3.19 (m, 1H), 3.40–3.52 (m, 1H), 3.84 (s, 3H), 4.04 (m, 1H), 5.71 (s, 1H), 6.88–6.95 (m, 1H), 7.04–7.08 (m, 1H), 7.29–7.37 (m, 1H), 7.31 (d, $J=0.5$ Hz, 1H), 7.59–7.64 (m, 1H), 11.76 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.83, 52.73, 65.80, 66.94, 114.11, 116.72, 117.91, 119.54, 127.24, 132.19, 152.67, 155.90, 170.10, 171.23.

4.1.15. (2″*S*,4″*R*)-2′-(2-Hydroxyphenyl)-4′-(2″,3″,4″,5″-tetra hydro)-[2″,4′]bisthiazolyl-4″-carboxylic acid methyl ester (8b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.11–3.19 (m, 1H), 3.40–3.52 (m, 1H), 3.83 (s, 3H), 4.27 (t, $J=6.6$ Hz, 1H), 5.94 (s, 1H), 6.88–6.95 (m, 1H), 7.04–7.08 (m, 1H), 7.22 (d, $J=0.9$ Hz, 1H), 7.29–7.37 (m, 1H), 7.59–7.64 (m, 1H), 11.95 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.42, 52.73, 64.64, 66.30, 112.62, 116.86, 117.80, 119.45, 127.13, 132.02, 152.67, 156. 75, 170.10, 171.83.

4.1.16. 2′-(2-Hydroxyphenyl)-thiazole-4′-*N*-methyl carboxamide (9). To a solution of **6** (469 mg, 1.76 mmol) in MeOH (50 mL) stirred at 23 °C was added portionwise NaH (220 mg of a 60% w/w dispersion in mineral oil, 132 mg, 5.52 mmol, 3.13 equiv). After 20 mn of gentle stirring, the mixture was carefully hydrolysed with saturated aqueous NH_4Cl (5 mL), diluted with water (50 mL) and extracted with EtOAc (50 mL). The organic layer was dried over Na_2SO_4, filtered and adsorbed onto silica gel before being filtered through a silica gel column (30 g SiO_2, cyclohexane/Et_2O: 1/1). The resulting pale yellow solid was then crystallised from hot cyclohexane/ethanol leading to the expected methylamide **9** (286 mg, yield: 70%) isolated as a white solid. Mp 172–174 °C, R_f 0.51 (CH_2Cl_2/MeOH: 95/5), IR (neat) 3397, 3113, 3050, 2949, 2735, 2577, 1650, 1602, 1486, 1457, 1412, 1378, 1322, 1306, 1278, 1261, 1247, 1209, 1157, 1107, 1036, 984, 948, 921, 847, 834, 802, 777, 749, 720, 693 cm^{-1}. UV (MeOH) 214 (28455), 223 (20855), 277 (10870), 323 (10678), ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (d, $J=5.0$ Hz, 3H), 6.86 (bs, 1H), 6.96 (ddd, $J=7.9$, 7.1, 1.1 Hz, 1H), 7.08 (dd, $J=8.3$, 0.8 Hz, 1H), 7.38 (ddd, $J=8.6$, 7.36, 1.5 Hz, 1H), 7.65 (dd, $J=7.9$, 1.7 Hz,

1H), 8.10 (s, 1H), 11.16 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.32, 116.39, 117.79, 120.05, 121.79, 127.60, 132.57, 148.00, 156.17, 162.00, 169.52. SM (ES+) *m/z* 235 ($M+H^+$, 26), 257 ($M+Na^+$, 100), 491 ($2M+Na^+$, 20), 522 (29). Anal. Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.96. Found: C, 56.51; H, 4.63; N, 11.73.

4.1.17. 2′-[2-(*t*-Butyldiphenylsilyloxy)-phenyl]-thiazole-4′-*N*-methylcarboxamide (17). To a solution of methylamide **9** (161 mg, 0.69 mmol) dissolved in a 3:1 mixture of CH_2Cl_2 and NEt_3 (4 mL) at 20 °C was added TBDPSCl (500 μL, 537 mg, 1.95 mmol, 2.84 equiv). After 16 h of gentle stirring, the mixture was evaporated under reduced pressure. The crude product was filtered through a silica gel column (20 g SiO_2, cyclohexane/EtOAc: 90/10 then cyclohexane/EtOAc: 80/20) leading to the expected protected phenol **17** (330 mg, yield: 100%) isolated as a white foam. Mp 130–133 °C, R_f 0.70 (Et_2O), IR (neat) 3643, 3340, 3076, 2950, 2932, 2856, 2161, 1978, 1655, 1636, 1579, 1539, 1496, 1449, 1427, 1404, 1392, 1361, 1290, 1239, 1222, 1189, 1163, 1112, 1052, 1031, 1017, 988, 944, 923, 890, 859, 826, 807, 775, 758, 746, 736, 706, 693, 681 cm^{-1}. UV (MeOH) 213 (50223), 289 (13000), 308 (12130), ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 9H), 3.06 (d, J=5.1 Hz, 3H), 6.55 (dd, J=7.9, 1.6 Hz, 1H), 6.89–6.99 (m, 2H), 7.36–7.46 (m, 6H), 7.53 (bs, 1H), 7.74–7.77 (m, 4H), 8.19–8.22 (m, 1H), 8.21 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.38, 26.03, 26.48, 120.52, 121.28, 123.15, 123.38, 128.01, 129.25, 130.16, 130.48, 131.87, 135.30, 149.22, 153.08, 162.15, 162.95. SM (ES+) *m/z* 473 ($M+H^+$, 50), 495 ($M+Na^+$, 91), 967 ($2M+Na^+$, 100). Anal. Calcd for $C_{27}H_{28}N_2O_2SSi$: C, 68.61; H, 5.97; N, 5.93. Found: C, 68.28; H, 6.22; N, 5.42.

4.1.18. 2′-[2-(*t*-Butyldiphenylsilyloxy)-phenyl]-4″,5″-dihydro-[2″,4′]bisthiazolyl-4″-carboxylic acid methyl ester (18). To a solution of **17** (330 mg, 0.71 mmol) in CH_2Cl_2 (5 mL) cooled to −30 °C, was added pyridine (218 μL, 215 mg, 2.71 mmol, 3.87 equiv). After 5 mn, Tf_2O (183 μL, 305 mg, 1.08 mmol, 1.55 equiv) was added dropwise by syringe. The mixture was allowed to warm to 21 °C and stirred gently for 2 h. The resulting orange solution was cooled down again to −30 °C before pyridine (218 μL, 215 mg, 2.71 mmol, 3.87 equiv) and *O*-methylcysteine hydrochloride (186 mg, 1.08 mmol, 1.55 equiv) were successively introduced. After 15 mn stirring at −30 °C, the mixture was warmed up to room temperature and stirred overnight. The mixture was then adsorbed onto silica gel before being purified by chromatography on a silica gel column (20 g SiO_2, cyclohexane/EtOAc: 8/2) leading to compound **18** (289 mg, yield: 74%) isolated as an unstable deliquescent colorless solid. R_f 0.86 (Et_2O), IR (neat) 3073, 2930, 2858, 1742, 1674, 1598, 1577, 1535, 1493, 1472, 1449, 1429, 1391, 1362, 1326, 1289, 1240, 1219, 1197, 1173, 1111, 1049, 1009, 973, 901, 882, 821, 804, 758, 735, 699, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (s, 9H), 3.70 (ABX, J_{AX}=9.0 Hz, J_{BX}=9.7 Hz, J_{AB}=11.3 Hz, 2H), 3.86 (s, 3H), 5.36 (t, J=9.3 Hz, 1H), 6.52 (dd, J=8.2, 1.1 Hz, 1H), 6.86–6.99 (m, 2H), 7.36–7.48 (m, 4H), 7.75–7.78 (m, 6H), 8.18 (s, 1H), 8.32 (dd, J=7.9, 2.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.14, 26.51, 34.95, 52.80, 78.51, 120.27, 121.37, 121.49, 123.12, 128.01, 129.71, 130.13, 130.40, 131.87, 135.32, 147.82, 152.97, 162.94, 166.50, 171.41. SM (ES+) *m/z* 559 ($M+H^+$, 100), 581 ($M+Na^+$, 20), 1117 ($2M+H^+$, 23), 1139 ($2M+Na^+$, 50).

4.1.19. 2′-(2-Hydroxyphenyl)-4″,5″-dihydro-[2″,4′]bis thiazolyl-4″-carboxylic acid methyl ester (19). To a stirred solution of **18** (840 mg, 1.50 mmol) in CH_2Cl_2 (40 mL), TBAF (1.70 mL, 1 M solution in THF, 1.70 mmol, 1.20 equiv) was added dropwise at 20 °C. After 20 mn, the mixture was adsorbed onto silica gel then filtered through a silica gel column (20 g SiO_2, pentane/Et_2O: 7/3 then pentane/Et_2O: 3/7). The phenolic compound **19** (376 mg, yield: 79%) was isolated as a white solid. Mp 100–102 °C, R_f 0.61 (Et_2O), IR (neat) 3296, 3123, 3067, 3009, 2954, 2160, 1978, 1719, 1622, 1600, 1584, 1506, 1478, 1443, 1427, 1330, 1304, 1258, 1211, 1172, 1158, 1110, 1030, 997, 971, 931, 896, 840, 827, 788, 743, 699, 684 cm^{-1}. UV (MeOH) 218 (41800), 281 (24600), 325 (16800), ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (ABX, J_{AX}=9.1 Hz, J_{BX}=9.7 Hz, J_{AB}=11.2 Hz, 2H), 3.86 (s, 3H), 5.32 (t, J=9.5 Hz, 1H), 6.93–6.96 (m, 1H), 7.08 (dd, J=8.2, 0.93 Hz, 1H), 7.36 (ddd, J=8.6, 7.3, 1.6 Hz 1H), 7.61 (dd, J=7.9, 1.5 Hz, 1H), 8.02 (s, 1H), 11.53 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.17, 52.88, 78.47, 116.23, 118.02, 118.68, 119.65, 127.32, 132.47, 147.36, 156.73, 164.82, 169.06, 171.02. SM (ES+) *m/z* 321 ($M+H^+$, 60), 343 ($M+Na^+$, 100), 641 ($2M+H^+$, 19), 663 ($2M+Na^+$, 83), 694 (9). Exact mass calcd for $C_{30}H_{31}N_2O_3S_2Si$ ($M+H^+$): 321.0368, found: 321.0406.

4.1.20. 2′-(2-Hydroxyphenyl)-4″,5″-dihydro-[2″,4′]bis thiazolyl-4″-carboxylic acid (*HPTT-COOH*) (4). To a solution of ester **19** (80 mg, 0.25 mmol) in THF (5 mL) and water (2 mL) was added powdered $LiOH\cdot H_2O$ (24 mg, 0.58 mmol, 2.30 equiv). After two hours of gentle stirring at room temperature (25 °C) the mixture was diluted with water (20 mL) and washed with Et_2O (20 mL) before being acidified to pH 2.0 with 0.5 N HCl solution. After evaporation under reduced pressure, the resulting pale yellow solid was extracted twice with hot ethanol. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting pale yellow powder was then recrystallised from MeOH yielding HPTT-COOH **4** (70 mg, yield: 91%) isolated as a light beige powder. Mp 220 °C (dec), R_f 0.69 (CH_2Cl_2/*i*PrOH/HCOOH: 66/33/1). IR (neat) 3514, 3379, 3094, 3028, 1713, 1672, 1616, 1502, 1475, 1394, 1361, 1314, 1292, 1271, 1251, 1224, 1194, 1180, 1165, 1141, 1053, 1036, 1019, 957, 941, 920, 900, 846, 826, 816, 783, 765, 757, 740, 703, 681, 661 cm^{-1}, ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 3.61 (ABX, J_{AX}=8.2 Hz, J_{BX}=9.7 Hz, J_{AB}=11.3 Hz, 2H), 5.29 (dd, J=8.2, 9.6 Hz, 1H), 6.95–7.01 (m, 1H), 7.08 (d, J=8.2 Hz, 1H), 7.34 (ddd, J=8.4, 7.1, 1.6 Hz 1H), 8.13 (dd, J=7.9, 1.6 Hz, 1H), 8.31 (s, 1H), 11.20 (bs, 1H), 12.99 (sl, 1H). ^{13}C NMR (75 MHz, CD_3SOCD_3) δ 34.32, 78.39, 116.46, 118.78, 119.62, 122.04, 127.37, 131.47, 146.78, 155.04, 162.93, 163.76, 171.86. SM (ES−): 305 ($M-H^+$, 100), 611 ($2M-2H^+$, 95).

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cis- and *trans*-*N*-(Benzylsulfinyl)hexahydrobenzoxazolidin-2-ones as novel chiral sulfinyl transfer reagents

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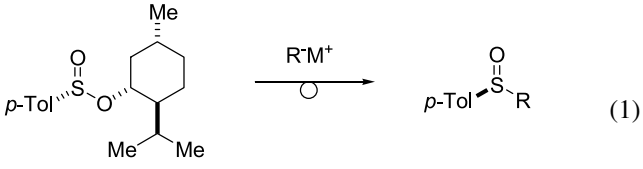
Abstract—The synthesis of *N*-benzylsulfinyl derivatives **5a–d** from both pairs of enantiomeric hexahydrobenzoxazolidin-2-ones **4a–d** is reported. The use of **5a–d** as effective chiral sulfinylating reagents in the preparation of enantiopure sulfoxides (e.e.>98%) is also reported.

1. Introduction

The interest in tricoordinated sulfur compounds in general, and sulfoxides in particular, has increased exponentially in the past two decades as consequence of the enormous potential of the chiral sulfinyl group as auxiliary in asymmetric synthesis.[1] Accordingly, the search for efficient and general methods in the preparation of chiral enantiopure sulfoxides continues to be a matter of great importance.

Salient methods for the preparation of non-racemic chiral sulfoxides can be divided into three classes: (1) kinetic resolution (either chemical[2a] or enzymatic[2b,c]) of racemic sulfoxides, (2) asymmetric oxidation of prochiral sulfides; especially with Sharpless reagent,[3a] with chiral oxaziridines,[3b] or in the presence of Noyori's BINOL ligand,[3c] and (3) stereospecific sufinylation of organometallic nucleophiles with chiral sulfinyl transfer reagents.[4–6]

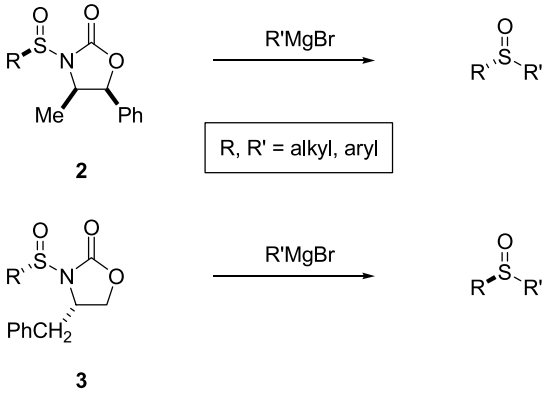
Special mention deserves the early (and still frequently used) Andersen method,[4a,b] which utilizes (*S*)-menthyl *p*-toluensulfinate (**1**) in the transfer of a chiral *p*-toluensulfinyl moiety to various types of nucleophilic organometallics with very high enantioselectivity (Eq. 1).



(1)

1

While the Andersen method (Eq. 1) is restricted to the synthesis of aryl-alkyl or diaryl sulfoxides, Evans et al.[6] reported in 1992 a new class of chiral sulfinyl transfer reagents, *N*-sulfinyloxazolidinones **2** and **3** (R = alkyl, aryl; Scheme 1). These sulfinylating agents were shown to react with Grignard reagents with inversion of configuration at the sulfur center to afford the derived chiral sulfoxides in high yields and enantioselectivities[6] (Scheme 1).



2

3

Scheme 1.

Keywords: Oxazolidinones; Sulfoxides; Sulfinyl transfer; Diastereoselective reactions; Enantioselective synthesis.

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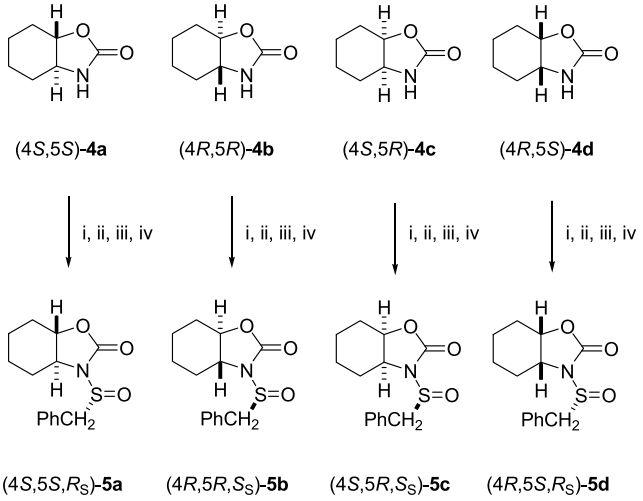
Recently, we reported a convenient procedure for the preparation of both pairs of enantiomeric hexahydrobenzoxazolidin-2-ones **4a–d** from inexpensive cyclohexene oxide and (*S*)-α-phenylethylamine.[7,8] We now report the use of *N*-benzylsulfinyl derivatives **5a–d** as effective chiral sulfinylating reagents in the preparation of enantiopure sulfoxides (Scheme 2).



Scheme 2. Conditions: i, *n*-BuLi, THF, 0 °C, 30 min. ii, $BnSSO_2Bn$, THF, −78 °C. iii, $NaIO_4$, $MeOH/H_2O$, 30–42 h, 0 °C. iv, Fractional recrystallization or preparative TLC.

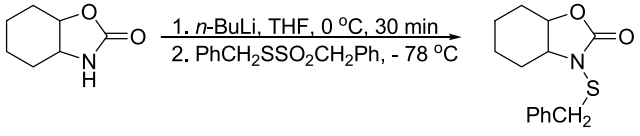
2. Results and discussion

2.1. Synthesis of *N*-(benzylsulfinyl)oxazolidinones 5a–d

The reaction of the lithiated oxazolidinones **4**-Li (obtained by treatment of **4a–d** with *n*-butyllithium at 0 °C)[9] with benzylthiosulfonate ester $PhCH_2SSO_2CH_2Ph$ proceeded in excellent yields to give crystalline products **6a–d** (Table 1).

Oxidation of *N*-sulfides **6a–b** to the desired *N*-sulfoxides **5a–b** was first attempted with *m*-chloroperoxybenzoic acid (*m*-CPBA), according to the conditions reported by Evans et al.[6] for the preparation of *N*-sulfinyloxazolidinones **3**. However, a ca. 1:1 mixture of diastereoisomeric sulfoxides **5** and *N*-sulfonyloxazolidinone **7** was produced under this condition (entry 1 in Table 2). Improved ratios of the *N*-sulfinyloxazolidinones **5** were obtained by the use of

Table 1. *N*-Thiobenzylation of hexahydrobenzoxazolidin-2-ones **4a–d** with benzylthiosulfonate ester

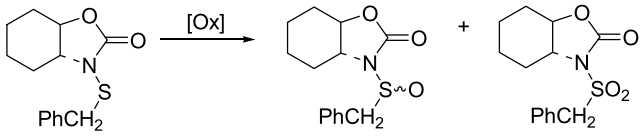


4a-d **6a-d**

Oxazolidinone	Product	Yield[a] (%)	Mp (°C)	$[\alpha]_D^{20b}$
(4*S*,5*S*)-**4a**	**6a**	91	102–103	−100.1
(4*R*,5*R*)-**4b**	**6b**	92	104–105	+98.1
(4*S*,5*R*)-**4c**	**6c**	90	50–51	−128.1
(4*R*,5*S*)-**4d**	**6d**	90	50–51	+131.1

[a] After purification by column chromatography.
[b] In $CHCl_3$, concentrations in Section 3.

Table 2. Oxidation of *N*-(thiobenzyl)oxazolidinones **6a** and **6b** with *m*-CPBA and $NaIO_4$



6a or **6b** **5** (mixture of diastereoisomers) **7**

Entry	[Ox] (equiv)	Time (h)	Temperature (°C)	Yield (%)	Product ratio[a] **5**:**7**
1	*m*-CPBA (1.5)	24	−20	66	50:50
2	*m*-CPBA (1.0)	3	−25	40	75:25
3	*m*-CPBA (1.0)	1.5	10	73	92:8
4	$NaIO_4$ (2.0)	48	25	70	98:2
5	$NaIO_4$ (3.0)	42	25	92	98:2

[a] Determined by ^1H NMR spectroscopy in the crude product.

1.0 equiv of *m*-CPBA (instead of 1.5 equiv; entries 2 and 3 in Table 2). Clearly, the use of a single equivalent of *m*-CPBA oxidant minimizes formation of the *N*-sulfonyl derivative **7**. Furthermore, faster reaction at 10 °C instead of −25 °C provided a better **5**:**7** ratio. Nevertheless, best results were observed with $NaIO_4$ as oxidant[10] (3.0 equiv, entry 5 in Table 2).

Once the optimum condition for the oxidation of *N*-sulfides **6** had been established, we proceeded to determine the diastereomeric ratios in the mixtures of sulfoxides **5**. The reaction of *N*-(thiobenzyl)hexahydrobenzoxazolidinone *trans*-(4*S*,5*S*)-**6a** in methanol with an aqueous solution of $NaIO_4$ at 0 °C afforded a 6:1 mixture of the expected diastereoisomeric *N*-sulfoxides. The major product was purified by fractional crystallization from methylene chloride–petroleum ether (5:95) (entry 1 in Table 3).

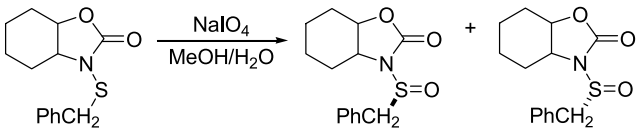
As expected, oxidation of *N*-sulfide *trans*-(4*R*,5*R*)-**6b** under the same conditions gave the enantiomeric sulfoxides in a similar 6:1 ratio (entry 2 in Table 3).

In contrast with the high diastereoselectivity observed in the oxidation of *trans*-*N*-(thiobenzyl)oxazolidinones **6a** and **6b**, the oxidation of *cis* congeners **6c** and **6d** proceeded with low, 1.6:1, diastereoselectivity (entries 3 and 4 in Table 3). Nevertheless, the major products **5c** and **5d** were easily purified by preparative TLC (petroleum ether–ethyl acetate, 2:1, eluent).

The absolute configuration at sulfur in sulfoxide (4*S*,5*S*,R_S)-**5a** was assigned by X-ray diffraction analysis from a suitable crystal of the major product from oxidation of sulfide (4*S*,5*S*)-**6a** (Fig. 1). Since the major diastereoisomeric sulfoxide product derived from enantiomeric (4*R*,5*R*)-**6b** presented same physical and spectroscopic properties but opposite sign of the optical rotation, its absolute configuration was assigned as (4*R*,5*R*,S_S)-**5b**.

The absolute configuration at the stereogenic sulfur in sulfoxide (4*R*,5*S*,R_S)-**5d** was similarly obtained by X-ray diffraction analysis from a suitable crystal (Fig. 2). Again, the major product from oxidation of (4*S*,5*R*)-**6c** was safely assigned as (4*S*,5*R*,S_S)-**5c** since it exhibited identical

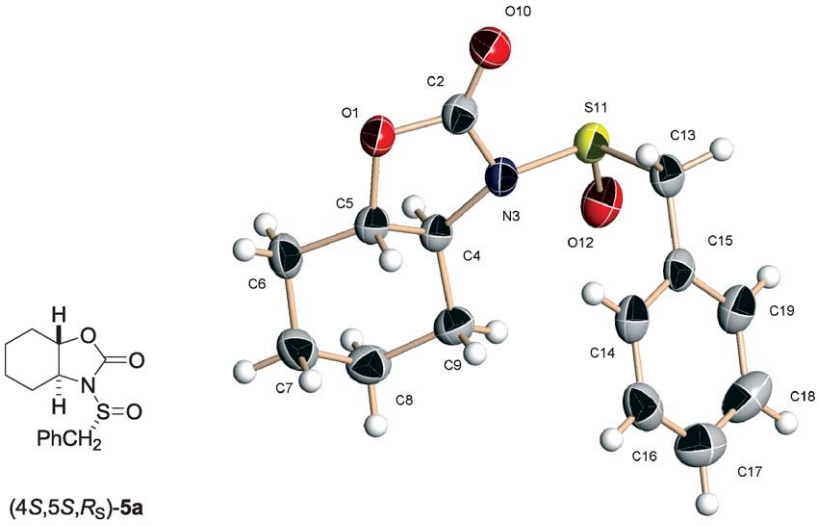
Table 3. Diastereoselectivity of the oxidation of *N*-(thiobenzyl)hexahydrobenzoxazolidinones **6** with $NaIO_4$



6 **5**

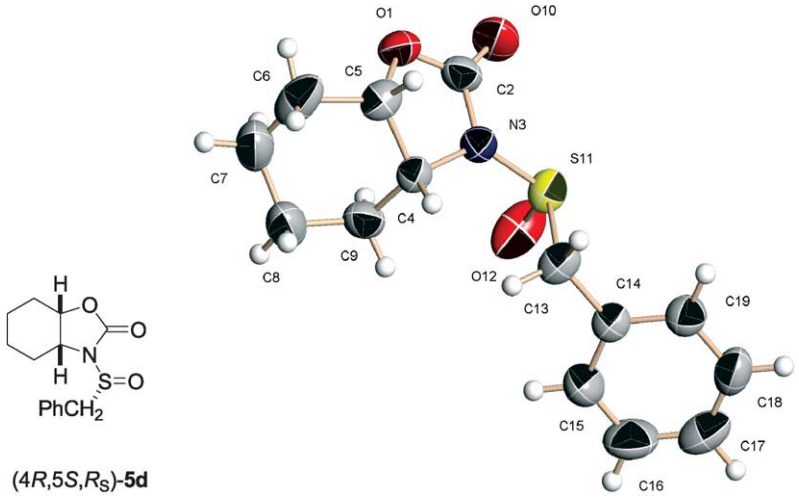
Entry	Substrate	Major product[a]	d.r. (R_S:S_S)	Yield[b] (%)	Mp[c] (°C)	$[\alpha]_D$[d]
1	(4*S*,5*S*)-**6a**	(4*S*,5*S*,R_S)-**5a**	6:1	90(50)	105–106	−30.6
2	(4*R*,5*R*)-**6b**	(4*R*,5*R*,S_S)-**5b**	1:6	90(55)	102–103	+32.0
3	(4*S*,5*R*)-**6c**	(4*S*,5*R*,S_S)-**5c**	1:1.6	80(46)	98–99	+185.3
4	(4*R*,5*S*)-**6d**	(4*R*,5*S*,R_S)-**5d**	1.6:1	80(43)	96–97	−184.1

[a] The configuration at sulfur was assigned by X-ray diffraction crystallography.
[b] Of the diastereoisomeric mixture (of the purified major isomer).
[c] Of the major product.
[d] In $CHCl_3$ concentrations in Section 3.



(4*S*,5*S*,R_S)-**5a**

Figure 1. Structure and solid-state conformation of (4*S*,5*S*,R_S)-*N*-(benzylsulfinyl)hexahydrobenzoxazolidin-2-one **5a**.[11]



(4*R*,5*S*,R_S)-**5d**

Figure 2. Structure and solid state conformation of (4*R*,5*S*,R_S)-*N*-(benzylsulfinyl)hexahydrobenzoxazolidin-2-one **5d**.[11]

melting points and NMR spectra, but an opposite sign in its optical rotation, upon comparison with (4*R*,5*S*,R_S)-**5d**.

2.2. Molecular modeling of the preferred conformations adopted by *N*-sulfides 6

The contrasting behavior of *cis*- and *trans*-*N*-(thiobenzyl)-hexahydrobenzoxazolidin-2-ones **6** can be understood with consideration of their most likely reactive conformations during oxidation, as predicted by HF/6-31G(d,p) ab initio calculations.

Indeed, the structure of lowest energy for *N*-sulfide **6b** (Fig. 3) clearly shows that steric hindrance should inhibit approach by the oxidant agent on the pro-(*R*) sulfur lone pair. As a consequence, the preferred pathway for oxidation involves the pro-(*S*) lone pair at sulfur, leading to formation of *S*-configurated *N*-sulfoxide (4*R*,5*R*,S_S)-**5b** as experimentally observed (entry 2 in Table 3).

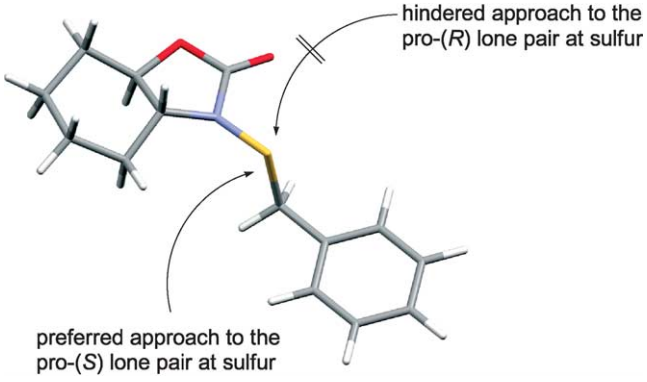


Figure 3. Lowest-energy structure calculated at HF/6-31G(d,p) ab initio level for (4*R*,5*R*)-**6b**.

Furthermore, the calculated electrostatic potential for the lowest-energy conformation of (4*R*,5*R*)-**6b** [DFT calculations[12,13] at the B3LYP/6-31G(d,p) level] shows increased electron density at the sulfur pro-(*S*) lone pair, which is in line with the experimentally observed S_S major sulfoxide product.

Along similar lines of thought, the optimized [HF/6-31G(d,p) level] structure for *cis*-*N*-(thiobenzyl)hexahydrobenzoxazolidin-2-one (4*R*,5*S*)-**6d** (Fig. 4) shows that both lone pairs at sulfur are accessible for approach by the oxidizing agent.

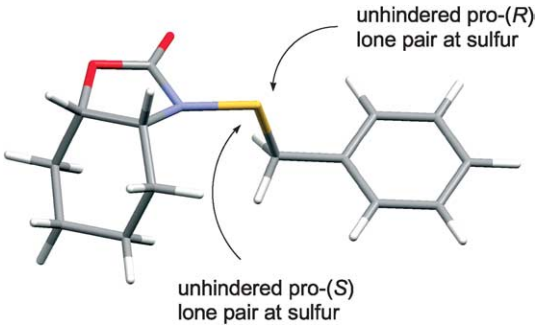


Figure 4. Lowest-energy structure calculated at the HF/6-31G(d,p) ab initio level for (4*R*,5*S*)-**6d**.

This observation is in line with the low selectivity found in the oxidation reaction (entries 3 and 4 in Table 3).

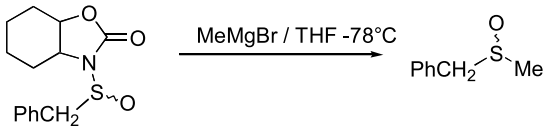
Finally, the calculated electrostatic potential for the lowest-energy conformation of (4*R*,5*S*)-**6d** [DFT calculations[12,13] at the B3LYP/6-31G(d,p) level] show similar electron density at both diastereotopic[14] sulfur lone pairs, which is in line with the observed comparable ratios of diastereoisomeric ratios of *N*-sulfoxide derivatives (entries 3 and 4 in Table 3).

2.3. Enantioselective chiral sulfinyl transfer reactions

To determine the ability of the *N*-benzylsulfinyl derivatives **5a–d** as effective chiral sulfinylating reagents in the preparation of enantiopure sulfoxides, the reaction with the Grignard reagent methylmagnesium bromide was carried out. It is known[6] that transfer of the sulfinyl group proceeds with inversion of configuration at sulfur.

To a solution of *N*-benzylsulfinyl derivatives **5a–d** in THF at −78 °C was added MeMgBr affording the chiral benzyl methyl sulfoxides **8a**,**b**. These sulfoxides and the recovered chiral auxiliary were purified by preparative TLC. Sulfoxides **8a**,**b** were obtained as white solids in 70–75% yield (Table 4). The assignment of configuration of the known benzyl methyl sulfoxides was achieved by chiral HPLC and optical rotation, respectively. The enantioselectivity measured was higher than 98% (Table 4) confirming the potential of *N*-sulfinyl derivatives **5** as effective sulfinylating agents in the preparation of enantiopure sulfoxides.

Table 4. *N*-Benzylsulfinyl derivatives **5a–d** as effective chiral sulfinylating reagents in the preparation of enantiopure sulfoxides **8a**,**b**



5a-d **8a,b**

Entry	Substrate	Yield (%)	$[\alpha]_D$[a]	Config.[b]	e.e. (%)[c]
1	(4*S*,5*S*,R_S)-**5a**	70	+51.1 (*c* 1.1)	(R_S)-**8a**	>98
2	(4*R*,5*R*,S_S)-**5b**	70	−49.1 (*c* 0.9)	(S_S)-**8b**	>98
3	(4*S*,5*R*,S_S)-**5c**	75	−49.3 (*c* 1.0)	(S_S)-**8b**	>98
4	(4*R*,5*S*,R_S)-**5d**	75	+50.1 (*c* 0.9)	(R_S)-**8a**	>99

[a] In $CHCl_3$.
[b] Assigned by comparison with the literature $[\alpha]_D = -55.0$ (*c* 0.9, $CHCl_3$) for (S_S)-benzyl methyl sulfoxide.[6,15]
[c] Determined by HPLC.

3. Experimental

3.1. General methods

All manipulations of organometallic compounds were carried out under an inert argon atmosphere. NMR spectra were obtained on a Jeol 400 MHz Fourier transform spectrometer. ^1H and ^{13}C NMR spectra were referenced to tetramethylsilane.

3.2. General procedure for the preparation of *trans*- and *cis*-*N*-(thiobenzyl)hexahydrobenzoxazolidin-2-one, 6a–d

To a solution of hexahydrobenzoxazolidin-2-ones **4a**–**d**[7] (0.2 g, 1.42 mmol) in THF (10 mL) was slowly added at 0 °C *n*-BuLi (0.98 mL, 1.56 mmol, 1.6 M in hexanes). The resulting mixture was stirred for 30 min at 0 °C, after which was cooled at −78 °C. The lithiated oxazolidinones **4**-Li were treated with commercially available (*S*)-benzyl phenyl-methanethiosulfonate (0.48 g, 1.70 mmol) in THF (2 mL). The resulting solution was stirred for 3 h, allowed to warm to rt, quenched with saturated aqueous NH_4Cl, extracted with 3×25 mL portions of dichloromethane, and the combined organic phases dried with sodium sulfate. The solvent was then removed under reduced pressure. The pale yellow solid obtained (0.36 g) was purified by column chromatography on silica gel (petroleum ether–EtOAc, 50:50, as eluent) to yield **6a**–**d**.

3.2.1. *trans*-(4*S*,5*S*)-*N*-(Thiobenzyl)hexahydrobenzoxazolidinone 6a. Mp 102–103 °C; 0.34 g (91% yield), $[\alpha]_D^{20}=-100.1$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) $\delta=0.90$ (m, 2H), 1.30 (m, 2H), 1.75 (m, 3H), 2.09 (m, 1H), 2.48 (td, $^3J=3.8$, 11.2 Hz, 1H), 3.61 (td, $^3J=3.8$, 11.2 Hz, 1H), 3.81 (d, $^3J=12.8$ Hz, 1H), 4.19 (d, $^3J=12.8$ Hz, 1H), 7.32 (m, 5H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) $\delta=23.3$, 23.8, 27.5, 28.5, 41.5, 66.2, 82.4, 127.6, 128.6, 129.5, 136.3, 159.5; IR (film) 3865, 3741, 3618, 3564, 2993, 2361, 1767, 1651, 1512, 1458, 1381, 1242, 1057 cm^{-1}; $C_{14}H_{17}NO_2S$ (263.36) calcd: 63.79% C, 6.45% H, 5.31% N; found: 63.63% C, 6.51% H, 5.34% N.

3.2.2. *trans*-(4*R*,5*R*)-*N*-(Thiobenzyl)hexahydrobenzoxazolidinone 6b. Mp 104–105 °C; 0.35 g (92% yield), $[\alpha]_D^{20}=+98.1$ (*c* 0.9, $CHCl_3$). ^1H and ^{13}C NMR spectra identical with those for **6a**.

3.2.3. *cis*-(4*R*,5*S*)-*N*-(Thiobenzyl)hexahydrobenzoxazolidinone 6d. Mp 50–51 °C; 0.34 g (90% yield), $[\alpha]_D^{20}=+131.1$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) $\delta=1.29$ (m, 2H), 1.45 (m, 2H), 1.65 (m, 4H), 3.10 (c, $^3J=5.6$, 12.1 Hz, 1H), 3.80 (d, $^3J=12.8$ Hz, 1H), 4.15 (d, $^3J=12.4$ Hz, 1H), 4.32 (c, $^3J=5.8$, 12.1 Hz, 1H), 7.30 (m, 5H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) $\delta=19.1$, 19.6, 25.7, 26.8, 41.8, 58.2, 74.4, 127.6, 128.6, 129.4, 135.6, 159.1; IR (film) 3865, 3741, 3618, 2993, 1759, 1512, 1381, 1242, 1057 cm^{-1}; HRMS-ES+*m*/*z* found 264.1068 $[(M+H)^+]$; calcd 264.1058 for $C_{14}H_{17}NO_2S+H^+$].

3.2.4. *cis*-(4*S*,5*R*)-*N*-(Thiobenzyl)hexahydrobenzoxazolidinone 6c. Mp 50–51 °C; 0.34 g (90% yield), $[\alpha]_D^{20}=-128.1$ (*c* 1.1, $CHCl_3$). ^1H and ^{13}C NMR spectra identical with those for **6d**.

3.3. General procedure for the preparation of *trans*- and *cis*-*N*-(benzylsulfinyl)hexahydrobenzoxazolidin-2-one, 5a–d

To a solution of *N*-(thiobenzyl)hexahydrobenzoxazolidin-2-one **6a**–**d** (0.31 g, 1.2 mmol) in MeOH (12 mL) was added $NaIO_4$ (0.77 g, 3.7 mmol) in H_2O (6 mL). The reaction mixture was stirred for 42 h at rt. The white solid formed was filtered, and the MeOH was removed under reduced pressure. The aqueous phase was extracted with 3×25 mL portions of dichloromethane, and the combined organic phase was dried with sodium sulfate. The solvent was removed under reduced pressure. The solid obtained from **6a**,**b** (0.30 g) was purified by fractional recrystallization from dichloromethane–petroleum ether (95:5) to yield the major diastereoisomers **5a**,**b** as white crystals. The solid obtained from **6c**,**d** (0.27 g) was purified by preparative TLC on silica gel (hexanes–EtOAc, 67:33, as eluent) to yield the major diastereoisomer **5c**,**d** as white crystals.

3.3.1. (4*S*,5*S*,R_S)-*trans*-*N*-(Benzylsulfinyl)hexahydrobenzoxazolidin-2-one, 5a. Mp 105–106 °C; 0.17 g (50% yield), $[\alpha]_D^{20}=-30.6$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) $\delta=1.3$–1.6 (m, 8H), 1.8–2.0 (m, 4H), 2.23 (m, 1H), 2.43 (m, 1H), 3.55 (dt, 1H, $^3J=3.6$, 11.6 Hz), 3.96 (dt, 1H, $^3J=3.6$, 11.2 Hz), 4.28 (dd, 2H, $^2J=13.0$ Hz), 7.2–7.4 (m, 5H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) $\delta=23.3$, 23.7, 28.7, 29.4, 61.0, 62.0, 81.8, 128.9, 129.9, 130.2, 158.0; IR (film) 3061, 2962, 2922, 2858, 1755, 1732, 1454, 1392, 1302, 1217, 1163, 1136, 1099, 1032, 762, 698 cm^{-1}; HRMS-ES+*m*/*z* found 302.0828 $[(M+Na)^+]$; calcd 302.0827 for $C_{14}H_{17}NO_3S+Na^+$].

3.3.2. (4*R*,5*R*,S_S)-*trans*-*N*-(Benzylsulfinyl)hexahydrobenzoxazolidin-2-one, 5b. Mp 102–103 °C; 0.18 g (55% yield), $[\alpha]_D^{20}=+32.0$ (*c* 0.9, $CHCl_3$). ^1H and ^{13}C NMR spectra identical with those for **5a**.

3.3.3. (4*S*,5*R*,S_S)-*cis*-*N*-(Benzylsulfinyl)hexahydrobenzoxazolidin-2-one, 5c. Mp 98–99 °C; 0.15 g (46% yield), $[\alpha]_D^{20}=+185.3$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) $\delta=0.92$ (m, 1H), 1.10 (m, 1H), 1.34 (m, 2H), 1.55 (m, 2H), 1.75 (m, 2H), 4.14 (c, 1H, $^3J=12.0$, 6.4 Hz), 4.34 (d, 1H, $^2J=12.8$ Hz), 4.44 (c, 1H, $^3J=11.6$, 5.6 Hz), 4.90 (d, 1H, $^2J=12.8$ Hz), 7.38 (m, 5H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) $\delta=19.1$, 19.3, 26.9, 27.5, 55.5, 59.0, 75.0, 128.4, 128.7, 128.8, 129.8, 154.8; IR (film) 1755, 1217 cm^{-1}; HRMS-ES+*m*/*z* found 302.0841 $[(M+Na)^+]$; calcd 302.0827 for $C_{14}H_{17}NO_3S+Na^+$].

3.3.4. (4*R*,5*S*,R_S)-*cis*-*N*-(Benzylsulfinyl)hexahydrobenzoxazolidin-2-one, 5d. Mp 96–97 °C; 0.14 g (43% yield), $[\alpha]_D^{20}=-184.1$ (*c* 1.0, $CHCl_3$). ^1H and ^{13}C NMR spectra identical with those for **5c**.

3.4. Preparation of (S_S) and (R_S)-benzyl methyl sulfoxides 8a,b

To a previously cooled solution at −78 °C of *N*-(benzylsulfinyl) hexahydrobenzoxazolidin-2-one **5a**–**d** (0.04 g, 0.143 mmol) in THF (10 mL) was added dropwise MeMgBr (1.4 M, in toluene–THF 75:25, 0.20 mL, 0.28 mmol). The reaction was quenched with saturated aqueous NH_4Cl (1 mL). The solvent was removed under reduced pressure, extracted with 3×25 mL portions of ethyl acetate, and the combined organic phase was dried with sodium sulfate. The solvent was then removed under reduced pressure. The product and the chiral auxiliary were purified by preparative TLC (petroleum ether–EtOAc, 33:67, as eluent). The recovered chiral auxiliary afforded 18 mg (90%).

3.5. Conditions for the analysis and assignment of configuration of the chiral sulfoxides 8a,b

Chiral HPLC: Chiralcel OD column 254 nm UV detector, diameter 0.46 cm, length 25 cm, 1.0 mL/min. Hexanes–*i*-PrOH, 90:10.

Specific rotations of the chiral sulfoxides were measured and compared with those reported on the literature to assign the configuration.[6,15]

3.5.1. (R_S)-Benzyl methyl sulfoxide 8a. The sulfoxide was obtained as a white solid (0.016 g, 75.0% yield); e.e.>98%; t_R=35.4 min, $[\alpha]_D^{20}=+50.1$ (*c* 1.0, $CHCl_3$).

3.5.2. (S_S)-Benzyl methyl sulfoxide 8b. The sulfoxide was obtained as a white solid (0.016 g, 75.0% yield); e.e.>98%; t_R=38.4 min, $[\alpha]_D^{20}=-49.3$ (*c* 1.1, $CHCl_3$). [Lit.[15] $[\alpha]_D^{20}=-55.0$ (*c* 0.9, $CHCl_3$)].

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Practical synthesis of 1-deoxy-D-xylulose and 1-deoxy-D-xylulose 5-phosphate allowing deuterium labelling

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Abstract—An optimised gram scale synthesis allows the production of 1-deoxy-D-xylulose and 1-deoxy-D-xylulose 5-phosphate with possible deuterium labelling at C-5. Such substrates are required for investigations on the mevalonate-independent 2-*C*-methyl-D-erythritol 4-phosphate pathway for isoprenoid biosynthesis in bacteria and chloroplasts of phototrophic eukaryotes and for the biosynthesis of vitamins B_1 (thiamine diphosphate) and B_6 (pyridoxol phosphate) in bacteria.

1. Introduction

1-Deoxy-D-xylulose 5-phosphate **3** (DXP, or 1-deoxy-D-*threo*-2-pentulose 5-phosphate) has been identified as precursor in three major metabolic pathways (Scheme 1): the biosynthesis of thiamine diphosphate (vitamin B_1)[1,2] and pyridoxol phosphate (vitamin B_6)[3] in bacteria and the formation of isopentenyl diphosphate **5** and dimethylallyl diphosphate **6** via the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway for isoprenoid biosynthesis in most bacteria and in the chloroplasts of phototrophic organisms.[4,5] DXP **3** is synthesized by the DXP synthase (DXS), a thiamine diphosphate dependant enzyme, catalysing the decarboxylation of pyruvate **1** and the condensation of the resulting (hydroxyethyl)thiamine diphosphate onto D-glyceraldehyde 3-phosphate **2** (Scheme 1).[6–8] In the MEP pathway for isoprenoid biosynthesis, DXP **3** is converted into MEP **4** by a two step process catalysed by the DXP reducto-isomerase (DXR): the rearrangement of DXP into 2-*C*-methyl-D-erythrose 4-phosphate and the concomitant NADPH-dependent reduction of the resulting aldehyde into MEP **4**.[9,10] All enzymes of the MEP pathway are potential targets for the development of new antibacterial agents,[11,12] and DXR is a most promising one. Indeed, fosmidomycin, a specific DXR inhibitor, presents an interesting antibacterial and antimalarial activity.[13–16] In order to determine the bioactivity of inhibitors, there is now a demand for larger quantities of DXP, the natural substrate of DXR. In contrast, for in vivo studies in bacteria, plant tissue cultures or whole plants or plant organs, free DX with stable isotope labelling is the material of choice, as the free pentulose is usually well incorporated into the isoprenoids after in vivo phosphorylation into DXP. In *Escherichia coli*, this phosphorylation is ATP-dependent and catalysed by a D-xylulokinase.[17]

Due to the biological importance of DX and DXP, many chemical and enzymatic syntheses, including labelling with stable or radioactive isotopes, have been developed over the last years. Efficient approaches to DX[18–22] or DXP[23–25] exploited either D-threitol or dimethyl D-tartrate derivatives using either benzylidene or 2,3-*O*-isopropylidene protection of the vicinal secondary hydroxy groups. Indeed, such commercially available starting materials already possess the required configuration at C-2 and C-3 corresponding to the two asymmetric centres of DX or DXP. These 7–8 steps reaction sequences afforded optically pure final products (DX or DXP) with 5–58% overall yields, but have not been really optimised, especially at the level of the number of steps. In addition, removal of the isopropylidene protecting group proved often critical, decreasing the overall yield and requiring purification of the final product.[18,19] Another approach started from carbohydrate derivatives: mannitol diacetonide for the synthesis of DX or DXP (5 or 6 steps, 25%),[26] isopropylidene-D-glyceraldehyde[1] (4 steps) or D-arabitol[1] (7 steps) affording DX as well as its C-3 epimer, which had to be separated, (1,2-isopropylidene-α-D-xylofuranose (5 steps, 48%),[27] D-arabinose (9 steps, 26%),[27] or 2,3-*O*-isopropylidene-D-erythrono-1,4-lactone (6 steps, 23%)[28] for the synthesis of DX and finally 2,3-*O*-isopropylidene-β-D-xylofuranose for the synthesis of methyl

Keywords: Biosynthesis; 1-Deoxy-D-*threo*-2-pentulose; 1-Deoxy-D-xylulose; 1-Deoxy-D-xylulose 5-phosphate; Isoprenoids; Deuterium labelling; 2-*C*-Methyl-D-erythritol 4-phosphate; Pyridoxol phosphate; Thiamine diphosphate.
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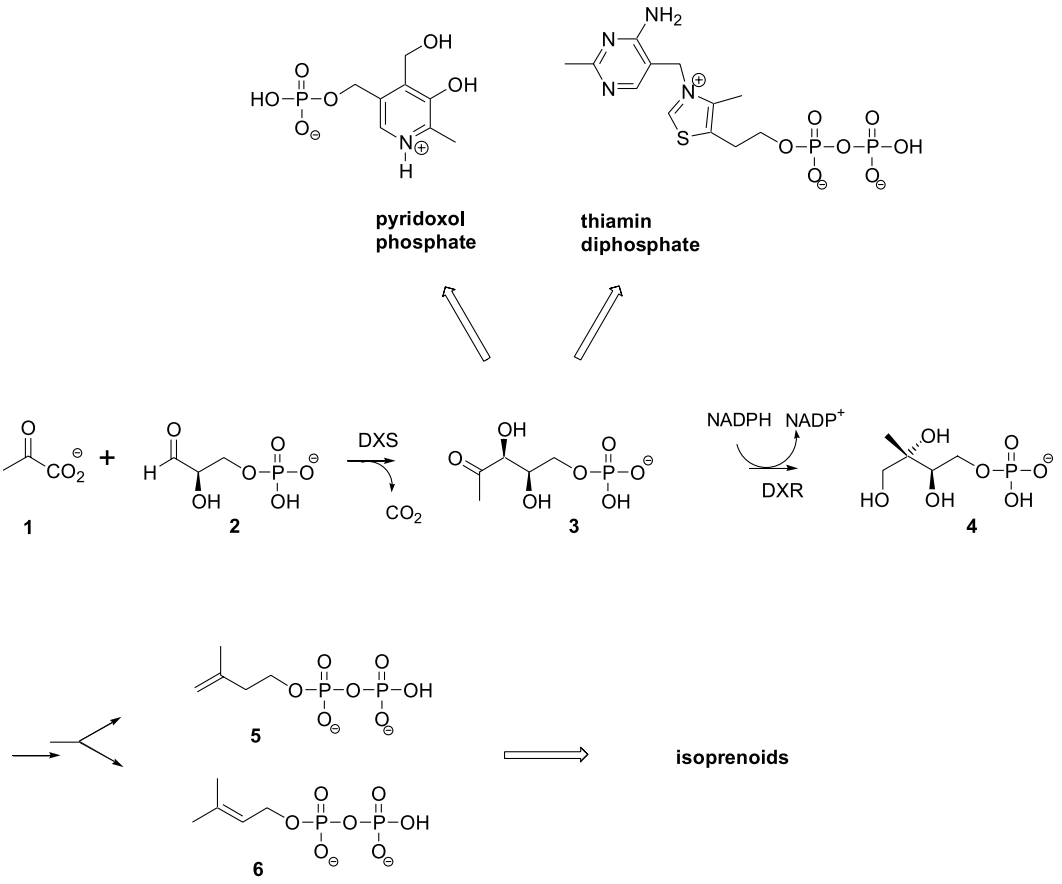


Scheme 1. 1-Deoxy-D-xylulose 5-phosphate **3** as precursors for isoprenoids via the methylerythritol phosphate pathway for isoprenoid, pyridoxol phosphate and thiamin diphosphate.

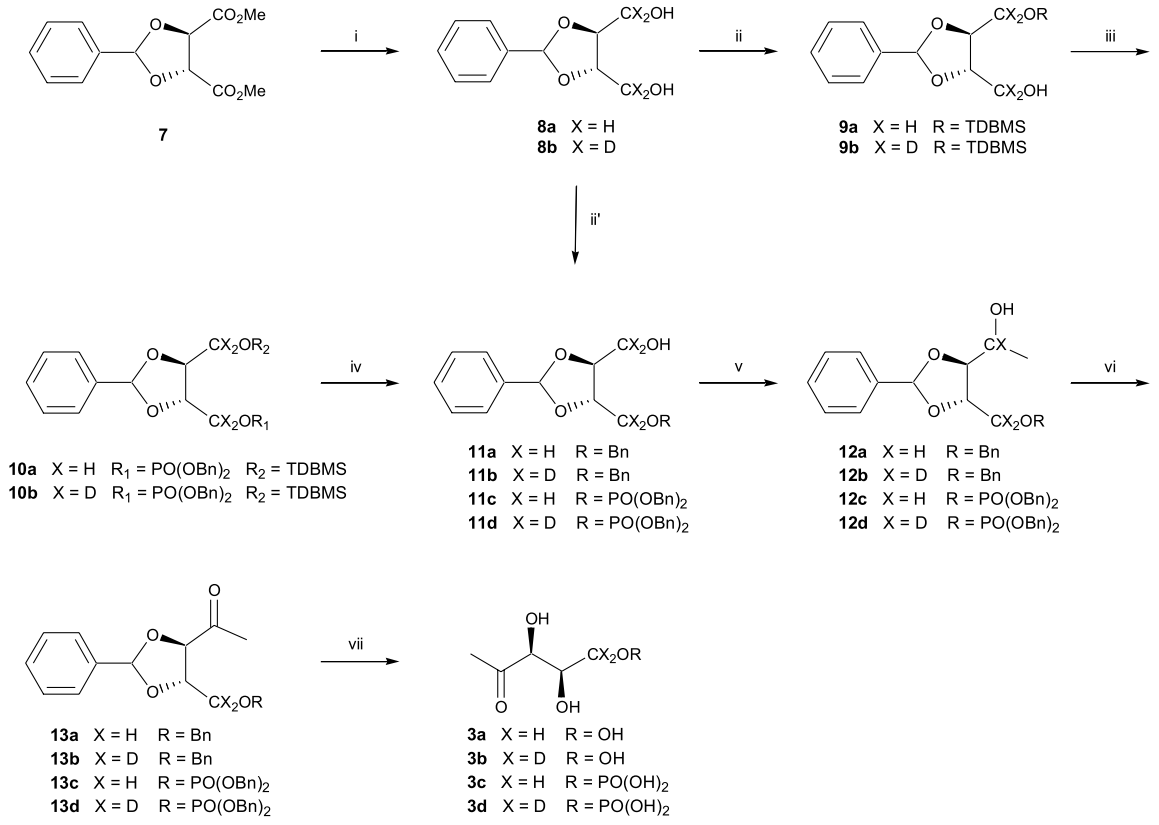
1-deoxy-α/β-D-xyluloside (3 steps, 24%).[29] These sequences also afforded optically pure final products. A third approach involved the synthesis of the C_5 DX/DXP carbon chain from achiral precursors. In this case, the stereogenic centres were generated by asymmetric dihydroxylation of an achiral α,β-unsaturated pentan-2-one derivative using a chiral osmium tetraoxide complex for DX (7 steps, 52%, optical rotation similar to literature value),[30] as well as DXP (5 steps, 22, 84% ee)[31] or from a chiral cyanohydrin obtained from acrolein using (*S*)-hydroxynitrile lyase from *Hevea brasiliensis* yielding in 5 steps DX with a maximal 86% ee and a 33% yield.[32]

Enzymatic synthesis using the recombinant bacterial DXP synthase (DXS) from *E. coli* or *Bacillus subtilis* represents an attractive alternative to chemical synthesis, once the enzyme is available, especially when DX or DXP with multiple ^{13}C labelling or with ^{14}C labelling are required.[23,33–36] DXS normally utilizes D-glyceraldehyde 3-phosphate **2** and pyruvate **1** as substrates to yield DXP **3** as sole reaction product (Scheme 1). Fortunately, DXS is not so substrate specific and it may be utilized for the synthesis of free DX. The enzyme accepts free D-glyceraldehyde as a substrate in the place of GAP, yielding free DX at a reasonable rate.[33,35] Although the enzymatic synthesis of DXP and DX is quite straightforward, producing amounts up to 3 mmol is difficult and recovery of the products from the reaction buffer requires purification by precipitation/redissolution of DXP[23] or chromatography and cation exchange,[34] procedures which require significant experimental technique.

In this paper, we describe an improved short method for large-scale synthesis of enantiopure DX or DXP from commercially available benzylidene D-threitol or benzylidene D-tartrate, with high yields and the possibility of introducing ^2H labelling at C-5. This route has been already adapted to the synthesis of (3*R*,4*S*)-3,4-dihydroxy-5-oxohexylphosphonic acid, an isosteric phosphonate analogue of DXP.[37]

2. Results and discussion

The chemical synthesis of DX and DXP was reinvestigated in order to reach a reaction sequence leading to both compounds that is as short, convenient and efficient as possible and allows labelling with a stable isotope such as deuterium. The commercial D-threitol benzylidene derivative **8a** is a convenient starting material (Scheme 2), possessing the required configurations at C-2 and C-3 that correspond to the stereogenic centres at C-3 and C-4 of DX/DXP. Despite the resulting complication of the NMR spectra, the benzylidene protection of the vicinal diol was preferred to the isopropylidene protection. Indeed, the benzylidene group may be simultaneously removed with other benzyl protecting groups at the very last step of the synthesis by one single catalytic hydrogenolysis, requiring no further purification of the final product. This feature is

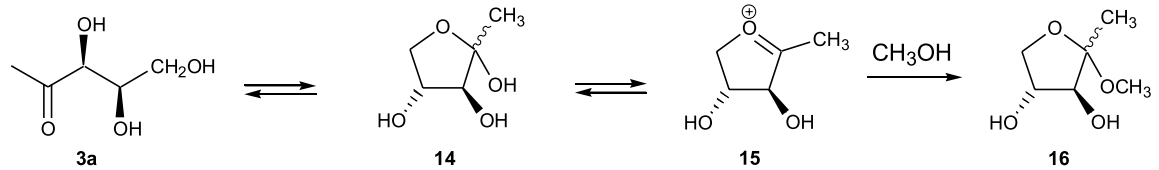


Scheme 2. Synthesis of DX **3a**,**b** and DXP **3c**,**d** from D-threitol **8**. (i) $LiAlD_4$, THF; (ii) TBDMSCl, NaH, DME; (ii′) BnBr, NaH, THF; (iii) $(BnO)_2P\text{-}NEt_2$, tetrazole, mCPBA, CH_2Cl_2; (iv) Bu_4NF, THF; (v) $(COCl)_2$, DMSO, NEt_3, MeMgCl, THF; (vi) TPAP, NMO, 3 Å molecular sieves, CH_2Cl_2, (vii) H_2, Pd/C.

most appreciable in the case of small, polar, water-soluble carbohydrates such as DX and DXP.

The first step of the synthesis is a benzyl monoprotection of a single hydroxyl group of the starting D-threitol **8a**. The reaction was achieved using sodium hydride and benzyl bromide to give **11a** with a yield of 85%. The oxidation of the resulting primary alcohol using the Swern oxidation modified by Ireland[38] led to the secondary alcohol **12a** as a mixture of four diastereomers with 88% yield. Under these conditions, the methyl group was directly introduced by addition of methyl magnesium chloride without isolation of the intermediate aldehyde. The resulting secondary alcohol was then oxidized using the mild TPAP/NMO conditions[39] and gave the protected 1-deoxy-D-xylulose **13a** in 86% yield. Hydrogenolysis of the benzylidene and benzyl groups quantitatively afforded DX **3a**.

A water solution of free DX **3a** contains a mixture of three forms in equilibrium, the straight chain ketone **3a** and the two anomeric α- and β-furanose hemiketals **14**, which may eliminate under certain conditions the anomeric hydroxyl group (Scheme 3).[18] When the final hydrogenolysis was performed in methanol, a less polar by-product accompanied DX **3** in significant amounts. The mass spectrum (chemical ionisation using *i*-butane as reactant gas of this product) showed signals at *m/z* 149 corresponding to the pseudo-molecular ion $(M+H)^+$ and two other signals at *m/z* 131 and 117 corresponding to the loss of water and methanol, respectively. This suggested the methyl glycoside structure **16** for this by-product, which may be formed by addition of methanol onto the oxocarbenium ion **15** (Scheme 3). In order to avoid this undesired reaction and to exclusively obtain the DX, the last hydrogenolysis step required a hindered protic solvent such as a mixture of *i*-propanol/water. The double deprotection of the benzylidene and benzyl groups was realized by catalytic hydrogenolysis using 20% of Pd/C in a mixture of *i*-propanol/water (8:2, v/v). After removing the catalyst by filtration over celite, DX **3a** was obtained without requiring further purification. This reaction sequence afforded DX **3a** in four steps with a 64% overall yield. This yield is comparable to



Scheme 3. Formation of methyl deoxyxyluloside **15** in hydrogenolysis conditions in the presence of methanol.

those reported for some of the former syntheses, but the reaction sequence is much shorter.

Synthesis of [5,5-$^2H_2$]DX **3b** was achieved starting from the commercially available benzylidene dimethyl D-tartrate **7**. Deuterium was introduced by reduction of the diester using lithium aluminium deuteride, which afforded [1,1,4,4-$^2H_4$]-2,3-*O*-benzylidene-D-threitol **8b** to 96% yield. The next steps leading to [5,5-$^2H_2$]DX were identical with those described for the synthesis of the natural abundance DX and the yields of the reactions performed on the deuterated compounds were the same as those found for the synthesis of the non-labelled products. [5,5-$^2H_2$]DX **3b** was thus obtained in five steps with a 61% overall yield, which is significantly better than those previously described.[20] Upon acetylation, [5-$^2H_2$]DX afforded one single triacetate with the straight chain in the place of the furanose anomers/straight chain mixture of free DX. The ^{13}C NMR spectrum of the triacetate was much simpler than that of the free DX, facilitating the localization of the deuterium by ^{13}C NMR. Only one single signal was detected for carbon atoms C-4, C-3 and C-2 with ^2H-induced shifts corresponding to β, γ and δ-shifts, respectively. No evidence of partial scrambling upon hydrogenolysis was observed as reported for the hydrogenolysis leading to [4-^2H]DX.[40]

For the synthesis of DXP **3c**, the phosphate was introduced from the beginning of the synthesis, serving as phosphotriester protecting group for one of the primary hydroxyls. Monophosphorylation of diol **8a** was attempted using different conditions (Table 1), but the monophosphorylated diol was nearly always accompanied by the corresponding biphosphate (Table 1). The use of one equivalent of ($P(OBn)_3/I_2$[41] or $ClPO(OBn)_2$[42]) in pyridine afforded a mixture of mono- and biphosphorylated compounds, which were very difficult to separate. Similar results were obtained by reacting of the mono-alcoholate formed with NaH or *n*-BuLi on an electrophilic phosphorus derivative like dibenzyl chlorophosphate or tetrabenzyl pyrophosphate (TBBP).[43] The best results were obtained with NaH and TBBP with a 7/1 ratio of mono-/biphosphorylated products (as determined by NMR). The monophosphate **11c** was isolated with a 76% yield starting from **8a**. To circumvent the multiple chromatographic purifications, diol **8a** was monoprotected by treatment with NaH and TBDMSCl in DME. The silyl ether **9a** was obtained with 96% yield and phosphorylated using dibenzyl-*N*,*N*-diethyl phosphoramidite, tetrazole and *m*-CPBA.[44] The phosphate **10a** was formed with 89% yield. The silyl group was removed with TBAF to give protected DXP **11c** (87% yield). This protective silylation, phosphorylation and deprotection sequence easily led to **11c** with an overall 74% yield in place of the 76% yield for the direct monophosphorylation of diol **8a** and avoiding a time consuming purification. A Swern oxidation using the conditions modified by Ireland followed by an oxidation with TPAP and a final hydrogenolysis afforded DXP **3c** in six steps with a 60% global yield, much better than the yields of former syntheses. Both ^1H and ^{13}C NMR spectra showed the presence of an intact phosphate group through the coupling of phosphorus with the two C-5 methylene protons as well as the C-5, C-4 and C-3 carbon atoms.

[5,5-$^2H_2$]DXP **3d** was synthesized starting from benzylidene dimethyl D-tartrate **7** [1,1,4,4-$^2H_4$]-2,3-*O*-benzylidene-D-threitol **8b** like [5,5-$^2H_2$]DX and was obtained from this deuterium labelled diol in seven steps with a global yield of 57% by following the same sequence as that used for the synthesis of natural abundance DXP.

The improved synthesis of free DX and of DXP has been successfully performed at the gram scale and may be scaled-up, if necessary. Purification of carbohydrates is often time consuming and associated with low yields. In our procedure, DX and DXP were obtained after the last hydrogenolysis step with a high degree of purity. No purification was required, and they were utilized as they were in enzyme tests with DXP reducto-isomerase or for incubations with plant systems. This synthesis thus represents a simple and practical alternative to the enzymatic synthesis, which usually requires the separation and purification of the reaction products from the incubation buffer.

3. Experimental

3.1. General

General methods and analytical procedures were identical with those previously described.[37,45] All compounds were found to be pure by ^1H and ^{13}C NMR spectroscopy. In the description of the NMR spectra of diastereomer mixtures, signals for different diastereomers are differentiated by a *, # or § sign added to the assignments. If only one signal is described, it is common to all diastereomers. For the benzylidene protected diol derivatives, evaluation of the diastereomer relative amounts was made by integration of the benzylic C*H*-Ph proton signal.

3.2. Synthesis

3.2.1. (+)-[1,1,4,4-$^2H_4$]-2,3-*O*-Benzylidene-D-threitol (8b). To a cold (0 °C) solution of (+)-dimethyl 2,3-*O*-benzylidene-D-tartrate **7** (1.4 g, 5.37 mmol, 1 equiv) in THF

Table 1. Monophosphorylation assays of 2,3-benzylidene D-threitol **8**

Reagent A	Reagent B	Mono-phosphate	Bi-phosphate	Monophosphate **5c** (% from **8**)
$P(OBn)_3$, I_2	Py	7	1	68
$ClPO(OBn)_2$	Py	5	1	41
$ClPO(OBn)_2$	NaH	1	0	40[a]
$ClPO(OBn)_2$	*n*-BuLi	6	1	63
TBPP	NaH	7	1	76
TBPP	*n*-BuLi	6	1	74

[a] Conversion determined by ^1H NMR (60% of starting material remaining). All other data correspond to isolated monophosphate **11**.

(40 mL) was slowly added lithium aluminium deuteride (0.45 g, 10.72 mmol, 2 equiv). After stirring for 1 h, the reaction mixture was diluted with diethyl ether (50 mL), and a saturated aqueous potassium sodium tartrate (50 mL) was slowly added. The mixture was extracted with diethyl ether. The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography. Colourless solid (mp: 70–71 °C) as a 1:1 mixture of two diastereomers obtained from **7** with 96%. R_f=0.31 (ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.82 (1/2 of 2H, s, OH), 2.89 (1/2 of 2H, s, OH*), 4.08 (4H, s, 2- and 3-H), 5.92 (1H, s, CH-Ph), 7.37–7.48 (5H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=broad signal centred at 61.5 (CD_2, quint, J_{C-D}=21.7 Hz, C-1 and C-4), 78.3 and 79.3 (CH, C-2 and C-3), 103.8 (CH, CH-Ph), 126.5, 128.4, 129.6, and 137.1 (aromatic C). ^{2}H NMR: δ= (61.4 MHz, $CHCl_3$) 3.79 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3387, 2191, 2079, 1602, 1487, 1461, 1403, 1377, 1288, 1096, 1068. MS (EI): M^+ 214.2. HRMS (EI): M^+ calcd for $C_{11}H_{10}D_4O_4$ 214.1143, found 214.1130.

3.2.2. General procedure for the monoprotection of the diols. To a cold (0 °C) solution of the diol **8a** or **8b** (1 equiv) in DME (2.5 mL/mmol), sodium hydride (1.1 equiv) was added in portions. The mixture was stirred at 0 °C for 15 min before addition of *t*-butyldimethylsilyl chloride (1.1 equiv) or benzyl bromide (1.1 equiv). When the starting material was consumed, the reaction was quenched by addition of a saturated aqueous ammonium chloride solution. The mixture was extracted with diethyl ether, and the combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by flash-chromatography.

3.2.3. (2*R*,3*S*)-*O*-Benzylidene-4-*O*-benzyl-D-threitol (11a). Colourless oil as a 1:1 mixture of two diastereomers obtained from **8a** with 85%. R_f=0.37 (ethyl acetate/cyclohexane, 60/40). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 2.19 (1H, s, OH), 3.76 (4H, m, 1- and 4-H), 4.36 (2H, m, 2- and 3-H), 4.61 (1/2 of 2H, s, CH_2Ph), 4.62 (1/2 of 2H, s, CH_2Ph*), 5.97 (1/2 of 1H, s, CH-Ph), 5.99 (1/2 of 1H, s, CH-Ph*), 7.33–7.50 (10H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=62.6 (CH_2, C-1), 70.0 (CH_2, C-4), 73.6 (CH_2, CH_2-Ph), 76.7 (CH, C-3), 77.8 (CH, C-3*), 79.8 (CH, C-2), 80.4 (CH, C-2*) 103.8 (CH, CH-Ph), 103.9 (CH, CH-Ph*), 125.9, 126.6, 126.7, 127.7, 127.8, 128.2, 128.3, 128.4, 128.5, 129.4, 129.5, 137.3, 137.4, 137.5, and 137.6 (aromatic C). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3387, 1602, 1495, 1454, 1400, 1361, 1275, 1096, 1071. MS (FAB^+): $(M+H)^+$ 301.1. HRMS (FAB^+): $(M+H)^+$ calcd for $C_{18}H_{21}O_4$ 301.1440, found 301.1439.

3.2.4. (2*R*,3*S*)-[1,1,4,4-$^2H_4$]-*O*-Benzylidene-4-*O*-benzyl-D-threitol (11b). Colourless oil as a 1:1 mixture of two diastereomers obtained from **8b** with 84% yield. R_f=0.44 (ethyl acetate/hexane, 60/40). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.37 (1/2 of 1H, s, OH), 2.43 (1/2 of 1H, s, OH*), 4.10 (1/2 of 1H, d, J_{2-3}=7 Hz, 2-H), 4.14 (1/2 of 1H, d, J_{2-3}= 7 Hz, 2-H*), 4.22 (1/2 of 1H, d, J_{2-3}=7 Hz, 3-H), 4.24 (1/2 of 1H, d, J_{2-3}=7 Hz, 3-H*), 4.61 (1/2 of 2H, s, CH_2Ph), 4.63 (1/2 of 2H, s, CH_2Ph*), 5.97 (1/2 of 1H, s, CH-Ph), 5.99 (1/2 of 1H, s, CH-Ph*), 7.31–7.53 (10H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=61.7 (CD_2, quint, J_{C-D}= 22.5 Hz, C-1, α shift: −805 ppb), 61.8 (CD_2, quint, J_{C-D}= 22.5 Hz, C-1*, α shift: −747 ppb), 69.2 (CD_2, quint, J_{C-D}=22.3 Hz, C-4, α shift: −854 ppb), 69.3 (CD_2, quint, J_{C-D}=22.3 Hz, C-4*, α shift: −780 ppb), 73.5 (CH_2, CH_2-Ph, γ shift: −148 ppb), 76.5 (CH, C-3, $\beta+\gamma$ shifts: −173 ppb), 77.6 (CH, C-3*, $\beta+\gamma$ shifts: −214 ppb), 79.6 (CH, C-2, $\beta+\gamma$ shifts: −246 ppb), 80.1 (CH, C-2*, $\beta+\gamma$ shifts: −222 ppb), 103.8 (CH, CH-Ph), 103.9 (CH, CH-Ph*), 126.5, 126.6, 126.7, 127.7, 128.2, 128.3, 128.4, 129.4, 129.5, 137.2, and 137.4 (aromatic C). ^{2}H NMR (61.4 MHz, $CHCl_3$): δ=3.69 (s), 3.77 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3387, 2191, 2079, 1602, 1496, 1456, 1409, 1370, 1288, 1098, 1069. MS (FAB^+): $(M+H)^+$ 305.1. HRMS (FAB^+): $(M+H)^+$ calcd for $C_{18}H_{17}D_4O_4$ 305.1691, found 305.1685.

3.2.5. (2*R*,3*S*)-*O*-Benzylidene-4-*O*-*t*-butyldimethylsilyl-D-threitol (9a). Colourless oil as a 1:1 mixture of two diastereomers obtained from **8a** with 96% yield. R_f=0.20 (ethyl acetate/hexane, 20/80). ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.08 (1/2 of 6H, s, 2×CH_3), 0.10 (1/2 of 6H, s, 2× CH_3*); 0.90 (1/2 of 9H, s, *t*-Bu), 0.92 (1/2 of 9H, s, *t*-Bu*), 2.25 (1/2 of 1H, dd, J_{1a-OH}=5.4 Hz, J_{1b-OH}=7.3 Hz, OH), 2.32 (1/2 of 1H, t, J_{1-OH}=6.1 Hz, OH*), 3.69–4.18 (6H, m, 1-, 2-, 3- and 4-H), 5.96 (1/2 of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.34–7.51 (5H, m, Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=−5.3 (2×CH_3), 18.4 (quaternary C, *t*-Bu), 26.0 (3×CH_3, *t*-Bu), 62.6 (CH_2, C-1), 63.0 (CH_2, C-1*), 63.6 (CH_2, C-4), 63.7 (CH_2, C-4*), 78.2 (CH, C-2), 79.3 (CH, C-2*), 80.4 (CH, C-3), 80.7 (CH, C-3*), 103.9 (CH, CH-Ph), 104.3 (CH, CH-Ph*), 126.1, 126.7, 128.5, 128.6, 129.1, 129.5, 137.6 and 137.8 (aromatic C). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3387, 1602, 1462, 1381, 1256, 1225, 1093, 839. MS (FAB^+): $(M+H)^+$ 325.3. HRMS (FAB^+): $(M+H)^+$ calcd for $C_{17}H_{29}O_4Si$ 325.1835, found 325.1822.

3.2.6. (2*R*,3*S*)-[1,1,4,4-$^2H_4$]-*O*-Benzylidene-4-*O*-*t*-butyldimethylsilyl-D-threitol (9b). Colourless oil as a 1:1 mixture of two diastereomers obtained from **8b** with 96% yield. R_f=0.52 (ethyl acetate/cyclohexane, 10/90). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.10 (1/2 of 6H, s, 2× CH_3), 0.12 (1/2 of 6H, s, 2×CH_3*); 0.91 (1/2 of 9H, s, *t*-Bu), 0.93 (1/2 of 9H, s, *t*-Bu*), 2.27 (1H, m, OH), 4.05 (1/2 of 1H, d, J_{2-3}=6.8 Hz, 3-H), 4.08 (1/2 of 1H, d, J_{2-3}= 6.8 Hz, 3-H*), 4.14 (1/2 of 1H, d, J_{2-3}=6.8 Hz, 2-H), 4.24 (1/2 of 1H, d, J_{2-3}=6.8 Hz, 2-H*), 5.96 (1/2 of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.35–7.51 (5H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=−5.5 (2×CH_3), −5.4 (2× CH_3*), 18.2 (quaternary C, *t*-Bu), 18.3 (quaternary C, *t*-Bu*), 25.8 (3×CH_3, *t*-Bu), 25.9 (3×CH_3, *t*-Bu*), broad signal centred at 62.4 (2×CD_2, m, C-1 and C-4), 77.8 (CH, C-2, $\beta+\gamma$ shifts: −351 ppb), 78.9 (CH, C-2*, $\beta+\gamma$ shifts: −303 ppb), 80.1 (CH, C-3, $\beta+\gamma$ shifts: −302 ppb), 80.4 (CH, C-3*, $\beta+\gamma$ shifts: −347 ppb), 103.7 (CH, CH-Ph), 104.1 (CH, CH-Ph*), 125.9, 126.4, 126.5, 126.6, 128.2, 128.3, 128.4, 128.9, 137.4 and 137.6 (aromatic C) ^{2}H NMR: δ= (61.4 MHz, $CHCl_3$) 4.05 (s), 4.20 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3438, 2194, 2089, 1602, 1492, 1462, 1406, 1382, 1288, 1098, 1069. MS (FAB^+): $(M+Na)^+$ 350.9. HRMS (FAB^+): $(M+Na)^+$ calcd for $C_{17}H_{24}D_4O_4NaSi$ 351.1906, found 351.1904.

3.2.7. General procedure for phosphorylation. To a

solution of alcohol (1 equiv) in CH_2Cl_2 (15 mL/mmol) were successively added dibenzyl-*N*,*N*-diethyl phosphoramidite (2.5 equiv) and tetrazole (2.5 equiv). After stirring for 1 h at room temperature, the resulting phosphite was oxidized by addition of solid *m*-CPBA (2.5 equiv) to yield the corresponding phosphate after one additional hour. The reaction mixture was diluted with diethyl ether and washed with a 10% aqueous $Na_2S_2O_5$ solution, a saturated aqueous $NaHCO_3$ solution, water and finally brine. The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography to afford a colourless oil.

3.2.8. (2*R*,3*S*)-*O*-benzylidene-4-*O*-*t*-butyldimethylsilyl butyl 1-dibenzylphosphate (10a). Colourless oil as a 1:1 mixture of two diastereomers obtained from **9a** with 89% yield. R_f=0.39 (ethyl acetate/hexane, 5/5). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.09 (1/2 of 6H, s, 2×CH_3), 0.10 (1/2 of 6H, s, 2×CH_3*); 0.89 (1/2 of 9H, s, *t*-Bu), 0.92 (1/2 of 9H, s, *t*-Bu*), 3.78 (2H, m, 1-H), 4.04 (1H, m, 3-H), 4.27 (3H, m, 2-H and 4-H), 5.03 (1/2 of 4H, d, J_{P-H}=8.1 Hz, 2×CH_2-Ph), 5.08 (1/2 of 4H, d, J_{P-H}=8.1 Hz, 2×CH_2-Ph*), 5.88 (1/2 of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.32–7.48 (15H, m, Ph). ^{13}C NMR: δ= (75 MHz, $CDCl_3$) −5.4 (2×CH_3), 18.2 (quaternary C, *t*-Bu), 25.8 (3×CH_3, *t*-Bu), 25.9 (3×CH_3, *t*-Bu*), 63.3 (CH_2, s, C-4), 63.4 (CH_2, s, C-4*), 67.3 (CH_2, d, J_{C1-P}=5.6 Hz, C-1), 67.4 (CH_2, d, J_{C1-P}=5.6 Hz, C-1*), 69.3 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.4 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 77.3 (CH, d, J_{C2-P}=8.6 Hz, C-2), 77.4 (CH, d, J_{C2-P}=8.0 Hz, C-2*), 78.0 (CH, C-3), 78.3 (CH, C-3*), 104.0 (CH, CH-Ph), 104.6 (CH, CH-Ph*), 126.6, 126.7, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 129.4, 135.7, 135.8, 137.2 and 137.3 (aromatic C). ^{31}P NMR: δ= (121.5 MHz, $CDCl_3$) 0.2 (s), 0.4 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1602, 1496, 1457, 1409, 1381, 1272, 1261, 1092, 1013, 839. MS (FAB^+): $(M+H)^+$ 585.2. HRMS (FAB^+): $(M+H)^+$ calcd for $C_{31}H_{42}O_7PSi$ 585.2437, found 585.2467.

3.2.9. (2*R*,3*S*)-[1,1,4,4-$^2H_4$]-*O*-Benzylidene-4-*O*-*t*-butyldimethylsilyl butyl 1-dibenzylphosphate (10b). Colourless oil as a 1:1 mixture of two diastereomers obtained from **9b** with 89% yield. R_f=0.18 (ethyl acetate/cyclohexane, 20/80). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.09 (1/2 of 6H, s, 2×CH_3), 0.10 (1/2 of 6H, s, 2×CH_3*); 0.89 (1/2 of 9H, s, *t*-Bu), 0.92 (1/2 of 9H, s, *t*-Bu*), 4.04 (1/2 of 1H, d, J_{2-3}=6.4 Hz, 3-H), 4.05 (1/2 of 1H, d, J_{2-3}=6.4 Hz, 3-H*), 4.25 (1/2 of 1H, dd, J_{2-3}=6.4 Hz, J_{H-P}=1.1 Hz, 2-H), 4.34 (1/2 of 1H, d, J_{2-3}=6.4 Hz, 2-H*), 5.04 (1/2 of 4H, d, J_{P-H}=8.1 Hz, 2×CH_2-Ph), 5.08 (1/2 of 4H, d, J_{P-H}=8.1 Hz, 2×CH_2-Ph*), 5.88 (1/2 of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.32–7.48 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=−5.5 (2×CH_3), −5.4 (2×CH_3*), 18.2 (quaternary C, *t*-Bu), 25.8 (3×CH_3, *t*-Bu), 25.9 (3×CH_3, *t*-Bu*), 62.8 (CD_2, m, C-4, α shift: ca. −510 and −490 ppb), 66.7 (CD_2, m, C-1, α shift: ca. −610 and −690 ppb), 69.3 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.4 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 77.1 (CH, d, J_{C2-P}=8.6 Hz, C-2, β+γ shifts: −156 ppb), 77.3 (CH, d, J_{C2-P}=8.0 Hz, C-2*, β+γ shifts: −172 ppb), 77.8 (CH, C-3, β+γ shifts: −189 ppb), 78.1 (CH, C-3*, β+γ shifts: −181 ppb), 104.0 (CH, CH-Ph), 104.6 (CH, CH-Ph*), 126.6, 126.7, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 129.4, 135.7, 135.8, 137.2 and 137.3 (aromatic C). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ=0.2 (s), 0.4 (s). ^{2}H NMR (61.4 MHz, $CHCl_3$): δ=4.08 (s), 4.13 (s), 4.50 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 2192, 2090, 1602, 1497, 1461, 1407, 1377, 1275, 1260, 1090, 1035, 1020, 836. MS (FAB^+): $(M+H)^+$ 589.0. HRMS (FAB^+): $(M+H)^+$ calcd for $C_{31}H_{38}D_4O_7SiP$ 589.2689, found 589.2683.

3.2.10. General procedure for desilylation. To a solution of silylated compound (1 equiv) in THF (10 mL/mmol) was added solid Bu_4NF (1.5 equiv). After stirring overnight at room temperature, the solvent was removed under vacuum, and the residue was purified by flash chromatography.

3.2.11. (2*R*,3*S*)-*O*-benzylidene-4-hydroxybutyl 1-dibenzylphosphate (11c). Colourless oil as a 1:1 mixture of two diastereomers obtained from **10a** with 87% yield. R_f=0.25 (ethyl acetate/hexane, 8/2). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.36 (1H, s, OH), 3.74 (1H, m, 1-H), 4.16 (3H, m, 2-, 3- and 4-H), 5.02 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph), 5.03 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph), 5.06 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.08 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.87 (1/2 of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.32–7.48 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=62.1 (CH_2, s, C-4), 62.2 (CH_2, s, C-4*), 66.7 (CH_2, d, J_{C1-P}=5.6 Hz, C-1), 66.8 (CH_2, d, J_{C1-P}=5.6 Hz, C-1*), 69.5 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.6 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 76.1 (CH, d, J_{C2-P}=7.4 Hz, C-2), 76.9 (CH, d, J_{C2-P}=8.0 Hz, C-2*), 78.4 (CH, C-3), 78.9 (CH, C-3*), 104.0 (CH, CH-Ph), 104.1 (CH, CH-Ph*), 126.6, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 129.4, 129.6, 135.4, 135.5, 137.2 and 137.3 (aromatic C). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ=0.2 (s), 0.4 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3376, 1602, 1496, 1456, 1409, 1380, 1272, 1104, 1016. MS (FAB^+): $(M+H)^+$ 471.1. HRMS (FAB^+): $(M+H)^+$ calcd for $C_{25}H_{28}O_7P$ 471.1573, found 471.1567.

3.2.12. (2*R*,3*S*)-[1,1,4,4-$^2H_4$]-*O*-benzylidene-4-hydroxybutyl 1-dibenzylphosphate (11d). Colourless oil as a 1:1 mixture of two diastereomers obtained from **10b** with 87% yield. R_f=0.22 (ethyl acetate/cyclohexane, 60/40). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.52 (1H, s, OH), 4.07 (1H, d, J_{2-3}=6.5 Hz 3-H), 4.23 (1H, d, J_{2-3}=6.5 Hz 2-H), 5.02 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph), 5.03 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph), 5.06 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.08 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.86 (1/2 of 1H, s, CH-Ph), 5.96 (1/2 of 1H, s, CH-Ph*), 7.30–7.48 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=61.4 (CD_2, m, C-4, α shift: ca. −750 and −710 ppb), 66.2 (CD_2, m, C-1, α shift: ca. −610 and −530 ppb), 69.4 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.5 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 75.9 (CH, d, J_{C2-P}=7.4 Hz, C-2, β+γ shifts: −140 ppb), 76.7 (CH, d, J_{C2-P}=8.0 Hz, C-2*, β+γ shifts: −144 ppb), 78.2 (CH, C-3, β+γ shifts: −197 ppb), 78.7 (CH, C-3*, β+γ shifts: −173 ppb), 104.0 (CH, CH-Ph), 104.1 (CH, CH-Ph*), 126.6, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 129.4, 129.6, 135.4, 135.5, 137.2 and 137.3 (aromatic C). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ=0.4 (s), 0.5 (s). ^{2}H NMR (61.4 MHz, $CHCl_3$): δ=3.98 (s), 4.40 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3397, 2198, 2100, 1602, 1497, 1457, 1406, 1377, 1273, 1215, 1093, 1019. MS

(FAB$^+$): (M+H)$^+$ 474.9. HRMS (FAB$^+$): (M+H)$^+$ calcd for $C_{25}H_{24}D_4O_7P$ 475.1824, found 475.1826.

3.2.13. General procedure for the Swern–Ireland reaction. To a stirred solution of oxalyl chloride (3.3 equiv) in THF (5 mL/mmol) at −78 °C was added dimethyl sulphoxide (3.5 equiv). The solution was allowed to warm up to −35 °C for 5 min and was cooled again to −78 °C. A solution of alcohol (1 equiv) in THF (5 mL/mmol) was then added to the reaction mixture via a canula. The resulting solution was allowed to warm up to −35 °C and after 15 min was treated with triethylamine (5 equiv). The reaction mixture was allowed to reach room temperature for 1 h and was then cooled to −78 °C. A 3 M diethyl ether solution of methyl magnesium chloride (4 equiv) was then added dropwise. The reaction was followed by TLC until the aldehyde had completely disappeared. The solution was immediately diluted with ethanol, saturated aqueous ammonium chloride solution, water and diethyl ether. The mixture was extracted with diethyl ether. The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography.

3.2.14. (3*R*,4*S*)-*O*-Benzylidene-5-*O*-benzylpentan-2-ol (12a). Colourless oil as a 6:5:2:1 mixture of four diastereomers obtained from **11a** with 88% yield. R_f=0.41 (ethyl acetate/cyclohexane, 50/50). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.25 (6/14 of 3H, d, J_{1-2}=6.4 Hz, 1-H), 1.29 (5/14 of 3H, d, J_{1-2}=6.4 Hz, 1-H*), 2.32 (2/14 of 1H, d, J_{OH-2}=5.5 Hz, OH), 2.36 (1/14 of 1H, s, OH*), 2.49 (5/14 of 1H, s, OH§), 2.54 (3/14 of 1H, s, OH$^{\#}$), 3.71 (2H, m, 5-H), 3.78 (1H, m, 2-H), 3.97 (1H, m, 3-H), 4.30 (1H, m, 4-H), 4.62 (6/14 of 2H, s, CH_2-Ph), 4.64 (5/14 of 2H, s, CH_2-Ph*), 5.96 (2/14 of 1H, s, CH-Ph), 5.97 (1/14 of 1H, s, CH-Ph*), 5.98 (3/14 of 1H, s, CH-Ph§), 5.99 (5/14 of 1H, s, CH-Ph$^{\#}$), 7.33–7.51 (10H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.7 (CH_3, C-1), 18.8 (CH_3, C-1*), 19.2 (CH_3, C-1§), 19.6 (CH_3, C-1$^{\#}$), 67.4 (CH, C-2), 67.9 (CH, C-2*), 68.1 (CH, C-2§), 70.1 (CH_2, C-5), 70.2 (CH_2, C-5*), 70.5 (CH_2, C-5§), 70.7 (CH_2, C-5$^{\#}$), 73.5 (CH_2, 2×CH_2-Ph), 73.6 (CH_2, 2×CH_2-Ph*), 73.7 (CH_2, 2×CH_2-Ph§), 76.6 (CH, C-4), 77.2 (CH, C-4*), 77.7 (CH, C-4§), 78.7 (CH, C-4$^{\#}$), 82.9 (CH, C-3), 83.0 (CH, C-3*), 83.2 (CH, C-3§), 83.3 (CH, C-3$^{\#}$), 103.6 (CH, CH-Ph), 103.7 (CH, CH-Ph*), 103.8 (CH, CH-Ph§), 126.5, 126.6, 126.7, 127.7, 127.8, 128.3, 128.4, 129.3, 129.5, 137.1, 137.4, 137.6 and 137.7 (aromatic C). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3380, 1602, 1489, 1454, 1396, 1364, 1274, 1096, 1068. MS (FAB$^+$): (M+H)$^+$ 315.1. HRMS (FAB$^+$): calcd for $C_{19}H_{23}O_4$ 315.1583, found 315.1591.

3.2.15. (3*R*,4*S*)-[2,5,5-$^2H_3$]-*O*-Benzylidene-5-*O*-benzylpentan-2-ol (12b). Colourless oil as a 7:4:2:1 mixture of four diastereomers obtained from **11b** with 88% yield. R_f=0.41 (ethyl acetate/hexane, 50/50). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.25 (9/14 of 3H, s, 1-H), 1.28 (5/14 of 3H, s, 1-H*), 2.41 (2/14 of 1H, d, J_{OH-4}=5.5 Hz, OH), 2.46 (1/14 of 1H, s, OH*), 2.57 (4/14 of 1H, s, OH§), 2.64 (7/14 of 1H, s, OH$^{\#}$), 3.39 (7/14 of 1H, d, J_{3-4}=6.8 Hz, 3-H), 3.88 (1/14 of 1H, d, J_{3-4}=6.4 Hz, 3-H*), 3.95 (4/14 of 1H, d, J_{3-4}=6.4 Hz, 3-H§), 3.99 (2/14 of 1H, d, J_{3-4}=6.4 Hz, 3-H$^{\#}$), 4.25 (1/14 of 1H, d, J_{3-4}=6.4 Hz, 4-H), 4.27 (2/14 of 1H, d, J_{3-4}=6.4 Hz, 4-H*), 4.34 (7/14 of 1H, d, J_{3-4}=6.4 Hz, 4-H§), 4.35 (4/14 of 1H, d, J_{3-4}=6.4 Hz, 4-H$^{\#}$), 4.62 (2/14 of 2H, s, CH_2-Ph), 4.63 (7/14 of 2H, s, CH_2-Ph*), 4.64 (4/14 of 2H, s, CH_2-Ph§), 4.65 (1/14 of 2H, s, CH_2-Ph$^{\#}$), 5.96 (7/14 of 1H, s, CH-Ph), 5.97 (4/14 of 1H, s, CH-Ph*), 5.99 (1/14 of 1H, s, CH-Ph§), 6.00 (2/14 of 1H, s, CH-Ph$^{\#}$), 7.32–7.53 (10H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.6 (CH_3, C-1, β shift: −140 ppb), 18.7 (CH_3, C-1*, β shift: −115 ppb), 19.1 (CH_3, C-1§, β shift: −145 ppb), 19.4 (CH_3, C-1$^{\#}$, β shift: −164 ppb), 67.3 (CD, m, C-2, α shift: −707 ppb), 69.7 (CD_2, m, C-5, α shift: −747 ppb), 73.4 (CH_2, 2×CH_2-Ph, γ shift: −115 ppb), 73.5 (CH_2, 2×CH_2-Ph*, γ shift: −115 ppb), 73.6 (CH_2, 2×CH_2-Ph§, γ shift: −139 ppb), 76.6 (CH, C-4, β+γ shifts: −90 ppb), 76.8 (CH, C-4*, β+γ shifts: −380 ppb), 77.5 (CH, C-4§, β+γ shifts: −197 ppb), 78.5 (CH, C-4$^{\#}$, β+γ shifts: −190 ppb), 82.7 (CH, C-3, β+γ shifts: −254 ppb), 82.9 (CH, C-3*, β+γ shifts: −148 ppb), 83.1 (CH, C-3§, β+γ shifts: −189 ppb), 103.5 (CH, CH-Ph), 103.7 (CH, CH-Ph*), 103.8 (CH, CH-Ph§), 103.9 (CH, CH-Ph$^{\#}$), 126.5, 126.6, 126.7, 127.5, 127.6, 127.7, 128.2, 128.3, 128.4, 129.3, 129.4, 137.1, 137.2, 137.4, 137.6 and 137.7 (aromatic C). ^{2}H NMR (61.4 MHz, $CHCl_3$): δ=3.69 (s), 3.84 (s), 3.98 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3378, 2194, 2089, 1602, 1494, 1455, 1406, 1373, 1291, 1097, 1061. MS (FAB$^+$): (M+H)$^+$ 318.1. HRMS (FAB$^+$): *m/z*: calcd for $C_{19}H_{20}D_3O_4$ 318.1785, found 318.1779.

3.2.16. (2*R*,3*S*)-*O*-Benzylidene-4-hydroxypentyl 1-dibenzylphosphate (12c). Colourless oil as a 1:9:6:6 mixture of four diastereomers obtained from **11c** with 89% yield. R_f=0.31 (ethyl acetate/hexane, 8/2). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.21 (14/22 of 3H, d, J_{5-4}=6.4 Hz, 5-H), 1.26 (8/22 of 3H, d, J_{5-4}=6.4 Hz, 5-H*), 2.40 (10/22 of 1H, s, OH), 2.54 (6/22 of 1H, s, OH*), 2.71 (6/22 of 1H, s, OH§), 3.88 (2H, m, 3-H and 4-H), 4.16 (3H, m, 1- and 2-H), 5.02 (6/22 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph), 5.03 (6/22 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph*), 5.07 (1/22 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph§), 5.08 (9/22 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph$^{\#}$), 5.86 (1/22 of 1H, s, CH-Ph), 5.87 (9/22 of 1H, s, CH-Ph*), 5.95 (6/22 of 1H, s, CH-Ph§), 5.96 (6/22 of 1H, s, CH-Ph$^{\#}$), 7.32–7.49 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.7 (CH_3, C-5), 19.1 (CH_3, C-5*), 19.2 (CH_3, C-5§), 19.9 (CH_3, C-5$^{\#}$), 66.9 (CH_2, J_{C1-P}=5.6 Hz, C-1), 67.1 (CH, C-4), 67.4 (CH, C-4*), 67.5 (CH, C-4§), 67.6 (CH_2, J_{C1-P}=5.6 Hz, C-1*), 67.7 (CH_2, J_{C1-P}=5.6 Hz, C-1§), 67.9 (CH, C-4$^{\#}$), 69.1 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.5 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 76.2 (CH, d, J_{C2-P}=7.4 Hz, C-2), 76.4 (CH, d, J_{C2-P}=7.4 Hz, C-2*), 77.9 (CH, d, J_{C2-P}=7.4 Hz, C-2§), 80.9 (CH, C-3), 81.5 (CH, C-3*), 81.9 (CH, C-3§), 82.1 (CH, C-3$^{\#}$), 103.7 (CH, CH-Ph), 103.9 (CH, CH-Ph*), 104.0 (CH, CH-Ph§), 104.1 (CH, CH-Ph$^{\#}$), 126.5, 126.6, 126.7, 127.9, 128.0, 128.3, 128.4, 128.5, 128.6, 128.7, 129.4, 129.5, 129.6, 135.5, 135.6, 135.7, 136.7, 136.8 and 137.3 (aromatic C). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ=0.2 (s), 0.4 (s), 0.5 (s), 0.6 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3376, 1602, 1496, 1456, 1409, 1380, 1272, 1104, 1016. MS (FAB$^+$): (M+H)$^+$ 485.1. HRMS (FAB$^+$): calcd for $C_{26}H_{30}O_7P$ 485.1729, found 485.1733.

3.2.17. (2*R*,3*S*)-[2,5,5-$^2H_3$]-*O*-Benzylidene-4-hydroxypentyl 1-dibenzylphosphate (12d). Colourless oil as a 1:5:3:7 mixture of four diastereomers obtained from **11d**

with 86% yield. R_f=0.21 (ethyl acetate/cyclohexane, 60/40). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.23 (3/16 of 3H, s, C-5), 1.25 (7/16 of 3H, s, C-5), 1.26 (5/16 of 3H, s, C-5), 1.27 (1/16 of 3H, s, C-5), 2.58 (1H, s, OH), 3.79 (5/16 of 1H, d, J_{2-3}=6.6 Hz, C-3), 3.84 (1/16 of 1H, d, J_{2-3}=6.6 Hz, C-3*), 3.85 (7/16 of 1H, d, J_{2-3}=6.6 Hz, C-3[§]), 3.88 (3/16 of 1H, d, J_{2-3}=6.6 Hz, C-3[#]), 4.23 (4/16 of 1H, d, J_{2-3}=6.6 Hz, C-2), 4.35 (5/16 of 1H, d, J_{2-3}=6.6 Hz, C-2*), 4.38 (7/16 of 1H, d, J_{2-3}=6.6 Hz, C-2[§]), 5.01 (3/16 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph), 5.03 (5/16 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph*), 5.07 (1/16 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph[§]), 5.08 (7/16 of 4H, d, J_{H-P}=8.2 Hz, CH_2-Ph[#]), 5.86 (7/16 of 1H, s, CH-Ph), 5.87 (3/16 of 1H, s, CH-Ph*), 5.95 (5/16 of 1H, s, CH-Ph[§]), 5.96 (1/16 of 1H, s, CH-Ph[#]), 7.32–7.48 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.6 (CH_3, C-5, β shift: −115 ppb), 18.9 (CH_3, C-5*, β shift: −123 ppb), 19.1 (CH_3, C-5[§], β shift: −115 ppb), 19.8 (CH_3, C-5[#], β shift: −139 ppb), broad signal centred at 67.2 (CD and CD_2, m, C-1 and C-4), 69.3 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.5 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 75.9 (CH, d, J_{C2-P}=7.4 Hz, C-2, β+γ shifts: −172 ppb), 76.2 (CH, d, J_{C2-P}=7.4 Hz, C-2*, β+γ shifts: −135 ppb), 77.8 (CH, d, J_{C2-P}=7.4 Hz, C-2[§], β+γ shifts: −156 ppb), 80.8 (CH, C-3, β+γ shifts: −115 ppb), 81.4 (CH, C-3*, β+γ shifts: −107 ppb), 81.8 (CH, C-3[§], β+γ shifts: −107 ppb), 82.0 (CH, C-3[#], β+γ shifts: −123 ppb), 103.7 (CH, CH-Ph), 103.9 (CH, CH-Ph*), 104.1 (CH, CH-Ph[§]), 126.5, 126.6, 126.7, 127.9, 128.0, 128.3, 128.4, 128.5, 128.6, 128.7, 129.4, 129.5, 129.6, 135.5, 135.6, 135.7, 136.7, 136.8 and 137.3 (aromatic C). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ=0.2 (s), 0.3 (s), 0.5 (s), 0.6 (s). ^{2}H NMR (61.4 MHz, $CHCl_3$): δ=4.17 (s), 4.40 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3390, 2201, 2099, 1602, 1497, 1457, 1406, 1377, 1273, 1093, 1018. MS (FAB^+): $(M+H)^+$ 487.9. HRMS (FAB^+): calcd for $C_{26}H_{27}D_3O_7P$ 488.1917, found 488.1929.

3.2.18. General procedure for oxidation. To a solution of the alcohol (1 equiv) in dichloromethane (2 mL/mmol) were added activated 4 Å molecular sieves, *N*-methylmorpholin-*N*-oxide (3.5 equiv) and solid TPAP (0.1 equiv). The mixture was stirred at room temperature and monitored by TLC until the starting material completely disappeared. The reaction mixture was then filtered through a layer of silica on a sintered-glass funnel. The solid cake was washed with ethyl acetate, and the filtrate was evaporated. The residue was purified by flash chromatography.

3.2.19. (3*R*,4*S*)-*O*-Benzylidene-5-*O*-benzylpentan-2-one (13a). Colourless oil as a 1:1 mixture of two diastereomers obtained from **12a** with 86% yield. R_f=0.43 (ethyl acetate/cyclohexane, 30/70). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.29 (1/2 of 3H, s, 1-H), 2.34 (1/2 of 3H, s, 1-H*), 3.78 (2H, m, 5-H), 4.37 (1H, m, 4-H), 4.43 (1/2 of 1H, d, J_{3-4}=6 Hz, 3-H), 4.50 (1/2 of 1H, d, J_{3-4}=6 Hz, 3-H*), 4.63 (1/2 of 2H, s, CH_2-Ph), 4.64 (1/2 of 2H, s, CH_2-Ph*), 5.96 (1/2 of 1H, s, CH-Ph), 6.09 (1/2 of 1H, s, CH-Ph*), 7.29–7.54 (10H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.5 (CH_3, C-1), 26.8 (CH_3, C-1*), 70.0 (CH_2, C-5), 70.4 (CH_2, C-5*), 73.5 (CH_2, 2×CH_2-Ph), 73.6 (CH_2, 2×CH_2-Ph*), 78.2 (CH, C-4), 78.3 (CH, C-4*), 82.1 (CH, C-3), 82.3 (CH, C-3*), 105.1 (CH, CH-Ph), 126.7, 126.8, 127.6, 127.7, 127.8, 128.4, 129.6, 129.7, 136.4, 136.6 and 137.7 (aromatic C), 207.8 (quaternary C, C-2), 208.4 (quaternary C, C-2*). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1719, 1602, 1495, 1453, 1359, 1272, 1096, 1071. MS (FAB^+): $(M+H)^+$ 313.1. HRMS (FAB^+): calcd for $C_{19}H_{21}O_4$ 313.1440, found 313.1437.

3.2.20. (3*R*,4*S*)-[5,5-$^2H_2$]-*O*-Benzylidene-5-*O*-benzylpentan-2-one (13b). Colourless oil as a 1:1 mixture of two diastereomers obtained from **12b** with 86% yield. R_f=0.46 (ethyl acetate/hexane, 30/70). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.30 (1/2 of 3H, s, 1-H), 2.35 (1/2 of 3H, s, 1-H*), 4.37 (1/2 of 1H, d, J_{3-4}=6.5 Hz, 3-H), 4.40 (1/2 of 1H, d, J_{3-4}=6.5 Hz, 3-H*), 4.45 (1/2 of 1H, d, J_{3-4}=6.5 Hz, 4-H), 4.51 (1/2 of 1H, d, J_{3-4}=6.5 Hz, 4-H*), 4.64 (1/2 of 2H, s, CH_2-Ph), 4.65 (1/2 of 2H, s, CH_2-Ph*), 5.98 (1/2 of 1H, s, CH-Ph), 6.10 (1/2 of 1H, s, CH-Ph*), 7.31–7.56 (10H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.5 (CH_3, C-1), 26.7 (CH_3, C-1*), 69.5 (CD_2, quint, J_{C-D}=20.2 Hz, C-5, α shift: −501 ppb), 69.6 (CD_2, quint, J_{C-D}=20.2 Hz, C-5*, α shift: −546 ppb), 73.4 (CH_2, 2×CH_2-Ph, γ shift: −131 ppb), 73.5 (CH_2, 2×CH_2-Ph*, γ shift: −148 ppb), 78.0 (CH, C-4, β shift: −197 ppb), 78.2 (CH, C-4*, β shift: −181 ppb), 81.9 (CH, C-3, γ shift: −98 ppb), 82.2 (CH, C-3*, γ shift: −90 ppb), 105.0 (CH, CH-Ph), 126.6, 126.7, 127.5, 127.6, 127.7, 128.4, 129.6, 129.7, 136.4, 136.6 and 137.7 (aromatic C), 207.7 (quaternary C, C-2), 208.3 (quaternary C, C-2*). ^{2}H NMR (61.4 MHz, $CHCl_3$): δ=3.76 (s), 3.78 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 2191, 2078, 1721, 1602, 1494, 1453, 1359, 1272, 1097, 1070. MS (FAB^+): $(M+H)^+$ 315.1. HRMS (FAB^+): calcd for $C_{19}H_{19}D_2O_4$ 315.1565, found 318.1561.

3.2.21. (2*R*,3*S*)-*O*-Benzylidene-4-oxopentyl 1-dibenzylphosphate (13c). Colourless oil as a 1:1 mixture of two diastereomers obtained from **12c** with 92% yield. R_f=0.53 (ethyl acetate/hexane, 8/2). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.26 (1/2 of 3H, s, 5-H), 2.30 (1/2 of 3H, s, 5-H*), 4.26 (3H, m, 1-, 2- and 3-H), 5.03 (1/2 of 4H, d, J_{H-P}=8.4 Hz, CH_2-Ph), 5.08 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.09 (1/2 of 2H, d, J_{H-P}=8.4 Hz CH_2-Ph*), 5.90 (1/2 of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.32–7.50 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.6 (CH_3, C-5), 26.7 (CH_3, C-5*), 66.6 (CH_2, d, J_{C1-P}=5.6 Hz, C-1), 66.9 (CH_2, d, J_{C1-P}=5.6 Hz, C-1*), 69.4 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph), 69.5 (CH_2, d, J_{C-P}=5.6 Hz, CH_2-Ph*), 76.8 (CH, d, J_{C2-P}=8.0 Hz, C-2), 76.9 (CH, d, J_{C2-P}=7.4 Hz, C-2*), 81.4 (CH, C-3), 81.6 (CH, C-3*), 105.0 (CH, CH-Ph), 105.1 (CH, CH-Ph*), 126.6, 126.7, 127.9, 128.0, 128.4, 128.5, 128.6, 129.7, 129.8, 135.5, 135.6, 135.9 and 136.2 (aromatic C), 206.8 (quaternary C, C-4), 207.7 (quaternary C, C-4*). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ=0.2 (s), 0.3 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1722, 1602, 1497, 1455, 1406, 1380, 1269, 1090, 1017. MS (FAB^+): $(M+H)^+$ 483.1. HRMS (FAB^+): calcd for $C_{26}H_{28}O_7P$ 483.1573, found 483.1573.

3.2.22. (2*R*,3*S*)-[5,5-$^2H_2$]-*O*-Benzylidene-4-oxopentyl 1-dibenzylphosphate (13d). Colourless oil as a 1:1 mixture of two diastereomers obtained from **12d** with 93% yield. R_f=0.38 (ethyl acetate/hexane, 60/40). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.26 (1/2 of 3H, s, 5-H), 2.30 (1/2 of 3H, s, 5-H*), 4.35 (2H, m, 2- and 3-H), 5.04 (1/2 of 4H, d, J_{H-P}=8.4 Hz, CH_2-Ph), 5.08 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.09 (1/2 of 2H, d, J_{H-P}=8.4 Hz, CH_2-Ph*), 5.90 (1/2

of 1H, s, CH-Ph), 5.97 (1/2 of 1H, s, CH-Ph*), 7.32–7.50 (15H, m, Ph). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=26.5$ (CH_3, C-5), 26.7 (CH_3, C-5*), broad signal centred at 66.4 (CD_2, C-1, m), 69.4 (CH_2, d, $J_{C-P}=5.6$ Hz, CH_2-Ph), 69.5 (CH_2, d, $J_{C-P}=5.6$ Hz, CH_2-Ph*), 76.7 (CH, d, $J_{C2-P}=8.0$ Hz, C-2, β shift: −148 ppb), 76.9 (CH, d, $J_{C2-P}=7.4$ Hz, C-2*, β shift: −156 ppb), 81.3 (CH, C-3, γ shift: −58 ppb), 81.6 (CH, C-3*, γ shift: −66 ppb), 104.9 (CH, CH-Ph), 105.0 (CH, CH-Ph*), 126.6, 126.7, 127.9, 128.0, 128.4, 128.5, 128.6, 129.7, 129.8, 135.5, 135.6, 135.9 and 136.2 (aromatic C), 206.8 (quaternary C, C-4, δ shift: −25 ppb), 207.7 (quaternary C, C-4*, δ shift: −17 ppb). ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta=0.2$ (s), 0.4 (s). ^{2}H NMR (61.4 MHz, $CHCl_3$): $\delta=4.43$ (s), 4.50 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 2204, 2101, 1720, 1602, 1497, 1457, 1406, 1380, 1275, 1097, 1021. MS (FAB^+): $(M+H)^+$ 484.9. HRMS (FAB^+): calcd for $C_{26}H_{26}D_2O_7P$ 485.1698, found 485.1705.

3.2.23. General procedure for hydrogenation. The protected compounds were hydrogenated in the presence of 10–15% Pd/C in *i*PrOH/H_2O (9:1) (20 mL/mmol) for **3a** and **3b** and MeOH/H_2O (9:1) (20 mL/mmol) for **3c**, **3d** at room temperature and atmospheric pressure. The mixture was filtered through celite, and the filtrate was evaporated to dryness.

3.2.24. 1-Deoxy-D-xylulose (3a). Colourless oil as a 1:1:4 mixture of respectively the cyclic α- and β-anomers and the open chain obtained from **13a** quantitatively. $R_f=0.19$ (chloroform/methanol, 90/10). $[\alpha]_D^{20}=+35$ (*c* 1.0, H_2O), lit.[18] $[\alpha]_D=+33.6$ (*c* 1.0, H_2O). ^{1}H NMR (300 MHz, CD_3OD): $\delta=1.38$ (s, CH_3, furanose anomer) 1.42 (s, CH_3, furanose anomer), 2.24 (s, 1-H, straight chain), 3.52 (m, furanose anomer), 3.58 (dd, $J_{5a-5b}=11.0$ Hz, $J_{4-5a}=6.6$ Hz, 5-Ha, straight chain), 3.64 (m, furanose anomer), 3.65 (dd, $J_{5a-5b}=11.0$ Hz, $J_{4-5a}=6.6$ Hz, 5-Hb, straight chain), 3.78 (m, furanose anomer), 3.99 (ddd, $J_{3-4}=2.2$ Hz, $J_{4-5a}=J_{4-5b}=6.6$ Hz, 4-H, straight chain), 4.08 (m, furanose anomer), 4.21 (dd, $J_{3-4}=2.2$ Hz, 3-H-open chain). ^{13}C NMR (75 MHz, CD_3OD): $\delta=21.8$ (CH_3, C-1), 25.1 (CH_3, C-1), 26.6 (CH_3, C-1), 63.7 (CH_2, C-5), 71.4 (CH_2, C-5), 73.3 (CH_2, C-5), 73.5 (CH, C-4), 77.4 (CH, C-4), 78.4 (CH, C-4), 78.1 (CH, C-3), 82.7 (CH, C-3), 83.0 (CH, C-3), 103.8 (quaternary C, C-2), 107.5 (quaternary C, C-2), 212.4 (quaternary C, C-2). IR (KBr) ν_{max} (cm^{-1}): 3428, 3104, 1792, 1716, 1240, 1202, 1176, 991. MS (ES^-) *m/z*: 133 ($M-H^+$).

3.2.25. [5,5-$^2H_2$]-1-Deoxy-D-xylulose (3b). Colourless oil as a 1:1:4 mixture of respectively the cyclic α- and the β-anomers and the open chain obtained from **13b** quantitatively. $R_f=0.18$ (chloroform/methanol, 90/10). $[\alpha]_D^{20}=+35$ (*c* 1, H_2O), lit.[19] $[\alpha]_D=+34.8$ (*c* 1.0, H_2O). ^{1}H NMR (300 MHz, CD_3OD); $\delta=1.38$ (s, CH_3, furanose anomer) 1.42 (s, CH_3, furanose anomer), 2.24 (s, 1-H, straight chain), 3.61 (m, furanose anomer), 3.79 (m, furanose anomer), 3.97 (s, 4-H, straight chain), 4.07 (s, furanose anomer), 4.21 (d, $J_{3-4}=2.2$ Hz, 3-H, straight chain). ^{13}C NMR (75 MHz, CD_3OD): $\delta=21.8$ (CH_3, C-1), 25.0 (CH_3, C-1), 26.5 (CH_3, C-1), broad signal centred at 63.2 (CD_2, m, C-5), 73.4 (CH, C-4, β shift: −82 ppb), 77.3 (CH, C-4, β shift: −92 ppb), 78.3 (CH, C-4, β shift: −76 ppb), 78.5 (CH, C-3, γ shift: −24 ppb), 82.8 (CH, C-3, γ shift: −34 ppb), 83.1 (CH, C-3, γ shift: −76 ppb), 103.7 (quaternary C, C-2, δ shift: −36 ppb), 107.5 (quaternary C, C-2, δ shift: −21 ppb), 212.3 (quaternary C, C-2, δ shift: −11 ppb). ^{2}H NMR (61.4 MHz, $CHCl_3$): $\delta=3.76$ (s), 3.86 (s). IR (KBr) ν_{max} (cm^{-1}): 3428, 3187, 2237, 2098, 1792, 1715, 1664, 1259, 1087, 1017, 873. MS (ES^-) *m/z*: 135 ($M-H^+$).

3.2.26. [5,5-$^2H_2$]-1-Deoxy-D-xylulose triacetate. After acetylation of **3b** with pyridine and acetic anhydride and subsequent preparative chromatography, the triacetate of [5,5-$^2H_2$]-1-deoxy-D-xylulose was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=2.05$, 2.07, 2.20 and 2.21 (12H, 4×CH_3), 5.23 (1H, d, $J_{3-4}=2.9$ Hz, 3-H), 5.57 (1H, d, $J_{3-4}=2.9$ Hz, 4-H). ^{13}C NMR (75 MHz, CD_3OD): $\delta=20.4$, 20.5, 20.6 (3×CH_3, Ac), 26.7 (CH_3, C-1), broad signal centred at 60.6 (CD_2, m, C-5, α shift: −700 ppb), 68.6 (CH, C-4, β shift: −98 ppb), 76.3 (CH, C-3, γ shift: −4 ppb), 169.7, 169.9, 170.3 (3×quaternary C, Ac), 201.4 (quaternary C, C-2, δ shift: −12 ppb).

3.2.27. 1-Deoxy-D-xylulose 5-phosphate (3c). Vitreous solid, which decomposed at 90 °C, obtained from **13c** quantitatively. $R_f=0.39$ (*i*-propanol/water/ethyl acetate, 6/3/1). $[\alpha]_D^{20}=+7$ (*c* 1.0, MeOH), lit.[29] $[\alpha]_D=+7.0$ (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, D_2O): $\delta=2.31$ (3H, s, 1-H), 3.96 (2H, m, 5-H), 4.39 (1H, ddd, $J_{3-4}=2$ Hz, $J_{4-5a}=1$ Hz, $J_{4-5b}=2$ Hz, 4-H), 4.50 (1H, d, $J_{3-4}=2$ Hz, 3-H). ^{13}C NMR (75 MHz, D_2O): $\delta=25.7$ (CH_3, C-1), 65.3 (CH_2, d, $J_{C-P}=5$ Hz, C-5), 70.0 (CH, d, $J_{C-D}=6.0$ Hz, C-4), 76.7 (CH, C-3), 212.8 (quaternary C, C-2). ^{31}P NMR (121.5 MHz, D_2O): $\delta=1.5$ (s). IR (KBr) ν_{max} (cm^{-1}): 3428, 1723, 1230, 1130, 1047, 972. MS (ES^-) *m/z*: 213 ($M-H^+$).

3.2.28. [5,5-$^2H_2$]-1-deoxy-D-xylulose 5-phosphate (3d). Vitreous solid, which decomposed at 90 °C, obtained from **13d** quantitatively. $R_f=0.41$ (*i*-propanol/water/ethyl acetate, 6/3/1). $[\alpha]_D^{20}=+7$ (*c* 1, MeOH), lit.[23] $[\alpha]_D=+24.0$ (*c* 1.1, H_2O). ^{1}H NMR (300 MHz, D_2O): $\delta=2.12$ (3H, s, 1-H), 4.16 (1H, d, $J=1.5$ Hz, 3-H), 4.33 (1H, d, $J=1.5$ Hz, 4-H). ^{13}C NMR (75 MHz, D_2O): $\delta=25.9$ (CH_3, C-1), 67.2 (CD_2, m, C-5, α shift: −1879 ppb), 70.4 (CH, d, $J_{C-P}=6.8$ Hz, C-4, β shift: −441 ppb), 76.9 (CH, C-3, γ shift: −273 ppb), 213.2 (quaternary C, C-2, δ shift: −370 ppb). ^{31}P NMR (121.5 MHz, D_2O): $\delta=1.4$ (s). ^{2}H NMR (61.4 MHz, H_2O): $\delta=4.14$ (s). IR (KBr) ν_{max} (cm^{-1}): 3391, 3238, 2223, 2102, 1716, 1243, 1125, 1077, 982. MS (ES^-) *m/z*: 215 ($M-H^+$).

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Synthesis of novel highly water-soluble 2:1 cyclodextrin/fullerene conjugates involving the secondary rim of β-cyclodextrin

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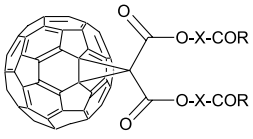
Abstract—Two novel fullerene[60]-cyclodextrin conjugates have been prepared, they display the highest solubility in water reported to date. This is the first synthesis of such conjugates in which the linker is attached to the secondary rim of β-CD.

1. Introduction

The recently described 2:1 cyclodextrin–fullerene conjugates **1** (Fig. 1)[1,2] display high solubility in water, an adequate property for their use in biological systems.[3]



1a R= NH-PMBCD ; X= $(CH_2)_{11}$

1b R= NH-PMBCD ; X= CH_2

1c R= NH-PMBCD ; X= CH_2-CH_2-O-CH_2

1d R= NH-PMGCD ; X= CH_2-CH_2-O-CH_2

Figure 1. The 2:1 CD-C_{60} conjugates linked via the primary rim of the β- or γ-CD.

Since cyclodextrins and permethylcyclodextrins are not toxic, these molecules seem well adapted to study the application to biological problems of the very attractive photo-, electro-chemical and physical properties of fullerenes.[3–5] It was postulated that these conjugates could be present in water equilibria between conformers such as **A**, **B** and **C** (Fig. 2); **A** and **B** could form micelle-like aggregates, while **C** could exist as a non-associated species.[1]

As expected, these compounds were very soluble in water: UV and NMR spectra showed the presence of aggregates; and the 'internal complexation' conformer **C** was not detected. Although this high solubility is convenient for application to biological systems, micellar aggregation may induce chemical,[6–9] electrochemical[10,11] or photophysical[12,13] properties differing from those of the isolated fullerene molecule (see however[14]). It thus seems worthwhile to try new structural modifications in order to obtain cyclodextrin–fullerene conjugates that would be highly water-soluble

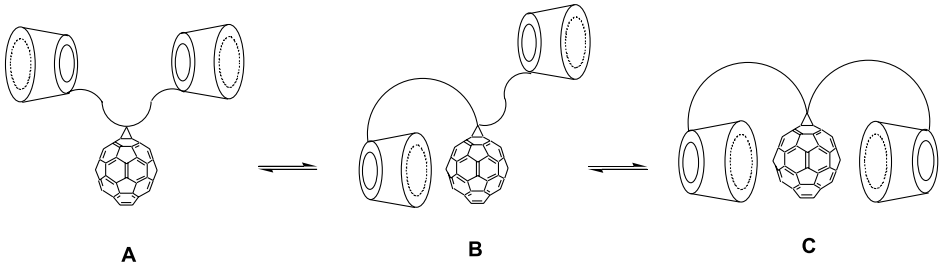


Figure 2. Postulated equilibria of the CD-C_{60} conjugates in water.

Keywords: C_{60}; Conjugate; Cyclodextrin; Fullerene; Synthesis.
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and, even at the highest concentration, would exist mainly in the form of the internal complex.

Because of the much easier availability of the primary carbon derivatives,[15] the two moieties of all the cyclodextrin–fullerene conjugates reported so far,[1,2,16,17] are linked via the primary external rim of the β- or γ-CD. As suggested previously,[1,2] a possible way to favour internal complexation vs. micelle formation could be to connect the fullerene to the cyclodextrin through the larger secondary rim, in order to favour a conformation D of the internal complex similar to the one (E) found by calculations on the (γ-CD)-fullerene 2:1 complex[18] (Fig. 3).

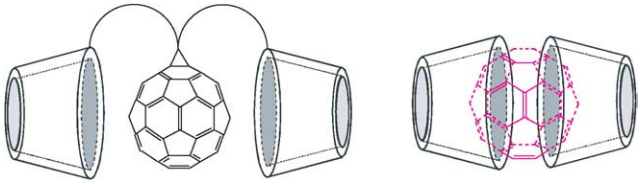
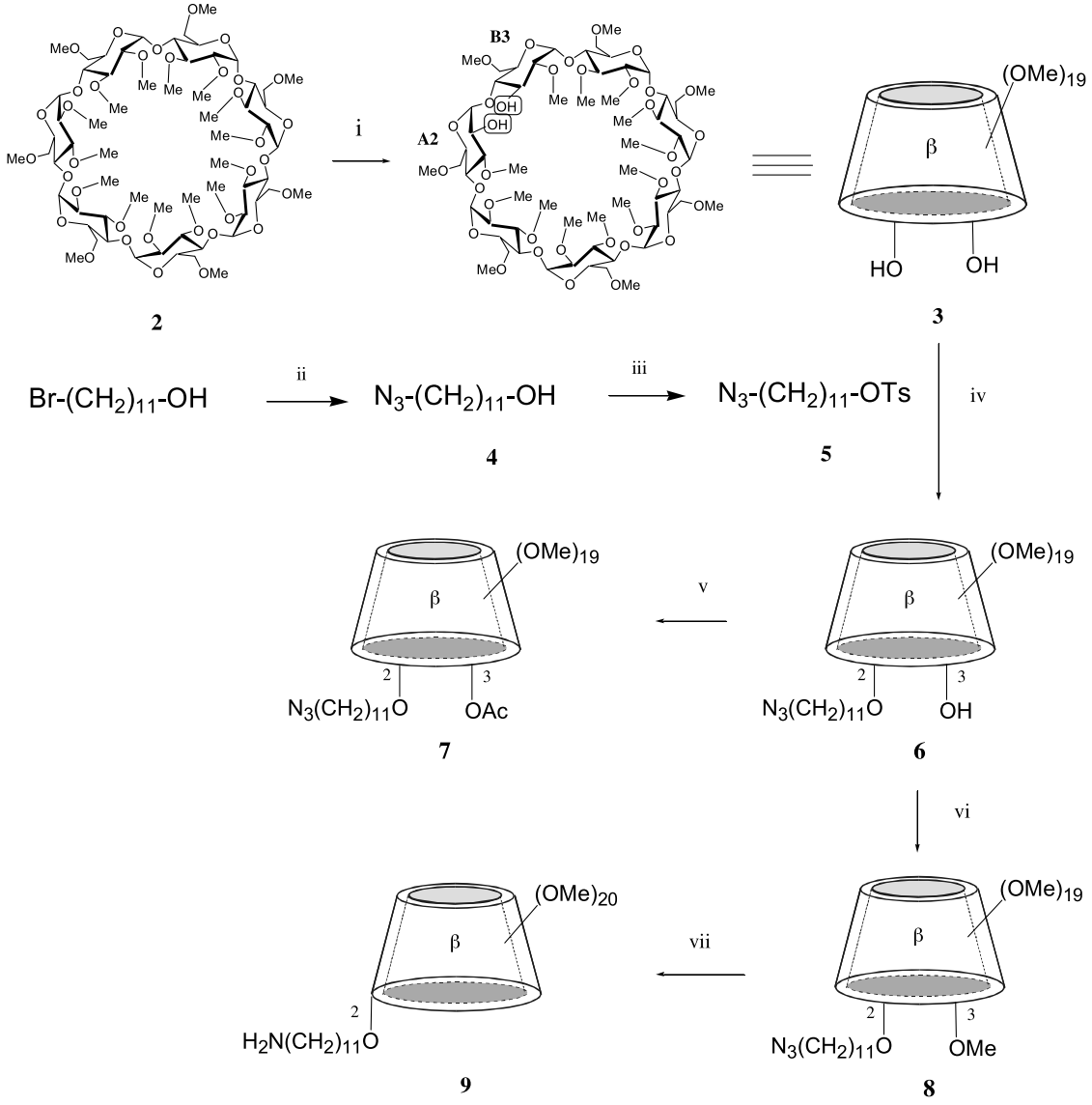


Figure 3. CD-C_{60} conjugate D and γ-CD/C_{60} 2:1 complex E.

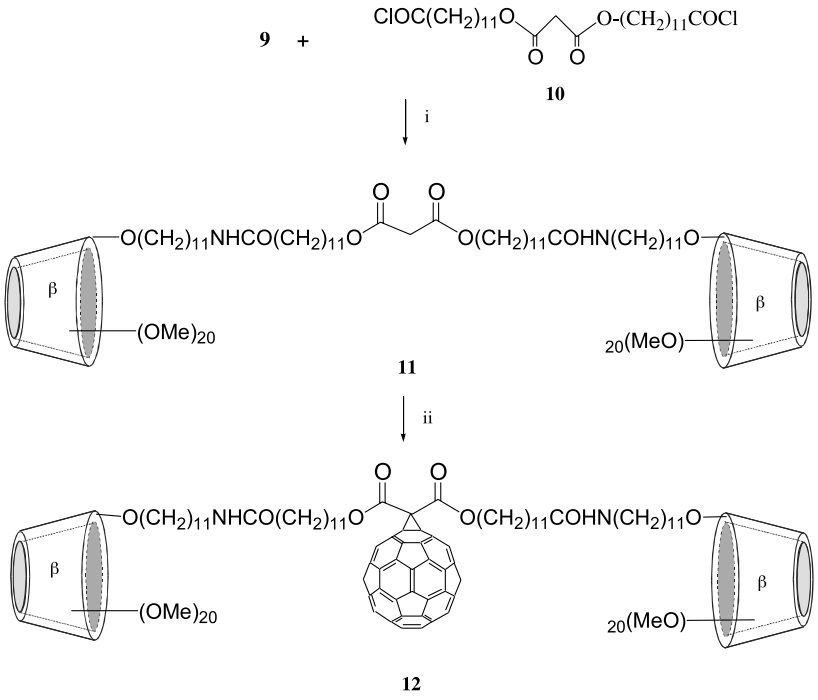
We present here two examples of fullerene–cyclodextrin conjugates in which the linker is attached to the secondary rim. This is indeed the first preparation of such conjugates through what may be a general method. In order to allow flexibility, we have chosen two long linkers consisting of 24 and 14 atoms, respectively. Permethylated β-cyclodextrin (PMBCD) **2** was selected for a better solubility in water compared to the native β-CD.

2. Results and discussion

The key intermediate of this synthesis is a methylated β-CD **3** having specifically located hydroxyl groups available on the secondary rim. The traditional methods for selectively



Scheme 1. Reagents and conditions: (i) DIBAL, 0 °C, 18 h (56%); (ii) NaN_3, DMF, 80 °C, 15 h (94%); (iii) TsCl, Et_3N, CH_2Cl_2, rt, 24 h (94%); (iv) NaH, DMF, 80 °C, 15 h (71%); (v) Ac_2O, Py, 40 °C, 15 h (68%); (vi) MeI, NaH, THF, 66 °C, 5 h (70%); (vii) $HS(CH_2)_3SH$, Et_3N, MeOH, rt, 28 h (95%).



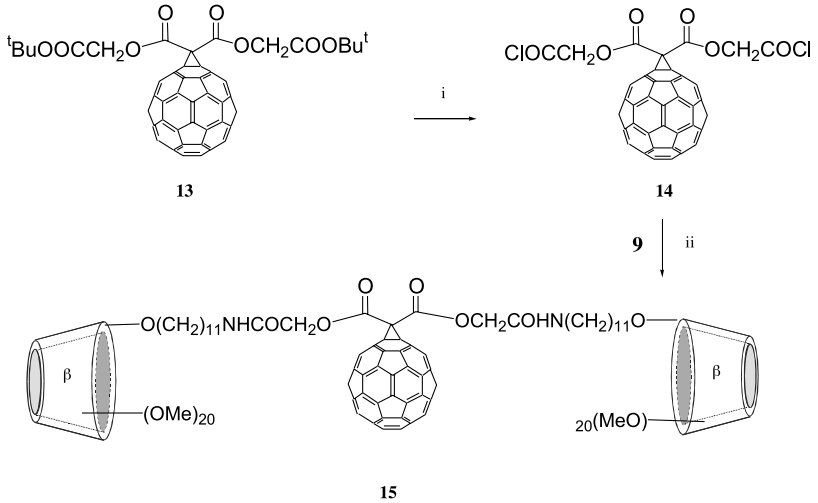
Scheme 2. Reagents and conditions: (i) Et_3N, CH_2Cl_2, rt, 7 h (84%); (ii) C_{60}, CBr_4, DBU, toluene, rt, 24 h (29%).

modified methylated CDs proceeds usually through a temporary regioselective protection of specific hydroxyl groups of the native CD, followed by *O*-methylation and final removal of the protective groups to unmask the required hydroxyl functions. We recently introduced a conceptually new way to obtain directly such compounds.[19–21] This alternative approach is based on the efficient selective de-*O*-alkylation of a fully alkylated α or β-CD, using commercially available diisobutylaluminum hydride (DIBAL-H) as a regioselective chemical 'scalpel'.[22]

Thus (Scheme 1) β-CD A2,B3-diol **3** was regioselectively prepared from the commercially available permethylated β-CD in 56% yield, and condensed with azidotosylate **5**, obtained from bromoundecanol through azidoalcohol **4**, to afford azidoalkyl β-CD **6** in good yield and as a single isomer. The structure of compound **6** was confirmed from the ^{1}H NMR spectrum of derivative **7**, readily obtained from **6** by acetylation; the H-3 of the glucose unit B displayed a deshielded signal at 5.41 ppm (dd, $J_{2,3}=J_{3,4}=10.0$ Hz), indicating that alkylation of **6** took place at position 2. The remaining OH of compound **6** was then methylated to give azidoalkyl permethylated β-CD **8**, which, after treatment by propane dithiol in the presence of triethyl amine,[23] gave aminoalkyl β-CD derivative **9** in 95% yield.

Condensation of **9** with malonic ester diacylchloride **10**[1] gave compound **11**, which through the Hirsch–Bingel reaction[24] with C_{60} afforded, after 24 h at room temperature, the target compound **12**, identified as a methanofullerene mono-adduct (Scheme 2).

The second conjugate was prepared in a slightly modified



Scheme 3. Reagents and conditions: (i) TFA, CH_2Cl_2, rt, 4 h; then $(COCl)_2$, CH_2Cl_2, reflux, 21 h. (ii) Et_3N, CH_2Cl_2, rt, 18 h (70%).

way: aminocyclodextrin **9** was reacted with the fullerene diacylchoride **14**, prepared from **13**[1] in the presence of triethylamine, to give conjugate **15** in 70% yield (Scheme 3).

The conjugates **12** and **15** are very soluble in dichloromethane and in chloroform and have a very high solubility in water at 20 °C, greater than 7×10^{-2} M for **12** and 9×10^{-2} M for **15**: clear solutions were obtained after dissolving **12** (32 mg) in water (100 μL), and **15** (35 mg) in water (100 μL). To our knowledge, these are the highest solubilities in neutral water for fullerene derivatives.[4,25]

As for the previously reported CD-C_{60} conjugates, aggregates are present in water solutions: the NMR spectra of **12** and **15** are much broader in water than in chloroform. The UV spectra of dichloromethane solutions of **12** and **15** are not distinguishable from those of **1c**. In water solution, these three compounds have slightly different UV spectra; none of these spectra display the absorption peak at 430 nm observed in dichloromethane solutions, a critical indication of the presence of aggregates.[26–28]

Similarly, water solutions (concentrations 10^{-4}–10^{-5} M) of these conjugates did not show any circular dichroism in the absorption bands of C_{60}, although induced circular dichroism has been observed for a γ-CD/C_{60} complex.[29,30]

3. Conclusion

We have reported here the first preparation of CD-C_{60} conjugates in which the connection is achieved through the secondary rim of the CD. These molecules display the highest solubility in water reported to date. Like most water-soluble fullerenes derivatives[31] (see however[25]) these conjugates are aggregated in water solution. Since it is possible that the affinity of β-CD for the fullerene moiety is not sufficient to induce this type of complexation, work is in progress towards conjugates connected to γ-CD, now[2] through the secondary rim.

4. Experimental

4.1. General procedures

Optical rotations were measured at 20±2 °C with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell. Chemical Ionisation Mass Spectra (CI-MS ammonia) and Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a JMS-700 spectrometer. Elemental analyses were performed by Service de Microanalyse de l'Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France. NMR spectra were recorded on a Bruker Avance 250 spectrometer or a Bruker DRX 400 spectrometer at ambient temperature. ^{1}H NMR chemical shifts are referenced to residual protic solvent ($CDCl_3$, $\delta_H=7.30$) or the internal standard TMS ($\delta_H=0.00$). ^{13}C NMR chemical shifts are referenced to the solvent signal ($\delta_c=77.0$ for the central line of $CDCl_3$). Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated silica gel 60 F_{254} plate (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulphuric acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

4.1.1. 11-Azido-1-undecanol (4). A mixture of the 11-bromo-1-undecanol (100 mg, 0.40 mmol), NaN_3 (78 mg, 1.20 mmol) in dry DMF (3 mL) was stirred at 80 °C overnight under argon. The DMF was removed by evaporation under reduced pressure, the residue was dissolved in CH_2Cl_2, washed with water and dried over $MgSO_4$. After evaporation of solvent, the residue was purified by chromatography on silica gel, eluted by CH_2Cl_2 to give the compound **4** as a yellowish syrup (80 mg, 94%). $R_f=0.39$ (CH_2Cl_2/MeOH 50:1); ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.61 (t, $J=6.5$ Hz, 2H, CH_2O), 3.25 (t, $J=6.9$ Hz, 2H, CH_2N_3), 2.12 (s, 1H, OH), 1.61–1.28 (m, 18H, 9×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 62.83 (CH_2–OH), 51.45 (CH_2–N_3), 32.73, 29.54, 29.44, 29.40, 29.12, 28.81, 26.68, 25.74 (9C, 9×CH_2); MS (ESI): *m/z* 235.8 (100%, M+Na^+); Anal. Calcd for $C_{11}H_{23}ON_3$: C, 61.92; H, 10.89; N, 19.70. Found: C, 61.79; H, 10.84; N, 19.86.

4.1.2. 11-Azido-1-undecanyl tosylate (5). To a solution of **4** (194 mg, 0.91 mmol), TsCl (262 mg, 1.37 mmol) in dry CH_2Cl_2 (4 mL) was added triethylamine (0.4 mL, 2.73 mmol) under argon, the reaction mixture was stirred at room temperature for 24 h. After diluted with CH_2Cl_2, washed with brine, water, dried over $MgSO_4$ and evaporated, the residue was purified by flash-chromatography, eluting with 1:1 cyclohexane/CH_2Cl_2 to offer **5** as a colourless syrup (313 mg, 94%): $R_f=0.39$ (cyclohexane/CH_2Cl_2 1:2); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.79 (d, 2H, $J_{a,b}=8.3$ Hz, 2×Ph-$H_{2,2'}$), 7.35 (d, 2H, $J_{a,b}=8.1$ Hz, 2×Ph-$H_{3,3'}$), 4.02 (t, $J=6.5$ Hz, 2H, CH_2O), 3.26 (t, $J=6.9$ Hz, 2H, CH_2N_3), 2.45(s, 3H, Ph-CH_3), 1.69–1.23 (m, 18H, 9×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.47 (Ph-C_1), 132.95 (Ph-C_4), 129.62 (Ph-$C_{2,2'}$), 127.64 (Ph-$C_{3,3'}$), 70.51 (CH_2–OH), 51.22 (CH_2–N_3), 29.18, 29.13, 29.11, 28.89, 28.66, 28.61, 28.57, 26.47, 25.09 (9C, 9×CH_2), 21.39 (Ph-CH_3); MS (ESI): *m/z* 390.0 (100%, M+Na^+); Anal. Calcd for $C_{18}H_{29}O_3N_3S$: C, 58.81; H, 7.97; N, 11.43. Found: C, 58.73; H, 7.99; N, 11.30.

4.1.3. Azidoalkyl β-CD (6). A mixture of **3** (423 mg, 0.30 mmol), NaH (60%, 18 mg, 0.45 mmol) in dry DMF (5 mL) under argon was stirred at room temperature for 1 h. Compound **5** (133 mg, 0.36 mmol) was dissolved with dry DMF (2 mL) and added to the above mixture at room temperature, then the reaction mixture was stirred at 80 °C overnight. MeOH was added dropwise to quench the reaction and the solvent was removed by evaporation. After dissolved with CH_2Cl_2, washed with brine and water, dried over $MgSO_4$ and concentrated, the crude product was purified by column chromatography (cyclohexane/acetone 3:2) to afford **6** (342 mg, 71%) as a white amorphous solid: $R_f=0.36$ (cyclohexane/acetone 3:2); $[\alpha]_D=+132$ (*c* 1.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.16–5.12 (m, 4H, 4×H_1), 5.11 (d, 1H, $J_{1,2}=3.6$ Hz, H_1), 5.08 (d, 1H, $J_{1,2}=3.6$ Hz, H_1), 4.97 (d, 1H, $J_{1,2}=3.6$ Hz, H_1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 101.19, 99.77, 99.68, 99.32, 98.99, 98.85, 98.76 (7C, 7×C_1), 83.32, 82.29, 82.19, 82.03, 81.97, 81.69, 81.64, 81.61, 81.59, 81.47, 81.45, 81.25, 81.19, 80.79, 80.36, 80.21, 80.09, 70.96, 70.91, 70.87, 70.82, 69.99

(28C, 7×C_2, C_3, C_4, C_5), 72.98, 71.40, 71.36, 71.30, 71.10 (8C, CH_2O+7×C_6), 61.81, 61.61, 61.48, 61.41, 61.38, 61.36, 59.00, 58.94, 58.91, 58.90, 58.56, 58.52, 58.49, 58.46, 58.41, 58.35 (19C, 19×OMe), 51.41 (CH_2N_3), 29.60, 29.47, 29.39, 29.37, 29.26, 29.06, 28.76, 26.64, 25.64 (9C, 9×CH_2); MS (FAB): *m/z* 1618.8 (75%, M+Na^+); Anal. Calcd for $C_{72}H_{129}O_{35}N_3$: C, 54.16; H, 8.14; N, 2.63. Found: C, 54.40; H, 8.42; N, 2.56.

4.1.4. Azidoalkyl permethylated β-CD (8). A mixture of **6** (90 mg, 0.056 mmol), NaH (60%, 11.3 mg, 0.28 mmol) in dry THF (2 mL) under argon was stirred at room temperature for 1 h. After CH_3I (17.5 μL, 0.28 mmol) added, the reaction mixture was stirred at 66 °C for 5 h, MeOH was added dropwise to quench the reaction and the solvent was removed by evaporation. The residue dissolved with CH_2Cl_2, washed with brine, water, dried ($MgSO_4$), concentrated, and purified by flash-chromatographed (eluent: cyclohexane/acetone 2:1) to give **8** (63 mg, 70%) as a white amorphous solid: R_f=0.42 (cyclohexane/acetone 3:2); $[\alpha]_D$=+122 (*c* 2.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19–5.16 (m, 4H, 4×H_1), 5.14 (2d, 2H, $J_{1,2}$=3.5 Hz, 2×H_1), 5.08 (d, 1H, $J_{1,2}$=3.4 Hz, H_1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 98.94, 98.93, 98.89, 98.86, 98.83 (7C, 7×C_1), 82.03, 81.95, 81.92, 81.77, 81.75, 81.68, 81.64, 81.61, 81.50, 80.86, 80.41, 80.36, 80.12, 80.02, 79.94, 79.81, 71.00, 70.87, 70.82, 70.74 (28C, 7×C_2,C_3,C_4,C_5), 71.49, 71.42, 71.35, 71.26, 71.16 (8C, CH_2O+7×C_6), 61.61, 61.49, 61.37, 61.34, 61.31, 58.93, 58.91, 58.90, 58.62, 58.58, 58.48, 58.39, 58.33 (20C, 20×OMe), 51.41 (1C, CH_2N_3), 30.09, 30.00, 29.53, 29.43, 29.41, 29.08, 28.76, 26.65, 25.90 (9C, 9×CH_2); MS (FAB): *m/z* 1632.9 (100%, M+Na^+); Anal. Calcd for $C_{73}H_{131}O_{35}N_3 \cdot H_2O$: C, 53.81; H, 8.25; N, 2.58. Found: C, 53.83; H, 8.40; N, 2.24.

4.1.5. Aminoalkyl permethylated β-CD (9). To a solution of **8** (285 mg, 0.18 mmol) in dry MeOH (8 mL) were added 1,3-propanedithiol (0.8 mL) and triethylamine (0.8 mL) under argon, the mixture was stirred at room temperature for 28 h. A white precipitate was formed. After filtration and washing with MeOH, the filtrate was concentrated. The residue was flash chromatographed, eluting with 6:1 ethyl acetate/MeOH, then 3:3:2 ethyl acetate/isopropanol/H_2O to afford **9** (265 mg, 95%) as a white amorphous solid: R_f=0.43 (ethyl acetate/isopropanol/H_2O 3:3:2); $[\alpha]_D$=+133 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19–5.16 (m, 4H, 4×H_1), 5.14 (2d, 2H, $J_{1,2}$=3.5 Hz, 2×H_1), 5.08 (d, 1H, $J_{1,2}$=3.5 Hz, H_1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 98.77, 98.75, 98.69, 98.67 (7C, 7×C_1), 81.85, 81.76, 81.72, 81.62, 81.59, 81.54, 81.32, 80.72, 80.22, 80.02, 79.90, 79.85, 79.69, 79.64, 70.93, 70.82, 70.74, 70.68, 70.6 (28C, 7×C_2,C_3,C_4,C_5), 71.38, 71.28, 71.19, 71.14, 71.02, 70.88 (8C, CH_2O+7×C_6), 61.49, 61.36, 61.35, 61.25, 61.21, 61.19, 58.84, 58.80, 58.54, 58.52, 58.50, 58.43, 58.31, 58.27 (20C, 20×OMe), 39.48 (CH_2NH_2), 29.89, 29.47, 29.43, 29.38, 29.30, 29.00, 28.18, 26.37, 25.83 (9C, 9×CH_2); MS (FAB): *m/z* 1606.8 (20%, M+Na^+), 1584.9 (35%, M+H^+); Anal. Calcd for $C_{73}H_{133}O_{35}N \cdot 3H_2O$: C, 53.49; H, 8.56; N, 0.85. Found: C, 53.41; H, 8.43; N, 1.06.

4.1.6. Malonic acid bis-(11-carboxy-undecyl) acid chloride (10). To a solution of Malonic acid bis-(11-carboxy-undecyl) acid (122 mg, 0.24 mmol) in dry CH_2Cl_2 (5 mL) in ice-bath under argon was added oxalyl chloride (0.063 mL, 0.73 mmol). The mixture was stirred under reflux for 18 h. After the solvent was removed in vacuum, the compound **10** (135 mg, dark blue solid) was obtained and used without further purification.

4.1.7. Permethylated β-CD dimer (11). To a solution of **9** (360 mg, 0.23 mmol) in dry CH_2Cl_2 (12 mL) in ice-bath under argon were added triethylamine (79 μL, 0.57 mmol) and **10** (61 mg, 0.11 mmol, dissolved with 3 mL CH_2Cl_2). The mixture was stirred at room temperature for 7 h. After removal of the solvent, the residue was flash chromatographed, eluting with 8:1 ethyl acetate/MeOH to provide **11** (343 mg, 84%) as a white amorphous solid. R_f=0.52 (EtOAc/isopropanol/H_2O 6:3:1); $[\alpha]_D$=+121 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.53 (t, 2H, *J*=5.5 Hz, 2×NH), 5.19–5.16 (m, 8H, 8×H_1), 5.14 (2d, 4H, $J_{1,2}$=3.6 Hz, 4×H_1), 5.08 (d, 2H, $J_{1,2}$=3.4 Hz, 2×H_1), 4.15 (t, 4H, *J*=6.8 Hz, 2×CH_2OOC); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.96, 166.62 (4C, 2×CO–NH, 2×CO–O), 98.90, 98.86, 98.84, 98.82, 98.80 (14C, 14×C_1), 82.00, 81.91, 81.88, 81.75, 81.72, 81.65, 81.61, 81.58, 81.47, 80.84, 80.38, 80.34, 80.11, 80.08, 79.99, 79.89, 79.78, 70.98, 70.85, 70.79, 70.71 (56C, 14×C_2,C_3,C_4,C_5), 71.47, 71.39, 71.31, 71.23, 71.21, 71.13, 66.42, 65.57 (18C, 2×OCH_2, 2×*C*H_2OCO, 14×C_6), 61.59, 61.46, 61.34, 61.31, 61.29, 58.91, 58.87, 58.59, 58.56, 58.46, 58.36, 58.30 (40C, 40×OMe), 41.62, 39.42, 36.81 (5C, OOC–*C*H_2–COO, 2×*C*H_2–NH–CO, 2×*C*H_2–CO–NH), 29.97, 29.61, 29.54, 29.50, 29.48, 29.43, 29.41, 29.36, 29.27, 29.24, 29.10, 28.35, 26.86, 25.89, 25.74, 25.68 (36C, 36×CH_2); MS (FAB): *m/z* 3655.6 (100%, M+Na^+); Anal. Calcd for $C_{173}H_{310}O_{76}N_2 \cdot H_2O$: C, 56.88; H, 8.63; N, 0.77. Found: C, 56.66; H, 8.51; N, 0.91.

4.1.8. 2:1 β-Cyclodextrin/fullerene[60] conjugate (12). To a solution of **11** (263 mg, 0.07 mmol), C_{60} (252 mg, 0.35 mmol) and CBr_4 (58 mg, 0.18 mmol) in dry toluene (25 mL) was added DBU (26 μL, 0.18 mmol) under argon, the mixture was stirred at room temperature for 24 h. The reaction mixture was directly flash chromatographed, eluting first with toluene to remove the excess C_{60}, then 3:2 cyclohexane/acetone to afford **12** as a dark-red solid (88 mg, 29%); $[\alpha]_D$=+10 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56 (t, 2H, *J*=5.6 Hz, 2×NH), 5.18–5.14 (m, 8H, 8×H_1), 5.13 (2d, 4H, $J_{1,2}$=3.5 Hz, 4×H_1), 5.07 (d, 2H, $J_{1,2}$=3.4 Hz, 2×H_1), 4.50 (t, 4H, *J*=6.5 Hz, 2×CH_2OOC); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.93, 163.60 (4C, 2×COO–, 2×CONH–), 145.28, 145.16, 145.09, 145.08, 144.78, 144.59, 144.57, 144.50, 143.78, 142.98, 142.92, 142.89, 142.10, 141.81, 140.84, 138.90 (C_{60}-sp^2C), 98.91, 98.87, 98.85, 98.83, 98.81 (14C, 14×C_1), 82.01, 81.92, 81.89, 81.75, 81.73, 81.65, 81.62, 81.58, 81.48, 80.85, 80.38, 80.35, 80.13, 80.08, 80.00, 79.90, 79.79, 70.98, 70.86, 70.79, 70.72 (56C, 14×C_2, C_3, C_4, C_5), 71.48, 71.40, 71.32, 71.24, 71.22, 71.14, 67.39, 39.42, 36.82 (23C, 14×C_6, 2×$COOCH_2$, 2×OCH_2, 2×C_{60}-sp^3C, 2×CH_2–NH, bridgehead C), 61.59, 61.45, 61.34, 61.31, 61.29, 58.91, 58.88, 58.59, 58.57, 58.55, 58.46, 58.36, 58.30 (40C, 40×OMe), 29.99, 29.63, 29.56, 29.50, 29.44, 29.33, 29.30, 29.26, 29.14, 28.50, 26.89, 26.81, 25.90, 25.76 (38C, 38×CH_2); MS (FAB): *m/z* 4375.0 (60%,

$M+Na^+$); Anal. Calcd for $C_{233}H_{308}O_{76}N_2 \cdot 10H_2O$: C, 61.72; H, 7.31; N, 0.62. Found: C, 61.44; H, 7.07; N, 0.96.

4.1.9. 2:1 β-Cyclodextrin/fullerene[60] conjugate (15). To a solution of **14** (20.5 mg, 0.021 mmol) in dry CH_2Cl_2 (2 mL) under argon in ice-bath was added triethylamine (11.8 μL) and **9** (66.6 mg, 0.042 mmol, dissolved with 3 mL of CH_2Cl_2). The mixture was stirred at room temperature for 18 h. After concentration at 30 °C, the residue was purified by flash chromatography, eluting with 3:2 cyclohexane/acetone to provide **15** (60 mg, 70%) as a dark-red solid. R_f= 0.5 (cyclohexane/acetone 1:1); $[\alpha]_D = +5$ (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.91 (t, 2H, J=5.7 Hz, 2× NH), 5.18–5.14 (m, 8H, 8×H_1), 5.13 (2d, 4H, $J_{1,2}$=3.5 Hz, 4×H_1), 5.06 (d, 2H, $J_{1,2}$=3.4 Hz, 2×H_1), 4.96 (s, 4H, 2× $OCCH_2COO$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.59, 162.44 (4C, 2×COO–, 2×CONH–), 145.27, 145.18, 144.92, 144.81, 144.69, 144.65, 144.40, 144.33, 143.79, 143.04, 142.93, 142.09, 141.70, 140.98, 139.01 (C_{60}–sp^2C), 98.89, 98.84, 98.82, 98.80, 98.76 (14C, 14×C_1), 81.99, 81.90, 81.87, 81.73, 81.63, 81.59, 81.55, 81.44, 80.84, 80.36, 80.34, 80.14, 80.05, 79.96, 79.85, 79.78, 70.97, 70.87, 70.84, 70.77, 70.70 (56C, 14×C_2, C_3, C_4, C_5), 71.47, 71.38, 71.30, 71.23, 71.20, 71.12, 66.50, 39.63 (23C, 14× C_6, 2×COO*C*H_2, 2×OCH_2, 2×C_{60}–sp^3C, 2×CH_2–NH, bridgehead C), 61.60, 61.45, 61.44, 61.33, 61.29, 61.27, 58.91, 58.86, 58.57, 58.55, 58.46, 58.34, 58.29 (40C, 40× OMe), 29.97, 29.56, 29.52, 29.50, 29.46, 29.44, 29.29, 26.92, 26.80, 25.89 (18C, 18×CH_2); MS (FAB): *m/z* 4094.5 (20%, $M+Na^+$); Anal. Calcd for $C_{213}H_{268}O_{76}N_2 \cdot 8H_2O$: C, 60.67; H, 6.79; N, 0.66. Found: C, 60.36; H, 6.95; N, 0.87.

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New polymers for catalytic carbene transfer: electropolymerization of tetrafluorenylporphyrinruthenium carbon monoxide

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Abstract—Condensation of pyrrole with 2-fluorenecarboxaldehyde yields *meso*-tetrafluorenylporphyrin as a new building block. After ruthenium insertion, oxidative electropolymerization of tetrafluorenylporphyrinruthenium (II) carbonyl complexes can be used to coat Pt electrodes with polymeric films. These insoluble polymeric materials are able to catalyze the heterogeneous cyclopropanations and 2,3 sigmatropic rearrangements with ethyl diazoacetate after being removed from the electrode.

1. Introduction

Organic polymers have attracted scientific attention due to their potential application in large area, such as the emerging field of nanoscience.[1] They also can be used for immobilizing metalloporphyrins under insoluble materials.[2]

Numerous methods for immobilizing metalloporphyrins under insoluble materials have been reported. They frequently involve fixation of metalloporphyrin catalysts on inorganic supports such as silica gel,[3–6] zeolites,[7] montmorillonite,[8,9] gold electrodes,[10] rhenium clusters[11] and solid state metal phosphonates.[12] Manganese porphyrin has been recently immobilized as a monolayer film by a combination of Langmuir–Blodgett and self-assembled monolayer techniques that use zirconium phosphonate linkages.[13] Organic polymers, such as poly(ethylene glycol),[14] ion-exchange resins,[15] isocyanide polymer[16] and polypeptides[17] have also been used to support metalloporphyrins. In this case, the polymer is functionalized and the porphyrin is attached through a covalent bond to the material.

Since the first report of Macor and Spiro,[18] the immobilization of metalloporphyrins onto electrodes has been carried out mainly by electropolymerization. Thus conducting metalloporphyrin polymers such as polypyrrole,[19] polythiophene,[20,21] polyaniline[22] and others[21,23] were prepared through electropolymerization. Application of these polymer-coated electrodes to electrocatalysis, photo-electrochemical devices and sensors are now well-developed.[7,19]

It was recently shown that poly(tetraspirobifluorenylporphyrin) complexed by manganese[24] or ruthenium[25] showed potential applications as heterogeneous catalyst. The present work will focus on carbene transfer to olefin and sulfides using poly(tetrafluorenylporphyrinruthenium) polymers as catalysts.

2. Results and discussion

2.1. Synthesis of ruthenium porphyrins

Due to the orthogonal position of the fluorene groups, 9,9′-spirobifluorene-metalloporphyrins polymerize along two perpendicular dimensions leading to a dense material. This situation may create active sites difficult to access. Furthermore spirobifluorenylporphyrins are quite tedious to prepare.[26] Thus we decided to target only porphyrins bearing four fluorene groups. In contrast to our previous

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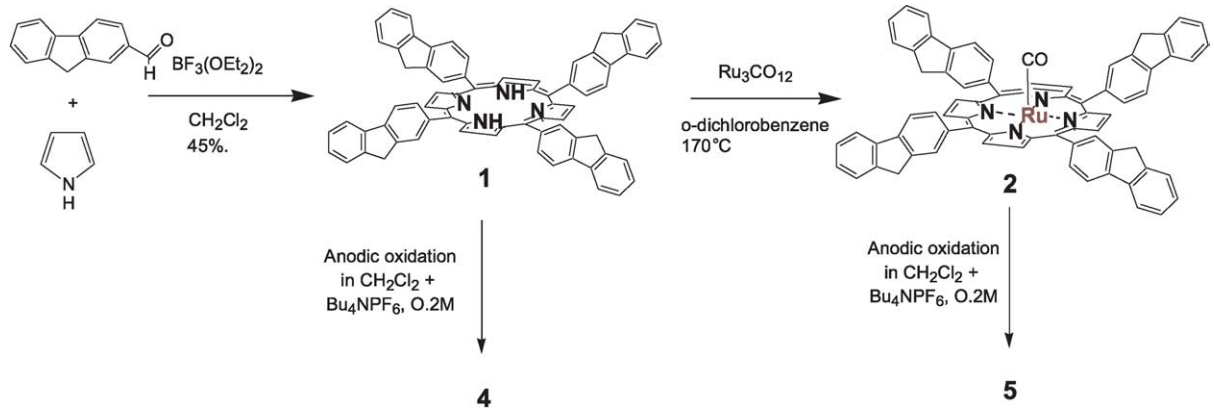


Figure 1. Syntheses of tetrafluorenylporphyrin ligand **1**, tetrafluorenylporphyrin-ruthenium complex **2** and their derived polymer **4** and **5**.

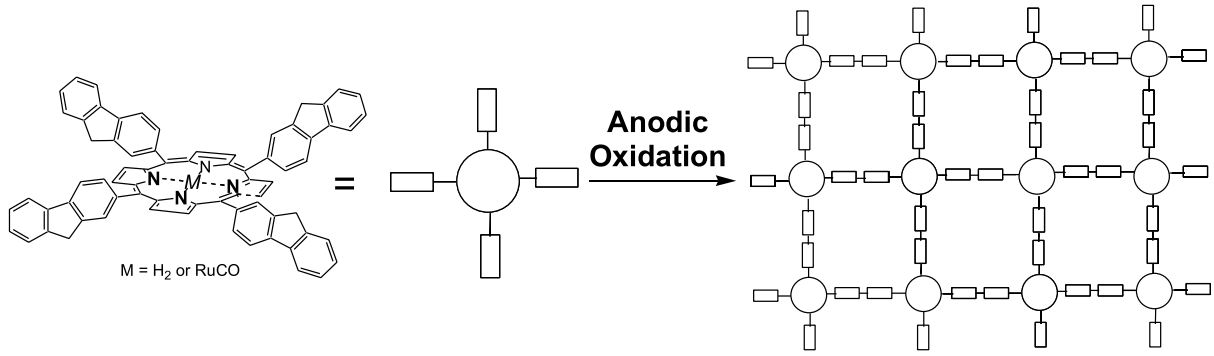


Figure 2. Schematic representation of the oxidative electropolymerization of tetrafluorenylporphyrin monomer to polytetrafluorenylporphyrin polymer.

work with spirobifluorene groups in which three polymerisable positions are available for each group, only one position is possible with the fluorene group. This new building block, porphyrin **1**, was prepared by condensation of pyrrole with fluorene aldehyde as described by Lindsey et al.[27] in 1986 for aromatic aldehydes (yield 45%). The ruthenium porphyrin monomer, compound **2** (Fig. 1) was prepared by treatment **1** with $Ru_3(CO)_{12}$ in *o*-dichlorobenzene at 170 °C (3 h) as previously reported for the ruthenium complex of the tetraspirobifluorenylporphyrinruthenium carbon monoxide.[28]

Then it was decided to study a possible atropisomerism through the complexation of two identical axial ligands. The ruthenium porphyrin monomer **2** reacts with an excess of *t*-butylisocyanide to form compound **3**. This new bis-(isocyanide) complex (TFP)Ru(*t*-BuNC)$_2$ **3** was studied by ^{1}H NMR to evaluate the possibility to form several atropisomers with the bulky fluorenyl substituents. However, the signal corresponding to the two *t*BuCN groups (6-CH_3) appeared as a singulet at −0.35 ppm in deuterated chloroform, according to two topologically identical faces. In contrast, the presence of four different conformers was detected by ^{1}H NMR for tetraspirobifluorenyporphyrinruthenium bis(*t*Bu-isocyanide).[26] In this later case, an equilibrium mixture corresponding to four atropisomers with the statistical composition: 1:2:4:1, respectively, for αααα, ααββ, αααβ, αβαβ, was observed. A likely explanation is that the fluorene arm does not hinder rotation and so the two faces of compound **3** are identical at room temperature.

2.2. Electropolymerization

The polymers **4** and **5** were prepared by anodic oxidation of the fluorene units of compound **1** and **2**, respectively. Porphyrin macrocycles **1** and **2** can be cross linked in four perpendicular positions (via the fluorene arms) (Fig. 2).

Figure 3a illustrates a typical voltammogram recorded during the anodic oxidation of (TFP)RuCO (2.3 10^{-3} M) in CH_2Cl_2 (0.2 M Bu_4NPF_6) between −0.15 and 1.02 V. As seen in this figure, two reversible and stable oxidation processes E^1 and E^2 occur with maxima at 0.43 and 0.81 V versus Fc, leading to the metalloporphyrin π-cation radical and dication, respectively. Similar voltammograms are observed for (TPP)RuCO. Potentials of these redox couples are summarized in Table 1 together with those of (TFP)H_2 **1** for comparison.

Figure 3b shows a typical voltammogram from −0.3 to 1.42 V. A third oxidation wave is visible with a maximum at $E^3 = 1.36$ V due to the irreversible oxidation of the fluorene units. Recurrent sweeps in that potential range lead to gradual modifications of the CVs. Thus, when scanning in a potential range including these three waves (Fig. 3c), the

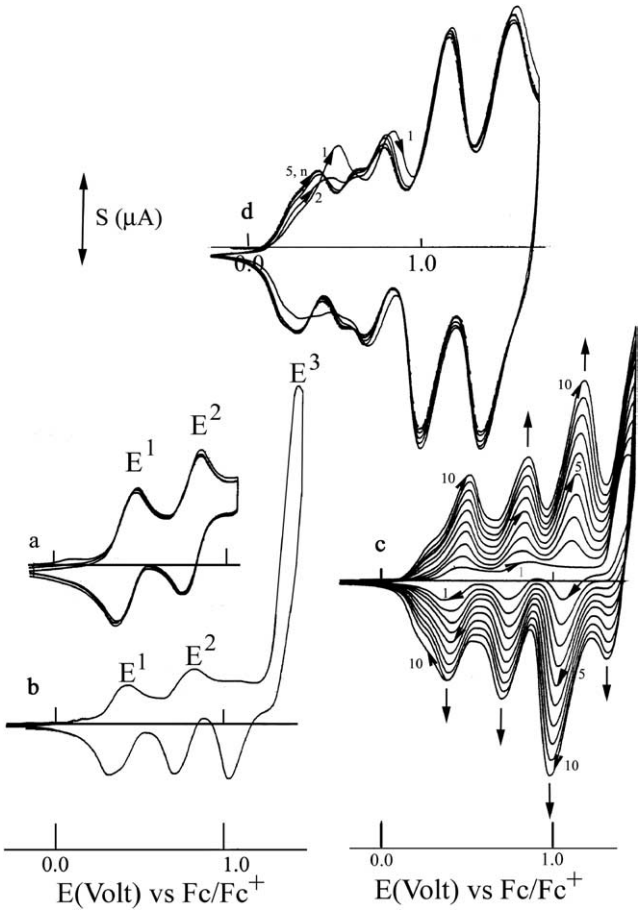


Figure 3. Cyclic voltammetry in CH_2Cl_2 (Bu_4NPF_6 0.2 M). In presence of **2** (RuCOTFP) (2.3 10^{-3} M); a: 3 cycles between −0.15 and 1.02 V. b: 1 cycle between −0.15 and 1.42 V. c: 10 cycles between −0.2 and 1.44 V; working electrode: platinum disk (d=1 mm). In a solution free of **2**; d: more than 5 cycles between −0.2 and 1.7 V; working electrode: platinum disk coated by **5** (poly(RuCOTFP)) during the CVs recorded in c. S: 3 μA in a; S: 6 μA in b and 16 μA in c and d, sweep-rate: 100 mV s^{-1}.

CVs show the appearance and the regular growth of a new reversible wave centered at about 1.1 V and the continuous increase in amplitude of the other peaks. This CVs modification indicates that an electrodeposition takes place on the electrode. Indeed, the electrode taken out of the electrochemical cell after the tenth sweep, rinsed in dichloromethane and used as working electrode in a new electrolytic solution free of any electroactive species presents the CVs shown in Figure 3d. These CVs show four main reversible waves whose maxima are at 0.5, 0.8, 1.1 and 1.55 V ranging in a potential range of the metalloporphyrin unit oxidation in **2** (0.43 and 0.81 V) and of the difluorenyl units oxidation in (1.1 and 1.55 V). These two last values fit with the oxidation potential of 9,9-dialkyl-difluorene in the same media: 0.9 and 1.3 V. The regular growth of the two first waves during the electrodeposition process shows that charge transfer occurs in all the polymer from difluorenyl unit to other even through the porphyrin units leading to a polymer being conducting since 0.1 V. The presence of shoulders closed to the two main first waves in Figure 3d is probably due to the existence of two electrochemical process under a same wave: the p-doping process of the polymer and the two electron oxidation of the porphyrin units. Additionally, Figure 3d shows the high electrochemical stability of the polymer between 0.0 and 1.7 V.

Polymer **5** is also obtained by oxidation of **2** at fixed potential (Epol: 1.45 V vs Fc/Fc^+). At this potential, the polymer is obtained at the electrode under its conducting oxidized form, the polymer is then reduced at 0.0 V leading to a neutral polymer. In order to study the behaviour of the polymer **5**, after this preparative electrosynthesis, the working electrodes were rinsed and the fine insoluble powder was removed from the electrode, and used for catalytic reaction after characterisation.

Analysis of the polymer **5** using scanning electron microscopy and electronic microanalysis gives a ratio C/Ru of about 74/1 in agreement with the conserved structure of monomers **2** in the material. As shown in supplementary data, electrochemical polymerization of **2** results in a structure with filaments grown from a thin layer of polymer. The thickness of this film was measured between 1–2 μm and the length of filaments is ∼70 μm. As expected, the IR spectrum of the polymer **5** showed a CO vibration at 1948 cm^{-1} in KBr similar to the value observed for the monomer **2**.

Anodic oxidation and electropolymerization of **1** gave similar results, leading to the unmetalled polymer **4** (see supplementary data). Polymerization leads to UV–vis spectral changes which are shown in Figure 4. UV–vis spectra of **1** in solution in CH_2Cl_2 is presented in straight lines. The electronic spectrum of oxidized polymer **4** obtained as a thin film on an ITO glass electrode showed four main absorption bands centered at 350, 432, 470 and

Table 1. Electrochemical data of monomer **1** and **2** and of their derived polymers **4** and **5**[a]

	E^1	E^2	E^3	E_{pol}
Compound **1**	0.58 V	0.9 V	1.5 V	1.55 V
Compound **2**	0.43 V	0.81 V	1.36 V	1.45 V
Polymer **4**	One reversible oxidation waves with maximum at 0.84 V		Threshold oxidation potential of **4:** 0.4 V	Stability of the polymer in a potential range between 0.4 and 1.1 V
Polymer **5**	Four reversible oxidation waves with maximum at 0.5; 0.8; 1.1 ; 1.55 V		Threshold oxidation potential of **5**: 0.1 V	Stability of the polymer in a potential range between 0.1 and 1.7 V

[a] All potentials were obtained during cyclic voltammetry investigations in 0.2 M Bu_4NPF_6 in CH_2Cl_2. For the compound **1** and **2**: monomer concentration, respectively: 4.37 10^{-3} M and 2.3 10^{-3} M. Platinum working electrode diameter 1 mm. For the polymer **4** and **5**: the study was performed using a modified platinum electrode previously coated by the polymer in solution of the monomers. Sweep-rate: 100 mV s^{-1}.

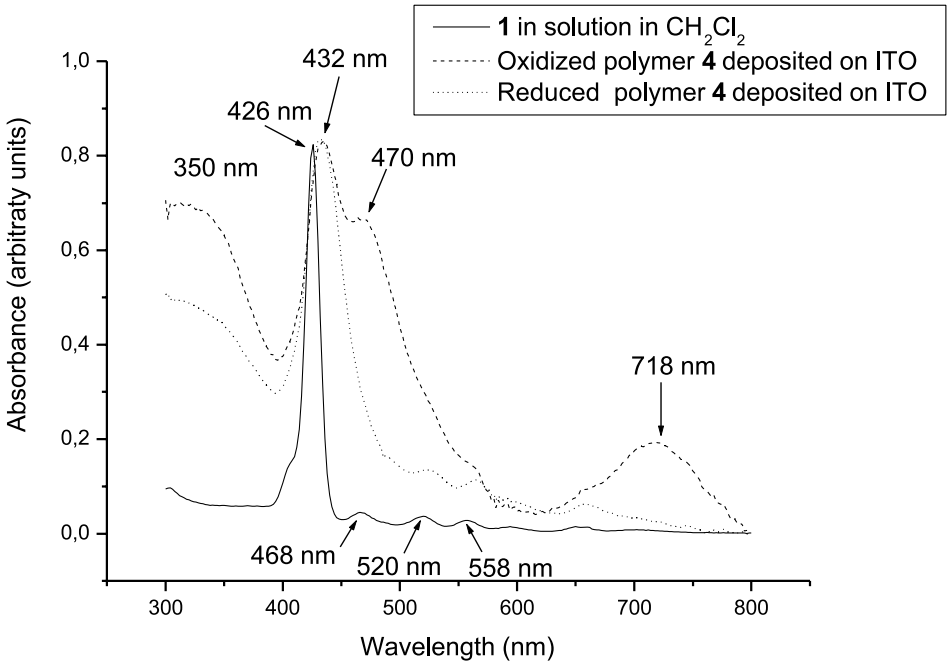


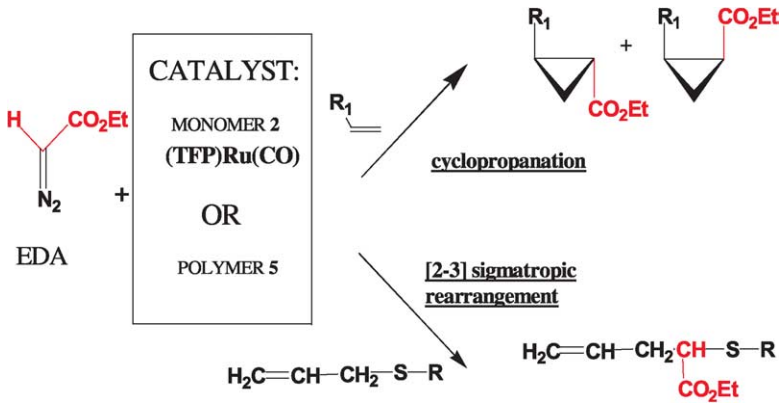
Figure 4. UV–vis spectra of poly((TFP)H_2) **4** and (TFP)H_2 **1**.

718 nm. Besides the bands at 432 and 470 nm which correspond to the Soret absorption band of the porphyrin group in the reduced and oxidized polymer, respectively, the two other bands are due to absorption of the aromatic fluorenyl units. The band at 350 nm corresponds to the main absorption band and the band at 718 nm is due to the existence of intermediate conducting bands in the oxidized polymer (polaron, bipolaron). After reduction of the polymer at −1.7 V, the two bands at 470 and 718 nm disappeared living the main aromatic band at about 350 nm and the Soret band at 432 nm of the reduced porphyrin (Q bands are also detected).

As described for the ruthenium complex, one free macrocycle can be cross linked in four perpendicular positions (via the fluorene arms). The redox potentials of the two monomers (**1** and **2**) and the polymers (**4** and **5**) are summarized in Table 1. In the case of poly((TFP)H_2) **4**, the threshold oxidation potential is 0.4 V, a value more positive than the value for the ruthenium polymer (0.1 V). Consequently, the oxidation of the porphyrin units in the polymer **4** occurs at higher potential together with the doping process of the polyfluorene and the potential range of stability of polymer **4** (free porphyrin) is less important than that of the polymer **5** containing RuCO groups.

2.3. Catalysis

Following the successful synthesis of the Ru porphyrin polymers **5**, their catalytic activity was initially tested in carbene transfer catalysis (Scheme 1). The catalytic essays involved the use of diazoethyl acetate as possible carbene precursors with styrene or methyl allyl sulfide as substrates. The activity of the polymer catalyst was first focused on styrene which has been frequently used in metalloporphyrin-catalyzed carbene transfer reactions and the results compared to the corresponding soluble monomer **2**. The reaction was monitored by gas chromatography and carbene insertion was found to be the major reaction in all cases. Treatment of styrene with ethyl diazoacetate at 25 °C in the



Scheme 1. Catalytic reaction of ethyl diazomethylacetate with styrene or allyl methyl sulfide.

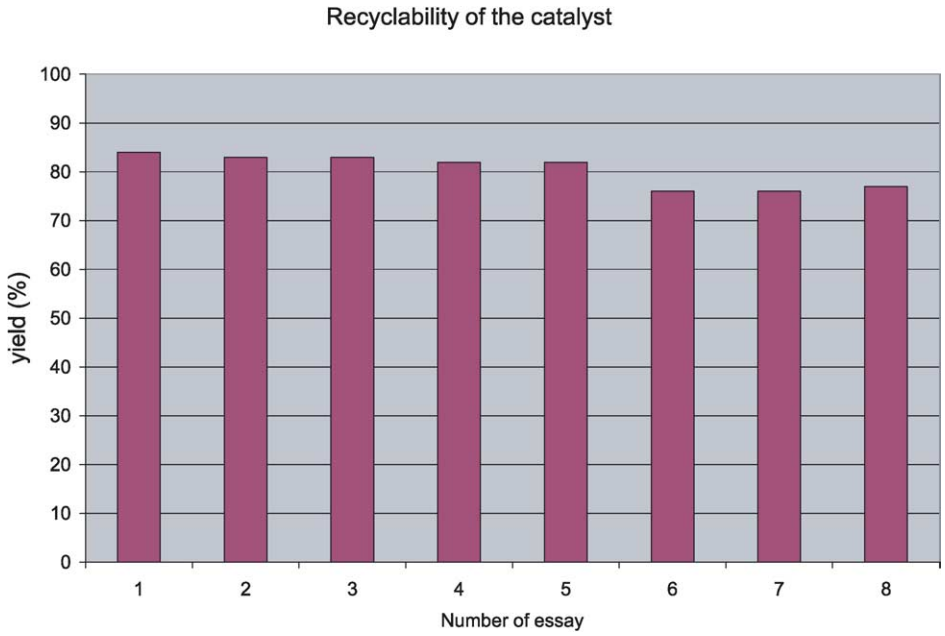


Figure 5. Recyclability of poly(TFP)RuCO **5** for styrene cyclopropanation.

presence of the ruthenium polymer **5** results in the formation of corresponding cyclopropane in 82% yield with an excess of the *trans* isomer (*trans*/*cis* ratio: 3/1). Similar results were obtained with the monomer but with a higher selectivity for the *trans* isomer (*trans*/*cis* ratio: 9/1) (Scheme 1). Polymer **5** also catalysed decomposition of ethyl diazoacetate in the presence of methyl allyl sulfide resulting in the formation of the corresponding ethyl 2-(methylthio)pent-4-enoate with 86% yield (Scheme 1). The formation of this compound derives from the [2,3]-sigmatropic rearrangement of the sulfur ylide.[25] Similar results were obtained with the monomer giving 85% yield (see Section 4 for details). The recovery and recyclability of polymer **5** have been also examined, leading to 8 recycling steps for cyclopropanation of styrene without decrease of activity (Fig. 5).

Similar yields and recyclability were obtained with poly-(tetraspirobifluoreneporphyrin) ruthenium carbon monoxide.[25] Actually, the two polymers seem very robust towards carbene transfer reaction. However we notice a significant decrease in the diastereoselectivity (*trans* to *cis* ratio) of the cyclopropanation reaction, decreasing from 9/1 to 3/1 with poly(tetraspirobifluoreneporphyrin) ruthenium carbon monoxide and **5**, respectively. A possible explanation for this different regioselectivity (also observed between the polymer and the monomer) could be the presence of ruthenium sites with different environment in the polymer **5** which would decrease the selectivity. A different conformational effect is also established by examining the ^{1}H NMR spectra of the two ruthenium complex precursors because we could not detect any atropisomerism with **2**.

3. Conclusion

In summary, we have shown that stable metalloporphyrin polymers, showing good electroactivity over practicable thickness (>100 μm) can be prepared by oxidative electropolymerization of metalloporphyrin complexes bearing fluorene groups. The ruthenium porphyrin sites are accessible, at least partially, as evidenced by the observed catalytic reaction, after removing the film from the electrode. Thus polymerization of Ru tetrafluorenylporphyrins leads to very efficient catalysts for carbene insertion that can be easily recovered and re-used. We anticipate that the present heterogeneous catalytic system would be potentially applicable to practical organic synthesis with a special focus on asymmetric catalysis using chiral polymers.

4. Experimental

4.1. General procedures

All reactions were performed under argon and were magnetically stirred. Solvents were distilled from appropriate drying agent prior to use: Et_2O and THF from sodium and benzophenone, toluene from sodium, CH_2Cl_2 from CaH_2, $CHCl_3$ from P_2O_5 and all other solvents were HPLC grade. Commercially available reagents were used without further purification unless otherwise stated. All reactions were monitored by TLC with Merck pre-coated aluminium foil sheets (Silica gel 60 with fluorescent indicator UV_{254}). Compounds were visualized with UV light at 254 nm and 365 nm. Column chromatography was carried out using silica gel from Merck (0.063–0.200 mm). ^{1}H NMR and ^{13}C NMR in $CDCl_3$ were recorded using Bruker 200 DPX and 300 DPX spectrometers. The assignments were performed by 2D NMR experiments: COSY (correlation spectroscopy), HMBC (heteronuclear multiple bond correlation) and HMQC (heteronuclear multiple quantum coherence). High-resolution mass spectra were recorded on a ZabSpec TOF Micromass spectrometer in FAB mode or ESI positif mode at the CRMPO.

Electrochemical experiments were performed using either a Pt disk electrode (diameter 1 mm), or platinum foils (area: 2 cm^2). The counter electrode was a vitreous carbon rod and the reference electrode was a silver wire in a 0.1 M $AgNO_3$ solution in CH_3CN. Ferrocene was added to the electrolyte solution at the end of a series of experiments. The ferrocene/ferrocenium (Fc/Fc^+) couple served as internal standard and all reported potentials were referenced to its reversible formal potential. Activated Al_2O_3 was added in the electrolytic solution to remove excess moisture. The three electrode cell was connected to a PAR Model 173 potentiostat monitored with a PAR Model 175 signal generator and a PAR Model 179 signal coulometer. The cyclic voltammetry traces (CVs) were recorded on an XY SEFRAM-type TGM 164.

Dichloromethane with less than 100 ppm of water (ref. SDS 02910E21) and tetrabutylammonium hexafluorophosphate from FLUKA were used without any purification. Aluminium oxide was obtained from Woëlm, activated by heating at 300 °C under vacuum for 12 h and used at once under argon pressure.

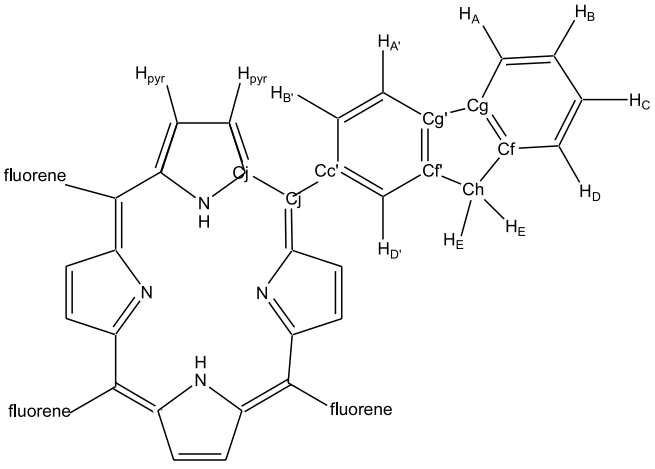
Liquid UV–visible spectra were recorded on a UVIKON XL from Biotech. Solid UV–visible spectra were recorded using, either a Guided Wave model 150 spectrophotometer with optical fibres, a concave platinum surface acted as a reflector for the optical beam, or a JASCO-V570 Spectrophotometer, the deposit being on ITO electrode. Scanning Electron Microscopy was performed on JEOL JSM 301F. Electronic Microanalysis was performed on JEOL JSM 6400 using an Energy Dispersive spectrometry (EDS) detector Oxford-Link Isis. Infra Red spectra were performed in KBr disk in a IFS 28 Bruker. All catalytic reactions were controlled on a Varian CP-3380 Gas Chromatograph equipped with a CP-Chirasil-Dex Column. TFP=tetrafluorenylporphyrin dianion, TPP tetraphenylporphyrin dianion.

4.2. Porphyrin synthesis

4.2.1. *meso*-Tetrakis-5,10,15,20-tetrakis(fluoren-2-yl)-porphyrin 1. Pyrrole (5 mmol), and fluorene-2-carbaldehyde (5 mmol) were allowed to react at room temperature in dry and degassed dichloromethane (1 L) under an argon atmosphere and protected from light with acid catalysis ($BF_3(OEt_2)_2$): 0.5 mmol). The reaction was stirred for 3 h. 3.8 mmol of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone was added to irreversibly oxidize the tetra-fluorenylporphyrinogene and the solution was stirred for 60 min. After addition of 1.5 mL of triethylamine, the solvent was removed under vacuum. The free base porphyrin **1** was purified twice by chromatography on silica gel using dichloromethane as eluant. Yield: 45%.

^{1}H NMR ($CDCl_3$): 8.98 (s, 8H, pyrrole), 8.46 (s, 4H, $H_{D'}$), 8.32 (d, $^3J_{HH}$=8.2 Hz, 4H, $H_{B'}$), 8.21 (d, $^3J_{HH}$=7.6 Hz, 4H, $H_{A'}$), 8.11 (d, $^3J_{HH}$=6.6 Hz, 4H, H_A), 7.76 (d, $^3J_{HH}$=6.8 Hz, 4H, H_D), 7.59 (t, $^3J_{HH}$=5.7 Hz, 4H, H_B), 7.49 (t, $^3J_{HH}$=6.8 Hz, 4H, H_C), 4.26 (s, 8H, H_E),

^{13}C NMR ($CDCl_3$): 143.8 (C_F), 141.59 ($C_{F'}$), 141.55 (C_G),141.26 ($C_{G'}$), 140.66 ($C_{C'}$), 133.5 ($C_{B'}$), 131.4 ($C_{D'}$), 131.12 ($C_{pyrrole}$), 129.1 (C_J), 127.0 (C_C), 127.1 (C_B), 125.3 (C_D), 120.6 (C_I), 120. (C_A), 117.9 ($C_{A'}$), 37.1 (C_E). UV–vis (CH_2Cl_2): $\lambda_{max/nm}$ (log ε): 426 nm (5.89), 522 (4.49), 557 (4.4), 598 (4.1) and 650 (4.15). MS (ESI): (m/z^+): calcd for $C_{72}H_{47}N_4$ (MH^+) 967.3801. Found: 967.3799.



4.2.2. *meso*-Tetrakis-5,10,15,20-tetrakis(fluoren-2-yl)porphyrinato ruthenium carbone monoxide 2. Free base porphyrin **1** (0.21 mmol, 0.2 g) was dissolved in distilled 1,2-dichlorobenzene (40 mL) and degassed for 15 min. The reaction mixture was heated at 180 °C and dodecacarbonyl triruthenium was added (0.31 mmol, 0.2 g) over a period of 2 h under an argon atmosphere. The mixture was stirred for an additional hour. The ruthenium insertion was followed by TLC and UV–vis spectroscopy. The solvent was removed under vacuum, the black-red residue was dissolved in dichloromethane and purified by chromatography on silica gel. The dodecacarbonyl triruthenium was eluted first with pentane and the desired ruthenium complex was eluted with a mixture hexane/dichloromethane (6:4). Yield: 50%.

^{1}H NMR: 8.97 (s, 8H, pyrrole), 8.42 (s, 4H, $H_{D'}$), 8.28 (d, $^3J_{HH}$=8.2 Hz, 4H, $H_{B'}$), 8.20 (d, $^3J_{HH}$=7.6 Hz 4H, $H_{A'}$), 8.10 (d, $^3J_{HH}$=6.6 Hz, 4H, H_A), 7.72 (d, $^3J_{HH}$=6.8 Hz, 4H, H_D), 7.56 (t, $^3J_{HH}$=5.7 Hz, 4H, H_B), 7.46 (t, $^3J_{HH}$=6.8 Hz, 4H, H_C), 4.23 (s, 8H, H_E), UV–visible: 426 nm (Soret band). MS (ESI): (m/z^+): calcd for $C_{74}H_{48}N_40_2$ ($M+CH_3OH$)$^+$ 1126.2842. Found: 1126.2830. IR (KBr, cm^{-1}) 1948 (ν_{CO}).

4.2.3. *meso*-Tetrakis-5,10,15,20-tetrakis(fluoren-2-yl)-porphyrinatoruthenium bis(tertiobutyl) isocyanide 3. To a solution of ruthenium carbonyl complex (0.01 mmol) in $CDCl_3$ in an NMR tube was added terbutylisocyanide (2 equiv) under an argon atmosphere. The solution was stirred at room temperature until the reaction was completed (5 min). The bis-ligation was checked by monitoring the UV–vis spectrum and then the NMR spectra were immediately recorded. ^{1}H NMR ($CDCl_3$): 8.51 (s, 8H, pyrrole), 8.35 and 8.33 (2s, 4H, $H_{D'}$), 8.20 and 8.17 (d, $^3J_{HH}$=8.2 Hz, 4H, $H_{B'}$), 8.09 (d, $^3J_{HH}$=7.6 Hz 4H, $H_{A'}$), 8.03 (d, $^3J_{HH}$=6.6 Hz, 4H, H_A), 7.67 (d, $^3J_{HH}$=6.8 Hz, 4H, H_D), 7.59 (t, $^3J_{HH}$=5.7 Hz, 4H, H_B), 7.49 (t, $^3J_{HH}$=6.8 Hz, 4H, H_C), 4.18 (broad s, 8H, H_E), −0.35 (broad s, 18H, $6CH_3$). ^{13}C NMR ($CDCl_3$): 143.8 (C_F), 141.59 ($C_{F'}$), 141.55 (C_G), 141.26 ($C_{G'}$), 140.66 ($C_{C'}$), 132.93 ($C_{B'}$), 130.85

($C_{D'}$), 131.22 (C_{pyrrol}), 129.1 (C_J), 127.0 (C_C), 126.93 (C_B), 126.72 (C_D), 120.6 (C_I), 120.07 (C_A), 117.44 ($C_{A'}$), 37.07 (C_E), 29.12 (C_{tBu}). UV–vis: 422 nm (Soret band), 538 and 575 nm.

4.3. Catalysis

Hogeneous catalysis: carbene insertion reactions of ethyl diazoacetate with styrene (or allylic sulfide) using **2** as catalyst: In a typical experiment, styrene (or methyl allyl sulfide) (2.5 mmol) and the ruthenium porphyrin complex (1/100: Substrate/EDA, 0.005 mmol) were dissolved in 200 μL of dry chloroform in a schlenk flask under argon. Ethyl diazoacetate (100 μL, 0.5 mmol) was added slowly at 20 °C. After the reaction was complete (4 h), during which time the reaction was monitored by GC-MS, the products were recovered by vacuum distillation. The product was identified by ^{1}H NMR studies and comparison to literature data.[29]

Heterogeneous catalysis: After scratching the film out of the electrode, the polymer was crushed to obtain a fine powder which is stored under nitrogen at 0 °C. The material was used without any further treatment for all catalytic experiments. To a mixture of **5** (3 mg) in dichloromethane (1 mL) is added 48 μL of styrene (500 μmol). Then 13 μL (100 μmol) of ethyl diazoacetate is slowly injected (3 h) to the stirred solution. In order to prevent the formation of diethyl fumarate and maleate, the concentration of the diazo compound was kept at low levels by slow addition of ethyl diazoacetate. The mixture is then stirred under an argon atmosphere for 1 h. Resulting products of cyclopropanation (*cis*/*trans* adducts) are monitored by gas chromatography. Fumarate and maleate resulting from the self condensation of the carbene are also monitored. The polymer is washed with dichloromethane, dimethyl sulfoxide and acetone dried under vacuum and used for another run in the same experimental conditions.

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Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.tet.2004.10.013

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Pyridazine derivatives. Part 39: Reactivity of 5-iodopyridazin-3(2*H*)-ones in palladium-catalysed reactions☆

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Abstract—In the search for novel antiplatelet agents, convenient and efficient methods for the preparation of 2,5-disubstituted pyridazin-3(2*H*)-ones are reported that utilise palladium-catalysed cross-coupling reactions. A post-coupling base-promoted isomerisation has been observed during Sonogashira alkynylation of 5-iodopyridazin-3(2*H*)-ones (**3**) with 1-phenyl-2-propyn-1-ol. Variable amounts of phthalazinones were isolated as by-products during the Heck alkenylation of **3**. The usefulness of the hydroxymethyl fragment as a protecting group during the synthesis of 5-substituted pyridazin-3(2*H*)-ones has been validated.

1. Introduction

In recent years, almost every part of the drug discovery processes has undergone a radical change. However, one of the few things that has not changed is the fact that the majority of medicines are still small organic molecules and a high proportion of these contain a heterocyclic ring.[2] As a consequence, low molecular weight heterocycles have a central role in the development of therapeutic agents. In this area the issues of bioavailability and toxicity must be addressed in addition to bioactivity. It is therefore of general interest to medicinal chemists to have straightforward synthetic methodologies that provide access to a large number of bioactive molecules. For these reasons, atom-efficient transformations and reactions that have high exploratory power are especially desirable during the processes of lead finding and lead optimisation.

In recent decades, the use of transition metal complexes in organic chemistry has fuelled a revolution in this field.[3] Such reactions have allowed the development of new transformations that were either difficult or impossible using previously available methods. Curiously, however, references describing the systematic use of these transformations as part of pharmacomodulation processes for bioactive prototypes have, to the best of our knowledge, not appeared. Palladium chemistry involving heterocycles has unique characteristics stemming from the inherently different structural and electronic properties of heterocycles in comparison to the corresponding carbocyclic aryl compounds.[4] The α and γ activation of heteroaryl halides means that Pd-catalysed chemistry may occur regioselectively at the activated positions, a phenomenon rarely seen in carbocyclic aryl halides.[5–7] Curiously, despite the useful nature of pyridazines, until a few years ago only a limited number of synthetic approaches to achieve substitution on these electron-deficient rings had been described.[8] A number of methods have recently been reported in the literature and, of these, reactions involving organometallics have proved to be powerful tools for the preparation of the desired compounds.[9]

In the last two decades, our research group has explored the chemistry[10] and pharmacology[11] of pyridazine derivatives. Initially, the well known properties of 3-hydrazinopyridazines as direct vasodilators attracted our attention[12] but, more recently, discovering of novel pyridazinone-based antiplatelet agents has become our goal.[13–16] These studies have recently involved the use of different

☆ For the previous paper in this series, see Ref. 1.

Keywords: Pyridazinones; Palladium; Platelets; Ene-adducts; Crystal structure.
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palladium-catalysed reactions,[17–20] which have demonstrated their versatility as tools to perform the pharmacomodulation and structural diversification at the 5-position of the 6-phenylpyridazin-3(2*H*)-one system.[16] We recently described the antiplatelet activity of compounds **I**[13] (Fig. 1) and the discovery of several 5-alkylidene-6-phenylpyridazin-3(2*H*)-ones **II**[14] and **III**[16] (Fig. 1), which are potent antiplatelet agents. Another interesting result concerning the biological activity of these derivatives is related to their mechanism of action, which is different to other antiplatelet agents that are already available. Recent experiments on these compounds suggested that their antiplatelet effect is related to their ability to affect protein phosphorylation.[13,14]

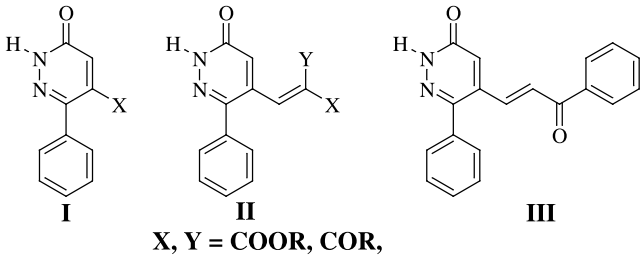


Figure 1.

Preliminary results from the structure–activity relationship (SAR) studies performed on this family of compounds have suggested that the presence of the phenyl group at position 6 and the presence of a free NH at position 2 of the heterocyclic ring (Fig. 1) may not be essential structural requirements for biological action in this new mechanism of action.[21] In order to validate this hypothesis, we became interested in the synthesis of 5-functionalised pyridazin-3(2*H*)-ones **IV**, which have different substituents at position 5 and the appropriate group at position 2 (Me, Bn, H) but do not incorporate the phenyl group at position 6 (Fig. 2). The approach selected to obtain 2,5-disubstituted pyridazin-3(2*H*)-ones **IV** involved the use of palladium-catalysed cross-coupling reactions[22] following the well known Suzuki,[23] Heck,[24] Stille[25] or Sonogashira[26] methodologies. These methods allowed the rapid and efficient introduction of a wide range of substituents at position 5 of the heterocycle.

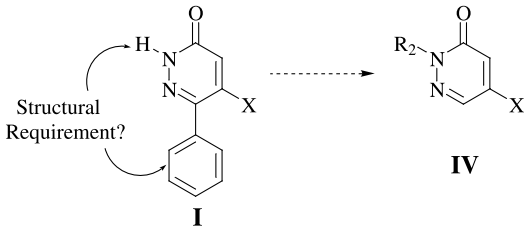


Figure 2.

2. Results and discussion

In order to achieve this objective, different 2-substituted 5-iodopyridazin-3(2*H*)-ones **3** were chosen as starting materials (Scheme 1). Some of these derivatives have been previously described by Mátyus[27a,b] (**3a** and **3b**) but, to the best of our knowledge, only very recently a detailed synthetic procedure to obtain **3a** been published.[27c] These derivatives were obtained by halogen exchange reactions followed by reductive dehalogenation.[27]

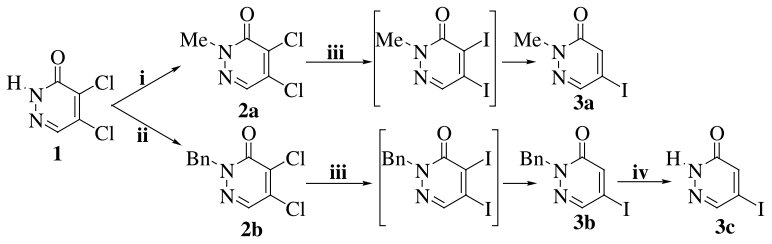
Commercially available 4,5-dichloropyridazin-3(2*H*)-one (**1**) was conveniently N-alkylated by treatment with methyl iodide or benzyl bromide to afford 2-substituted 4,5-dichloropyridazin-3(2*H*)-ones **2** (Scheme 1).[28] Treatment of **2** with a large excess of 57% hydriodic acid under reflux during 24 h yielded the corresponding 2-substituted 5-iodopyridazin-3(2*H*)-ones **3** (Scheme 1). It is worth mentioning here that, as reported by Mátyus,[27] the main intermediates during these transformations are the corresponding 2-substituted 4,5-diiodopyridazin-3(2*H*)-ones, which can be isolated in high yields if reaction times are shorter (3 h) or on using dioxane as the solvent (see Section 3). 5-Iodopyridazin-3(2*H*)-one **3c** was obtained by removing the benzyl group in **3b** by treatment with anhydrous aluminium chloride in dry toluene.[29]

Once a small subset of 5-iodopyridazin-3(2*H*)-ones had been obtained, we proceeded to study the functionalisation of the 5 position of the heterocyclic scaffolds **3** (Scheme 2) using Suzuki, Heck, Stille or Sonogashira cross-coupling reactions. First, the palladium-catalysed transformations were studied for the 2-benzyl- and 5-iodo-2-methylpyridazin-3(2*H*)-ones **3a** and **3b**, which have a non-tautomeric carbonyl group (Schemes 2–5).

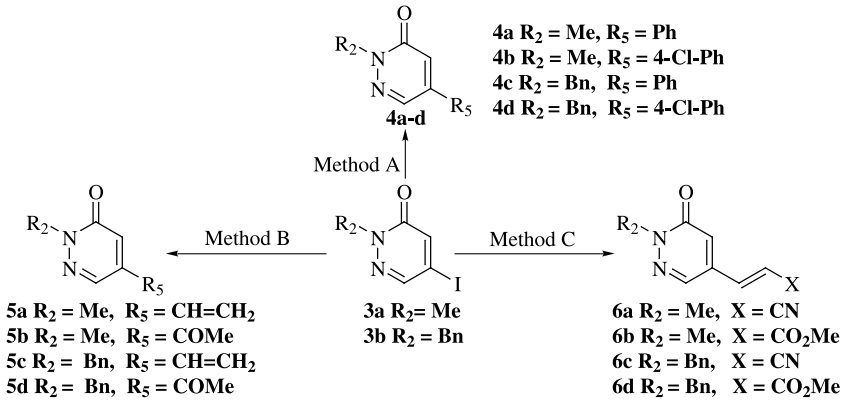
Arylation of compounds **3a** and **3b** was smoothly performed by reaction with the 4-chlorophenyl- or phenylboronic acids in the presence of sodium carbonate as a base and tetrakis(triphenylphosphine)palladium as a palladium source in a 3:1 mixture of dimethoxyethane/water (Scheme 2). This process afforded the corresponding 2-substituted 5-arylpyridazin-3(2*H*)-ones **4a–f** in excellent yields (Table 1). While our work was in progress, a paper was published by Mátyus et al. concerning the synthesis of compound **4a** and other 5-(aryl)-2-methylpyridazin-3(2*H*)-ones as part of the synthesis of new pyridazino[4,5-*b*]indoles.[30]

Pyridazinones **5** were obtained in excellent yields (Table 1) by Stille cross-coupling of **3a–b** with tributyl vinyl stannane or tributylethoxyvinyl stannane at room temperature (Scheme 2). The 5-acetyl-derivatives **5b** and **5d** were prepared in a one-pot procedure by cleavage of the corresponding enol-ether intermediate with 3 N hydrochloric acid.

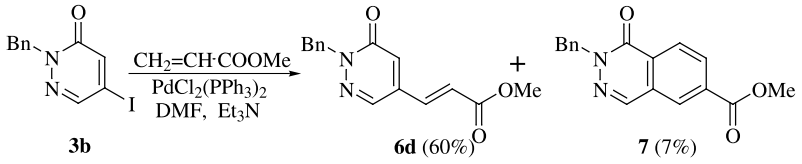
The introduction of alkenyl groups at position 5 in **3a–b** was achieved by coupling with methyl acrylate or acrylonitrile in dimethylformamide under the basic conditions provided by triethylamine (Scheme 2). Different palladium sources were tested for the Heck alkenylation of the heterocycle. Firstly, methods were studied that employ cocktails of palladium catalysts/phosphines [$Pd(OAc)_2$/PPh_3, $PdCl_2(PPh_3)_2$, $PdCl_2[P(o\text{-Tolyl})_3]_2$] and, secondly, the use of palladium on charcoal under phosphine-free conditions was investigated.[24] In all experiments, the expected 5-alkenylpyridazin-3(2*H*)-ones **6** were obtained but it was found that $PdCl_2[P(o\text{-Tolyl})_3]_2$ was a superior



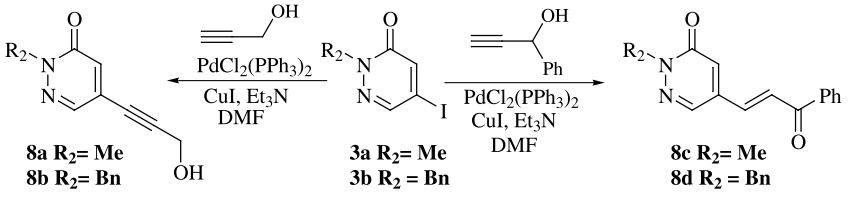
Scheme 1. (i) Me-I/K_2CO_3/Bu_4NBr/acetonitrile, (ii) Bn-Br/K_2CO_3/Bu_4NBr/acetonitrile, (iii) 57% HI, (iv) $AlCl_3$/toluene.



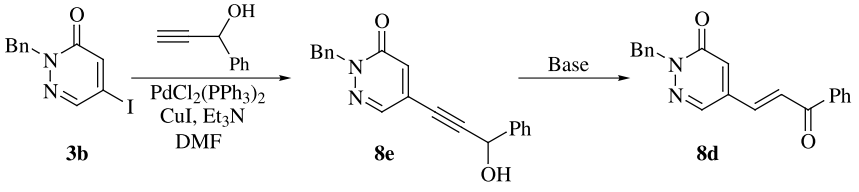
Scheme 2. Method A: $ArB(OH)_2$/$Pd(PPh_3)_4$/Na_2CO_3/DME–H_2O. Method B: R-$Sn(Bu)_3$/$PdCl_2(PPh_3)_2$/Et_3N/DMF/for compounds **5b** and **5d** then 3 N HCl. Method C: CH_2═CH–X/ $PdCl_2[P(o\text{-Tolyl})_3]_2$/$Et_3N$/DMF.



Scheme 3.

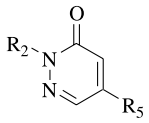


Scheme 4.



Scheme 5.

Table 1. Structure of the obtained 2,5-substituted pyridazin-3(2*H*)-ones



Compound	R_2	R_5	Yield (%)	Compound	R_2	R_5	Yield (%)
4a	Me	Ph	91	**6d**	Bn	CH=CH–CO_2Me	68
4b	Me	4-Cl–Ph	90	**8a**	Me	≡–CH_2-OH	70
4c	Bn	Ph	94	**8b**	Bn	≡–CH_2-OH	93
4d	Bn	4-Cl–Ph	90	**8c**	Me	CH=CH–CO-Ph	89
5a	Me	CH=CH_2	90	**8d**	Bn	CH=CH–CO–Ph	70
5b	Me	CO–Me	70	**8e**	Bn	≡–CH(OH)–Ph	30
5c	Bn	CH=CH_2	78	**10a**	H	CH=CH_2	65
5d	Bn	CO–Me	68	**10b**	H	Ph	60
6a	Me	CH=CH–CN	60	**10c**	H	≡–CH_2-OH	88
6b	Me	CH=CH–CO_2Me	63	**10d**	H	CH=CH–CO-Ph	60
6c	Bn	CH=CH–CN	60	**10e**	H	CH=CH–CO_2Me	62

catalyst for these processes and gave the corresponding functionalised alkenes **6** in yields in the range 40–70%. In contrast to similar experiments on the 5-bromopyridazin-3(2*H*)-ones,[1] we did not find any evidence of dehalogenation during Heck alkenylation of **3a–b**, most probably due to the superior reactivity of the iodo-substituent.

Although the procedures described above give the 5-alkenylpyridazin-3(2*H*)-ones **6a–d** as the main products, an exhaustive investigation of the transformation allowed the isolation of small amounts (5–15%) of phthalazinones as by-products. For instance, the Heck alkenylation of **3b** with methyl acrylate gave, in addition to the expected acrylate **6d** (60%), the phthalazinone **7** (7%) (Scheme 3).

The structure of the unexpected phthalazinones was unambiguously established by analytical and spectroscopic methods (see Section 3). The presence of the methoxycarbonyl group at position 6 of phthalazinone **7** suggests that a highly regioselective process is operating during its formation.

Preliminary mechanistic proposals to explain the formation of phthalazinones during this reaction have recently been put forward.[1] It is thought that this process involves a tandem reaction that initially follows a Heck sp^2 cascade due to a second insertion of another olefin molecule into the previously formed σ-alkylpalladium complex. Further experiments are now in progress to study the mechanism of such a transformation in greater detail and to evaluate the scope of this reaction in the synthesis of 2,6-disubstituted-1-phthalazinones.

Standard Sonogashira conditions[26] were employed to perform the alkynylation at position 5 of the heterocycle (Scheme 4). Although these optimised conditions proved to be applicable to 2-propyn-1-ol (Scheme 4, compounds **8a–b**), the cross-coupling of **3a–b** with 1-phenyl-2-propyn-1-ol (Scheme 4) did not give the expected 2-substituted 5-(3-hydroxy-3-phenylprop-1-yn-yl)pyridazin-3(2*H*)-ones. Instead, the isomeric *E*-chalcones **8c–d** were obtained in good yields (Table 1).

The structure of the heterocyclic chalcones **8c–d** was established on the basis of the analytical and spectroscopic data (see Section 3). The ^{1}H NMR spectra of these products contain doublets with a coupling constant of 15–16 Hz, which confirms a *trans* stereochemistry for the double bond. It is worth noting that this reaction produces the chalcones **8c–d** with an *E*-selectivity greater than 95% after isolation and purification by column chromatography. Other NMR experiments and, in particular, the data extracted form X-ray crystallography on a monocrystal of compound **8c** confirmed our assignment (Fig. 3).

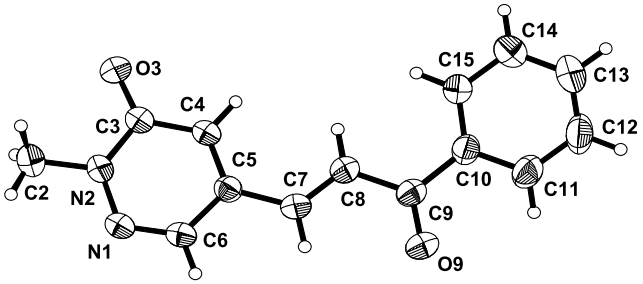


Figure 3. Plot showing the crystal structure and atomic numbering scheme for **8c**. Displacement ellipsoids are drawn at 50% probability level for non-H atoms.

The crystal structure of **8c** is essentially planar and shows a *trans* configuration (Fig. 3). The crystal structure is stabilised by means of weak intermolecular interactions (Fig. 4) of the type C–H···O [C4···O3 = 3.404(3) Å and C6···O9 = 3.332(3) Å]. A weak intramolecular interaction of the type C–H···O is also present [C7···O9 = 2.788(3) Å].

Formation of chalcones **8c–d** under these conditions is dissimilar to results described in previous papers concerning the Sonogashira coupling between 1-phenyl-2-propyn-1-ol and different electron-deficient halides.[31–35] Several authors initially proposed that chalcone formation during these transformations could be related to the participation of organo-palladium intermediates,[31–33] but some recent results[35] (and our own findings[20]) have confirmed that a base-catalysed isomerisation of the expected phenyl-substituted propargyl alcohol is a more likely explanation for this transformation.

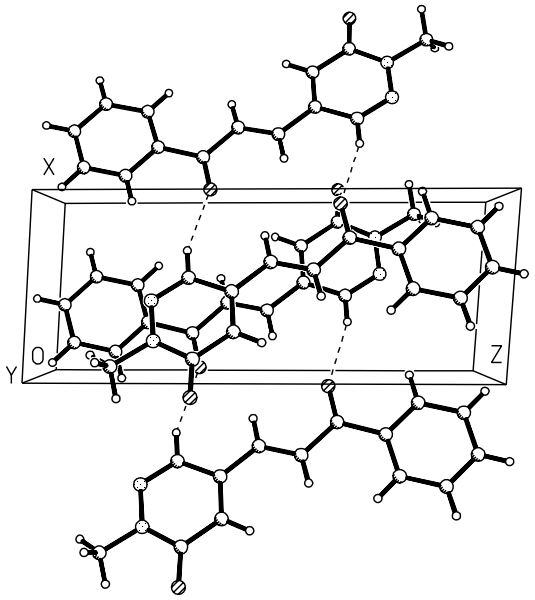


Figure 4. Packing of molecules of **8c** in the unit cell along the [010] crystallographic direction. The most important intermolecular interactions are denoted with dashed lines.

The detailed study of the Sonogashira coupling of **3b** with 1-phenyl-2-propyn-1-ol [by carrying out the reaction at room temperature (25 °C) and after a careful work up] enabled the isolation of the expected phenyl-substituted propargyl alcohol **8e** (30%) together with **8d** (67%) (Scheme 5). Identification of intermediate **8e** is supported by both analytical and spectroscopic data (see Section 3).

Quantitative isolation of **8d** after stirring **8e** in the presence of a base (triethylamine or *N*,*N*-diisopropylethylamine) in a range of solvents (DCM, MeOH, THF, DMF)—even at room temperature—showed that chalcone formation occurs as a consequence of the base-catalysed isomerisation of the phenyl-substituted propargyl alcohol **8e**. This process could be facilitated by the electron-deficient nature of the pyridazinone system, which increases the acidity of the propargylic proton.[36]

The mechanistic pathway proposed for this transformation is outlined in Figure 5. Sonogashira coupling of **3b** with 1-phenyl-2-propyn-1-ol afforded the substituted propargyl alcohol **8e**, which, upon deprotonation at the propargyl centre with triethylamine, led to a propargyl-allenyl anion. Protonation of this species afforded the allene and, finally, the allenol–enone tautomerism furnished the *trans*-configured enone **8d** (Fig. 5).

Our previous work on the 5-bromo-6-phenylpyridazin-3(2*H*)-one showed the low reactivity of this compound in palladium-catalysed reactions.[17–20] However, the high reactivity of the 2-substituted 5-iodopyridazin-3(2*H*)-ones **3a**–**b** (some of the reactions described here can be performed at room temperature) led us to examine such transformations on the 5-iodopyridazin-3(2*H*)-one **3c** on the hypothesis that the change in the halogen (Br→I) could produce an increase in reactivity during the cross-coupling reactions.

Unfortunately, although not completely unexpected, a quick screening experiment with 5-iodopyridazin-3(2*H*)-one **3c** as

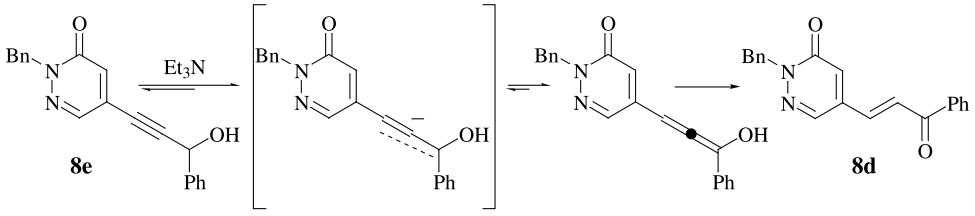
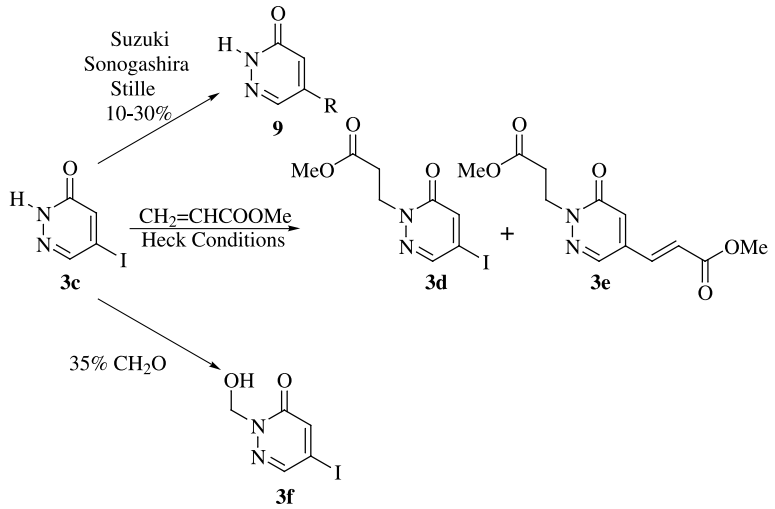


Figure 5.



Scheme 6.

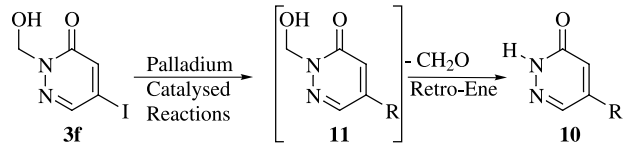
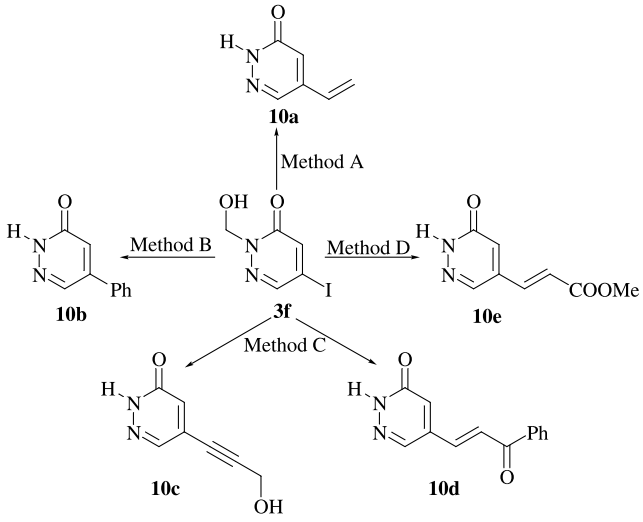


Figure 6.



Scheme 7. Method A: $Bu_3Sn–CH=CH_2/PdCl_2(PPh_3)_2/Et_3N/DMF$. Method B: $PhB(OH)_2$ $Pd(PPh_3)_4/Na_2CO_3/DME–H_2O$. Method C: HC≡C–CH(OH)R/$PdCl_2(PPh_3)_2$/CuI/Et_3N/DMF. Method D: $CH_2=CH–COOMe/PdCl_2[P(o\text{-Tolyl})_3]_2/Et_3N$/DMF.

the starting material under different experimental procedures showed that the degree of transformation was less than 30% during Suzuki, Stille or Sonogashira coupling and most of the starting material was recovered (Scheme 6). All attempts to perform Heck alkenylation of **3c** employing methyl acrylate as the olefin led to formation of *N*-alkyl derivatives **3d** and **3e**. Isolation of these derivatives results from Michael addition of position 2 of the heterocycle to the highly activated and sterically unhindered methyl acrylate. In this transformation, after alkylation at position 2 has been achieved, the Heck alkenylation of **3d** yields the corresponding 5-alkenyl-2-alkylpyridazin-3(2*H*)-one **3e** (Scheme 6).

These results are in accordance with previous studies[17–20] and confirm that a critical factor to ensure the successful coupling is the presence of a group at position 2 of the heterocyclic ring that is able to block the enolisable carbonyl group.

5-Substituted pyridazin-3(2*H*)-ones **9** were prepared using a synthetic strategy recently described by our group that uses a hydroxymethyl group as a thermolabile protecting group for position 2 of the pyridazinone during cross-coupling reactions (Fig. 6).[37]

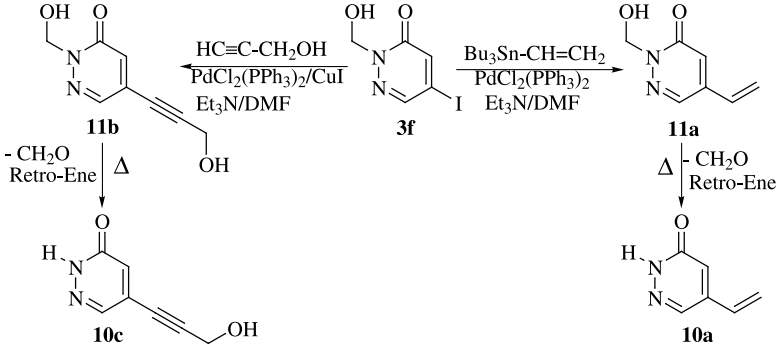
Straightforward hydroxymethylation of **3c** by treatment with 35% formaldehyde solution afforded 2-hydroxymethyl-5-iodopyridazin-3(2*H*)-one **3f** (90%) (Scheme 6). Since pyridazinone **3c** is not reactive under these conditions, the proposed pathway to explain this sequence would involve the initial cross-coupling reactions on **3f** to afford a 5-substituted ene-adduct **9**, which would subsequently lose formaldehyde in a process that may be regarded as a retro-ene fragmentation (Fig. 6).

The excellent reactivity of the 1-O, 3-N, 5-O ene-adduct[38] **3f** toward Suzuki, Sonogashira, Stille or Heck conditions was readily demonstrated by the efficient preparation of different 5-aryl-, 5-alkynyl- or 5-alkenylpyridazin-3(2*H*)-ones **10** in a one-pot procedure (Scheme 7, Table 1).

These results confirm the versatility and usefulness of the hydroxymethyl group as a convenient thermolabile group to protect position 2 of halopyridazinones during palladium-catalysed reactions.

The reactivity of the iodine atom in the ene-adduct **3f** allowed to confirm that formation of compounds **10** is not a concerted process. The isolation and identification of intermediates **11a** and **11b** during some of these transformations, when performed at room temperature, are completely consistent with the pathway shown in Fig. 6 (Scheme 8). Thus, when the starting pyridazinone **3f** was submitted to Stille or Sonogashira cross-coupling reaction conditions at room temperature during 2–3 h, the 5-substituted 2-hydroxymethylpyridazin-3(2*H*)-ones **9** could be isolated from the reaction mixtures. Reactions times greater than 24 h led to 5-substituted pyridazin-3(2*H*)-ones **10**.

Compared to 5-bromo-2-methoxymethyl-6-phenylpyridazin-3(2*H*)-one, the 2-substitued 5-iodopyridazin-3(2*H*)-ones **3** proved to be much more reactive toward



Scheme 8.

palladium-catalysed reactions. This superior reactivity could be due to the change in the halogen, the absence of the phenyl group at position 6 of the heterocycle (which would reduce steric hindrance) or, most probably, a combination of these effects.

In summary, practical and efficient palladium-assisted procedures to perform the structural diversification of the 5-position of 2-substituted pyridazinones have been developed. The palladium-mediated alkynylation of **3** using 1-phenyl-2-propyn-1-ol affords *E*-chalcones in excellent yields. A study of this transformation allowed the isolation of an intermediate and confirmed the electron-deficient nature of the starting 5-iodopyridazin-3(2*H*)-one to be the key factor during the base-catalysed isomerisation process. Furthermore, part of this work has demonstrated the synthetic utility of the 2-hydroxymethyl unit as a convenient protecting group for the lactam function during couplings on 5-iodopyridazin-3(2*H*)-one.

3. Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured using a Perkin–Elmer 1640 FTIR spectrophotometer with samples as potassium bromide pellets. The NMR spectra were recorded on Bruker AM300 and XM500 spectrometers. Chemical shifts are given as δ values against tetramethylsilane as internal standard and J values are given in Hz. Mass spectra were obtained on a Varian MAT-711 instrument. High-resolution mass spectra were obtained on an Autospec Micromass spectrometer. Elemental analyses were performed on a Perkin–Elmer 240B apparatus at the Microanalysis Service of the University of Santiago de Compostela. The reactions were monitored by TLC with 2.5 mm Merck silica gel GF 254 strips, and the purified compounds each showed a single spot; unless stated otherwise, iodine vapour and/or UV light were used for detection of compounds. Commercially available starting materials and reagents were purchased and used without further purification.

The X-ray crystallographic determination of **8c** was performed on a Siemens P4 four-circle diffractometer with graphite monochromated Cu K_α radiation. The intensity data were collected using 2θ–ω scans, with ω scan width equal to the difference of the background and the high ωbackground plus the separation between the $K_{\alpha1}$ and $K_{\alpha2}$ positions; 2792 reflections measured ($3.71<\theta<68.87°$, $-1<h<6$, $-9<k<9$, $-16<l<16$), 2111 unique (merging $R=0.0599$) and 1462 observed [$F^2\geq 2\sigma(F)^2$] reflections. Empirical absorption correction via ψ scans was applied.[39] Three standard reflections were monitored every 100 reflections (intensity decay: 3%).

The crystal structure of **8c** was solved by direct methods and Fourier synthesis. Non-H atoms were refined anisotropically by full-matrix least-squares techniques. H atoms were calculated geometrically and included in the refinement, but were restrained to ride on their parent atoms. The isotropic displacement parameters of the H atoms were fixed to 1.3 times *Ueq* of their parent atoms. Data collection: XSCANS.[40] Cell refinement: XSCANS.[40] Data reduction: XSCANS.[40] Program used to solve structure: SIR92.[41] Program used to refine structure: SHELXL97.[42] Molecular graphics: DIAMOND.[43] Software used to prepare material for publication: PLATON.[44]

2-Substituted 4,5-dichloropyridazin-3(2*H*)-ones **2** were obtained by following previously described procedures.[28]

3.1. Synthesis of 2-substituted 5-iodopyridazin-3(2*H*)-ones 3. General procedure

A solution of 2-substituted 4,5-dichloropyridazin-3(2*H*)-one **2** (28 mmol) in 57% hydriodic acid (41 mL) was stirred and heated under reflux (oil bath 140 °C) until the starting material had been consumed (24 h). After cooling, the solution was treated with 30% sodium thiosulphate and then extracted with dichloromethane. The organic phase was dried over sodium sulphate, and concentrated to dryness under reduced pressure. The residue was purified twice by column chromatography on silica gel. Further purification was achieved by recrystallisation from the appropriate solvent.

3.1.1. 5-Iodo-2-methylpyridazin-3(2*H*)-one 3a. Purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent. Mp 179–180 °C (isopropanol). Yield 81%. IR (KBr): ν_{max}/cm^{-1} 1654 (CO). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.89 (d, $J=1.9$ Hz, 1H, H_6), 7.44 (d, $J=1.9$ Hz, 1H, H_4), 3.71 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 159.0, 142.0, 138.2, 102.2, 40.3. MS (70 eV) *m/z* (%): 236 (M^+, 100), 208 (42), 165 (82), 127 (55). Anal. Calcd for $C_5H_5IN_2O$, C 25.45, H 2.14, N 11.87; found, C 25.47, H 2.15, N 11.96.

3.1.2. 2-Benzyl-5-iodopyridazin-3(2*H*)-one 3b. Purification by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. Mp 132–133 °C (isopropanol). Yield 88%. IR (KBr): ν_{max}/cm^{-1} 1654 (CO), 1560 (aromatics). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.91 (d, $J=2.0$ Hz, 1H, H_6), 7.46 (d, $J=2.0$ Hz, 1H, H_4), 7.40 (m, 2H, aromatics), 7.33 (m, 3H, aromatics), 5.25 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 159.0, 142.3, 138.8, 135.9, 129.2, 129.0, 128.5, 102.8, 55.4. MS (70 eV) *m/z* (%): 312 (M^+, 24), 165 (38), 125 (42), 111 (64), 97 (100). Anal. Calcd for $C_{11}H_9IN_2O$, C 42.33, H 2.91, N 8.98; found, C 42.43, H 3.01, N 9.07.

2-Substituted 4,5-diiodopyridazin-3(2*H*)-ones can be successfully obtained employing the general procedure previously described for the 2-substituted 5-iodopyridazin-3(2*H*)-ones **3** but shorting reaction times to 3 h.

3.1.3. 4,5-Diiodo-2-methylpyridazin-3(2*H*)-one. Purification by column chromatography on silica gel using AcOEt/hexane (1:6) as eluent. Mp 156–157 °C (isopropanol). Yield 75%. IR (KBr): ν_{max}/cm^{-1} 1650 (CO). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.80 (s, 1H, H_6), 3.76 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 159.0, 141.4, 121.0, 116.5, 41.8. MS (70 eV) *m/z* (%): 362 (M^+, 88), 333 (28), 270 (100).

3.1.4. 2-Benzyl-4,5-diiodopyridazin-3(2*H*)-one. Purification by column chromatography on silica gel using

AcOEt/hexane (1:8) as eluent. Mp 97–98 °C (isopropanol). Yield 68%. IR (KBr): ν_{max}/cm^{-1} 1651 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.58 (s, 1H, H_6), 7.44 (m, 2H, aromatics), 7.30 (m, 3H, aromatics), 5.32 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 158.6, 145.4, 136.1, 135.5, 135.3, 129.5, 129.1, 128.7, 56.2. MS (70 eV) *m/z* (%): 438 (M^+, 14), 347 (25), 242 (58).

3.1.5. 5-Iodopyridazin-3(2*H*)-one 3c. To a suspension of anhydrous aluminium chloride (0.66 g, 5.03 mmol) in dry toluene (8 mL) under an argon atmosphere was slowly added the 2-benzyl-5-iodopyridazin-3(2*H*)-one **3b** (0.31 g, 1.0 mmol) and the mixture was stirred and heated (oil bath 120 °C) until the starting material had been consumed. After cooling, ice-water was added, the mixture stirred for 10 min and then extracted with dichloromethane. The organic phase was dried over sodium sulphate and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using AcOEt/hexane (1:10) as eluent. Further purification was achieved by recrystallisation from isopropanol. Mp 147–148 °C (isopropanol). Yield 90%. IR (KBr): ν_{max}/cm^{-1} 3004 (NH), 1651 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 12.57 (bs, 1H, NH), 8.00 (d, $J=1.9$ Hz, 1H, H_6), 7.54 (d, $J=1.9$ Hz, 1H, H_4). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 161.1, 143.6, 139.1, 105.3. MS (70 eV) *m/z* (%): 222 (M^+, 100), 194 (20), 127 (41). Anal. Calcd for $C_4H_3IN_2O$, C 21.64, H 1.36, N 12.62; found, C 21.64, H 1.45, N 12.66.

3.2. Reaction of methyl acrylate with 3c under Heck conditions (synthesis of 3d and 3e)

A mixture of 5-iodopyridazin-3(2*H*)-one **3c** (1.00 mmol), bis(tri-*o*-tolyl-phosphine)palladium(II) dichloride (0.10 mmol), triethylamine (1.52 mmol) and methyl acrylate (2.00 mmol) in DMF (10 mL) in a sealed tube was heated under reflux (oil bath 110 °C) under argon until the starting material had been consumed. The mixture was allowed to cool to room temperature, filtered through a pad of Celite and the filtrate was evaporated to dryness to give a brown oily residue. The residue was purified by column chromatography (AcOEt/hexane, 1:4) to give **3d** and **3e**. Further purification was achieved by recrystallisation.

3.2.1. Methyl-3-(4-iodo-6-oxo-pyridazin-1(6*H*)-yl)propanoate 3d. Mp 58–60 °C (isopropanol). Yield 9%. IR (KBr): ν_{max}/cm^{-1} 1730 (COO), 1640 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.91 (d, $J=1.9$ Hz, 1H, H_6), 7.46 (d, $J=1.9$ Hz, 1H, H_4), 4.38 (t, $J=7.1$ Hz, 2H, CH_2), 3.68 (s, 3H, OCH_3), 2.81 (t, $J=7.1$ Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 171.6, 158.9, 142.3, 138.6, 103.0, 52.3, 47.7, 39.6. MS (70 eV) *m/z* (%): 308 (M^+, 34), 249 (49), 222 (100). HRMS *m/z* calcd for $C_8H_9IN_2O_3$ (M^+): 307.9658, found: 307.9660.

3.2.2. Methyl (2*E*)-3-[1-(3-methoxy-3-oxopropyl)-6-oxo-1,6-dihydropyridazin-4-yl]acrylate 3e. Mp 120–122 °C (isopropanol). Yield 85%. IR (KBr): ν_{max}/cm^{-1} 1733 (COO), 1636 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.89 (d, $J=2.1$ Hz, 1H, H_6), 7.40 (d, $J=16.1$ Hz, 1H, CH), 6.90 (d, $J=2.1$ Hz, 1H, H_4), 6.53 (d, $J=16.1$ Hz, 1H, CH), 4.41 (t, $J=7.0$ Hz, 1H, H_2), 3.82 (s, 3H, CH_3), 3.68 (s, 3H, CH_3), 2.83 (t, $J=7.0$ Hz, 1H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 171.7, 165.9, 160.3, 138.2, 134.3, 128.2, 125.5, 52.7, 52.3, 47.8, 32.8, 31.3. MS (70 eV) *m/z* (%): 266 (M^+, 55), 234 (45), 207 (40), 179 (100). HRMS *m/z* calcd for $C_{12}H_{14}IN_2O_5$ (M^+): 266.0903, found: 266.0907.

3.2.3. 2-Hydroxymethyl-5-iodopyridazin-3(2*H*)-one 3f. A mixture of **3c** (1.5 g, 6.75 mmol) and 35% formaldehyde (50 mL) was flushed with argon for 5 min. The suspension was stirred and heated under reflux (oil bath 110 °C) under argon until the starting material had been consumed. The mixture was cooled and the suspension was concentrated to dryness under reduced pressure. The resulting solid was purified by column chromatography on silica gel (AcOEt/hexane, 1:3). Further purification was achieved by recrystallisation from isopropanol. Mp 140–141 °C (isopropanol). Yield 90%. IR (KBr): ν_{max}/cm^{-1} 3261 (OH), 1651 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.13 (d, $J=2.1$ Hz, 1H, H_6), 7.59 (d, $J=2.1$ Hz, 1H, H_4), 6.77 (t, $J=7.6$ Hz, 1H, OH), 5.25 (d, $J=7.6$ Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 158.3, 142.2, 138.3, 105.7, 73.5. MS (70 eV) *m/z* (%): 252 (M^+, 20), 236 (30), 223 (100). HRMS *m/z* calcd for $C_5H_5IN_2O_2$ (M^+): 251.9396, found: 251.9399.

3.3. General procedure for the Suzuki coupling of 3

The 2-substituted 5-iodopyridazin-3(2*H*)-one **3** (1.7 mmol) was mixed with the corresponding arylboronic acid (2.2 mmol), $Pd(PPh_3)_4$ (0.016 mmol) and K_2CO_3 (5.08 mmol) in a 3:1 mixture of DME/H_2O (15 mL) and flushed with argon for 5 min. The mixture was then stirred and heated under reflux (oil bath 100 °C) under argon until the starting material had been consumed. After cooling, the solution was filtered through a pad of Celite and the filtrate was evaporated to dryness to give an oily residue, which was purified by column chromatography on silica gel. Further purification was achieved by recrystallisation from the appropriate solvent.

3.3.1. 2-Methyl-5-phenylpyridazin-3(2*H*)-one 4a. Purification by column chromatography on silica gel using AcOEt/hexane (1:1) as eluent. Mp 119–120 °C (isopropanol). Yield 91%. IR (KBr): ν_{max}/cm^{-1} 1658 (CO), 1590 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.00 (d, $J=2.2$ Hz, 1H, H_6), 7.55 (m, 2H, aromatics), 7.46 (m, 3H, aromatics), 7.00 (d, $J=2.2$ Hz, 1H, H_4), 3.83 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.3, 144.2, 136.0, 134.3, 130.6, 129.8, 127.2, 124.7, 40.3. MS (70 eV) *m/z* (%): 186 (M^+, 40), 158 (28), 130 (10), 115 (100). Anal. Calcd for $C_{11}H_{10}N_2O$, C 70.95, H 5.41, N 15.04; found, C 71.05, H 5.43, N 15.04.

3.3.2. 5-(4′-Chlorophenyl)-2-methylpyridazin-3(2*H*)-one 4b. Purification by column chromatography on silica gel using AcOEt/hexane (1:1) as eluent. Mp 167–168 °C (isopropanol). Yield 90%. IR (KBr): ν_{max}/cm^{-1} 1654 (CO), 1589 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.98 (d, $J=2.3$ Hz, 1H, H_6), 7.52–7.48 (m, 4H, aromatics), 7.00 (d, $J=2.3$ Hz, 1H, H_4), 3.81 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 161.0, 143.0, 137.0, 135.5, 132.7, 130.1, 128, 5, 124.7, 40.4. MS (70 eV) *m/z* (%): 220 (M^+, 48), 192 (20), 149 (100). Anal. Calcd for

$C_{11}H_9ClN_2O$, C 59.88, H 4.11, N 12.70; found, C 60.10, H 4.15, N 12.72.

3.3.3. 2-Benzyl-5-phenylpyridazin-3(2*H*)-one 4c. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 126–127 °C (isopropanol). Yield 94%. IR (KBr): ν_{max}/cm^{-1} 1655 (CO), 1590 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.00 (d, $J=2.0$ Hz, 1H, H_6), 7.55–7.46 (m, 7H, aromatics), 7.36–7.27 (m, 3H, aromatics), 7.00 (d, $J=2.0$ Hz, 1H, H_4), 5.37 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.8, 143.9, 136.6, 136.3, 134.2, 130.6, 129.7, 129.1, 129.0, 128.3, 127.2, 125.2, 55.2. MS (70 eV) *m/z* (%): 262 (M^+, 35), 220 (10), 158 (20), 115 (100). Anal. Calcd for $C_{17}H_{14}N_2O$, C 77.84, H 5.38, N 10.68; found, C 77.90, H 5.56, N 10.67.

3.3.4. 2-Benzyl-5-(4-chlorophenyl)pyridazin-3(2*H*)-one 4d. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 145–146 °C (isopropanol). Yield 90%. IR (KBr): ν_{max}/cm^{-1} 1656 (CO), 1588 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.00 (d, $J=2.2$ Hz, 1H, H_6), 7.51–7.43 (m, 7H, aromatics), 7.32 (m, 2H, aromatics), 7.00 (d, $J=2.2$ Hz, 1H, H_4), 5.36 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.6, 142.8, 136.8, 136.5, 135.9, 132.7, 130.0, 129.2, 129.0, 128.5, 128.4, 125.3, 55.3. MS (70 eV) *m/z* (%): 312 (M^+, 24), 165 (38), 125 (42), 111 (64), 97 (100). Anal. Calcd for $C_{17}H_{13}ClN_2O$, C 68.81, H 4.42, N 9.44; found, C 69.14, H 4.54, N 9.53.

3.4. General procedure for the Stille coupling of 3

A mixture of 2-substituted 5-iodopyridazin-3(2*H*)-one **3** (3.40 mmol), bis(triphenylphosphine)palladium(II) dichloride (0.16 mmol) and the corresponding tributylstannane (3.73 mmol) in anhydrous toluene (20 mL) was heated under reflux under argon until the starting material had been consumed. The mixture was allowed to cool to room temperature, filtered through a pad of Celite and the filtrate was evaporated to dryness to give a yellow oily residue. For the synthesis of compounds **5b** and **5d** the oily residue containing the corresponding enol-ether was heated under reflux in 3 N hydrochloric acid during 12 h. After extraction with dichloromethane, the organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography and further purification was achieved by recrystallisation from the appropriate solvent.

3.4.1. 2-Methyl-5-vinylpyridazin-3(2*H*)-one 5a. Purification by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. Mp 101–102 °C (isopropanol). Yield 90%. IR (KBr): ν_{max}/cm^{-1} 1654 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.83 (d, $J=2.0$ Hz, 1H, H_6), 6.72 (d, $J=2.0$ Hz, 1H, H_4), 6.48 (dd, $J=6.7$, 10.9 Hz, 1H, CH), 5.93 (d, $J=16.6$ Hz, 1H, CH), 5.58 (d, $J=10.9$ Hz, 1H, CH), 3.74 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 161.3, 140.6, 134.4, 131.7, 124.8, 122.1, 40.1. MS (70 eV) *m/z* (%): 136 (M^+, 100), 108 (26). Anal. Calcd for $C_7H_8N_2O$, C 61.75, H 5.92, N 20.58; found, C 61.79, H 5.94, N 20.57.

3.4.2. 5-Acetyl-2-methylpyridazin-3(2*H*)-one 5b. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 145–146 °C (isopropanol). Yield 70%. IR (KBr): ν_{max}/cm^{-1} 1654 (CO), 1560 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.15 (d, $J=2.2$ Hz, 1H, H_6), 7.30 (d, $J=2.2$ Hz, 1H, H_4), 3.80 (s, 3H, CH_3), 2.54 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 195.9, 160.9, 137.8, 133.7, 129.5, 40.7, 26.9. MS (70 eV) *m/z* (%): 152 (M^+, 100), 124 (14), 109 (70). Anal. Calcd for $C_7H_8N_2O_2$, C 55.26, H 5.30, N 18.41; found, C 55.32, H 5.42, N 18.46.

3.4.3. 2-Benzyl-5-vinylpyridazin-3(2*H*)-one 5c. Purification by column chromatography on silica gel using AcOEt/hexane (1:6) as eluent. Mp 60–62 °C (isopropanol). Yield 78%. IR (KBr): ν_{max}/cm^{-1} 1653 (CO), 1586 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.91 (d, $J=2.0$ Hz, 1H, H_6), 7.39 (m, 2H, aromatics), 7.28 (m, 3H, aromatics), 6.72 (d, $J=2.0$ Hz, 1H, H_4), 6.44 (dd, $J=6.7$, 11.0 Hz, 1H, CH), 5.88 (d, $J=16.6$ Hz, 1H, CH), 5.54 (d, $J=11.0$ Hz, 1H, CH), 5.27 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.9, 140.5, 136.7, 134.8, 131.7, 129.0, 128.9, 128.2, 125.4, 122.3, 55.1. MS (70 eV) *m/z* (%): 212 (M^+, 75), 91 (62), 65 (100). HRMS *m/z* calcd for $C_{13}H_{12}N_2O$ (M^+): 212.0901, found: 212.0903.

3.4.4. 5-Acetyl-2-benzylpyridazin-3(2*H*)-one 5d. Purification by column chromatography on silica gel using AcOEt/hexane (1:4) as eluent. Mp 105–106 °C (isopropanol). Yield 68%. IR (KBr): ν_{max}/cm^{-1} 1661 (CO), 1547 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.17 (d, $J=2.1$ Hz, 1H, H_6), 7.44 (d, $J=2.1$ Hz, 1H, H_4), 7.40 (m, 2H, aromatics), 7.30 (m, 3H, aromatics), 5.33 (s, 2H, CH_2), 2.53 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 196.0, 160.2, 137.3, 135.9, 134.0, 130.2, 129.2, 129.0, 128.6, 55.9, 26.9. MS (70 eV) *m/z* (%): 228 (M^+, 100), 186 (20), 124 (20). Anal. Calcd for $C_{13}H_{12}N_2O_2$, C 68.41, H 5.30, N 12.27; found, C 68.63, H 5.34, N 12.28.

3.5. General procedure for the Heck coupling of 3

A degassed (argon) mixture of the 2-substituted 5-iodopyridazin-3(2*H*)-one **3** (1.00 mmol), bis(tri-*o*-tolylphosphine)-palladium(II) dichloride (0.10 mmol), triethylamine (1.52 mmol) and the corresponding alkene (2.00 mmol) in DMF (10 mL) in a sealed tube was heated under reflux (oil bath 110 °C) under argon until the starting material had been consumed. The mixture was allowed to cool to room temperature, filtered through a pad of Celite and the filtrate was evaporated to dryness to give a brown oily residue. The residue was purified by column chromatography and further purification was achieved by recrystallisation from the appropriate solvent.

3.5.1. (2*E*)-3-(1-Methyl-6-oxo-1,6-dihydropyridazin-4-yl)acrylonitrile 6a. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 155–156 °C (isopropanol). Yield 63%. IR (KBr): ν_{max}/cm^{-1} 2230 (CN), 1670 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.87 (d, $J=2.1$ Hz, 1H, H_6), 7.18 (d, $J=16.7$ Hz, 1H, CH), 6.90 (d, $J=2.1$ Hz, 1H, H_4), 6.07 (d, $J=16.7$ Hz, 1H, CH), 3.77 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.3, 144.3, 136.9, 135.4, 132.8, 127.6, 116.4, 40.4.

MS (70 eV) *m/z* (%): 161 (M^+, 20), 133 (16), 90 (43), 58 (100). Anal. Calcd for $C_8H_7N_3O$, C 59.62, H 4.38, N 26.07; found, C 59.67, H 4.42, N 26.12.

3.5.2. Methyl (2*E*)-3-(1-methyl-6-oxo-1,6-dihydropyridazin-4-yl)acrylate 6b. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 194–196 °C (isopropanol). Yield 60%. IR (KBr): ν_{max}/cm^{-1} 1723 (COO), 1636 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.87 (d, $J=1.9$ Hz, 1H, H_6), 7.40 (d, $J=16.0$ Hz, 1H, CH), 6.91 (d, $J=1.9$ Hz, 1H, H_4), 6.52 (d, $J=16.0$ Hz, 1H, CH), 3.76 (s, 3H, CH_3), 3.74 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 166.0, 160.6, 138.3, 138.0, 134.0, 127.8, 125.3, 52.6, 40.4. MS (70 eV) *m/z* (%): 194 (M^+, 100), 163 (24), 135 (60). Anal. Calcd for $C_9H_{10}N_2O_3$, C 55.67, H 5.19, N 14.43; found, C 55.71, H 5.19, N 14.45.

3.5.3. (2*E*)-3-(1-Benzyl-6-oxo-1,6-dihydropyridazin-4-yl)acrylonitrile 6c. Purification by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. Mp 123–125 °C (isopropanol). Yield 68%. IR (KBr): ν_{max}/cm^{-1} 1654 (CO), 1560 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.82 (d, $J=2.2$ Hz, 1H, H_6), 7.52–7.38 (m, 5H, aromatics), 7.12 (d, $J=16.7$ Hz, 1H, CH), 6.89 (d, $J=2.2$ Hz, 1H, H_4), 6.03 (d, $J=16.7$ Hz, 1H, CH), 5.29 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 159.9, 144.3, 136.8, 136.3, 136.0, 130.2, 129.2, 129.0, 128.5, 128.0, 115.3, 55.4. MS (70 eV) *m/z* (%): 237 (M^+, 54), 209 (13), 181 (9), 91 (100). Anal. Calcd for $C_{14}H_{11}N_3O$, C 70.87, H 4.67, N 17.71; found, C 70.89, H 4.88, N 17.75.

3.5.4. Methyl (2*E*)-3-(1-benzyl-6-oxo-1,6-dihydropyridazin-4-yl)acrylate 6d. Purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent. 112–113 (isopropanol). Yield 60%. IR (KBr): ν_{max}/cm^{-1} 1726 (COO), 1654 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.90 (d, $J=2.2$ Hz, 1H, H_6), 7.44–7.25 (m, 6H, 5H aromatics + CH), 6.92 (d, $J=2.2$ Hz, 1H, H_4), 6.50 (d, $J=16.1$ Hz, 1H, CH), 5.31 (s, 2H, CH_2), 3.82 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 165.3, 160.0, 138.3, 136.3, 134.3, 129.1, 129.0, 128.5, 128.4, 128.3, 125.3, 55.5, 52.7. MS (70 eV) *m/z* (%): 270 (M^+, 31), 211 (14), 91 (100). Anal. Calcd for $C_{15}H_{14}N_2O_3$, C 66.66, H 5.22, N 10.36; found, C 66.68, H 5.24, N 10.49.

3.5.5. Methyl 2-benzyl-1-oxo-1,2-dihydrophthalazin-6-carboxylate 7. Purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent. Mp 156–157 °C (isopropanol). Yield 7%. IR (KBr): ν_{max}/cm^{-1} 1725 (COO), 1645 (CO), 1494 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.50 (d, $J=8.50$ Hz, 1H, CH), 8.37–8.33 (m, 2H, aromatics), 8.23 (s, 1H, CH), 7.48–7.41 (m, 2H, aromatics), 7.34–7.25 (m, 3H, aromatics), 5.41 (s, 2H, CH_2), 3.99 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 166.2, 159.5, 138.2, 137.0, 134.7, 132.0, 131.3, 130.5, 129.1, 129.0, 128.3, 128.2, 127.8, 55.2, 53.2. MS (70 eV) *m/z* (%): 294 (M^+, 19), 266 (5), 190 (85). Anal. Calcd for $C_{17}H_{14}N_2O_3$, C 69.38, H 4.79, N 9.52; found, C 69.45, H 4.87, N 9.55.

3.6. General procedure for the Sonogashira coupling of 3

To a degassed (argon) suspension of 2-substituted 5-iodopyridazin-3(2*H*)-one **3** (1.0 mmol), $PdCl_2(PPh_3)_2$ (0.02 mmol) and CuI (0.02 mmol) in DMF (10 mL) was added triethylamine (2.1 mmol) and the corresponding alkyne (1.50 mmol). The mixture was stirred at room temperature under argon until the starting material had been consumed. The mixture was cooled to room temperature, diluted with dichloromethane and filtered through Celite. The filtrate was concentrated in vacuo and the residue purified by column chromatography on silica gel. Further purification was achieved by recrystallisation from the appropriate solvent.

3.6.1. 2-Methyl-5-(3-hydroxyprop-1-yn-1-yl)pyridazin-3(2*H*)-one 8a. Purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent. Mp 134–135 °C (isopropanol). Yield 70%. IR (KBr): ν_{max}/cm^{-1} 3342 (OH), 2210 (C≡C), 1650 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.68 (d, $J=1.9$ Hz, 1H, H_6), 6.99 (d, $J=1.9$ Hz, 1H, H_4), 4.50 (bs, 1H, OH), 3.80 (s, 2H, CH_2), 3.75 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.5, 137.7, 130.5, 128.6, 98.0, 79.1, 51.3, 40.6. MS (70 eV) *m/z* (%): 164 (M^+, 100), 136 (35). Anal. Calcd for $C_8H_8N_2O_2$, C 58.53, H 4.91, N 17.06; found, C 58.61, H 4.89, N 16.97.

3.6.2. 2-Benzyl-5-(3-hydroxyprop-1-yn-1-yl)pyridazin-3(2*H*)-one 8b. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 125–126 °C (isopropanol). Yield 93%. IR (KBr): ν_{max}/cm^{-1} 1640 (CO), 1579 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.64 (d, $J=1.8$ Hz, 1H, H_6), 7.40–7.34 (m, 2H, aromatics), 7.33–7.25 (m, 3H, aromatics), 6.97 (d, $J=1.8$ Hz, 1H, H_4), 5.27 (s, 2H, CH_2), 4.42 (t, $J=6.3$ Hz, 1H, OH), 3.94 (d, $J=6.3$ Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.1, 138.1, 136.0, 131.1, 129.2, 129.0, 128.6, 128.4, 98.4, 79.2, 55.7, 51.2. MS (70 eV) *m/z* (%): 240 (M^+, 44), 213 (8), 184 (14), 156 (10), 136 (23), 104 (36), 91 (100). Anal. Calcd for $C_{14}H_{12}N_2O_2$, C 69.99, H 5.03, N 11.66; found, C 70.06, H 5.01, N 11.67.

3.6.3. 2-Methyl-5-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-one 8c. Purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent. Mp 193–194 °C (MeOH). Yield 89%. IR (KBr): ν_{max}/cm^{-1} 1666 (CO), 1645 (CO), 1586 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.09 (d, $J=1.9$ Hz, 1H, H_6), 7.70 (m, 2H, aromatics), 7.31 (d, $J=15.5$ Hz, 1H, CH), 7.21–6.68 (m, 5H, 3H, aromatics, 1H, CH, 1H, H_4), 3.69 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 189.3, 160.8, 138.6, 137.6, 137.4, 134.3, 134.1, 129.3, 129.0, 128.6, 127.9, 40.5. MS (70 eV) *m/z* (%): 240 (M^+, 100), 211 (40). Anal. Calcd for $C_{14}H_{12}N_2O_2$, C 69.99, H 5.03, N 11.66; found, C 70.07, H 5.01, N 11.66.

X-ray structure analysis. Crystals of **8c** were grown by slow evaporation from a methanol solution. *Crystal data*. $C_{14}H_{12}N_2O_2$, $M=240.26$, triclinic, $a=5.5013(4)$ Å, $b=7.8346(5)$ Å, $c=13.6808(9)$ Å, $\alpha=87.116(6)°$, $\beta=86.082(6)°$, $\gamma=85.108(5)°$, $V=585.54(7)$ Å^3 [by least-squares refinement on diffractometer angles for 34 automatically centered reflections with $10.73<\theta<27.74°$, $\lambda=1.54178$ Å, $T=293(2)$ K], space group $P\bar{1}$, $Z=2$, $D_c=1.363(1)$ g cm^{-3}, $\mu=0.758$ mm^{-1}.

A prism-like colourless crystal ($0.46\times0.06\times0.02\ mm^3$) was used for the analysis. CCDC 231679 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

3.6.4. 2-Benzyl-5-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-one 8d. Purification by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. Mp 174–175 °C (isopropanol). Yield 70%. IR (KBr): ν_{max}/cm^{-1} 1646 (CO), 1629 (CO), 1578 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 8.15 (d, $J=2.0$ Hz, 1H, H_6), 7.92 (d, $J=8.2$ Hz, 2H, aromatics), 7.54 (d, $J=15.5$ Hz, 1H, CH), 7.42 (m, 5H, aromatics), 7.10 (m, 4H, 3H aromatics + H_4), 6.75 (d, $J=15.5$ Hz, 1H, CH), 5.01 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 189.3, 160.3, 138.5, 137.6, 137.4, 136.3, 134.7, 134.1, 129.3, 129.2, 129.1, 129.0, 128.6, 128.5, 128.4, 55.44. MS (70 eV) *m/z* (%): 316 (M^+, 72), 197 (58), 184 (100). Anal. Calcd for $C_{20}H_{16}N_2O_2$, C 75.93, H 5.10, N 8.86; found, C 75.95, H 5.09, N 8.85.

3.6.5. 2-Benzyl-5-(3-hydroxy-3-phenylprop-1-yn-1-yl)pyridazin-3(2*H*)-one 8e. This compound was obtained by following the general procedure described above for the Sonogashira alkynylation of compounds **3** but at room temperature. Careful work up and purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent afforded **8e** (30%) and **8d** (67%). **8e**: Mp 133–134 °C (isopropanol). IR (KBr): ν_{max}/cm^{-1} 3254 (OH), 2218 (C–≡–C), 1645 (CO), 1581 (aromatics). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.64 (d, $J=1.9$ Hz, 1H, H_6), 7.53 (m, 2H, aromatics), 7.36–7.12 (m, 8H, aromatics), 6.96 (d, $J=1.9$ Hz, 1H, H_4), 5.65 (d, $J=6.0$ Hz, 1H, CH), 5.25 (s, 2H, CH_2), 3.72 (d, $J=6.0$ Hz, 1H, OH). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 159.8, 139.9, 137.7, 136.1, 131.4, 129.2, 129.1, 129.0, 128.4, 128.0, 126.9, 90.0, 80.2, 65.0, 55.1, 31.3. MS (70 eV) *m/z* (%): 316 (M^+, 100), 91 (75). Anal. Calcd for $C_{20}H_{16}N_2O_2$, C 75.93, H 5.10, N 8.86; found, C 75.98, H 5.12, N 8.90.

Base-promoted isomerisation of intermediate **8e**. A mixture of 2-benzyl-5-(3-hydroxy-3-phenylprop-1-yn-1-yl)pyridazin-3(2*H*)-one **8e** (50 mg), MeOH (7 mL) and a catalytic amount of triethylamine was heated under reflux until the starting material had been completely transformed into the chalcone **8d**. The solvent was evaporated in vacuo and the resulting residue was purified by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. The compound obtained had identical physical and spectroscopic properties to **8d**. Mp 174–175 °C (isopropanol).

Compounds **11a–b** and **10a–c** were obtained by following the general procedures previously described for the Suzuki, Heck, Stille or Sonogashira reactions but starting from the 2-hydroxymethyl-5-iodopyridazin-3(2*H*)-one **3f**.

3.6.6. 5-Vinylpyridazin-3(2*H*)-one 10a. Purification by column chromatography on silica gel using AcOEt/hexane (1:3) as eluent. Mp 279–280 °C (dec) (isopropanol). Yield 65%. IR (KBr): ν_{max}/cm^{-1} 1664 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 12.30 (bs, 1H, NH), 7.93 (d, $J=1.8$ Hz, 1H, H_6), 6.77 (d, $J=1.8$ Hz, 1H, H_4), 6.50 (dd, $J=6.7$, 11.0 Hz, 1H, CH), 5.96 (d, $J=16.6$ Hz, 1H, CH), 5.62 (d, $J=11.0$ Hz, 1H, CH). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 163.0, 135.8, 131.8, 131.5, 125.4, 122.9. MS (70 eV) *m/z* (%): 122 (M^+, 84), 58 (100). HRMS *m/z* calcd for $C_6H_6N_2O$ (M^+): 122.0554, found: 122.0556.

3.6.7. 5-Phenylpyridazin-3(2*H*)-one 10b. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 193–194 °C (isopropanol). Yield 60%. IR (KBr): ν_{max}/cm^{-1} 1662 (CO), 1534 (aromatics). ^1H NMR (DMSO-d_6 300 MHz), δ (ppm): 13.10 (bs, 1H, NH), 8.29 (d, $J=2.1$ Hz, 1H, H_6), 7.83–7.78 (m, 2H, aromatics), 7.52–7.48 (m, 3H, aromatics), 7.11 (d, $J=2.1$ Hz, 1H, H_4). ^{13}C NMR (DMSO-d_6 75 MHz), δ (ppm): 162.3, 143.6, 136.2, 133.9, 130.6, 129.6, 127.5, 124.5. MS (70 eV) *m/z* (%): 172 (M^+, 100), 144 (45), 115 (85). Anal. Calcd for $C_{10}H_8N_2O$, C 69.76, H 4.68, N 16.27; found, C 69.77, H 4.89, N 16.34.

3.6.8. 5-(3-Hydroxyprop-1-yn-1-yl)pyridazin-3(2*H*)one 10c. Purification by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. Mp 179–180 °C (isopropanol). Yield 68%. IR (KBr): ν_{max}/cm^{-1} 3500–300 (NH), 2226 (C≡C), 1640 (CO). ^1H NMR (DMSO-d_6 300 MHz), δ (ppm): 13.14 (bs, 1H, NH), 7.79 (d, $J=1.9$ Hz, 1H, H_6), 6.91 (d, $J=1.9$ Hz, 1H, H_4), 5.51 (t, $J=5.44$ Hz, 1H, OH), 3.71 (d, $J=5.44$ Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 160.2, 137.4, 130.9, 128.5, 98.9, 78.8, 49.7. MS (70 eV) *m/z* (%): 150 (M^+, 100), 121 (85), 94 (50). Anal. Calcd for $C_7H_6N_2O$, C 56.00, H 4.03, N 18.66; found, C 56.13, H 4.11, N 18.72.

3.6.9. 5-[(1*E*)-3-Oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-one 10d. Purification by column chromatography on silica gel using AcOEt/hexane (1:2) as eluent. Mp 193–194 °C (MeOH). Yield 60%. IR (KBr): ν_{max}/cm^{-1} 1660 (CO), 1615 (CO), 1576 (aromatics). ^1H NMR (DMSO-d_6 300 MHz), δ (ppm): 13.42 (bs, 1H, NH),, 8.10 (d, $J=8.12$ Hz, 2H, aromatics), 8.01 (d, $J=1.9$ Hz, 1H, H_6), 7.53 (d, $J=15.5$ Hz, 1H, CH), 7.46 (m, 3H, aromatics), 7.28 (d, $J=15.5$ Hz, 1H, CH), 7.06 (d, $J=1.9$ Hz, 1H, H_4). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 189.2, 161.5, 138.7, 137.7, 137.5, 134.4, 134.2, 129.4, 129.1, 128.7, 128.0. MS (70 eV) *m/z* (%): 226 (M^+, 100), 197 (84), 105 (48). Anal. Calcd for $C_{13}H_{10}N_2O_2$, C 69.02, H 4.46, N 12.38; found, C 69.11, H 4.42, N 12.48.

3.6.10. Methyl (2*E*)-3-(6-oxo-1,6-dihydropyridazin-4-yl)acrylate 10e. Purification by column chromatography on silica gel using AcOEt/hexane (1:1) as eluent. Mp 205–207 °C (MeOH). Yield 62%. IR (KBr): ν_{max}/cm^{-1} 1719 (COO), 1657 (CO). ^1H NMR (DMSO-d_6 300 MHz), δ (ppm): 13.07 (bs, 1H, NH), 8.27 (d, $J=1.8$ Hz, H_6), 7.45 (d, $J=16.2$ Hz, 1H, CH), 7.15 (d, $J=1.8$ Hz, H_4), 6.90 (d, $J=16.2$ Hz, 1H, CH), 3.34 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 166.0, 161.2, 139.0, 138.5, 135.1, 129.0, 125.3, 52.7. MS (70 eV) *m/z* (%): 180 (M^+, 100). Anal. Calcd for $C_8H_8N_2O_3$, C 53.33, H 4.48, N 15.55; found, C 53.42, H 4.56, N 15.56.

3.6.11. 2-Hydroxymethyl-5-vinylpyridazin-3(2*H*)-one 11a. Purification by column chromatography on silica gel using AcOEt/hexane (1:5) as eluent. Mp 91–93 °C (isopropanol). Yield 65%. IR (KBr): ν_{max}/cm^{-1} 1660 (CO). ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm): 7.91 (d, $J=1.8$ Hz, 1H, H_6), 6.77 (d, $J=1.8$ Hz, 1H, H_4), 6.52 (dd, $J=6.7$, 11.0 Hz, 1H, CH), 5.97 (d, $J=16.6$ Hz, 1H, CH), 5.63 (d, $J=11.0$ Hz, 1H, CH), 5.52 (d, $J=6.1$ Hz, 2H, CH_2), 5.01 (bs, 1H, OH). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 161.9, 141,9, 135.1, 131.5, 125.5, 123.0, 76.67. MS (70 eV) *m/z* (%): 152 (M^+, 42), 122 (100), 58 (52). HRMS *m/z* calcd for $C_7H_8N_2O_2$ (M^+): 152.0586, found: 152.0594.

3.6.12. 2-Hydroxymethyl-5-(3-hydroxyprop-1-yn-1-yl)pyridazin-3(2*H*)-one 11b. Purification by column chromatography on silica gel using AcOEt/hexane (1:8) as eluent. Mp 118–120 °C (isopropanol). Yield 70%. IR (KBr): ν_{max}/cm^{-1} 1640 (CO). ^1H NMR (DMSO-d_6 300 MHz), δ (ppm): 7.879 (d, $J=1.9$ Hz, 1H, H_6), 7.01 (d, $J=1.9$ Hz, 1H, H_4), 6.76 (t, $J=7.6$ Hz, 1H, OH), 5.52 (t, $J=5.1$ Hz, 1H, OH), 5.27 (d, $J=7.6$ Hz, 2H, CH_2) 4.34 (d, $J=5.1$ Hz, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 159.1, 137.1, 131.0, 128.5, 98.7, 78.7, 73.9, 49.7. MS (70 eV) *m/z* (%): 180 (M^+, 76), 150 (100), 121 (55). HRMS *m/z* calcd for $C_8H_8N_2O_3$ (M^+): 180.0535, found: 180.0551.

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Lytophilippines A–C: novel macrolactones from the Red Sea hydroid *Lytocarpus philippinus*

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Abstract—Lytophilippines A–C, new chloro-containing macrolactones, were isolated from the Red Sea hydroid *Lytocarpus philippinus* and their structures were elucidated by IR, UV, MS, ^{1}H and ^{13}C NMR, and by chemical degradation. The compounds gave positive results in the crown gall tumor inhibition test and brine shrimp toxicity assay.

1. Introduction

Marine natural products are produced by marine invertebrates, microorganisms and plants that have stimulated interdisciplinary studies by chemists and biologists for marine drug discovery.[1–3] The Red Sea is a unique habitat, which is characterized by extreme changes in environmental conditions such as oxygen availability, salinity, and temperature.[4] Organisms, which inhabit the Red Sea, are excellently adapted to environmental stress and offer an enormous potential as a natural source of novel biologically active molecules.[5,6]

The Hydrozoans (hydroids) are mostly all bottom dwelling animals, in a polypoid shape, and can easily be mistaken for plants. Stinging hydroids such as *Lytocarpus philippinus* in the Indo-Pacific area and Red Sea,[7] have harmless-looking, feather-like plumes that can inflict a rather nasty sting on the softer areas of human skin.[8] Hydroids are an interesting group of marine invertebrates for chemical studies but only the following papers have been published: Hydrallmanol A, a diphenyl-*p*-menthane derivative, has been isolated from the marine hydroid *Hydrallmania falcate*.[9] Four Mediterranean hydroids contain uncommon Δ^5C_{26}-sterols, but cholesterol was the principal compound.[10] The polyhydroxylated steroid, cholest-4-en-4,16β,18,22*R*-tetrol-3-one 16,18-diacetate, was found in the hydroid *Eudendrium* sp.[11] A series of Δ^5-C_{26-29} sterols, and the corresponding stanols have also been identified from the hydroid *Dynamena pumila*.[12] Four biologically active polyhalogenomonoterpenes have been isolated from four species of marine hydroids.[13] Trimethyl amine oxide, dimethyl amine and choline chloride have been isolated from the marine hydroid *Tubularia larynx*.[14]

In the course of our investigation of the chemical composition of marine invertebrates[6,15–17] we have examined one hydroid *Lytocarpus philippinus* from the Red Sea, collected in the Gulf of Aqaba, Eilat, Israel. Three novel macrolide derivatives, with unusual multi-branched chain, have been isolated from the extract. According to the literature data, we could not find any natural products isolated from this sea organism. Here we report the structure elucidation of these new chloro-containing compounds, based mainly on their spectral characteristics.

2. Results and discussion

The fireweed (hydroid) *Lytocarpus philippinus* Kirchen. (Order Hydroida, Family Plumulariidae) was collected in the Red Sea, Aqaba Gulf, Eilat, Israel. The fireweed was extracted with butanol and the extract was separated on Sephadex LH-20. The fractions were further purified by RP-HPLC to give three compounds lytophilippines A–C (**1**,**10**,**11**, see Fig. 1), which were identified by their IR, UV,

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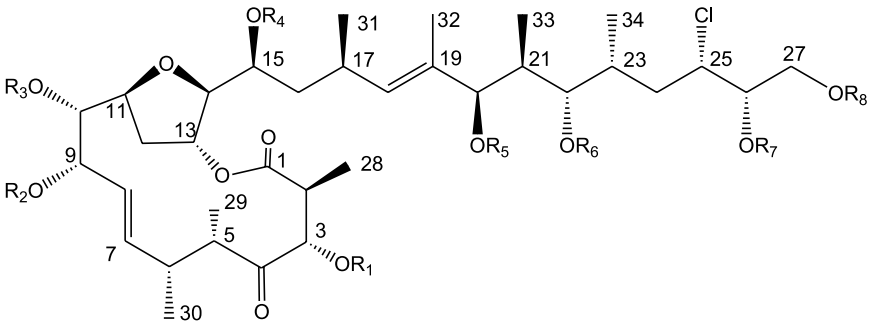

1 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = R_8 = H$
2 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = H$, R_8 = Piv
3 $R_1 = R_4 = R_7 = H$, R_8 = Piv, $R_2,R_3 = R_5,R_6$ =
4 $R_1 = R_4 = R_7$ = Me, R_8 = Piv, $R_2,R_3 = R_5,R_6$ =
5 $R_1 = R_4 = R_7$ = Me, R_8 = Piv, $R_5 = R_6 = H$, R_2,R_3 =
6 $R_1 = R_4 = R_7$ = Me, R_8 = Piv, $R_5 = R_6$ = p-ClBz, R_2,R_3 =
7 $R_1 = R_4 = R_7$ = (*R*)-MTPA, R_8 = Piv, $R_2,R_3 = R_5,R_6$ =
8 $R_1 = R_4 = R_7$ = (*S*)-MTPA, R_8 = Piv, $R_2,R_3 = R_5,R_6$ =
10 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = H$, R_8 =
11 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = H$, R_8 =

Figure 1. Lytophilippine A–C (**1**, **10**, **11**), a new chlorinated macrolide from the Red Sea hydroid *Lytocarpus philippinus* and its derivatives **2**–**8**.

MS, and ^{1}H and ^{13}C NMR spectroscopic data and by chemical degradation.

The molecular formula of lytophilippine A (**1**) was established as $C_{34}H_{57}{}^{35}ClO_{12}$ from the observation of a pseudomolecular ion in the HRFABMS (715.3751, $[M+Na]^+$) consistent with both the ^{13}C and ^{1}H NMR spectra. The presence of seven hydroxymethine groups (broad absorption at 3490 cm^{-1} in IR spectrum), one oxymethylene group, a saturated ester or lactone (δ_C 174.5; IR 1735 cm^{-1}), and one di- and one trisubstituted double bond were evident from the IR and NMR spectra. Further, ^{1}H and ^{13}C NMR data (Table 1) disclosed the existence of a ketone, 20 methines (three of them bearing two double bonds), four methylenes, and seven methyls (one of them attached to an olefin). Since four out of six unsaturations were accounted, lytophilippine A (**1**) was inferred to contain two rings and suggesting the possibility of a highly oxygenated macrolactone.

Detailed analyses of the ^{1}H–^{1}H COSY spectrum disclosed three proton networks from H-2 to H-3 and H-28, from H-5 to H-17 (including H-31), and from H-20 to H-27 (Fig. 2). Eight hydroxyl protons and their corresponding methines (and one methylene) were also clearly coupled in ^{1}H–^{1}H COSY spectra. Long-range couplings were observed between H-2 (δ 2.53) and C-1 (δ 174.5), between H_2-13 (δ 5.10) and C-l, and between H-2 and C-3 (δ 84.0). From these connectivities and chemical shifts, the existence of unsaturated 14-membered lactone was revealed.

HMBC correlations of H-2/H-3 and H-5/H-17 to a ketone carbonyl carbon (δ 211.3, C-5) suggested that C-3 and C-5 were linked through the ketone carbonyl. The existence of an ester linkage between C-1 and C-13 was implied by HMBC correlations of H_3-28 and H_2-13 to C-1 (δ 174.5). A series of alternating oxymethines and high field methylene or methine groups were characterized in structure. At one end of the structure (C-25), the methine carbon was considerably shielded (δ 53.0) compared to the other oxymethines carbons, consistent with its presence as a halogen atom. Also a vinyl proton at δ 5.05 (C-18) was vicinally coupled to a methine at δ 2.81 (C-17) and allylically coupled to a vinyl methyl group at δ 1.60 (H-32). An alternation of oxymethines and high field methylene groups was characterized in structure as well.

The geometry of disubstituted olefin at $\Delta^{9,10}$ was assigned as *E* by ^{1}H–^{1}H coupling constants ($J_{H\text{-}9/H\text{-}10}$ = 15.2 Hz), while *E*-geometry of a trisubstituted olefin at $\Delta^{18,19}$ was revealed by NOESY cross-peaks for H-17/H_3-32 and H-18/H-20. Thus, the gross structure of lytophilippine A was elucidated to be **1**.

The relative stereochemistry of **1** was elucidated by chemical (Fig. 3) and spectral means. The compound **1**

Table 1. ^{1}H and ^{13}C NMR data of lytophilippine A (**1**)

No.	^{1}H	^{13}C
1	—	174.5
2	2.53 (1H, qd, J=7.0, 8.8 Hz)	38.5
3	4.39 (1H, dd, J=8.8, 1.7 Hz)	84.0
4	—	211.3
5	2.84 (1H, dqd, J=7.8, 6.9, 1.7 Hz)	44.9
6	2.48 (1H, dqdd, J=7.8, 6.0, 9.4, 3.0 Hz)	33.8
7	5.55 (1H, ddd, J=9.4, 15.2, 2.0 Hz)	133.4
8	5.38 (1H, ddd, J=15.2, 5.7, 3.0 Hz)	130.6
9	4.12 (1H, ddd, J=5.7, 15.2, 2.0 Hz)	72.4
10	3.74 (1H, dd, J=15.2, 4.5 Hz)	78.0
11	3.95 (1H, dtd, J=4.5, 8.0, 1.5 Hz)	69.8
12a	2.11 (1H, dt, J=13.8, 8.0 Hz)	29.8
12b	1.87 (1H, dt, J=13.8, 8.0 Hz)	
13	5.10 (1H, tdd, J=8.0, 6.6, 1.5 Hz)	69.6
14	3.66 (1H, dd, J=9.0, 6.6 Hz)	81.1
15	4.02 (1H, ddd, J=9.0, 5.0, 10.0 Hz)	66.3
16a	1.39 (1H, ddd, J=5.0, 4.9, 14.0 Hz)	39.2
16b	1.22 (1H, ddd, J=10.0, 8.9, 14.0 Hz)	
17	2.81 (1H, ddqd, J=8.9, 4.9, 6.8, 9.6 Hz)	27.9
18	5.05 (1H, brd, J=9.6 Hz)	134.3
19	—	131.7
20	4.45 (1H, d, J=1.8 Hz)	72.5
21	2.57 (1H, dqd, J=1.8, 6.8, 9.7 Hz)	41.6
22	4.00 (1H, dd, J=9.7, 2.6 Hz)	74.8
23	1.84 (1H, qddd, J=6.5, 2.6, 2.1, 10.6 Hz)	38.4
24a	1.88 (1H, ddd, J=2.1, 4.7, 13.6 Hz)	32.6
24b	1.92 (1H, ddd, J=10.6, 8.8, 13.6 Hz)	
25	4.24 (1H, ddd, J=4.7, 8.8, 5.9 Hz)	53.0
26	4.22 (1H, ddd, J=5.9, 2.4, 7.1 Hz)	80.3
27a	4.01 (1H, dd, J=12.0, 2.4 Hz)	65.5
27b	4.26 (1H, dd, J=12.0, 7.1 Hz)	
28	1.31 (3H, d, J=7.0 Hz)	8.5
29	0.94 (3H, d, J=6.9 Hz)	11.6
30	1.07 (3H, d, J=6.0 Hz)	17.1
31	0.99 (3H, d, J=6.8 Hz)	20.7
32	1.60 (3H, s)	11.5
33	0.98 (3H, d, J=6.8 Hz)	10.4
34	1.08 (3H, d, J=6.5 Hz)	15.7

was converted to pivaloyl ester (**2**), protecting the primary hydroxy group. Further reaction with 2,2-dimethoxypropane gave *O*-isopropylidene derivative (**3**). The ^{1}H NMR spectrum (see Table 2) and FABMS data (*m*/*z* 857 and 859 $[M+H]^{+}$) of **3** indicated that **3** was a di-*O*-isopropylidene derivative of **2**. The positions of three free hydroxyls in **3** were determined by the chemical shifts of oxymethine protons [δ 4.39 (H-3), 4.02 (H-15), and 4.27 (H-26)] assigned by COSY experiments, revealing that **3** is the 9,10:20,22-di-*O*-isopropylidene derivative of **2**.

The relative stereochemistry at C-2 and C-3 was determined as *threo* based on the NOESY spectrum of methine H-2 and

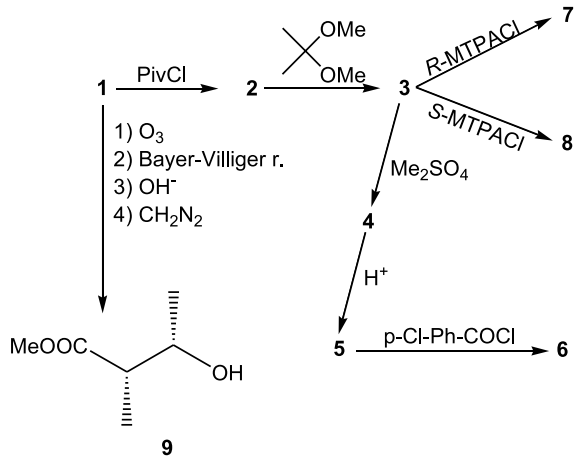

Figure 3. Reaction scheme for the preparation of the lytophilippine A derivatives **2**–**9**.

H-3 protons, since NOESY cross-peak was clearly observed for H-3/H_3-28. Further, the relative stereochemistry at C-5/C-6 was determined from vicinal $^{3}J_{H\text{-}5/H\text{-}6}$, which was approximately 8 Hz and the torsion angle corresponds to *threo* configuration and thence it follows that relative configuration is 2*S**,3*S**,5*S**,6*R**.

The relative stereochemistry of C9/C10 in **3** was determined to be *syn* based on NOESY correlations and vicinal coupling constants of **3**, while the stereochemistry of substituents about the tetrahydrofuran ring was determined using ROESY and selective 1D NOE experiments. Through space interactions were observed between H-11 and H-14, H-11 and H-12b, H-12b and H-13, and H-13 and H-14 (Fig. 4).

The relative configurations at C-14, C-15, and C-17 were elucidated on the basis of ^{1}H–^{1}H and ^{1}H–^{13}C coupling constants in addition to NOESY correlations. For the C-14/C-15 bond (Fig. 5), the $^{3}J_{H\text{-}14,H\text{-}15}$ 6.5 Hz suggested that this bond underwent a conformational change between *anti* and *gauche*. Furthermore, the $^{2}J_{C\text{-}15,H\text{-}14}$ showed a medium value 2.0 Hz, also implying that H-14 had both *anti* and *gauche* relations to OH-15 due to rotation of the bond. The values for $^{3}J_{C\text{-}16,H\text{-}17} \sim 0$ Hz obtained from the hetero half-filtered TOCSY spectrum indicated that H-14 and H-15 were *gauche* to C-16. Of the six possible rotamers arising from *erythro* and *threo*, only one pair of *threo* relationship satisfied all of these coupling data. NOESY correlations also

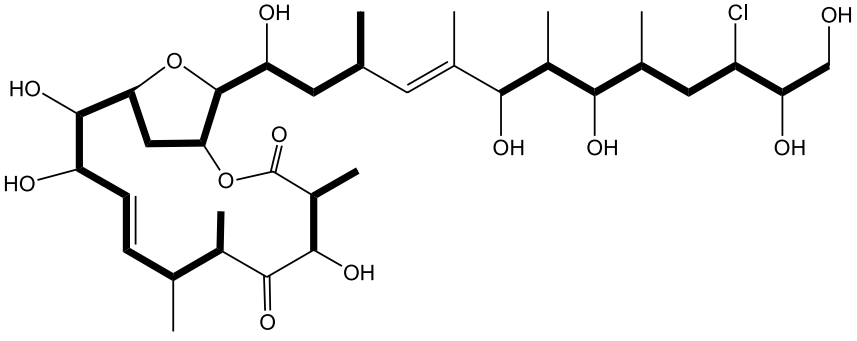

Figure 2. The ^{1}H–^{1}H COSY correlations of lytophilippine A.

Table 2. ^{1}H and ^{13}C NMR data of compound **3**

No.	^{1}H	^{13}C
1	—	174.5
2	2.53 (1H, qd, J=7.0, 8.8 Hz)	38.5
3	4.39 (1H, dd, J=8.8, 1.7 Hz)	84.0
4	—	211.3
5	2.84 (1H, dqd, J=7.8, 6.9, 1.7 Hz)	44.9
6	2.48 (1H, dqdd, J=7.8, 6.0, 9.4, 3.0 Hz)	33.8
7	5.55 (1H, ddd, J=9.4, 15.2, 2.0 Hz)	133.4
8	5.41 (1H, ddd, J=15.2, 5.7, 3.0 Hz)	130.2
9	4.52 (1H, ddd, J=5.7, 15.2, 2.0 Hz)	78.2
10	4.24 (1H, dd, J=15.2, 4.5 Hz)	82.3
11	4.06 (1H, dtd, J=4.5, 8.0, 1.5 Hz)	67.7
12a	2.13 (1H, dt, J=13.8, 8.0 Hz)	30.1
12b	1.87 (1H, dt, J=13.8, 8.0 Hz)	
13	5.10 (1H, tdd, J=8.0, 6.6, 1.5 Hz)	69.6
14	3.66 (1H, dd, J=9.0, 6.6 Hz)	81.1
15	4.02 (1H, ddd, J=9.0, 5.0, 10.0 Hz)	66.3
16a	1.39 (1H, ddd, J=5.0, 4.9, 14.0 Hz)	39.2
16b	1.22 (1H, ddd, J=10.0, 8.9, 14.0 Hz)	
17	2.81 (1H, ddqd, J=8.9, 4.9, 6.8, 9.6 Hz)	27.9
18	5.05 (1H, brd, J=9.6 Hz)	134.3
19	—	131.7
20	4.72 (1H, d, J=1.8 Hz)	73.2
21	2.63 (1H, dqd, J=1.8, 6.8, 9.7 Hz)	39.9
22	4.38 (1H, dd, J=9.7, 2.6 Hz)	75.3
23	1.89 (1H, qddd, J=6.5, 2.6, 2.1, 10.6 Hz)	37.3
24a	1.88 (1H, ddd, J=2.1, 4.7, 13.6 Hz)	32.6
24b	1.92 (1H, ddd, J=10.6, 8.8, 13.6 Hz)	
25	4.24 (1H, ddd, J=4.7, 8.8, 5.9 Hz)	53.2
26	4.27 (1H, ddd, J=5.9, 2.4, 7.1 Hz)	83.6
27a	4.43 (1H, dd, J=12.0, 2.4 Hz)	69.4
27b	4.61 (1H, dd, J=12.0, 7.1 Hz)	
28	1.31 (3H, d, J=7.0 Hz)	8.5
29	0.94 (3H, d, J=6.9 Hz)	11.6
30	1.07 (3H, d, J=6.0 Hz)	17.1
31	0.99 (3H, d, J=6.8 Hz)	20.7
32	1.60 (3H, s)	11.7
33	0.98 (3H, d, J=6.8 Hz)	10.9
34	1.08 (3H, d, J=6.5 Hz)	16.0
35	—	107.2
36	1.37 (3H, s)	26.1
37	1.39 (3H, s)	26.1
38	—	100.3
39	1.41 (3H, s)	24.6
40	1.36 (3H, s)	24.8
41	—	174.1
42	—	39.7
43	1.24 (3H, s)	23.8
44	1.24 (3H, s)	23.8
45	1.24 (3H, s)	23.8

supported the *threo* relation for the C-14/C-15 bond. For the C-15/C-16 bond, $^{3}J_{\text{H-15,H-16b}}$ was a typical value 2.2 Hz for *gauche* relationships, while $^{3}J_{\text{H-15,H-16a}}$ 10.0 Hz was indicative of an *anti* relation for H-15/H-16a. Combination of two-bond ^{1}H–^{13}C coupling constants of C-15/H-16a (5.0 Hz) and C-15/H-16b (~0 Hz) with NOESY correlations for H-14/H-16a and H-14/H-16b suggested that H-16a was

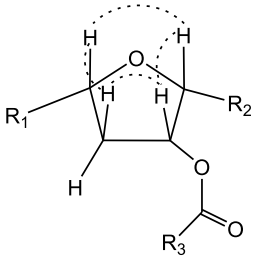

Figure 4. Key NOE correlations for protons on the tetrahydrofuran ring.

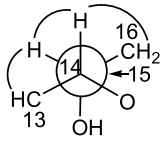

Figure 5. Rotation model for C-14–C-15 bond of lytophilippine A. NOESY correlations are illustrated by continuous curve.

gauche to C-14 and 12-OR and that H-16b was *gauche* to C-14 and *anti* to 12-OR. Therefore, the 1,3-chiral center of C-15/C-17 was elucidated to have a *syn* relation and relative configuration is 9*S**, 10*S**, 11*S**, 13*R**, 14*S**, 15*S**, 17*R**.

The relative configuration at C-20/C-22 was established as 20,22-*anti* by Rychnovsky's method,[18] because the two acetonide methyl carbon atoms and one ketal carbon appeared at δ 24.6, 24.8, and 100.3 ppm, respectively, in the ^{13}C NMR spectrum of **3**. The *anti*-acetonide exists in the twist boat conformation owing to the 1,3-diaxial interaction. The vicinal coupling constants $J_{20,21}$=1.8 Hz and $J_{21,22}$=9.7 Hz in the ^{1}H NMR spectrum of **3** indicate that the three substituents at C-20, C-21, and C-22 are *threo* and *erythro* disposed, respectively. The relative configuration at C-22/C-23 was determined by the vicinal coupling constant $J_{22,23}$=2.6 Hz. This value showed that the substituents were 22,23-*threo*. From these results, the relative configurations of **3** were determined to be 20*R**, 21*S**, 22*S**, and 23*R**.

J-based configuration analysis, a powerful method recently developed by Murata[19] for the elucidation of relative stereochemistry in acyclic structures using $^{3}J_{\text{H,H}}$ values was successfully applied to our molecule. Using ^{1}H NMR homonuclear and heteronuclear *J* values of the functionalized portions C-25/C-26 were successfully determined (Table 2) and evaluated. For the 1,2-methine systems along C25–C26, the coupling constant data were consistent and sufficient to determine their relative configurations. In fact, for *threo* rotamer, the hydrogens linked to the two gauche carbons should come within the range of NOE, while in the case of C/C-*anti* conformation (*erythro* rotamer), no NOE should be observed between them. In our case, NOE experiments revealed spatial proximity for H-25 and H-26 and we identified the right rotamers along C25/C26 as *threo* and the configuration of the fragment C25/C26 must be 25*S*, 26*S* or its enantiomer (25*R*, 26*R*).

Absolute configuration at C-5 and C-6 was determined after ozonolysis and following Bayer–Villiger reaction. Lytophilippine A (**1**) was treated by ozone and subsequent Baeyer–Villiger reaction was performed by treatment of the crude mixture of **1** with trifluoroperoxyacetic acid. The mixture was hydrolysed and treated with an excess of diazomethane. As result of the all these reactions, the methyl ester of nilic acid was isolated and give $[\alpha]_{D}$=+30.2, which agree with methyl (2*S*,3*S*)-2-methyl-3-hydroxy butyrate (**9**) (lit.[20,21] for this compound give $[\alpha]_{D}$=+36.8 or +27.8, respectively).

Further, this methyl ester (**9**) was also compared with *S*,*S* enantiomer, synthesized by means of chiral gas–liquid chromatography (see Experimental). From retention times it is evident that both carbons, C-5 and C-6 have the *S*,*S* absolute configurations.

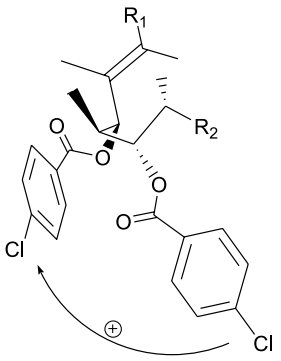

Figure 6. Stereostructure of *p*-chlorbenzoates (**6**) of lytophilippine A (**1**). Positive chirality between the two chromophores (two *p*-chlorbenzoate groups) of **6**.

The compound **3** was methylated and permethylated derivative (**4**) was hydrolyzed at heating in methanol with acetic acid according to Sviridov et al.[22] The major product (**5**), i.e. the compound with a five membered acetonide was reacted with *p*-chlorbenzoyl chloride afforded the 20,22-bis-*p*-chlorobenzoate (**6**). Consequently, the CD exciton chirality method[23] was applied and the CD spectrum of **6** showed a large positive first Cotton effect at 253 nm ($\Delta\varepsilon$ +8.6) and a second negative Cotton effect at 228 nm ($\Delta\varepsilon$ −7.1), indicating that the absolute configurations of **1** were 20*R*,22*S*, respectively, see Figure 6.

To determine the full stereochemistry of the remaining isolated asymmetric centers at C-3, C-15 and C-26, compound **3** was treated with *R*(−) and *S*(+) MTPA chloride in pyridine solution[24] at room temperature for 2 h to give ester derivatives **7** [from *R*(−)MTPA] and **8** [from *S*(+)-MTPA] (Fig. 7), respectively, that were believed to be appropriate for the application of the Mosher method,[24] because of the absence of mutual influence between the three introduced MTPA groups. ^{1}H NMR resonances of the esters **7** and **8** were assigned by an extensive analysis of 1D and 2D NMR spectra. Significant $\Delta\delta$ values ($\delta_{S\text{-MTPA-ester}} - \delta_{R\text{-MTPA-ester}}$) for the protons near to the derivatized chiral centers C-3, C-15, and C-26 were observed. Inspection of the molecular models of the MTPA esters **7** and **8** indicated that there is no steric hindrance to all the MTPA groups adopting the 'ideal conformation' having trifluoromethyl, ester carbonyl, and carbinol methine proton coplanar. These results enabled the absolute configurations at C-3, C-15, and C-26 in **1** to be determined as *S*, *S*, and *S*, respectively. The results from MM2 calculations and molecular modeling, together with those described above, indicated that the most stable conformation of **1** is depicted in Figure 8, i.e. the final absolute stereochemistry is 2*S*, 3*S*, 5*S*, 6*R*, 7*E*, 9*S*, 10*S*, 11*S*, 13*R*, 14*S*, 15*S*, 17*R*, 18*E*, 20*S*, 21*S*, 22*S*, 23*R*, 25*S*, and 26*S*.

The structure of lytophilippine B (**10**) was elucidated to be the same as that of lytophilippine A except that long chain acyl was inserted in the molecule of lytophilippine A. The structure was easily assigned to a saturated fatty acyl chain; however, NMR analysis alone was not able to establish its chain length. This was determined by catalyzed transesterification in HCl–MeOH and analysis of the resultant methyl ester by GC–MS. A single peak was observed which was identical in retention time and fragmentation pattern with methyl palmitate.

The distinctive ^{1}H and ^{13}C shifts of C-27 versus those at C-26, C-22, C-20, etc. (Table 3) indicated that an ester group was attached at this position. A HMBC correlation between the H-27 proton and ester carbonyl at (C-1′) δ 173.7 confirmed this assignment. HMBC correlations from H-2′ to C-1′, C-3′ and the overlapped methylene envelope at ~30 ppm additionally confirmed the presence of this fatty acyl chain. In the acyl moiety, the key signals relative to

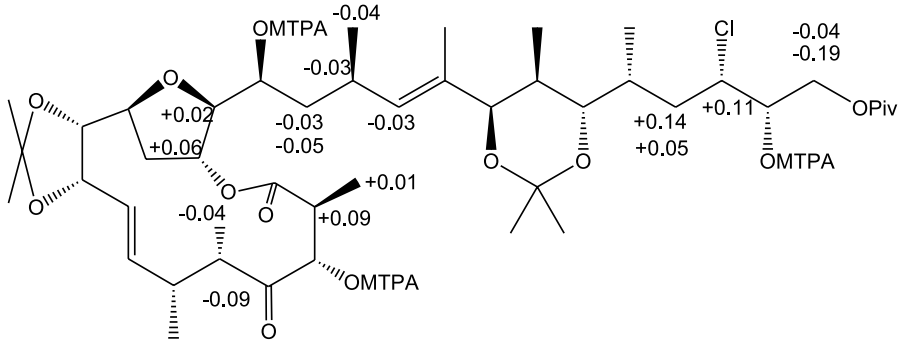

Figure 7. $\Delta\delta=(\delta_S-\delta_R)$ values (ppm) obtained for the MTPA esters.

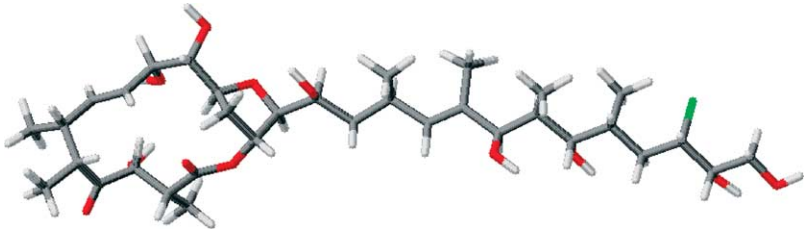

Figure 8. 3D-MM2 model of compound **1**.

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of compound **10** and **11**

No.	^{1}H of **10**	^{1}H of **11**	^{13}C of **10**	^{13}C of **11**
1′	—	—	173.7	173.8
2′	2.24 (2H, t, J=7.3 Hz)	2.10 (2H, t, J=7.5 Hz)	26.5	26.5
3′	1.53 (2H, m)	1.43 (2H, m)	30.3	30.3
4′–7′	1.32 (8H, m)	1.30 (8H, m)	30.3	30.3
8′, 11′	1.32 (4H, m)	1.96 (4H, m)	30.3	27.3
9′, 10′	1.32 (4H, m)	5.39 (2H, m)	30.3	131.7
12′–15′	1.32 (8H, m)	1.30 (8H, m)	30.3	30.3
16′	1.32 (2H, m)	1.30 (2H, m)	32.5	32.5
17′	1.23 (2H, m)	1.23 (2H, m)	23.1	23.1
18′	0.88 (3H, t, J=7.0 Hz)	0.88 (3H, t, J=7.0 Hz)	14.0	14.0
27	5.32 (2H, q, J=6.5 Hz)	5.32 (2H, q, J=6.5 Hz)	70.4	70.4

protons located at the ends of the alkyl chain clearly indicated its unbranched nature. The structure of compound **10** is 27-O-palmitate of lytophilippine A.

The NMR spectra of lytophilippine C (**11**) indicated that the structure of this compound is very similar to **10**, apart from for the presence of the chemical shifts at δ 5.39 (^{1}H NMR) and 131.7 (^{13}C NMR) in the long acyl chain. Thus, the structure of lytophilippine C was elucidated to be the same as that of lytophilippine B except that one ethylenylidene (C_2H_2=26 mass units) was inserted in the hexadecyl side chain of lytophilippine B. The geometrical configuration of the double bond was assumed to be *Z* from the *J* value (7.2 Hz) of the neighboring methy1ene,[25] although a spin–spin coupling was not observed between olefinic protons. The fragmentation of the *Z*-alkenyl side chain in lytophilippine C appeared to resemble that of N-mono-unsaturated fatty acyl pyrro1idide.[26,27] The structure of **11** is 27-O-oleate of lytophilippine A.

The marine dinoflagellates of the genus *Amphidinium* are a rich source of a series of polyketide macrolides known as the amphidinolides,[28–30] which possess cytotoxic properties. These compounds are very similar to the lytophilippines A–C, which are presented in this paper.

The presence of Cl or Br in the macrolides causes significant changes in the physico-chemical characteristics, increasing their reactivity and demonstrated antibacterial, antiviral and other activities.[31] According to these suggestions were by us discovered new chloro-containing 15-membered macrolides, namely lytophilippines A–C **1**–**3**, which show a modest activity against different microorganisms (see Table 4). They are active against *Escherichia coli*, but inactive against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

Table 4. Bioactivities of lytophilippines A–C (**1**, **10**, **11**)

Test organism	**1**	**2**	**3**
Staphylococcus aureus[a]	0	0	0
Bacillus subtilis[a]	0	0	0
Escherichia coli[a]	26.3	20.4	19.5
Saccharomyces cerevisiae[a]	0	0	0
Artemia salina[b,c]	3.2	6.4	4.8
Agrobacterium tumefaciens[c,d]	28±3[e]	68±6	65±7

[a] Samples (10 μg) were applied on 50.8 mm paper disks, values are diameters (mm) of inhibitory zones.
[b] In μg/mL (minimum lethal doses).
[c] The details in Section 3.
[d] Presented values are means of three determinations.
[e] Percentage of crown gall tumor inhibition (±SD).

The crown gall tumor inhibition test has been used for the active antitumor agents produced in vivo by organisms and is also used to evaluate extracts for different pharmacological activities. The isolated compounds were evaluated by their ability to inhibit the growth of crown gall tumors on potato discs inoculated with *Agrobacterium tumefaciens* carrying a tumor-inducing plasmid. All compounds showed significant inhibition of the growth of crown gall tumors on potato disks, suggestive of in vivo antitumor activity. All the extracts assayed demonstrated crown gall tumor inhibition, ranging from 32% for compound **2** to 72% for compound **1**.

The second test, i.e. brine shrimp lethality test showed that all three compounds were active against *Artemia salina*. As has been proposed,[31] macrolide have an allelopathic role in hydroids. Their potent cytotoxicity could play a role in hydroids defence against marine organisms, particularly active predators: tropical fishes, starfishes, sea urchins and/or nudibranchs. It is known that hydroid species as well as ascidians or sponges are conspicuous members of marine fouling and benthic communities. Their soft-bodied morphology provides hydroids with little obvious structural defense from predation. No information able about hydroid's defence compounds, but it is know that marine sponges and/or acsidians have been a rich source of natural products, and the ecological roles of these metabolites have been investigated in a few studies only.[32,33]

3. Experimental

3.1. General experimental procedures

UV–VIS spectra were measured in MeOH within the range of 220–550 nm in a Cary 118 (Varian) apparatus. A Perkin–Elmer (Perkin–Elmer, Norwalk, CT, USA) model 1310 IR spectrophotometer was used for scanning IR spectroscopy as neat films. Circular dichroism (CD) measurement was carried out under dry N_2 on a Jasco-500A spectropolarimeter at 24 °C. A Perkin–Elmer Model 1310 (Perkin–Elmer, Norwalk, CT, USA) IR spectroscope was used. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H), 125.7 MHz (^{13}C). High- and also low-resolution MS were recorded using a VG 7070E-HF spectrometer (70 eV). HRFABMS (positive ion mode)

were obtained with a PEG-400 matrix. GC–MS of the fatty acid methyl esters were done using a Finnigan 1020 B (Finnigan MAT, San Jose, CA, USA) single-state quadrupole GC–MS instrument in the EI mode. Gas chromatography analysis was in a Hewlett Packard HP 5980 gas chromatograph (Hewlett Packard, Czech Republic).

3.2. Chromatography of fatty acid derivatives

The fatty acid composition was determined by GC–MS after transmethylation with 5% HCl in methanol. The fatty acid methyl esters and pyrrolidides were chromatographed by a fused silica capillary column of chemically bonded liquid phase (Supelcowax 10, 0.2 mm ID, 60 m length, Supelco) and helium carrier gas at a flow rate of 0.35 mL min^{-1}. The column temperature was programmed from 50 °C held for 1 min, to 100 °C at a rate of 10 °C min^{-1}, and then raised to the final hold temperature of 270 °C at a rate of 5 °C min^{-1}. The mass spectra of methyl esters and pyrro1idide agreed with previously published data.[26,27]

3.3. Chiral chromatography

FS capillary column HYDRODEX β-3P ID 0.25 mm, length 25 m, with the stationary phase [heptakis-(2,6-di-*O*-metyl-3-*O*-pentyl)-β-cyclodextrine] from Macherey-Nagel GmbH & Co. KG, Düren, Germany was used. Oven temperature: 50–150 °C at 2 °C/min, then to 240 °C at 5 °C/min, carrier gas helium, 20 cm/s, detector FID, 300 °C, injection of 1 μL mixture in methylene chloride (for standards: containing 0.5 mg/mL of each analyte), split (100:1), 300 °C.

3.4. Animal materials

The fireweed (hydroid) *Lytocarpus philippinus* Kirch. (Order Hydroida, Family Plumulariidae) was collected by hand from rocks (from 10 to 15 m deep), on 31 June 2003 in the Red Sea, Aqaba Gulf, Eilat, Israel. The voucher specimens are deposited in the collection of the third author (V.M. Dembitsky). Fresh hydroid was put into ethanol and stored at −10 °C under nitrogen.

3.5. Isolation

Hydroid was extracted three times by butanol and the extracts were further chromatographed by means of the Sephadex LH-20 column with chloroform–methanol 7:3 and then separated by RP-HPLC on a Discovery C18 column (Supelco) particle size 5 μm, length×I.D. (250 mm×21.2 mm) using a linear gradient from 20% H_2O and 80% acetonitrile to 1% water and 99% acetonitrile over 25 min, with a flow rate of 9.9 mL/min and monitored by a variable wavelength detector at 208 nm was used to separate of compounds **1** (28.9 mg), **10** (4.1 mg) and **11** (3.5 mg) in the crude extract.

3.5.1. Pivaloyl ester (2). Lytophilippine A (11.5 mg) was dissolved in pyridine (0.5 mL), and pivaloyl chloride (12 μL) was added. After 15 min the reaction solvents were evaporated to dryness. The residue was subjected to TLC (benzene–ethyl acetate, 1:1) to yield 10.7 mg of pivaloyl ester **2**. HRFABMS calcd. for $C_{39}H_{65}{}^{35}ClO_{13}$ $[M+Na]^+$ 799.4011, found 799.4016.

3.5.2. Bis-9,10:20,22-*O*-isopropylidene derivative (3). Compound **2** (10.5 mg) was treated with dimethoxypropane (0.3 mL) and pyridinium *p*-toluenesulfonate (0.3 mL) in CH_2Cl_2 (2.0 mL) at room temperature for 12 h. After evaporation the solvent, the residue was purified on a silica gel TLC (hexane–EtOAc, 6:11) to give diacetonide **3** (yield 9.2 mg). HRFABMS calcd for $C_{45}H_{73}{}^{35}ClO_{13}$ $[M+Na]^+$ 879.4637, found 879.4641; ^1H and ^{13}C NMR data see Table 2.

3.5.3. Methyl ether (4). To a stirred solution of 1 mL of methyl triflate in 2 mL of di-*tert*-butylpyridine was added 7.0 mg of acetonide (**3**) in 2 mL of chloroform. A condenser was then fixed to the flask, and the solution was brought to reflux. After 15 h of stirring, the solution was allowed to cool and 0.5 mL of concentrated ammonium hydroxide was added. After a further 2 h of stirring, the mixture was poured into water and extracted with dichloromethane. The combined organic layers were then washed with three 5 mL portions of 10% hydrochloric acid. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resulting oil could be purified by chromatography eluting with 15% ethyl actate in hexanes to give the methyl ether **4** as clear oil, yield 6.4 mg. HRFABMS calcd for $C_{48}H_{79}{}^{35}ClO_{13}$ $[M+Na]^+$ 921.5423, found 921.5418.

3.5.4. 9,10-*O*-Isopropylidene derivative (5). To the solution of compound **4** (6.2 mg) in 1 mL of methanol was added 250 μL of acetic acid and mixture was kept at 40 °C 2 h. The solution was cooled and 0.5 mL of concentrated of sodium hydrogen carbonate was added, the mixture was poured into water and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resulting oil could be purified by chromatography eluting with 15% ethyl acetate in hexanes to give 3.3 mg of the acetonide (**5**). HRFABMS calcd for $C_{45}H_{75}{}^{35}ClO_{13}$ $[M+Na]^+$: 881.4793, found 881.4800.

3.5.5. *p*-Chlorobenzoate (6). Acetonide **5** (3.1 mg) was dissolved in pyridine (1.0 mL), and 4-chlorobenzoyl chloride (20 μL) and a catalytic amount of 4-dimethylaminopyridine were added. After 18 h methanol (2.0 mL) and hexane (1 mL) were added, and the reaction solvents were evaporated to dryness. The residue was subjected to TLC (benzene–EtOAc, 9:1) to yield **6** (yield 3.7 mg). HRFABMS calcd for $C_{59}H_{81}{}^{35}Cl_3O_{15}$ $[M+Na]^+$ 1157.4540, found 1157.4544; CD (EtOH) λ_{ext} ($\Delta\varepsilon$) 253 nm (+8.6), 236 (~0), 228 (−7.1).

3.5.6. (*S*)-MTPA Esters (7). To a stirred solution of ~1.0 mg of the hydroxy compound (**1**) in 0.3 mL dry pyridine was added 20 μL of (−)-MTPA chloride. The mixture was stirred under N_2 at room temperature for 1 h and the solvent was then removed by blowing with N_2. The residue was redissolved in 2 mL of EtOAc–hexane and filtered through a Sep-Pak silica column. After removing the solvent under vacuum, the residue was separated by RP-HPLC (ODS column, 100% acetonitrile) to yield ~1.0 mg of *S* ester as a colorless gum. HRFABMS calcd for $C_{75}H_{94}{}^{35}ClF_9O_{19}$ $[M+Na]^+$ 1527.6148, found 1527.6141; ^1H NMR data, see Figure 7.

3.5.7. (*R*)-MTPA Esters (8). Prepared as described for *S* esters. An amount of ~1.0 mg of compound (**1**) and 20 μL of (+)-MTPA chloride gave 0.9 mg of *R* esters as a colorless gum. HRFABMS calcd for $C_{75}H_{94}{}^{35}ClF_9O_{17}$ $[M+Na]^+$ 1495.5932, found 1495.5936.

3.5.8. Methyl 3-hydroxy-2-methyl-butyrate (9). A stream of 4% ozone was passed through a solution of the mixture lytophilippine A (**1**, 15 mg) in dichloromethane (0.5 mL) at −78 °C for 5 min. The solution was flushed with nitrogen and concentrated. The residue was dissolved in CH_2Cl_2 (0.2 mL), and treated with TFPA prepared by adding trifluoroacetic anhydride (0.1 mL) to a 30% aqueous H_2O_2 (0.1 mL) in CH_2Cl_2 (10 mL) at 4 °C for 12 h and evaporated. After gentle heating, the mixture was heated under reflux for 70 min. The mixture was concentrated and the residue was dissolved in methanol (0.5 mL), hydrolyzed for 10 min at 60 °C with 1 mL of 1 M KOH, cooled and acidified to pH 2.5 and treated with excess diazomethane in ether. The resulting solution was distilled (up to 90 °C/10 mmHg) to give **9** with $[\alpha]_D^{23}=+30.2°$. The distillate was also further separated by chiral GC. Mass spectrum was identical with synthesized methyl ester (**9**), see bellow. Retention time was 32.83 min.

3.5.9. Methyl (2*S*,3*S*)-3-hydroxy-2-methyl-butyrate (9). This compound was synthesized from 260 mg of methyl (3*S*)-3-hydroxybutyrate (from Sigma-Aldrich) according to Frater et al.[34] The product was distilled at 77 °C and 10 mmHg (77–78/10 mmHg)[34], yield 180 mg (62%). The literature[34] describe yield 67.8%. $[\alpha]_D^{22}=+29.3$ (MeOH; $c=0.54$), lit.[34,35] give $[\alpha]_D^{22}=+19.1$ and/or +27.8, respectively; IR ν_{max}: 3420, 3020, 1725, 1460 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.03 (1H, m), 3.67 (3H, s), 2.55 (1H, m), 1.21 (3H, d), 1.18 (3H, d). ^{13}C NMR ($CDCl_3$): δ 175.3 (C-1), 68.9 (C-3), 51.2 (OCH_3), 46.5 (C-2), 19.8 (C-4), 11.3 (2-Me); EI-MS: *m/z* 132 $[M]^+$, 114 $[M-H_2O]^+$, 101 $[M-OCH_3]^+$, 88 $[M-CH_3CHO]^+$. The chiral chromatography showed four peaks, one major (93.8%) with retention time 32.87 min and three minor (1.2, 1.9 and 3.1%, respectively).

3.6. Transesterification and preparation of pyrrolidide

The fatty acyl components were obtained as their methyl esters by reaction of the lytophilippines B and C with methanolic HCl followed by column chromatography including elution with *n*-hexane–diethyl ether (9:1).

The methyl oleate was dissolved in freshly distilled pyrrolidine (0.5 mL), acetic acid (0.1 mL) was added, and the mixture was heated at 100 °C for 1 h. Excess pyrrolidine was blown off in a stream of nitrogen at 50 °C, and then the residue was taken up in hexane–diethyl ether (1:1, v/v; 2 mL) and was washed three times with water (1 mL portions). After drying over anhydrous sodium sulfate, the product was injected to GC–MS.

3.6.1. Lytophilippine A (1). Colorless powder (28.9 mg). $[\alpha]_D^{23}=-45.8$ (*c* 0.036, MeOH). UV λ_{max} (MeOH, nm) (log ε): 282 (2.07). IR (film, cm^{-1}): ν_{max} 3490 (OH), 2900, 1735 (C=O), 1680. HRFABMS (*m/z*): 715.3751 $[M+Na]^+$, calcd for $[C_{34}H_{57}^{35}ClNaO_{12}+Na]^+$ 715.3748; NMR spectra see Tables 1 and 2.

3.6.2. Lytophilippine B (10). Colorless powder (4.1 mg). $[\alpha]_D^{23}=-37.4$ (*c* 0.009, MeOH). UV λ_{max} (MeOH, nm) (log ε): 282 (1.98). IR (film, cm^{-1}): ν_{max} 3600 (OH), 2900, 1735 (C=O), 1680. HRFABMS (*m/z*): 953.5737 $[M+Na]^+$, calcd for $[C_{50}H_{87}^{35}ClNaO_{13}+Na]^+$ 953.5732; NMR spectra see Tables 1 and 2.

3.6.3. Lytophilippine C (11). Colorless powder (3.5 mg), $[\alpha]_D^{23}=-44.2$ (*c* 0.008, MeOH). UV λ_{max} (MeOH, nm) (log ε): 282 (2.07). IR (film, cm^{-1}): ν_{max} 3600 (OH), 2900, 1735 (C=O), 1680. HRFABMS (*m/z*): 979.5893 $[M+Na]^+$, calcd for $[C_{52}H_{89}^{35}ClNaO_{13}+Na]^+$ 979.5889; NMR spectra see Tables 1 and 2.

3.7. Antibacterial tests

The test organisms were *Bacillus subtilis*, *Staphyloccocus aureus Escherichia coli* and *Saccharomyces cerevisiae* (Czechoslovak Collection of Microorganisms, Brno). Antibacterial assays were carried out according to the literature.[36] The amounts used were 50 μg of compound per test disk (see Table 4).

3.8. Brine shrimp toxicity bioassay

The sample (~0.05 mg) was dissolved in 50 μL of DMSO and added to a test vial of artificial seawater (3.0 mL). Approximately 20 brine shrimp, *Artemia salina*, were added to the vial. The brine shrimp were observed periodically over a 24 h period. A positive assay was the death of all brine shrimp.

3.9. Crown gall tumors on potato disks test

The *Agrobacterium tumefaciens* potato disc assay for tumor/antitumor induction was performed according to the procedure described in literature.[37] The potatoes were sterilized by immersion in ethanol 70% for 2 min and in 50% sodium hypochlorite solution (active chlorine 30 g/l) for 30 min. Then, the potatoes were rinsed several times with sterilized distilled water, in the laminar flow hood. A core of tissue was extracted from each tuber with a sterilized 1.5 cm cork borer. Discs of 0.5 cm were cut with a scalpel. The potato discs were placed in 1.5% agar Petri dishes. To each potato disc was applied 0.05 mL of a solution containing 2 mL of a broth culture of *A. tumefaciens* (48 h culture of ca. 109 cells/mL), 1.5 mL of sterile H_2O and 0.5 mL of the solution test extract (8 mg of extract in 2 mL of DMSO filtered through 0.22 mm filters). Control discs were prepared with sterile DMSO instead of test extract. A minimum of three Petri dishes (5 disks/dish) ($n=15$–25) was used for each test compound and the control. Following preparation, the Petri dishes were placed in an incubator at 27 °C for 12–21 days. To determine the number of tumors, the potato discs were stained with a solution of I_2 (1 g) and KI (2 g) in 300 mL distilled H_2O. Significant activity was indicated when two independent assays gave 20% or greater inhibition.

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Stemmosides C and D, two novel unusual pregnane glycosides from *Solenostemma argel*: structural elucidation and configurational study by a combined NMR-quantum mechanical strategy

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Abstract—Stemmosides C and D, two novel pregnane glycosides characterized by an unusual C-17α side chain were isolated from the pericarps of *Solenostemma argel*. In addition, stemmoside D displays an uncommon 14β proton configuration, apparently being the first pregnane isolated from plants known to have a 15 keto, *cis* CD ring junction. Their structures have been established by ESIMS and NMR experiments. The relative configuration of the molecules was determined using a strategy based on the simulation of ^{1}H, ^{13}C, and *J* coupling NMR parameters. DFT calculations of ^{1}H and ^{13}C chemical shifts, and of the ^{1}H homonuclear spin–spin coupling constants were performed with the mPW1PW91 functional using the 6-31G(d,p) basis set on the fully optimized geometries of all the possible stereoisomers.

1. Introduction

Plants belonging to the family Asclepiadaceae are frequently used in traditional medicine and have been reported to be rich in steroidal glycosides.[1,2] *Solenostemma argel* Hayne (Asclepiadaceae) is an Egyptian wild perennial erect shrub growing in the eastern desert and along the Nile in South Egypt,[3] whose leaves are commonly used in traditional medicine as a purgative, antipyretic, expectorant, antispasmodic and in cases of bile congestion.[4] Previous studies have reported the occurrence of monoterpenes,[5] pregnane glycosides including stemmosides A and B,[6,7] and acylated phenolic glycosides in the leaves,[8] and 14,15-secopregnane glycosides in the pericarps.[9]

Here we report the occurrence of two novel pregnane glycosides namely stemmosides C (**1**) and D (**2**) from the pericarps of *S. argel*. The structures of these compounds were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D NMR experiments (DQF-COSY, HSQC, HMBC, ROESY and HOHAHA) as well as ESIMS analysis. The interest of these two compounds is suggested by the antitumor and cytotoxic activities previously reported for a number of pregnane glycosides,[10–14] and for the structural features of stemmosides C and D. In fact, stemmoside C possesses an unusual C-17α side chain, while stemmoside D displays in addition an uncommon 14β proton configuration, apparently being the first pregnane isolated from plants known to have a 15 keto, *cis* CD ring junction. A few other naturally occurring steroids possessing a 15-keto, *cis* CD ring junction have only been isolated from marine sponges,[15–19] although for 15-dehydro-14β-anosmagenin, a steroidal aglycone isolated from the saponins of the plant *Solanum vespertilio*, there was considerable doubt by the authors whether the 14β configuration exists in the natural product or was formed by epimerization during the workup.[20]

To our knowledge, besides stereoselective synthesis and X-ray diffraction methods, the determination of the relative configuration of side chains at C-16 and C-17 and/or the C/D junctions in steroids mainly relies on comparison with NMR literature data,[10,21,22] on the analysis of NMR 2D-NOESY and ROESY spectra,[23,24] and on the biosynthetic pathways analysis.[25] However, due to the stereochemical properties (dihedral angle and distance) of the ring

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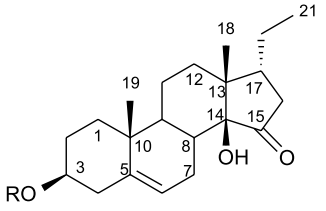
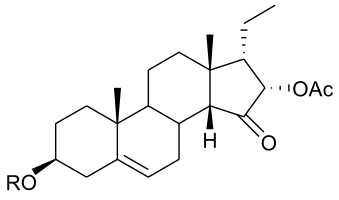
D protons and to the limited number of flexible side chains in naturally occurring steroids, the determination of the relative configuration by these methods is not always reliable. On the basis of this affirmation and on the fact that quantum mechanical (QM) calculation of NMR parameters has been shown to be a powerful tool in the structure determination of complex compounds and in the interpretation of their spectra,[26–28] we established the relative configuration of the ring D of compounds **1** and **2** by combination of the NMR data with quantum mechanical calculations of the geometries, ^{1}H and ^{13}C chemical shifts, and ^{1}H homonuclear spin–spin coupling constants. Recently, two new methodologies based on GIAO (gauge including atomic orbitals) quantum-mechanical ^{13}C chemical shift calculations have shown their efficiency as a support in the analysis of the NMR data of organic molecules.[29,30] Although several examples of computational methods rely on the comparison between calculated and experimental chemical shifts,[31–33] only a few applications regard the use of QM *J*'s for the resolution of conformational and/or configurational problems related to medium sized organic molecules.[34,35] In the progress of our continuing studies of natural products, we have envisaged the possibility of improving the NMR based strategies for the stereostructure determination of steroids by means of a combined use of calculated NMR chemical shifts and *J*'s as a support for the interpretation of the experimental data.



1



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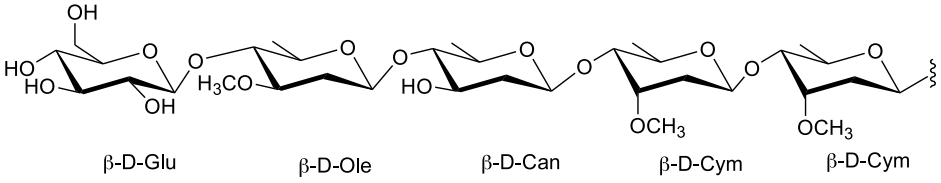


identical in the two compounds. In particular for the sugar portion, compound **1** showed in the ^{1}H NMR spectrum signals corresponding to four doublet methyls at δ 1.43 (3H, d, J=6.1 Hz), 1.32 (3H, d, J=6.1 Hz), 1.25 (3H, d, J=6.1 Hz) and 1.22 (3H, d, J=6.1 Hz), three methoxy groups at δ 3.51 (3H, s), 3.46 (3H, s) and 3.45 (3H, s), as well as signals for five anomeric protons at δ 4.90 (1H, dd, J=9.2, 2.0 Hz), 4.83 (1H, dd, J=9.6, 2.0 Hz), 4.67 (2H, dd, J=9.6, 2.0 Hz) and 4.49 (1H, d, J=7.5 Hz) (see Table 1). All these data indicated that the sugar chain of compound **1** consisted of five sugars, four of them being 2,6-dideoxy sugars. The chemical shifts of all the individual protons of the five sugar units were ascertained from a combination of 2D-HOHAHA and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their attached carbons could be assigned unambiguously from the HSQC spectrum (see Table 1). These data showed the presence of two β-D-cymaropyranosyl units (δ 4.90 and 4.83), one β-D-canaropyranosyl unit (δ 4.67), one β-D-oleandropyranosyl unit (δ 4.67) and one β-D-glucopyranosyl unit (δ 4.49). Glycosidation shifts were observed for C-4_{cymI} (δ 83.7), C-4_{cymII} (δ 83.7), C-4_{can} (δ 88.5) and C-4_{ole} (δ 83.2) suggesting that β-D-glucopyranosyl was a terminal unit. Direct connectivity information was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signals at δ 4.90 (H-1_{cymI}) and the carbon resonances at δ 78.9 (C-3), 4.83 (H-1_{cymII}) and 83.7 (C-4_{cymI}), 4.67 (H-1_{can}) and 83.7 (C-4_{cymII}), 4.67 (H-1_{ole}) and 88.5 (C-4_{can}), and the proton signal at δ 4.49 (H-1_{glc}) and the carbon resonance at δ 83.2 (C-4_{ole}). It is worthwhile to note that these results are in accordance with the fact that C-1 of β-D-cymarose characteristically resonates upfield (~97.0 ppm) when linked at C-3 of the aglycone in opposition to the resonance at ~101.0 ppm when it is linked to the hydroxyl group of a different sugar.[36] Thus, the sugar sequence was established as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

2. Results and discussion

2.1. Extraction and isolation

The dried pericarps of *S. argel* were extracted with EtOH 80% and fractionated on Sephadex LH-20. The fractions containing pregnane glycosides were chromatographed by reversed-phase HPLC to yield two new compounds **1** and **2** (see Section 4 for details).

2.2. Determination of the plain structure of stemmosides C and D (1, 2)

A detailed comparison of the sugar region NMR data (^{1}H, ^{13}C, HSQC, HMBC, DQF-COSY, 2D-HOHAHA) of compounds **1** and **2** showed that the saccharide chain was

Compound **1** showed a quasimolecular ion peak at *m/z* 1079 $[M+Na]^+$ and significant fragments at *m/z* 917 $[M+Na-162]^+$, *m/z* 773 $[M+Na-162-144]^+$, *m/z* 643 $[M+Na-162-144-130]^+$ in the positive ESIMS, while the molecular formula was unequivocally established to be $C_{54}H_{88}O_{20}$ by HRMALDI mass spectrometry (*m/z*

Table 1. ^{13}C and ^{1}H NMR data δ (ppm) of the sugar portions of compounds **1** and **2** (CD_3OD, 600 MHz)

	β-D-cymI	β-D-cymI
1	96.9	4.90 dd (9.2, 2.0)
2	36.8	2.08 m, 1.57 m
3	78.3	3.88 br m
4	83.7	3.25 dd (9.5, 3.0)
5	69.6	3.85 dq (9.5, 6.1)
6	18.3	1.22 d (6.1)
OMe	58.1	3.46 s
	β-D-cymII	β-D-cymII
1	100.9	4.83 dd (9.6, 2.0)
2	36.2	2.17 m, 1.62 m
3	78.3	3.88 br m
4	83.7	3.30 dd (9.5, 3.0)
5	69.6	3.85 dq (9.5, 6.1)
6	18.3	1.25 d (6.1)
OMe	58.1	3.45 s
	β-D-can	β-D-can
1	102.1	4.67 dd (9.6, 2.0)
2	39.4	2.23 ddd (13.0, 4.0, 2.0), 1.52 ddd (13.0, 9.6, 9.0)
3	70.5	3.63 ddd (9.5, 9.0, 4.0)
4	88.5	3.04 dd (9.5, 9.5)
5	71.4	3.39 dq (9.5, 6.1)
6	17.9	1.32 d (6.1)
	β-D-ole	β-D-ole
1	102.1	4.67 dd (9.6, 2.0)
2	37.4	2.43 ddd (13.0, 4.0, 2.0), 1.49 ddd (13.0, 9.6, 9.0)
3	79.7	3.49 ddd (9.5, 9.0, 4.0)
4	83.2	3.37 dd (9.5, 9.5)
5	72.7	3.55 dq (9.5, 6.1)
6	18.1	1.43 d (6.1)
OMe	58.0	3.51 s
	β-D-glc	β-D-glc
1	103.9	4.49 d (7.5)
2	75.3	3.21 dd (9.0, 7.5)
3	77.9	3.37 dd (9.0, 9.0)
4	71.5	3.27 dd (9.0, 9.0)
5	78.1	3.28 dd (9.0, 9.0)
6	62.7	3.90 dd (12.0, 2.5), 3.67 dd (12.0, 4.5)

1079.5760 $[M+Na]^+$, calcd for $C_{54}H_{88}O_{20}Na$, 1079.5766). IR absorptions implied the presence of hydroxy (3431 cm^{-1}) and ketone functionalities (1756 cm^{-1}). The ^{1}H NMR spectrum of the aglycone portion showed signals for two tertiary methyl groups at δ 1.01 (3H, s) and 1.08 (3H, s), a primary methyl signal at δ 0.97 (3H, t, $J=7.0$ Hz) and one olefinic proton at δ 5.45 (1H, dd, $J=3.3$, 2.7 Hz) characteristic of Δ^5-pregnanes (Table 2). In addition, the ^{13}C NMR spectrum showed for the aglycone moiety 21 signals suggesting the presence of a pregnane skeleton. The ^{13}C NMR chemical shifts of all the hydrogenated carbons could be assigned unambiguously by the HSQC spectrum. In particular, the analysis of the ^{13}C NMR spectrum on the basis of the HSQC correlations clearly showed the occurrence of one olefinic quaternary carbon (δ 139.9), one olefinic methine (δ 123.4), one secondary oxygenated carbon (δ 78.9), one tertiary oxygenated carbon (δ 82.5) and one carbonyl carbon (δ 218.0) (see Table 2). The three six-membered rings of a pregnane skeleton were established from the analysis of the strong HMBC correlations of the protons of the angular methyls Me-18 and Me-19. Long-range correlations from the proton signal at δ 1.01 (Me-19) to the carbon resonances at δ 38.0 (C-1), 139.9 (C-5), 46.7 (C-9) and 36.9 (C-10), revealed the connectivity of the A and B rings. Similarly, the HMBC spectrum indicated correlations between the proton signals at δ 1.08 (Me-18) and the carbon resonances at δ 29.3 (C-12), δ 45.7 (C-13), δ 82.5 (C-14), and δ 43.5 (C-17) establishing the connectivity between the rings B and C. Finally, the HMBC spectrum showed key correlation peaks between the proton at δ 0.97 (Me-21) and the carbon resonances at δ 22.4 (C-20) and δ 43.5 (C-17), and between the proton at δ 1.77 (H-16α) and the carbon resonances at δ 43.5 (C-17) and δ 218.0 (C-15) allowing us to deduce the ring D. Further evidence of the placement of the ketone in position 15 was provided by the deshielded chemical shift of the proton resonance of H-7α (δ 3.03) which was ascribable to a neighboring group effect

Table 2. ^{13}C and ^{1}H NMR data of the aglycone moieties of compounds **1** and **2** (CD_3OD, 600 MHz)

	1		**2**	
		δ_H (J in Hz)		δ_H (J in Hz)
1	38.0	α 1.05 m, β 1.88 m	37.9	α 1.08 m, β 1.88 m
2	30.4	α 1.32 m, β 1.53 m	30.2	α 1.32 m, β 1.55 m
3	78.9	3.53 m	78.8	3.53 m
4	39.7	α 2.37 m, β 2.20 m	39.7	α 2.37 m, β 2.21 m
5	139.9		140.3	
6	123.4	5.45 dd (3.3, 2.7)	123.2	5.42 dd (3.2, 2.4)
7	24.2	α 3.03 m, β 2.04 m	27.9	α 2.99 ddd (13.5, 15.5, 3.2), β 1.81 dd (15.5, 2.4)
8	38.0	1.88 m	29.5	1.93 m
9	46.7	1.21 m	44.6	1.30 m
10	36.9		36.6	
11	21.9	α 1.63 m, β 1.45 m	21.7	α 1.61 m, β 1.37 m
12	29.3	α 0.97 m, β 1.50 m	30.1	α 1.44 m, β 1.15 m
13	45.7		42.3	
14	82.5		58.7	2.38 m
15	218.0		214.6	
16	40.0	α 1.77 dd (18.0, 9.0), β 2.62 dd (18.0, 9.0)	72.3	5.51 d (10.1)
17	43.5	2.17 m	51.5	2.08 dd (10.1, 1.7)
18	15.3	1.08 s	21.4	1.22 s
19	19.6	1.01 s	19.6	1.01 s
20	22.4	1.12 m, 1.55 m	16.2	1.44 m, 1.51 m
21	13.4	0.97 t (7.0)	13.2	0.92 t (7.0)
*C*OMe			171.9	
CO*Me*			20.3	2.14 s

from a C-15 ketone.[16] Thus, these data showed the occurrence of a pregnane with a ketone group at C-15 and a hydroxyl group at C-14.

Compound **2** showed a quasimolecular ion peak at *m/z* 1121 $[M+Na]^+$ and significant fragments at *m/z* 1061 $[M+Na-60]^+$, *m/z* 959 $[M+Na-162]^+$, *m/z* 815 $[M+Na-162-144]^+$, *m/z* 685 $[M+Na-162-144-130]^+$ in the positive ESIMS. The molecular formula was unequivocally established to be $C_{56}H_{90}O_{21}$ by HRMALDI mass spectrometry (*m/z* 1121.5889 $[M+Na]^+$, calcd for $C_{56}H_{90}O_{21}Na$, 1121.5872). Also in this case, the IR spectrum showed a diagnostic absorption band corresponding to carbonyl functionalities (1735 cm^{-1}). The ^1H NMR spectrum of the aglycone portion showed signals for two tertiary methyl groups at δ 1.01 (3H, s) and 1.22 (3H, s), a primary methyl signal at δ 0.92 (3H, t, $J=7.0$ Hz), an acetyl methyl signal at δ 2.14 (3H, s), one olefinic proton at δ 5.42 (1H, dd, $J=3.2$, 2.4 Hz) and one signal at 5.51 (1H, d, $J=10.1$ Hz) corresponding to a secondary oxygenated carbon. The ^{13}C NMR spectrum showed for the aglycone moiety 23 signals, two of them corresponding to an acetyl group, suggesting the presence of an acetylated pregnane skeleton. The ^{13}C NMR chemical shifts of all the hydrogenated carbons could be assigned unambiguously by the HSQC spectrum. The analysis of the ^{13}C NMR spectrum on the basis of the HSQC correlations, showed the occurrence of one olefinic quaternary carbon (δ 140.3), one olefinic methine (δ 123.2), two secondary oxygenated carbons (δ 78.8 and 72.3) and two carbonyl carbons (δ 214.6 and 171.9) (see Table 2). Once again, the complete elucidation of the aglycone moiety of **2** was established by the HMBC experiment. Strong long-range correlations of the proton signal at δ 1.01 (Me-19) and the carbon resonances at δ 37.9 (C-1), 140.3 (C-5), 44.6 (C-9) and 36.6 (C-10) suggested the connectivity between the rings A and B. Finally, diagnostic correlation peaks between the proton signals at δ 1.22 (Me-18) and the carbon resonances at δ 30.1 (C-12), δ 42.3 (C-13), δ 58.7 (C-14) and δ 51.5 (C-17); the proton at δ 0.92 (Me-21) and the carbon resonances at δ 16.2 (C-20) and δ 51.5 (C-17), and between the proton signals at δ 5.51 (H-16) and the carbon resonances at δ 42.3 (C-13), δ 171.9 (OCOMe), and δ 214.6 (C-15), allowed us to deduce the occurrence of a 15-ketone pregnane with an acetoxy group at C-16. Also in this case, the presence of the ketone group at C-15 was corroborated by the deshielded chemical shift of H-7α (δ 2.99).[16]

2.3. Determination of the relative configuration of stemmosides C and D (1, 2)

In order to determine the configuration of stereocenter C-17 of **1**, we firstly inspected the *J* coupling values between H-17 and H-16α, and H-17 and H-16β, but the experimental values of 9.0 Hz, unusually both large, were not in accordance with the data present in literature for H-17α or H-17β pregnane compounds.[11,12] Moreover, compound **1** did not show any crucial cross peak in the ROESY spectrum necessary to determine the relative configuration of C-17. In fact, it is remarkable that a careful examination of the 3D structures of the two possible stereoisomers, obtained as outlined below, indicated for H-17 and Me-18 a distance of 2.86 Å for the H-17β isomer (**1a**) and of 3.38 Å for the H-17α isomer (**1b**) (Fig. 1); this observation suggested that a ROESY correlation between H-17 and Me-18 could be expected for both the H-17α and the H-17β configurations.

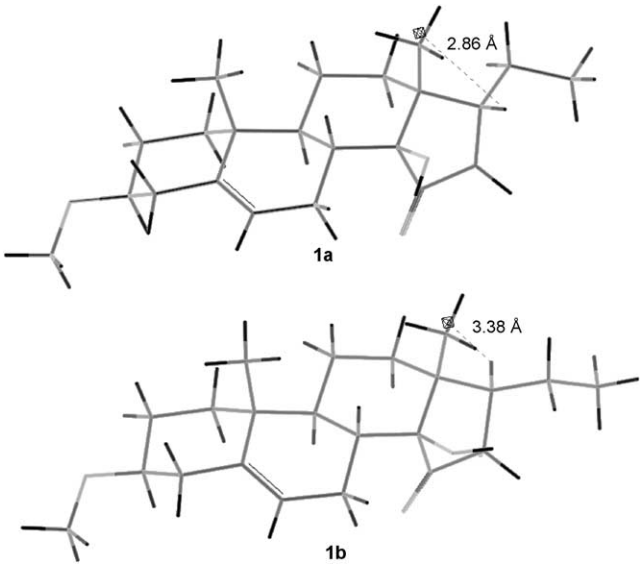
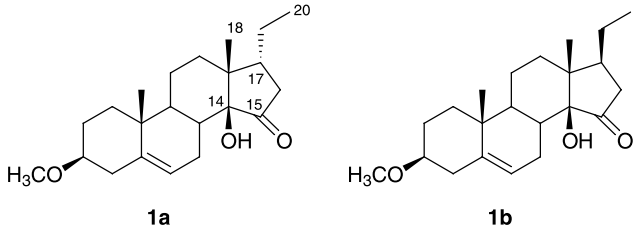


Figure 1. Measured distances H-17/Me-18 for the H-17β isomer (**1a**, 2.86 Å) and for the H-17α isomer (**1b**, 3.38 Å).

These considerations prompted us to apply a strategy based on the quantum mechanical calculation of the NMR properties of the two possible stereoisomers, and their comparison with the experimental data. In particular, our approach may be outlined as follows: (a) building all the possible relative stereoisomers of the molecule under investigation; (b) conformational search and preliminary geometry optimization of all the significant conformers of each stereoisomers; (c) final geometry optimization of all the species at DFT level; (d) GIAO ^{13}C and ^1H NMR calculation of all the so-obtained structures at DFT level; (e) spin–spin coupling constants calculation using the ONIOM method; (f) comparison of the ^{13}C c.s., the ^1H c.s. and the *J* values calculated for each stereoisomer, with the corresponding experimental values of the natural product.

In particular, for compound **1**, we first built the two possible stereoisomers differing at C-17 (**1a** and **1b**), simplified by the substitution of the sugar moiety with a methyl group. Such a simplification may be considered a good compromise between a reduction in the calculation time and the accuracy in the reproduction of the NMR parameters of interest that, as we will see below, mainly concern ring D and ring C. Subsequently, a conformational search, performed by molecular mechanics and dynamics calculations (Discover module, InsightII Software Package), provided a minimum energy conformer for each stereoisomer.



In order to obtain an accurate prediction of the spectral properties,[37] the two structures were further optimized at MPW1PW91 level,[38] using the 6-31G(d) basis set, and single point GIAO calculations using the same functional and the 6-31G(d,p) basis set provided us with the ^{13}C and ^{1}H theoretical values (Tables S1 and S2). Furthermore, an ONIOM calculation using the MPW1PW91 functional and the 3-21G (low level, rings A–B) and 6-31G(d,p) (high level, ring C–D) basis sets was executed on the two stereoisomers providing the theoretical J values for ring D. The obtained calculated ^{1}H and ^{13}C c.s. were then compared with the experimental data in order to determine which of the two stereoisomers best fitted the NMR data of compound **1**. In particular, for what concerns ^{13}C calculated results, preliminary considerations based on the $\Delta\delta$ parameter, i.e. the difference of the experimental vs calculated ^{13}C NMR c.s, and the MAE parameter (mean average error, $MAE=\sum[|(\delta_{exp}-\delta_{calcd})|]/n$, summation through n of the absolute values of the differences of the corresponding experimental and calculated ^{13}C chemical shifts, normalized to the carbon atom number of the molecule, Table S1) pointed to stereoisomer **1a**, displaying a MAE of 1.63 vs 2.48 of stereoisomer **1b**. Moreover, a careful analysis was carried out taking into consideration, singularly, the ^{13}C calculated values for ring C and D, which were expected to experience the larger variations upon inversion of configuration at C-17. In fact, due to the high level of structural similarity of rings A and B for the two stereoisomers **1a** and **1b**, the corresponding calculated ^{1}H and ^{13}C chemical shift values resulted very similar (see Table S1 and S2) and therefore were not considered diagnostic in our analysis. As shown in Table 3, very large differences between the $\Delta\delta$ ^{13}C c.s. values of **1a** and **1b** were observed in C-12 and C-20 (−0.2 vs −8.7 and −1.8 vs −8.7, respectively), suggesting again the exclusion of stereoisomer **1b**.

The same observations could be derived from the analysis of the calculated ^{1}H chemical shifts for **1a** and **1b** (Table S2). For the sake of simplicity, we have shown in Table 4 only the calculated and experimental ^{1}H values for protons of ring D; a straightforward analysis of the $\Delta\delta$ values shows that the chemical shift values for H-16α, H16β, and H-17 of **1a** are in very good agreement with the experimental ones, while the corresponding results obtained for compound **1b** display relatively large differences with respect to the experimental data.

Table 3. Crucial ^{13}C NMR values for stemmoside C (**1**), GIAO ^{13}C NMR c.s. (δ) for the stereoisomers **1a** and **1b**, and $\Delta\delta^{a}$ values for **1a** and **1b**

Atom	1	1a	1b	Δδ 1a	Δδ 1b
C-8	38.0	36.1	35.4	1.9	2.6
C-11	21.9	22.9	23.1	−1.0	−1.3
C-12	29.3	29.5	38.0	−0.2	*−8.7*
C-13	45.7	46.2	45.1	−0.5	0.6
C-14	82.5	79.9	80.0	2.6	2.5
C-16	40.0	39.6	39.0	0.4	1.0
C-17	43.5	42.1	44.6	1.4	−1.1
C-18	15.3	15.4	15.3	−0.1	0.0
C-20	22.4	24.2	31.1	−1.8	*−8.7*
C-21	13.4	15.1	15.4	−1.7	−2.0

[a] $\Delta\delta=\delta_{exp}-\delta_{calcd}$, differences for experimental vs calculated ^{13}C NMR c.s.

Table 4. Significant ^{1}H NMR c.s. for **1**, corresponding GIAO ^{1}H NMR c.s. calculated for stereoisomers **1a** and **1b**, and $\Delta\delta^{a}$ values for **1a** and **1b**

Atom	1	1a	1b	Δδ 1a	Δδ 1b
H16α	1.77	1.74	2.33	0.03	−0.56
H16β	2.62	2.60	2.09	0.02	0.53
H17	2.17	2.28	1.33	−0.11	0.84

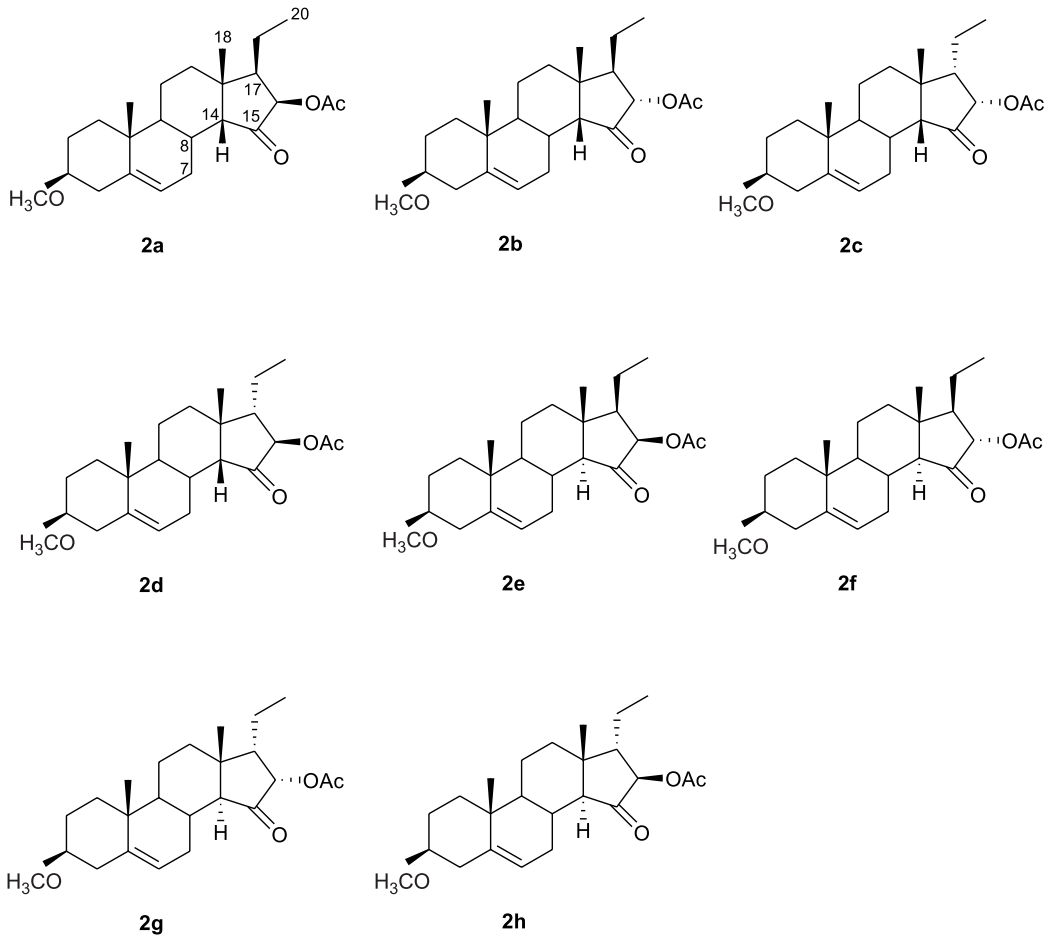
[a] $\Delta\delta=\delta_{exp}-\delta_{calcd}$, differences for experimental vs calculated ^{1}H NMR c.s.

Finally, the spin–spin coupling constant J_{H-H} values reported in Table 5 for compound **1a**, both large, fitted very well the unusual experimental values, while for compound **1b** a small (3.3 Hz) and a large J coupling (9.0 Hz) reproduce a pattern which is in accordance with examples previously cited in the literature,[10,21] but allows us to exclude the stereostructure of **1b** for stemmoside C (**1**). Based on the above evidence, the orientation of the side chain at C-17 was established to be α as in **1a**, hence stemmoside C was defined as the new compound 3β,14β-dihydroxy-17Hβ-pregnan-5-en-15-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside (**1**).

Following the encouraging results obtained for stemmoside C (**1**), we applied the same strategy to stemmoside D (**2**). In this case we had to consider the two stereogenic centers at C-16 and C-17, and, after a preliminary investigation of the ROESY spectra, also the position of H-14 resulted not clearly defined. In accordance to the above observation, we built all the possible stereoisomers differing for C-14, C-16, and C-17 (**2a–2h**, also in this case simplifying the substitution of the sugar moiety with a methyl group), which were submitted to a conformational search, performed by molecular mechanics and dynamics calculations, providing minimum energy conformations for each stereoisomer. The eight structures were further optimized at mPW1PW91 level, and single point GIAO calculations (see Section 4.2) provided us with the ^{1}H and ^{13}C theoretical values (Tables S3 and S4). Finally, an ONIOM calculation using the mPW1PW91 functional (see Section 4.2) was executed on the eight stereoisomers **2a–2h** providing the theoretical $^{3}J_{HH}$ values for ring D. As seen for **1**, the obtained calculated ^{1}H and ^{13}C c.s. of **2a–2h** were then compared with the experimental data for compound **2**.

Table 5. Comparison between experimental (**1**) and calculated (stereoisomers **1a** and **1b**) $^{3}J_{HH}$ values of ring D, in Hz

	1	1a	1b
H16α–H17	9.0	7.7	9.7
H16β–H17	9.0	8.9	3.3



In particular, for what concerns ^{13}C and ^{1}H calculated results, we firstly took in consideration the MAE values (mean average error, $MAE=\sum[|(\delta_{exp}-\delta_{calcd})|]/n$) of the absolute values of the differences of the corresponding experimental and calculated ^{13}C and ^{1}H chemical shifts, see Tables S3 and S4) which indicated, for both ^{13}C and ^{1}H, the lowest values for stereoisomer **2c**, as briefly outlined in Table 6. It is worthwhile to note that, also in this case, particular attention was dedicated to rings C and D, due to the structural similarity of rings A and B for all the eight stereoisomers **2a**–**2h**.

Subsequently, we analyzed the calculated $^{3}J_{HH}$ values for ring D considering each of the eight possible stereoisomer **2a**–**2h**, and then we compared them to the corresponding experimental value. As it is evident in Table 7, the value of 9.7 Hz, corresponding to the $^{3}J_{HH}$ value of H16–H17, of stereoisomer **2c** displays the best agreement with the experimental value of 10.1 Hz; this result is aligned and consistent with the results obtained for the ^{1}H and ^{13}C calculated chemical shifts, thus suggesting the configuration indicated in **2c** for stemmoside D.

We have also carried out a retrospective analysis of the NMR data of compound **2** in order to corroborate the unusual 14β proton configuration and relative configuration of H-16 and H-17 obtained from our QM calculations. In particular, it has been described that the presence of a 15-ketone functionality exerts a deshielding effect on 7α or 7β proton, depending on the C-14 configuration. Based on this, the 7β proton resonates at low field in *trans* CD 15-keto steroids, while in *cis* CD 15-keto steroids, the 7α proton resonates at low field. Moreover, it was noted that *trans* CD 15-keto steroids display in the ^{1}H NMR spectrum the signal for C18 methyl singlet in the range 0.70–1.00 ppm, whereas the corresponding signal in *cis* CD 15-keto steroids ranges between 1.10 and 1.30 ppm.[22] In stemmoside D, the signal at δ 2.99 displayed two large couplings, the first one, 15.5 Hz related to the geminal proton at 1.81 ppm, while the

Table 6. ^{13}C and ^{1}H MAE[a] values for **2a**–**2h**

	2a	2b	2c	2d	2e	2f	2g	2h
MAE ^{13}C	2.64	2.66	1.81	2.14	2.87	3.20	2.59	2.56
MAE ^{1}H	0.16	0.14	0.13	0.15	0.15	0.15	0.16	0.15

[a] Mean average error, MAE $^{13}C=\sum(|(\delta_{exp}-\delta_{calcd})|]/n$, summation through n of the absolute values of the differences of the corresponding experimental and calculated ^{13}C chemical shifts, normalized to the carbon atom number of the molecule; MAE $^{1}H=\sum[((\delta_{exp}-\delta_{calcd})|]/n$, summation through n of the absolute values of the differences of the corresponding experimental and calculated ^{1}H chemical shifts, normalized to the hydrogen atom number of the molecule.

Table 7. Comparison between experimental (**2**) and calculated (stereoisomers **2a** and **2h**) $^{3}J_{HH}$ values of ring D, in Hz

	2	2a	2b	2c	2d	2e	2f	2g	2h
H16–H17	10.1	5.4	2.6	9.7	8.4	9.1	3.3	8.9	1.9

second one, 13.5 Hz revealed a 1,2 diaxial position with respect to H-8. This allowed us to assign this low field signal to H-7α, thus corroborating the *cis* CD arrangement. Furthermore, the occurrence of C18 methyl signal at δ 1.22 confirmed the 14β proton configuration.

In addition, the observation of ROESY correlations between Me-18 (δ 1.22) and H-17 (δ 2.08), H-17 and H-16 (δ 5.51), H-16 and H-14 (δ 2.38), H-14 and H-7β (δ 1.81), and H-14 and Me-18 were in complete accordance with the configuration **2c** as suggested above by the methodology described in this paper. Thus, the structure of stemmoside D was established as 16α-acetoxy-3β-hydroxy-14Hβ-17Hβ-pregn-5-en-15-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside (**2**).

3. Conclusions

Stemmosides C and D have been extensively characterized by means of a combined NMR-Quantum Mechanical approach. This methodology, based on the combined use of QM calculations of ^{1}H, ^{13}C and *J* values, has allowed the determination of an unusual C-17 α side chain for both compounds and the uncommon 14β proton configuration for stemmoside D. In particular, the proposed procedure has been shown to be crucial whereas the analysis of NMR 2D-NOESY and ROESY spectra did not allow us to unambiguously establish the position of protons belonging to ring D, as observed for stemmoside C. Moreover, this straightforward method may be applied to obtain a de novo configurational assignment of new natural and synthetic steroids, or to corroborate the assignment suggested by biosynthetic consideration and/or comparison with literature data.

4. Experimental

4.1. General

Optical rotations were measured on a Jasco DIP 1000 polarimeter IR measurements were obtained on a Bruker IFS-48 spectrometer. Exact masses were measured by a Voyager DE mass spectrometer (Applied Biosystems, Foster City, CA, USA). Samples were analysed by matrix assisted laser desorption ionization (MALDI) mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18–39) of at 2465,1989 Da and Angiotensin III at 931,5154 Da as internal standard. ESIMS were performed on a Finnigan LC-Q Deca Ion Trap mass spectrometer scanned from 150 to 1200 Da. The mass spectral data were acquired and processed using Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump at a flow rate of 3 μL/min. The capillary voltage was 5 V, the spray voltage 5 kV and the tube lens offset 50 V. The capillary temperature was 220 °C. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (Time Proportional Phase Increment) used to achieve frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, PE-COSY, 2D-TOCSY, HSQC, HMBC and ROESY spectra. The spectra were acquired at 600 MHz. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software. Column chromatography was performed over Sephadex LH-20 (Pharmacia), and HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC_{18} column, and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

4.2. Computational details

Molecular mechanics/dynamics calculations on each of the compounds under examination were performed on Silicon Graphics Indigo2 using the CVFF force field[39] and the INSIGHT II/Discover package.[40] MD calculations (500 K, 50 ps) were executed in order to allow a full exploration of the conformational space. The Verlet algorithm was used to integrate the equation of motions. The methanol solution phase was mimicked through the value of the corresponding dielectric constant. All the structures so obtained were minimized using the steepest descent and Newton-Raphson algorithms (maximum derivative less than 0.05 kcal/mol). This led to the selection of the lowest energy minimum conformers. The geometry of the above, as well as that of tetramethylsilane (TMS), were subsequently optimized at DFT level. QM calculations were carried out using the Gaussian03W software package.[41] Structures and energies of the considered species were optimized at mPW1PW91 level using the 6-31G(d) basis set. Single point ^{13}C c.s. calculations, carried out using as inputs the mPW1PW91/6-31G(d) optimized structures, were performed employing the same functional combined with the 6-31G(d,p) basis set. The calculated values of the chemical shifts were referred to the theoretical tetramethylsilane ^{13}C c.s. value, computed at the same level of theory. ONIOM calculations using the mPW1PW91 functional and the 3-21G (low level, rings A–B) and 6-31G(d,p) (high level, ring C–D) were executed on all compounds, taking into account the contributions of the following interactions: Fermi contact (FC), paramagnetic spin-orbit (PSO), diamagnetic spin-orbit (DSO), and spin-dipole (SD), and so providing the theoretical *J* values for ring D.

4.3. Plant material

Fresh samples of *S. argel* pericarps were collected at Allaqi (South-East of Aswan, Egypt) in May 2002 and identified by one of the authors (A.I.H.).

4.4. Extraction and isolation

The dried pericarps (1.5 kg) were extracted with EtOH 80% yielding 50 g of extract. Part of this extract (2.3 g) was fractionated on Sephadex LH-20 (100×5 cm) using MeOH as the mobile phase. Ninety-five fractions (8 mL) were obtained. The fractions containing pregnane glycosides

(frs. 18–39, 450 mg) were chromatographed by HPLC (Refractive index detector), on a Waters (XTerra Prep MSC_{18}) column (300×7.8 mm) using MeOH–H_2O 73:27 as mobile phase (flow rate 2.5 mL/min) to yield compound **1** (1.9 mg) and **2** (2.5 mg), respectively.

4.4.1. Stemmoside C (1). White amorphous powder; $[\alpha]_D^{24} = -11.3$ (*c* 0.2, MeOH); IR (KBr) ν_{max} 3431, 2919, 1756, 1513, 1461, 1102, 1060 cm^{-1}; ^1H and ^{13}C NMR data sugar moiety, see Table 1; ^1H and ^{13}C NMR data aglycone moiety, see Table 2; diagnostic 2D-HMBC correlations (see also Section 2) H-16α/C-15, H-16α/C-17, Me-18/C-12, Me-18/C-13, Me-18/C-14, Me-18/C-17, Me-19/C-1, Me-19/C-5, Me-19/C-9, Me-19/C-10, Me-21/C-17, Me-21/C-20, $H\text{-}1_{cymI}$/C-3, $H\text{-}1_{cymII}$/$C\text{-}4_{cymI}$, $H\text{-}1_{can}$/$C\text{-}4_{cymII}$, $H\text{-}1_{ole}$/$C\text{-}4_{can}$, $H\text{-}1_{glc}$/$C\text{-}4_{ole}$, $Me\text{-}6_{cymI}$/$C\text{-}5_{cymI}$, $Me\text{-}6_{cymI}$/$C\text{-}4_{cymI}$, $Me\text{-}6_{cymII}$/$C\text{-}5_{cymII}$, $Me\text{-}6_{cymII}$/$C\text{-}4_{cymII}$, $Me\text{-}6_{can}$/$C\text{-}5_{can}$, $Me\text{-}6_{can}$/$C\text{-}4_{can}$, $Me\text{-}6_{ole}$/$C\text{-}5_{ole}$, $Me\text{-}6_{ole}$/$C\text{-}4_{ole}$, OMe_{cymI}/$C\text{-}3_{cymI}$, OMe_{cymII}/$C\text{-}3_{cymII}$, OMe_{ole}/$C\text{-}3_{ole}$; diagnostic 2D-ROESY correlations (see also Section 2) H-6/H-4α, H-6/H-7α, H-6/H-8, Me-18/H-8, Me-18/H-12β, Me-18/H-17, Me-19/H-1β, Me-19/H-2β, Me-19/H-4β, Me-21/H-16α, Me-21/H-17, $H\text{-}1_{cymI}$/H-3, $H\text{-}1_{cymII}$/$H\text{-}4_{cymI}$, $H\text{-}1_{can}$/$H\text{-}4_{cymII}$, $H\text{-}1_{ole}$/$H\text{-}4_{can}$, $H\text{-}1_{glc}$/$H\text{-}4_{ole}$; ESIMS *m/z* 1079 $[M+Na]^+$ (100), 917 (26), 773 (13), 643 (53); HRMALDIMS *m/z* 1079.5760 $[M+Na]^+$, calcd for $C_{54}H_{88}O_{20}Na$, 1079.5766.

4.4.2. Stemmoside D (2). White amorphous powder; $[\alpha]_D^{24} = -12.1$ (*c* 0.2, MeOH); IR (KBr) ν_{max} 3410, 2940, 1735, 1461, 1098, 1059 cm^{-1}; ^1H and ^{13}C NMR data sugar moiety, see Table 1; ^1H and ^{13}C NMR data aglycone moiety, see Table 2; diagnostic 2D-HMBC correlations (see Section 2): H-4/C-3, H-16/C-13, H-16/C-15, H-16/*C*OMe, H-8/C-7, H-8/C-14, Me-18/C-12, Me-18/C-13, Me-18/C-14, Me-18/C-16, Me-19/C-1, Me-19/C-5, Me-19/C-9, Me-19/C-10, Me-21/C-17, Me-21/C-20, CO*Me*/*C*OMe, sugar moiety correlations identical to those observed for compound **1**; diagnostic 2D-ROESY correlations (see also Section 2) H-6 /H-3, H-6/H-4α, H-6/H-7α, H-14/H-7β, H-14/Me-18, H-16/H-17, H-16/H-14, Me-18/H-8, Me-18/H-17, Me-19/H-1β, Me-19/H-2β, Me-19/H-4β, Me-21/H-17, Me-21/H-20a, Me-21/H-20b, sugar moiety correlations identical to those observed for **1**; ESIMS *m/z* 1121 $[M+Na]^+$ (100), 1061 (30), 959 (26), 815 (13), 685 (53); HRMALDIMS *m/z* 1121.5889 $[M+Na]^+$, calcd for $C_{56}H_{90}O_{21}Na$, 1121.5872.

Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.tet.2004.10.021

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The synthesis of [2,3,4-$^{13}C_3$]glycitein

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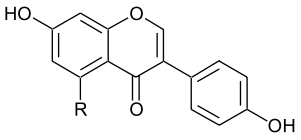
Abstract—Glycitein is one of the soy isoflavones which have attracted considerable interest in recent years due to their possible beneficial effects on human health. However, glycitein has been much less studied than other members of this family due to the lack of good methods for its synthesis. Herein we report a short high yielding synthesis of a multiply ^{13}C-labelled glycitein derivative, [2,3,4-$^{13}C_3$]glycitein, which has been employed as an internal standard in LC–MS analysis. A key feature is a rapid and efficient synthesis of 2,4-dihydroxy-5-methoxy-[1′,2′-$^{13}C_2$]acetophenone via acetylation of isovanillin with [$^{13}C_2$]acetyl chloride followed by a Baeyer–Villiger reaction, selective hydrolysis and finally a BF_3 catalysed Fries rearrangement. An aldol reaction using 4-benzyloxy-[carbonyl-^{13}C]benzaldehyde gave a chalcone and then thallium(III) mediated oxidative rearrangement, deprotection and cyclisation provided the [2,3,4-$^{13}C_3$]glycitein. The overall yield for the 8 step reaction sequence, based on [$^{13}C_2$]acetyl chloride, was 57%.

1. Introduction

The soy isoflavones, including daidzein **1** (R=H), genistein **1** (R=OH) and glycitein **2**, and their glucosides are associated with important health protective properties including anti-cancer activity, plasma cholesterol reduction and reductions in postmenopausal bone loss.[1] There have been many studies on the biological activity of daidzein and genistein, whereas the first studies on glycitein have appeared only recently.[2] Glycitein was shown to have weak estrogenic activity comparable to that of daidzein and genistein, although on an equal mole basis glycitein actually produced a stronger estrogenic response than genistein and daidzein in the mice uterine enlargement assay. It has also been found that glycitein is actually more bioavailable than daidzein in humans.[3] Therefore, although glycitein accounts for only 5–10% of the total isoflavones in soybeans, its biological activities and potential health effects cannot be neglected.



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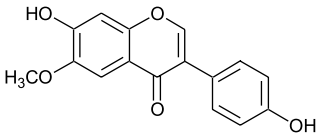

2

In order to better understand, quantify and evaluate the biological activities of glycitein, there is a need for an efficient synthesis of an isotopically labelled derivative, which would be of great value as an internal standard for analysis and for metabolic studies. We have previously synthesised a number of [2-^{13}C]isoflavones,[4,5] which have been employed in metabolic studies on menopausal women[6] and a number of multiply ^{13}C-labelled phytoestrogens such as [2,3,8-$^{13}C_3$]daidzein,[7] which have been fully validated as internal standards for analysis by both LC–MS[8,9] and GC–MS[10] methods. For isoflavone type structures a minimum of three extra mass units is required to produce an optimum internal standard which has a large enough mass difference to nullify the effect of natural abundance heavy isotopes in the analyte.[7] Herein we describe the synthesis of multiply ^{13}C-labelled glycitein derivative, which allows glycitein to be added to the range of analytes with its own ^{13}C-labelled internal standard. The synthesis involves a Baeyer–Villiger reaction followed by a key Fries rearrangement to generate one of the ^{13}C-labelled precursors and a thallium(III) mediated oxidative rearrangement of a chalcone to construct the isoflavone.

2. Results and discussion

The general synthetic route towards the ^{13}C-labelled isoflavones in our previous work (Scheme 1) involved the condensation of an appropriate phenol **3** with either a substituted phenylacetic acid **4** or a benzyl nitrile **5** to give a deoxybenzoin **6** which can then undergo formylation and finally cyclisation to give the isoflavonoid **7**.[4,5,7]

This methodology worked well for daidzein and genistein, and their methylated derivatives formononetin and

Keywords: Isoflavones; Glycitein; Phytoestrogens; ^{13}C-labelling.
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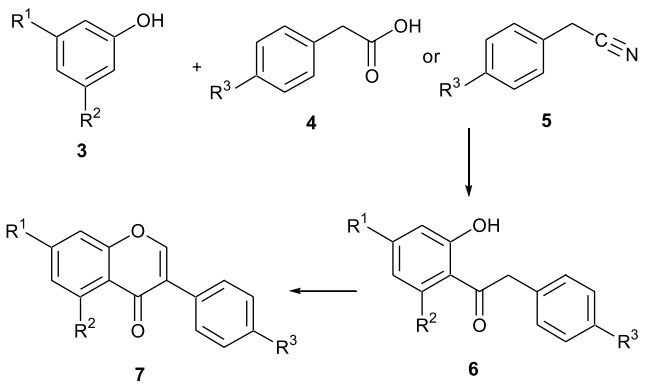
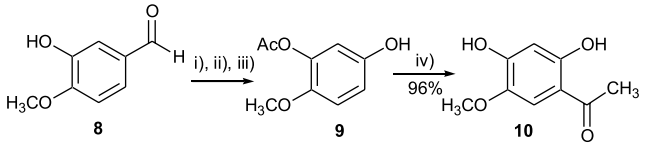


Scheme 1. Deoxybenzoin route to isoflavones.

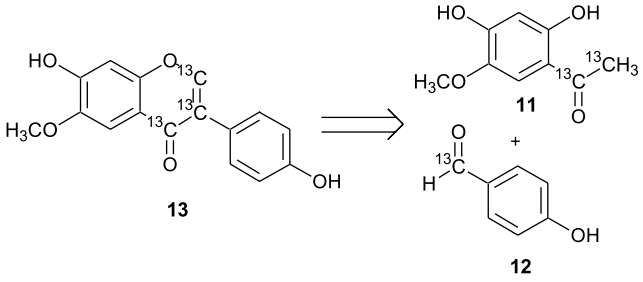
biochanin A and the ^{13}C-atoms were incorporated into either or both fragments. It was thus decided to adopt a similar approach for the glycitein synthesis, especially as recent work reported the preparation of glycitein in 26% yield using a microwave-mediated cyclisation of the corresponding deoxybenzoin.[11] The required phenol, 4-methoxyresorcinol, however, is not commercially available. An initial synthesis involving the hydrolysis of the diazonium salt of 5-amino-2-methoxyphenol was found to be very poor yielding and gave a difficult to purify product.[12] An alternative procedure is the Baeyer–Villiger oxidation of isovanillin.[13–15] Using a literature procedure[15] the hydroxy group of isovanillin **8** was first protected by acetylation with acetic anhydride and oxidative rearrangement of the aldehyde to formate group carried out using *m*CPBA (Scheme 2). Partial hydrolysis of the oxidised product in EtOH–5% aq $NaHCO_3$ gave 3-acetoxy-4-methoxyphenol **9** in good yield. Further hydrolysis of 3-acetoxy-4-methoxyphenol in aq ammonia led to 4-methoxyrecinol as expected.



Scheme 2. (i) Ac_2O, (90%); (ii) *m*CPBA, CH_2Cl_2; (iii) EtOH–5% aq $NaHCO_3$ (86% over two steps); (iv) $BF_3{\cdot}Et_2O$, 70 °C.

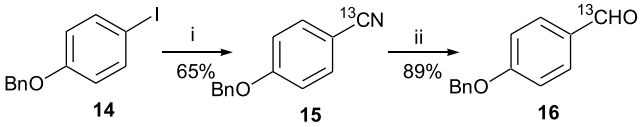
In an attempt to shorten the synthesis the 3-acetoxy-4-methoxyphenol was employed directly in the coupling reaction to form the deoxybenzoin, by reaction with 4-hydroxyphenylacetic acid in the presence of $BF_3{\cdot}OEt_2$ at 70 °C. However, no trace of the expected deoxybenzoin was found and the only isolated product was 2,4-dihydroxy-5-methoxyacetophone. It was then discovered that treatment of 3-acetoxy-4-methoxyphenol with $BF_3{\cdot}OEt_2$ gave 2,4-dihydroxy-5-methoxyacetophone **10** in quantitative yield implying that a Fries rearrangement had taken place.

This observation led to a change in the synthetic strategy. An alternative synthesis of isoflavones involves an aldol condensation of an acetophenone and a benzaldehyde to give a chalcone which can then undergo a thallium mediated oxidative rearrangement to give the isoflavone. This method has been previously used for the synthesis of glycitein in good yield.[16] Since [$^{13}C_2$]acetyl chloride is commercially available, our methodology would allow the rapid synthesis of 2,4-dihydroxy-5-methoxy-[1′,2′-$^{13}C_2$]acetophenone **11** which could then be incorporated into the glycitein. Using protected 4-hydroxy-[carbonyl-^{13}C]benzaldehyde **12** as the other building block would afford [2,3,4-$^{13}C_3$]glycitein **13** (Scheme 3).



Scheme 3. Retrosynthesis for [2,3,4-$^{13}C_3$]glycitein.

Firstly the required ^{13}C-labelled benzaldehyde, was prepared by a simple two-step procedure. Firstly cyanation of 4-benzyloxyiodobenzene **14** using potassium [^{13}C]cyanide and a palladium(II) acetate catalyst in DMF under basic conditions[7] (calcium hydroxide) introduced the ^{13}C-atom. Reduction of the aromatic nitrile **15** with DIBAL-H. then gave 4-benzyloxy-[*carbonyl*-^{13}C]benzaldehyde **16** (Scheme 4).



Scheme 4. (i) K^{13}CN, $Ca(OH)_2$, $Pd(OAc)_2$, DMF; (ii) DIBAL-H, THF.

The presence of the ^{13}C atoms in the labelled compounds was demonstrated using a combination of ^{13}C NMR spectroscopy, IR spectroscopy and mass spectrometry. In the case of 4-benzyloxybenzo[^{13}C]nitrile an enhanced signal was clearly observed in the ^{13}C NMR spectrum at 119.3 ppm and the mass spectrum showed the expected increase of one mass unit compared to the unlabelled compound giving a molecular ion at 210 mass units. In the IR spectrum the effect of ^{13}C-labelling was also visible. The characteristic nitrile vibration is shifted due to the effect of the increase in mass, such that $\nu_{^{13}C\equiv N}$ is observed at 2169 s cm^{-1} in **15** compared with $\nu_{C\equiv N}$ 2221 s cm^{-1} in the unlabelled molecule. In the 4-benzyloxybenzaldehyde a similar effect was seen on the carbonyl stretch, giving $\nu_{^{13}C=O}$ 1723 cm^{-1}, compared to $\nu_{C=O}$ 1772 cm^{-1} in the unlabelled aldehyde. In all the ^{13}C-labelled compounds where $\nu_{C=O}$ was present in the IR spectrum a shift of around 40 cm^{-1} was observed due to the isotopic labeling.

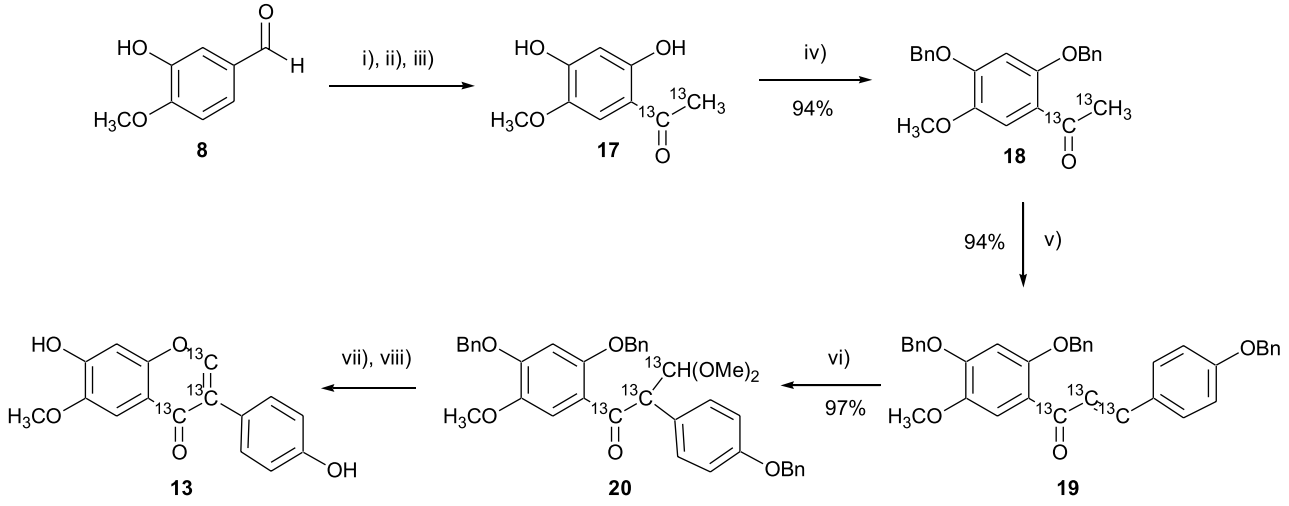
Synthesis of the ^{13}C-labelled acetophenone fragment began with acetylation of isovanillin with [$^{13}C_2$]acetyl chloride to give the 3-[1,2-$^{13}C_2$]acetoxy-4-methoxybenzaldehye in 94% yield. The Bayer–Villager reaction with *m*CPBA followed by selective hydrolysis of the formate gave 3-[1,2-$^{13}C_2$]acetoxy-4-methoxyphenol in 83% yield over the two steps. The Fries rearrangement catalysed by $BF_3{\cdot}Et_2O$ was a very clean reaction and after work up

gave a 96% yield of 2,4-dihydroxy-5-methoxy-[1′,2′-$^{13}C_2$]-acetophenone **17**. The two ^{13}C-atoms in **17** were visible in the ^{13}C NMR spectrum at 202.5 ppm for the carbonyl and 26.60 ppm for the methyl group and appeared as the expected doublets with a coupling constant of 43 Hz. With this compound, as with a number of other ^{13}C-labelled compounds, it was difficult to see all the unlabelled carbon atoms in the NMR spectrum due to a combination of poor solubility and suppression of these signals as a result of the enhanced signals for the ^{13}C-enriched positions. In the ^{1}H NMR spectrum the 2-OH group was observed at 12.6 ppm due to strong intramolecular hydrogen bonding between the 2-OH group and the carbonyl group. This resonance was also split into a doublet, with $J=0.9$ Hz, presumably due to coupling with the ^{13}C-labelled carbonyl group.

Formation of the chalcone intermediate required an aldol condensation between the acetophenone and the benzaldehyde. Preliminary work with unlabelled material showed that the reaction did not occur without protection of the hydroxyl groups of the acetophenone, despite some literature precedent that claimed otherwise.[17] Therefore, 2,4-hydroxy-5-methoxy-[1′,2′-$^{13}C_2$]acetophenone **17** was fully benzylated using benzyl bromide in acetone with anhydrous potassium carbonate to give 2,4-dibenzyloxy-5-methoxy-[1′,2′-$^{13}C_2$]-acetophenone **18** in 94% yield. The aldol condensation between **16** and **18** was first attempted in EtOH–50% aq NaOH at reflux as described in literature,[16] however even after extended reaction times there was always some starting material left and the by-products made purification problematic. As a result of the poor yields the conditions were modified. Reaction in refluxing MeOH–50% aq KOH aq gave 58% pure chalcone after recrystallisation and refluxing EtOH–NaOEt did not improve the yield much, giving 69%. It was observed that while the aldehyde dissolves in EtOH or MeOH well upon heating, the solubility of acetophenone is limited and the addition of aq NaOH, or aq KOH, made the solubility even poorer. Bearing this in mind, a mixed solvent of MeOH/THF (8:3). was used to improve the solubility. Once the starting materials had completely dissolved at reflux, anhydrous KOH was added in one portion with stirring and this dissolved completely after about 10 min. The clear yellow solid of the product precipitated out soon after the disappearance of the solid KOH. Simple work up by filtration followed by washing with cold MeOH, copious water to remove KOH, cold MeOH, cold Et_2O and drying gave the product as a pure yellow solid in excellent yield. (93% for unlabelled chalcone, 94.5% for ^{13}C-labelled chalcone **19**). The ^{13}C NMR spectrum confirmed the presence of the labelled carbon atoms. The carbonyl carbon gave a doublet at 189.4 ppm ($J_{1,2}=56$ Hz. For the alkene carbons, there was a doublet at 142 ppm ($J_{2,3}=70$ Hz) and a double doublet at 125.4 ($J_{1,2}=56$ Hz, $J_{2,3}=70$ Hz). The electrospray mass spectrum showed the expected increase in mass of 3 units compared to the unlabelled compound giving $MH^+=560$ (Scheme 5).

Reaction of the chalcone in MeOH in the presence of trimethyl orthoformate with 1.13 equiv of thallium nitrate gave the ^{13}C-labelled acetal **20** in an excellent 97% yield. The work up involved a simple suction filtration followed by washing with cold MeOH, copious water to remove thallium salts, 5% $NaHCO_3$ to neutralize the trace of HNO_3 produced in the reaction, cold MeOH and cold Et_2O successively to get a pure white product.

The final step involved deprotection of benzyl groups and cyclisation in methanol and concd HCl to give [2,3,4-^{13}C]glycitein in 95% yield. The ^{13}C-labelled carbons were observed as enhanced signals in the ^{13}C NMR spectrum at 174.5, 150.9 and 123.0 ppm with the expected ^{13}C–^{13}C coupling(dd, enhanced, $J_{C,H}=72.0$, 54.5 Hz, *C*-3), while electrospray mass spectrometry (in negative ion mode) gave $M^-=286$ as expected. To confirm its purity the [2,3,4-$^{13}C_3$]glycitein **13** was also subject to microanalysis, UV absorption and HPLC analysis. All these data compared satisfactorily with the literature.[2,11] The compound has been used as internal standard for the analysis of glycitein in plasma samples and it appears to be an excellent standard.



Scheme 5. (i) [$^{13}C_3$]Acetyl chloride, Et_3N, THF/Et_2O, (94%); (ii) *m*CPBA, CH_2Cl_2, reflux, then $NaHCO_3$, EtOH (83%); (iii) $BF_3\cdot Et_2O$, 70 °C (96%); (iv) BnBr, K_2CO_3, acetone, reflux; (v) **16**, KOH, MeOH/THF, reflux; (vi) $Tl(NO_3)_2\cdot 3H_2O$, $HC(OMe)_3$, MeOH; (vii) H_2, 5% Pd/C, MeOH/Acetone (93%); (viii) MeOH, Concd HCl, reflux (95%).

In conclusion, we have developed an efficient synthetic method for [2,3,4-$^{13}C_3$]glycitein by employing the Baeyer–Villiger oxidation, Fries rearrangement, aldol condensation and thallium oxidative rearrangement. Each step gave an excellent yield of the corresponding product. The total yield of [2,3,4-$^{13}C_3$]-glycitein over an 8 step reaction sequence was 57% based on the starting reagent [$^{13}C_2$]-acetyl chloride.

3. Experimental

3.1. General

Chemical and reagents were obtained from Aldrich and Lancaster and used without further purification. Chemical reactions were monitored by thin layer chromatography using MN precoated silica gel G/UV254 (0.2 mm thickness) plates. Silica Gel 60A (35–70 units) was utilized for column chromatography. THF was freshly distilled from sodium/benzophenone under nitrogen. DCM was refluxed over powdered calcium hydride and distilled under nitrogen. Methanol was heated to reflux over magnesium and distilled under nitrogen. Infrared spectra were recorded as KBr pellets in the range of 400–220 cm^{-1} on a Perkin–Elmer system 2000 spectrometer, UV spectra on a Kontron UVIKON spectrophotometer. ^{1}H NMR spectra (300 MHz) and ^{13}C NMR (75 MHz) on a Varian Gemini 2000 spectrometer with the residue peak of $CHCl_3$ (7.27 ppm) and the central peak of $CDCl_3$ (77.2 ppm) as reference, respectively. Chemical shifts are given in δ and J values in Hz. Due to the poor solubility of the target glycitein, the smallest amount of d^6-DMSO was used in addition to $CDCl_3$ for NMR spectra recording. HREIMS were measured on a Finnigan VG AutoSpec instrument, HRESMS on a Waters LCT-ES spectrometer. Microanalysis was performed by the St Andrews University Service within this Department. HPLC spectra were obtained from a Waters 600 Multisolvent Delivery System using Kingsorb 3μ C-18 silica-gel on a 150×4.60 mm^2 3 micron sized column eluted with a mixture of acetonitrile/water (75:25) at a run rate of 0.6 mL/min.

3.1.1. 3-[1,2-$^{13}C_2$]Acetoxy-4-methoxybenzaldehyde. To a solution of isovanillin (3.75 g, 24.64 mmol) in dry THF (40 mL) was added Et_3N (5 mL, 36 mmol). The mixture was cooled to 0 °C. [$^{13}C_2$]Acetyl chloride (2.0 g, 24.8 mmol) in Et_2O (8 mL) was added dropwise over 30 min. The reaction mixture was stirred at room temperature for 14 h and then the solvent was removed at reduced pressure. The residue was taken up in water (200 mL) to dissolve the salt. Filtration followed by successive washings with water, cold MeOH and cold Et_2O afforded the first crop of product. The filtrates were then extracted with EtOAc. The extracts were combined, washed with brine, dried ($MgSO_4$) and the solvent removed at reduced pressure. The residue was subject to flash column chromatography (SiO_2 eluted with gradient solvent CH_2Cl_2/EtOAc=20:1 to 10:1) to give the second crop of the product. Total yield: 4.75 g, 94%. ν_{max} (KBr disc)/cm^{-1} 1723 (^{13}C=O); δ_H (300 MHz, $CDCl_3$) 9.89 (2H, s, C*H*O), 7.77 (1H, dd, $J_{5,6}$=8.7 Hz, $J_{2,6}$=2.0 Hz, *H*-6), 7.60 (1H, d, $J_{2,6}$=2.0 Hz, *H*-2), 7.09 (1H, d, $J_{5,6}$=8.7 Hz, *H*-5), 3.93 (3H, s, CH_3O), 2.34 (3H, dd, $J_{C,H}$=130.3, 7.0 Hz, $^{13}CH_3^{13}CO$); δ_C (75 MHz, $CDCl_3$) 190.1 (*C*HO), 168.8 (d, enhanced, $J_{C,C}$=60.0 Hz, ^{13}COCH$_3$), 156.5 (*C*-1), 140.4 (*C*-3), 130.3 (*C*-6), 130.1 (*C*-4), 123.6 (*C*-2), 112.2 (*C*-5), 56.4 (*C*H$_3$O), 20.7 (d, enhanced, $J_{C,C}$=60.0 Hz, $^{13}CH_3^{13}CO$); *m*/*z* (EI) 196.0640 (M^+, $C_8{}^{13}C_2H_{10}O_4$ requires 196.0646).

3.1.2. 3-[1,2-$^{13}C_2$]Acetoxy-4-methoxyphenol. A clear solution of 3-[1,2-$^{13}C_2$]acetoxy-4-methoxybenzaldehyde (4.52 g, 23.0 mmol) and *m*-chloroperbenzoic acid (*m*CPBA) (7.95 g, 46.1 mmol) in dry CH_2Cl_2 (60 mL) was heated under reflux with stirring for 5 h. The reaction mixture was then cooled to room temperature. Filtration and removal of the solvent at reduced pressure afforded an oily residue which was diluted with EtOAc and washed with 5% $NaHCO_3$ and brine. The residue was then dissolved in EtOH (75 mL), after addition of 5% $NaHCO_3$ aq (100 mL) and the solution was stirred for 17 h at room temp. The reaction mixture was acidified to pH 2 with 2 M HCl, salted out and extracted with EtOAc. The extracts were washed with brine, 5% aq $NaHCO_3$ and brine successively, dried over anhydrous $MgSO_4$ and filtered. After removal of the solvent at reduced pressure, the residue was purified by flash column chromatography on silica eluting with CH_2Cl_2/AcOEt (40:1 to 20:1) and then recrystallised from 4:1 CH_2Cl_2/petroleum ether (bp 40–60) to give colourless needles (3.54 g, 83%); ν_{max} (KBr disc)/cm^{-1} 1700 (^{13}C=O); δ_H (300 MHz, $CDCl_3$) 6.80 (1H, d, $J_{5,6}$=9 Hz, *H*-5), 6.60 (1H, dd, $J_{5,6}$=9 Hz, $J_{2,6}$=3 Hz, *H*-6), 6.52 (1H, d, $J_{5,6}$=3 Hz, *H*-2), 5.81 (1H, br s, O*H*), 3.76 (3H, s, CH_3O), 2.31 (3H, dd, $J_{C,H}$=130.3, 6.8 Hz, $^{13}CH_3^{13}C$); δ_C (75 MHz, $CDCl_3$) 170.0 (d, enhanced, $J_{C,C}$=59.9 Hz, $^{13}CH_3^{13}CO$), 150.0 (*C*-1), 145.1 (*C*-4), 140.1 (*C*-3), 113.8 (*C*-5), 113.4 (*C*-6), 111.0 (*C*-2), 56.7 (*C*H$_3$O), 20.9 (d, enhanced, $J_{C,C}$=59.9 Hz, $^{13}CH_3^{13}CO$); *m*/*z* (EI) 184.0644 (M^+, $C_7{}^{13}C_2H_{10}O_4$ requires 184.0646).

3.1.3. 2,4-Dihydroxy-5-methoxy-[1,2-$^{13}C_2$]acetophenone (17). To 3-[1,2-$^{13}C_2$]acetoxy-4-methoxyphenol (3.30 g, 17.9 mmol) was added neat boron trifluoride diethyl etherate $BF_3 \cdot Et_2O$ (10 mL, 48.4 mmol). The mixture was stirred at 70 °C for 2 h and then cooled to room temperature. The suspension was taken up in saturated aq NaOAc (50 mL) and saturated aq $NaHCO_3$ was added until no further CO_2 was evolved. The suspension was then extracted with EtOAc/Et_2O (1:1). The extracts were washed with brine, dried ($MgSO_4$) and the solvent removed at reduced pressure to give the product (3.16 g, 96%); ν_{max} (KBr disc)/cm^{-1} 1634 (^{13}C=O); δ_H (300 MHz, $CDCl_3$) 12.58 (1H, d, $^2J_{C-O\cdots H}$=0.9 Hz, D_2O exchangeable, O*H*-2), 7.06 (1H, d, $^3J_{C,H}$=4.3 Hz, *H*-6), 6.53 (1H, d, $J_{C,H}$=1.2 Hz, *H*-3), 6.23 (1H, s, D_2O exchangeable, O*H*-4), 3.92 (3H, s, CH_3O), 2.57 (3H, dd, $J_{C,H'}$=127.8 Hz, $J_{C,H}$=5.8 Hz, $^{13}CH_3^{13}C$); δ_C (75 MHz, $CDCl_3$) 202.5 (d, enhanced, $J_{C,C}$=43.2 Hz, $CH_3{}^{13}CO$), 56.9 (*C*H$_3$O), 26.60 (d, enhanced, $J_{C,C}$=43.2 Hz, $^{13}CH_3^{13}CO$); *m*/*z* (EI) 184.0650 (M^+, $C_7{}^{13}C_2H_{10}O_4$ requires 184.0646).

3.1.4. 2′,4′-Dibenzyloxy-5-methoxy-[1,2-$^{13}C_2$]acetophenone (18). To **17** (3.50 g, 19.0 mmol), 18-crown-6 (0.50 g, 1.90 mmol) and anhydrous K_2CO_3 (30 g, 217 mmol) in acetone (200 mL) was added benzyl bromide (5.0 mL, 42.9 mmol). The reaction mixture was heated to

reflux for 5 h and then cooled to room temperature. Removal of solvent at reduced pressure and recrystallisation from acetone/Et_2O and petroleum ether afforded the first (4.93 g) crop of product as colourless needles. The mother liquors were subject to column chromatography [SiO_2, petroleum ether (bp 40–60)/Et_2O=6:1 to 3:1] to give more product (1.57 g). Total yield: 6.50 g, 94%. ν_{max} (KBr disc)/cm^{-1} 1623 (^{13}C=O); δ_H (300 MHz, $CDCl_3$) 7.45 (1H,d, $J_{C,H}$=3.9 Hz, *H*-6), 7.45–7.33 (10H, m, Ph*H*), 6.55 (1H, d, $J_{C,H}$=1.4 Hz, *H*-3), 5.17, 5.02 (4H, 2×s, 2×CH_2Ph), 3.89 (3H, s, CH_3O), 2.57 (3H, dd,, $J_{C,H}$=128.3 Hz, $^2J_{C,H}$= 6.0 Hz, $^{13}CH_3{}^{13}C$); δ_C (75 MHz, $CDCl_3$) 197.6 (d, enhanced, $J_{C,C}$=42.6 Hz, $^{13}CH_3{}^{13}C$O), 154.3, 152.9, 143.9, 136.4, 128.9, 128.4, 128.3, 127.7, 127.3, 120.5 (d, *C*-1), 113.2, 100.6, 71.7 (*C*H_2), 71.3 (*C*H_2), 56.6 (*C*H_3O), 32.5 (d, enhanced, $J_{C,C}$=42.6 Hz, ^{13}CH$_3{}^{13}$CO); *m*/*z* (ES) 387.1480 (MNa^+, $C_{21}{}^{13}C_2H_{22}NaO_4$ requires 387.1484).

3.1.5. 4-Benzyloxybenzo-[1-^{13}C]nitrile (15). To a solution of 4-benzyloxyphenyl iodide (3.71 g, 11.96 mmol), $Ca(OH)_2$ (0.443 g, 5.98 mmol) and $Pd(OAc)_2$ (0.27 g, 1.12 mmol) in anhydrous DMF (40 mL) was added potassium [^{13}C]cyanide (0.79 g, 12.0 mmol). The reaction mixture was heated to reflux for 16 h and then cooled to room temperature. After filtration washing with CH_2Cl_2 and acetone, the filtrates were concentrated, diluted with copious water (500 mL) and extracted with EtOAc. The extracts were washed with brine and dried over anhydrous $MgSO_4$. Removal of the solvent at reduced pressure afforded a brown solid, which was purified by column chromatography (SiO_2, petroleum ether (bp 40–60)/Et_2O= 10:1) to give the product as a white solid (1.63 g, 65%); ν_{max} (KBr disc)/cm^{-1} 2169 (^{13}CN); δ_H (300 MHz, $CDCl_3$) 7.59 (2H, dd, $J_{2,3}={}^3J_{5,6}$=8.7 Hz, $J_{C,H}$=4.8 Hz, *H*-2 and 6), 7.45–7.35 (5H, m, Ph*H*), 7.03 (2H, d, $J_{2,3}=J_{5,6}$=8.7 Hz, *H*-3 and 5), 5.13 (2H, s, CH_2Ph); δ_C (75 MHz, $CDCl_3$) 162.1 (*C*-*4*), 135.9 (*C*-*1*′), 134.1 (d, $J_{C,C}$=2.0 Hz, *C*-3 and 5), 128.9 (*C*-2′ and 6′), 128.5 (*C*-4′), 127.6 (*C*-3′ and 5′), 119.3 (enhanced, ^{13}CN), 115.71 (d, $J_{C,C}$=5.7 Hz, C-2 and 6), 104.3 (d, $J_{C,C}$=74.0 Hz, *C*-1), 70.4 (*C*H_2Ph); *m*/*z* (EI) 210.0871 (M^+, $C_{13}{}^{13}CH_{11}NO$ requires 210.0874).

3.1.6. 4-Benzyloxy-[*carbonyl*-^{13}C]benzaldehyde (16). To a solution of 4-benzyloxybenzo[^{13}C]nitrile **15** (1.62 g, 7.72 mmol) in THF (50 mL) was added diisobutylaluminum hydride (DIBAL-H, 1.0 M in THF, 15.4 mL, 15.4 mmol). The reaction mixture was stirred at room temperature overnight and then quenched with water and 2 M HCl until pH=1. Extraction with CH_2Cl_2, drying over anhydrous $MgSO_4$ and removal of solvent gave the off-white product. (1.46 g, 89%); ν_{max} (KBr disc)/cm^{-1} 1653 (^{13}C=O); δ_H (300 MHz,$CDCl_3$) 9.86 (1H, d, $J_{C,H}$=172.2 Hz, ^{13}C*H*O), 7.81 (2H, dd, $J_{2,3}=J_{5,6}$=8.7 Hz, $J_{C,H}$=4.1 Hz, *H*-2 and 6), 7.42–7.31 (5H, m, Ph*H*), 7.05 (2H, d, $J_{2,3}=J_{5,6}$=8.7 Hz, *H*-3 and 5), 5.13 (2H, s, CH_2Ph); δ_C (75 MHz, $CDCl_3$) 190.9 (enhanced, ^{13}CHO), 162.1 (*C*-4), 136.1 (*C*-1′), 132.1 (d, $J_{C,C}$=3.4 Hz, *C*-2 and 6), 130.3 (d, $J_{C,C}$=56.4 Hz, *C*-1), 128.9 (*C*-3′ and 5′), 128.4 (*C*-4′), 127.6 (*C*-3′ and 5′), 115.3 (d, $J_{C,C}$=4.6 Hz, *C*-3 and 5), 70.5 (*C*H_2Ph); *m*/*z* (EI) 213.0870 (M^+, $C_{13}{}^{13}CH_{12}O_2$ requires 213.0871).

3.1.7. 3-(4-Benzyloxyphenyl)-1-[2,4-bis(benzyloxy)-5-methoxyphenyl]-[1,2,3-$^{13}C_3$]-2-propen-1-one (19). To a solution of **18** (2.35 g, 6.45 mmol) and 4-benzyloxy-[*carbonyl*-^{13}C]benzaldehyde **16** (1.46 g, 213 mmol) in a mixture of THF (20 mL) and MeOH (60 mL) was added anhydrous KOH (3.6 g, 64.3 mmol). The solution was then heated to reflux for 6 h during which time the product precipitated out as bright yellow solid. After cooling in an ice bath, the suspension was filtered washing successively with cold MeOH, water, cold MeOH and cold Et_2O to give the product as tiny yellow crystals (3.40 g, 94%); ν_{max} (KBr disc)/cm^{-1} 1603, 1589, 1572, 1533, 1521, 1507; δ_H (300 MHz, $CDCl_3$) 7.89–7.74 (m, 1H, 1/2 C*H*=C*H*), 7.41 (1H, d, $J_{C,H}$=4.3 Hz, H-6′), 7.38–7.15 (18H, m, Ph*H*+1/2 C*H*=C*H*+*H*-2″ and 6″), 6.78 (d, 2H, $J_{2'',3''}=J_{5'',6''}$=8.7 Hz, H-3″ and 5″), 6.54 (d, 1H, $J_{C,H}$=1.4 Hz, H-3′), 5.14, 5.01, 5.00 (3×s, 3×2H, 3×CH_2Ph), 3.84 (3H, s, CH_3O); δ_C (75 MHz, $CDCl_3$) 189.4 (d, enhanced, $J_{1,2}$=56.4 Hz, ^{13}C=O), 141.98 (d, enhanced, $J_{2,3}$=70.2 Hz, ^{13}C-3), 125.4 (dd, enhanced, $J_{1,2}$=56.4 Hz, $J_{2,3}$=70.2 Hz, ^{13}C-2); *m*/*z* (ES) 560.2436 (MH^+, $C_{34}{}^{13}C_3H_{33}O_5$ requires 560.2430).

3.1.8. 2-(4-Benzyloxyphenyl)-1-[2,4-bis(benzyloxy)-5-methoxyphenyl]-3,3-dimethoxy-[1,2,3-$^{13}C_3$]-1-propanone (20). To a suspension of **19** (1.12 g, 2.00 mmol) in dry MeOH (10 mL) and trimethyl orthoformate (7 mL, 64.0 mmol) was added $Tl(NO_3)_3\cdot 3H_2O$ (1.00 g, 2.25 mmol). The mixture was stirred for 24 h at room temperature and then cooled in an ice bath. Filtration washing with cold MeOH, copious H_2O, 5% $NaHCO_3$ aq, H_2O, cold MeOH and Et_2O successively afforded the product as a white solid. (1.20 g, 97%); ν_{max} (KBr disc)/cm^{-1} 1627 (^{13}C=O); δ_H (300 MHz, $CDCl_3$) 7.42–7.28 (m, 16 H, Ph*H*+*H*-6′), 7.11 (dd, 2H, $J_{2'',3''}=J_{5'',6''}$=8.7 Hz, $J_{C,H}=J_{C,H}$=3.4 Hz, *H*-2″ and 6″), 6.80 (d, 2H, $J_{2'',3''}=J_{5'',6''}$=8.7 Hz, *H*-3″ and 5″), 6.44 (d, 1H, $J_{C,H}$=1.4 Hz, *H*-3′), 5.16 (dm, 1H, $J_{C,H}$=130.7 Hz, *H*-2′), 5.09 (s, 2H, CH_2Ph), 5.04 (dd, $J_{C,H}$=166.9 Hz, $J_{H,H}$=7.7 Hz, *H*-3), 5.01 (d, 1H, $J_{H,H}$=12 Hz, OC*H*^{a}Ph-2′), 4.99 (s, 2H, CH_2Ph), 4.92 (d, 1H, $J_{H,H}$=12 Hz, OC*H*^{b}Ph-2′), 3.86 (s, 3H, CH_3O-5′), 3.39, 3.05 (2×d, 2×3H, $^{13}CH(OCH_3)_2$, $J_{C,H}$=4.8 Hz). δ_C (75 MHz, $CDCl_3$) 197.5 (d, enhanced, ^{13}C=O, $J_{1,2}$=41.4 Hz), 107.0 (d, enhanced, $J_{2,3}$=47.2 Hz, ^{13}C-3), 58.7 (dd, enhanced, $J_{1,2}$=41.4 Hz, $J_{2,3}$=47.2 Hz, ^{13}C-2); *m*/*z* (ES) 644.2616 (MNa^+, $C_{36}{}^{13}C_3H_{38}O_7Na$ requires 644.2617).

3.1.9. 1-(2,4-Dihydroxy-5-methoxyphenyl)-2-(4-hydroxyphenyl)-3,3-dimethoxy-[1,2,3-$^{13}C_3$]-1-propanone. A solution of the acetal **20** (1.13 g, 1.81 mmol) in methanol/acetone (1:1, 120 mL) was hydrogenated over palladium on charcoal (0.56 g, 5%). The mixture was stirred overnight at room temperature. Filtration through a celite pad and removal of solvent afforded the product as an off-white foam (0.57 g, 93%); ν_{max} (nujol)/cm^{-1} 1636 (^{13}C=O); δ_H (300 MHz, d^6-acetone) 12.66 (1H, s, O*H*-2′), 8.59 (2H, br s, O*H*-4′ and O*H*-4″), 7.53 (1H, d, $J_{C,H}$=4.0 Hz, *H*-6′), 7.37 (2H, dd, $J_{2''3''}=J_{5'',6''}$=8.6 Hz, $J_{C,H}$=3.3 Hz, *H*-2″ and 6″), 6.80 (2H, d, $J_{2'',3''}=J_{5'',6''}$= 8.6 Hz, *H*-3″ and 5″), 6.34 (1H, d, $J_{C,H}$=1.1 Hz, *H*-3′), 5.09 (1H, dd, $J_{C,H}$=166.1 Hz, $J_{2,3}$=8.2 Hz, *H*-3), 4.87 (1H, ddt, $J_{C,H}$=130.0 Hz, J=4.3 Hz, $J_{2,3}$=8.2 Hz, *H*-2), 3.86 (3H, s, CH_3O-5′), 3.38, 3.18 (6H, 2×d, $J_{C,H}$= 4.4 Hz, $^{13}CH(OCH_3)_2$); δ_C (75 MHz, $CDCl_3$) 203.2

(d, enhanced, $J_{C,C}=42.6$ Hz, ^{13}C=O), 107.1 (d, enhanced, $J_{2,3}=47.2$ Hz, ^{13}C-3), 58.7 (dd, enhanced, $J_{1,2}=42.6$ Hz, $J_{2,3}=47.2$ Hz, ^{13}C-2); *m/z* (ES^-) 350.1233 (M^-, $C_{15}{}^{13}C_3H_{19}O_7$ requires 350.1233).

3.1.10. [2,3,4-$^{13}C_3$]Glycitein (13). A solution of the hydroxyacetal (0.59 g, 1.68 mmol) in a mixture of methanol (20 mL) and concentrated hydrochloric acid (2.0 mL) was heated to reflux for 4 h and then cooled to room temperature. Filtration followed by washing with cold MeOH, water, 5% aq $NaHCO_3$, water, cold MeOH and diethyl ether successively afforded the crude product as off-white solid. Recrystallisation from EtOH gave a white solid (0.455 g, 95%); (Found: C, 66.81; H, 4.08, $C_{13}^{13}C_3H_{11}O_5$ requires C, 66.90; H 4.21%); λ_{max} (EtOH)/nm 260 (ε/30,225 $dm^3\,mol^{-1}\,cm^{-1}$, and 320 (ε/13,134 $dm^3\,mol^{-1}\,cm^{-1}$); literature[2] unlabelled glycitein in MeOH 257 (ε/31,622 $dm^3\,mol^{-1}\,cm^{-1}$); ν_{max} (KBr disc)/cm^{-1} 3405, 1614, 1548, 1515; δ_H (300 MHz, $CDCl_3$ and d^6-DMSO) 9.58 (1H, br s, O*H*-7), 8.66 (1H, br s, O*H*-4′), 7.51 (1H, dt, $J_{C,H}=194.0$, 6.5 Hz, *H*-2), 7.09 (1H, d, $J_{C,H}=3.9$ Hz, *H*-5), 6.93 (2H, dd, $J_{2',3'}=J_{5',6'}=8.7$ Hz, $J_{C,H}=3.4$ Hz, *H*-2′ and 6′), 6.50 (1H, d, $J_{C,H}=1.9$ Hz, *H*-8), 6.42 (2H, d, $J_{2',3'}=J_{5',6'}=8.7$ Hz, H-3′ and 5′), 3.52 (3H, s, OCH_3); δ_C (75 MHz, ($CDCl_3$ and d^6-DMSO) 174.5 (d, enhanced, $J_{C,H}=54.5$ Hz, *C*-4), 150.9 (d, enhanced, $J_{C,H}=72.0$ Hz, *C*-2), 123.0 (dd, enhanced, $J_{C,H}=72.0$, 54.5 Hz, *C*-3); *m/z* (ES^-) 286.0712 (M^-, $C_{13}{}^{13}C_3H_{11}O_5$ requires 286.0707).

Acknowledgements

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Phosphonates containing sulfur and selenium. Synthesis of vinylphosphonates bearing α-sulfenyl, α-selenenyl, α-sulfinyl and α-seleninyl moieties and studies on nucleophilic addition

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Abstract—The selenenylation of racemic and optically active α-phosphoryl sulfoxides is a key step leading efficiently to α-phosphorylvinyl sulfoxides or α-phosphorylvinyl selenides depending on the reaction conditions. Oxidation of α-phosphorylvinyl selenides and subsequent thermolysis of selenoxides afford alkynylphosphonates. Studies of the stereochemical course of nucleophilic addition to α-phosphoryl sulfoxides show high facial stereoselectivity of the reaction, however, epimerisation at the α-carbon atom leads to mixtures of diastereomers.

1. Introduction

Functionalized vinylphosphonates have attracted much interest in synthetic chemistry and their synthetic applications have been widely investigated in the last two decades.[1,2] In recent years, special attention has been devoted to α-heterosubstituted vinylphosphonates as useful intermediates for the synthesis of natural products.[3–5]

Recently, we have designed[6] and investigated a new type of vinylphosphonate with a sulfinyl substituent as a stereocontrol element. These vinylphosphonates were found to easily undergo Michael reaction, Diels–Alder cycloaddition and cyclopropanation by reaction with sulfur ylides and diazoalkanes. Various types of asymmetric reaction using these α-phosphorylvinyl sulfoxides have been investigated[6b,7] and found to proceed in some cases with considerably high stereoselectivity, which has been attributed to differentiation of the π-faces induced by the sulfinyl group. In this paper, we wish to describe the full account of our studies on the synthesis of our target compounds and investigations into nucleophilic addition.

2. Results and discussion

2.1. Synthesis

One of the best methods to introduce a C–C double bond is the selenenylation and subsequent oxidative selenoxide elimination. In a classical procedure PhSeX (X=Cl, Br, SePh) is used for introduction of the selenenyl moiety. An alternative selenenylation method, developed by Liotta[8] for selenenylation of ketones, involves addition of elemental selenium to enolate anions. This method seems to be a cheaper and easier procedure. In order to find a general methodology for the synthesis of vinylphosphonates, we decided to define the scope and limitations of the selenium addition procedure.

We have found that the α-lithioalkanephosphonates, obtained from the phosphonates **1** and *n*-butyllithium in THF solution at −78 °C, react with elemental selenium, which is simply added to the reaction mixture as a powder.[9] Dissolution of selenium was observed to take place at −30 °C and above affording the corresponding lithium selenoates. The latter, without isolation, were easily converted into the corresponding methyl selenides **2** by treatment with methyl iodide (Scheme 1).

Phosphonates **2a**,**b** possessing β-hydrogen atoms were converted into vinylphosphonates **3a**,**b** by oxidation to the corresponding selenoxides followed by their spontaneous elimination under the reaction conditions. The utility of this

Keywords: Selenenylation; α-Phosphoryl-vinyl sulfoxides; α-Phosphoryl-vinyl selenoxides; Nucleophilic addition.
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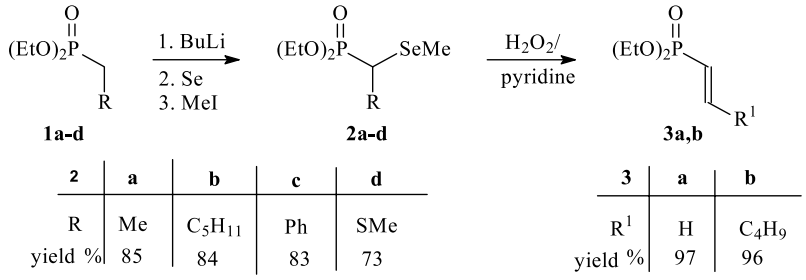
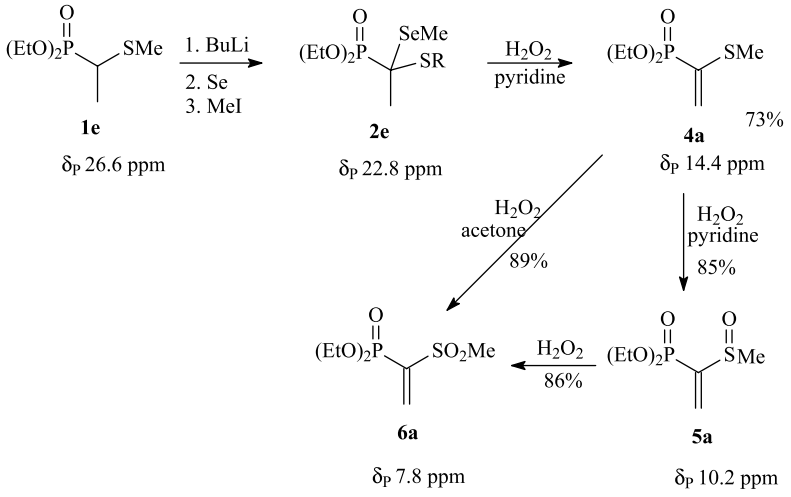


2	a	b	c	d
R	Me	C_5H_{11}	Ph	SMe
yield %	85	84	83	73

3	a	b
R^1	H	C_4H_9
yield %	97	96

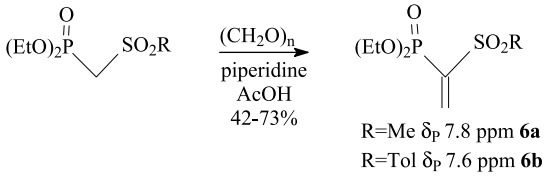
Scheme 1.



Scheme 2.

approach for vinylphosphonate synthesis was demonstrated by the synthesis of diethyl α-methylthiovinylphosphonate **4a** (Scheme 2).

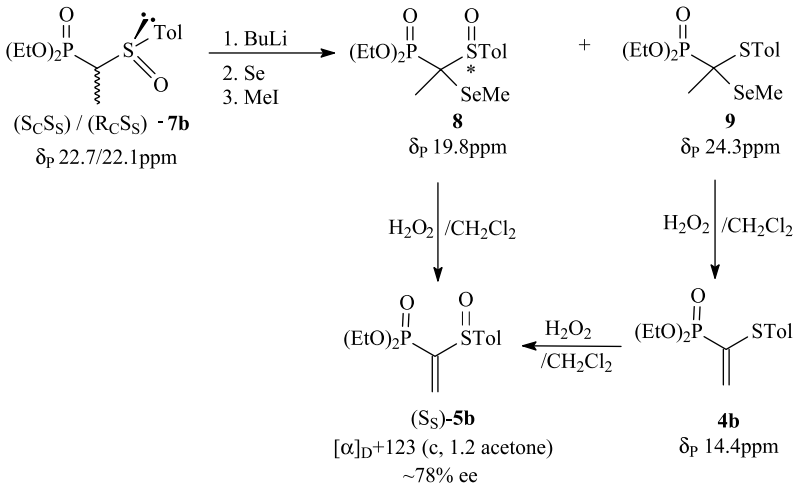
The synthesis of α-phosphorylvinyl sulfides **4** in Scheme 2 is complementary to the earlier syntheses of these compounds, that is, the addition of methanesulfenyl chloride to diethyl vinylphosphonate followed by hydrochloride elimination[10a] and the Peterson reaction of α-silyl α-phosphoryl sulfides.[10b] α-Phosphoryl vinyl sulfides **4** easily undergo oxidation and they can be transformed into the vinyl sulfones **6** using H_2O_2 in acetone solution (Scheme 2). An alternative method for the introduction of the methylene moiety at the carbon atom possessing strongly electron-withdrawing groups is the Mannich reaction. The reaction of α-phosphoryl sulfone with paraformaldehyde in the presence of a catalytic amount of piperidine and acetic acid also afforded the vinyl sulfone **6** in moderate to high yield (42–73%) (Scheme 3).



Scheme 3.

On the other hand, selective oxidation of the vinyl sulfide **4a** by means of H_2O_2 in pyridine solution leads to the corresponding vinyl sulfoxide **5a**, however, this reaction affords the desired compound only in racemic form. Because our interest is focused on the utilization of chiral sulfoxides in asymmetric synthesis we elaborated a new procedure for the synthesis of the optically active sulfoxides **5** starting from the optically active sulfoxides **7**,[11] which are mixtures of two diastereomers $(S_CS_S)/(R_CS_S)$ in a 2:1 ratio (Scheme 4). Thus, the lithium salt of the α-phosphoryl sulfoxide **7b** was found to undergo the reaction with elemental selenium and after methylation and oxygenation afforded the vinyl sulfoxide **5b** in above 50% yield. Unfortunately, it turned out that the addition of selenium does not occur cleanly as in the case of simple phosphonates **1** and in the reaction mixture, in addition to the desired α-phosphorylselenothioketal *S*-oxide **8**, different side products were found. One of them was identified as the corresponding selenothioketal **9**—a sulfoxide reduction product. The latter in the next step undergoes oxidation leading to racemic **5b** (path b). Because oxidative elimination of selenenic acid must be performed on a crude reaction mixture (to avoid sulfenic acid elimination), this reduction–oxidation process decreases the optical purity of the sulfoxide **5b** $[\alpha]_D+123$ (*c*, 1.2 acetone) (Scheme 4). The mechanism of this reduction is not clear.

The efficient and highly stereoselective synthesis of the enantiopure sulfoxide **5b** was accomplished using



Scheme 4.

phenylselenenyl bromide as the selenenylating agent. Thus, the α-carbanion of α-diethoxyphosphorylethyl *p*-tolyl sulfoxide **7b**, formed on treatment with *n*-butyllithium in THF at −78 °C, reacted with PhSeBr and after 3 min the reaction mixture was quenched with cold aqueous solution of $NaHCO_3$. Extraction with ethyl ether afforded the PhSe-substituted sulfoxide **10**, of unknown diastereomeric ratio, since only one broad signal in the ^{31}P NMR spectrum was visible. To complete the synthesis of α-phosphorylvinyl sulfoxide **5b**, oxidation of the selenide moiety was performed using H_2O_2 in CH_2Cl_2 solution at 0 °C which caused benzeneselenenic acid elimination and formation of the desired product (Scheme 5).

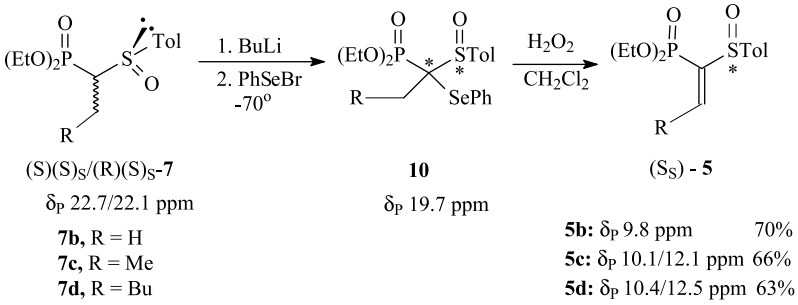
This methodology was extended to the synthesis of β-substituted α-phosphorylvinyl sulfoxides **5c** and **5d.** They were obtained from α-phosphorylpropyl *p*-tolyl sulfoxide **7c** and α-phosphorylhexyl *p*-tolyl sulfoxide **7d** (optically pure at the sulfur atom) according to the procedure described above (Scheme 5).

It is interesting to underline that the oxidative selenoxide elimination affords vinyl sulfoxides **5c**,**d** as mixtures of *E* and *Z* isomers in about 10:1 ratio. After purification by column chromatography both isomers were separated, however, the minor *Z*-isomer was contaminated with a small amount of the starting material. The configuration of the α,β-unsaturated sulfoxides **5c**,**d** was determined[12] based on the $^3J_{P-H}$ coupling constants values which were 41.4 and 42 Hz for *E* and 23.3 and 23.1 Hz for *Z* isomers, respectively.

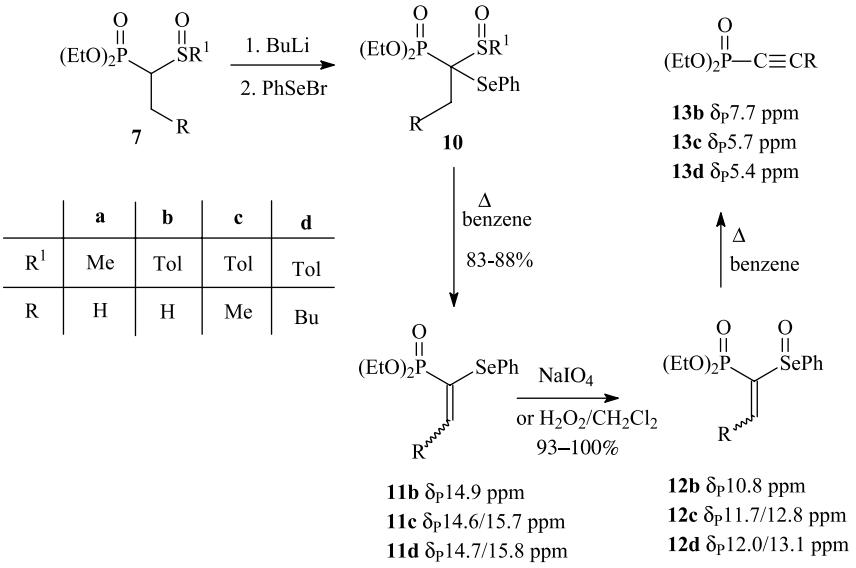
The PhSe-substituted phosphoryl sulfoxides **10**, which upon oxidation undergo transformation to α-phosphorylvinyl sulfoxides **5**, can also afford α,β-unsaturated selenides **11** (Scheme 6). When **10** was heated in a benzene solution, elimination of sulfenic acid took place resulting in the formation of α-phosphorylvinyl selenides **11** in high yields (72–88%). Elimination of *p*-toluenesulfenic acid from **10b**,**c**,**d** occurs quite easily and the full conversion requires heating at 80 °C during the course of a few hours (4–5 h). The conversion of **10a** to **11a**, where the loss of methanesulfenic acid takes place, requires a longer time (ca. 10 h) at the same temperature.

From a preparative point of view, it is worth noting that sulfoxide elimination from **10c**,**d** was found to take place at room temperature when they were subjected to chromatography on a silica gel column. In contrast to stereoselective selenoxide elimination of **10**, the sulfoxide elimination leads to a 1:1 mixture of *E* and *Z* isomers, which were easily separated by column chromatography.

Oxidation of vinyl selenides **11** yields selenoxides **12** in very high yields. It was found that α-phosphorylvinyl selenoxides *E* and *Z*-**12** differ in stability. Whereas the isomer *E* can be stored for a few days, the isomer *Z* easily loses its oxygen (1 day, rt), reverting to starting selenide and



Scheme 5.



	a	b	c	d
R^1	Me	Tol	Tol	Tol
R	H	H	Me	Bu

Scheme 6.

cannot be purified by chromatography. The better stability of the isomer *E*-**12**, where the substituent at the α-carbon atom is on the same side of a double bond as the bulky phosphoryl group, can be attributed to hydrogen bonding between the selenoxide oxygen and the vinyl hydrogen.[13]

Owing to the presence of the selenoxide moiety, the α-phosphoryl vinyl selenoxides **12** were found to undergo further *syn*-elimination. Hence, the thermolysis of **12b**, and *E*-**12c** and *E*-**12d** performed in refluxing (benzene solution affords α-phosphorylalkynes **13** as the only products. In this way a new synthetic approach to this class of compounds was elaborated.[14]

2.2. Nucleophilic addition

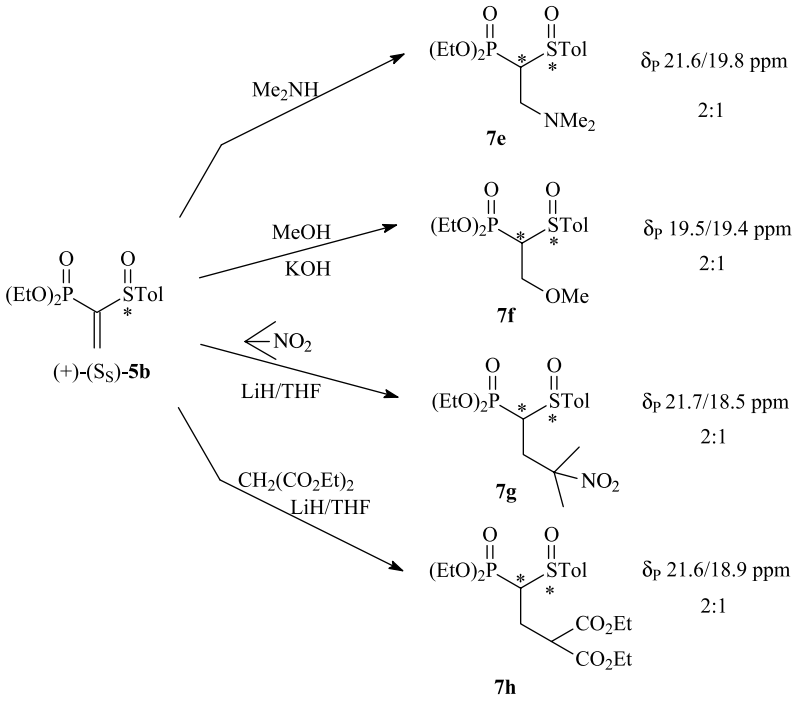
α-Phosphorylvinyl sulfoxides **5** as well as α-phosphoryl-vinyl selenoxides **12** having two electron-withdrawing groups are effective acceptors in conjugate addition of nucleophiles. Taking into account an easy way of elimination of selenenic and sulfenic acid, the sulfoxide **5** and selenoxide **12** can be considered as equivalents of α-phosphoryl alkynes in nucleophilic addition. On the other hand, as we mentioned before, nucleophilic addition to chiral sulfoxides **5** should occur under stereochemical control by the sulfinyl group.

Our first experiments of nucleophilic addition were perfomed using the vinyl sulfoxide **5b** and various heteronucleophiles: dimethylamine, ethyl mercaptan in the presence of Et_3N and methanol in the presence of KOH. In all cases, addition occurred easily at room temperature affording the desired products as a mixture of diastereomers in around 2:1 ratio. This ratio is determined by thermodynamic factors which was established by equilibration using the isolated pure diastereomers. The reaction with the lithium salt of diethyl malonate also affords the corresponding addition product as a mixture of diastereomers in the same ratio (Scheme 7).

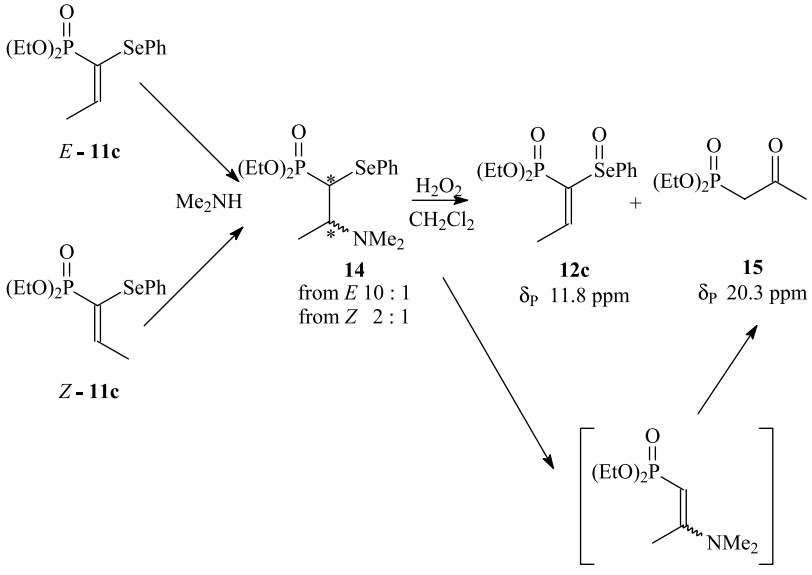
Since the chirality of the α-carbon atom in α-phosphoryl sulfoxide would be destroyed in further transformations (after the Horner reaction or desulfurization), much more interesting from the synthetic point of view is the chirality on the β-carbon atom. In order to establish the stereochemical course of nucleophilic addition to vinylphosphonates with β-substituent, some reactions with different nucleophiles were performed using α-phosphorylvinyl sulfoxides **5c**, **5d** and **5e** and α-phosphorylvinyl selenides and selenoxides **11c** and **12c** as Michael acceptors.

The addition of Me_2NH to *E* and *Z* selenides **11c** afforded the adducts **14** as mixtures of diastereomers in a 10:1 and 2:1 ratio, respectively (Scheme 8). Typical oxidation of **14** with H_2O_2 in CH_2Cl_2 gives two major products, whose ratio depends on the reaction conditions. At room temperature, the only product was the *E*-vinyl selenoxide **12c**. Probably, this temperature favours oxidation of the amine moiety to the corresponding amine oxide, which after elimination, gives rise to a rather stable vinyl selenoxide. With decreasing temperature the selenoxide elimination takes place forming enamine, which, however, undergoes hydrolysis under the reaction and work-up conditions affording β-ketophosphonate **15** as the major product. For this reason no information about the steric course of the reaction can be drawn from these experiments.

Since nucleophilic addition to the selenide **11c** gave no answer concerning the steric course of the addition, the next experiments were performed using the vinyl selenoxide **12c** as starting material. Addition of the malonate anion to α-phosphorylvinyl selenoxide *E*-**12c** occurs easily, but affords the product of isomerization i.e., the β,γ-unsaturated phosphonate **16**. Probably, in this case the equilibrium between α,β and β,γ-isomers is shifted to the latter. To exclude the possibility of this isomerization 2-nitropropane was used in our further studies. The reaction with the potassium salt of 2-nitropropane with *E*-**12c** afforded only the *Z*-isomer of the vinylphosphonate **17** and allylic alcohol **18c** as a side reaction product. The latter was undoubtedly formed as a result of α,β to β,γ-isomerization and allylic



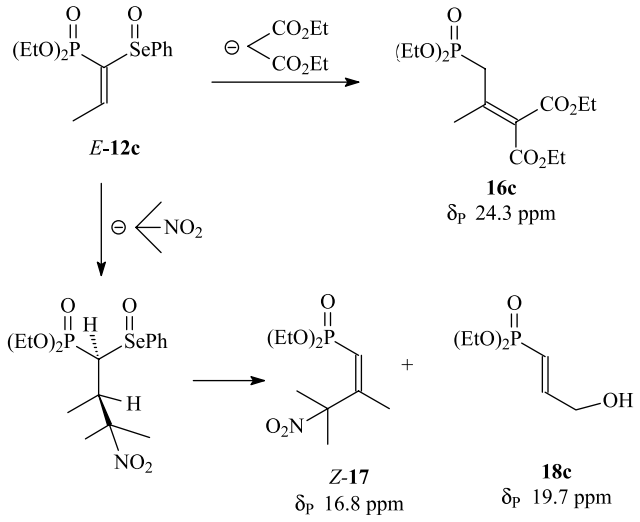
Scheme 7.



Scheme 8.

rearrangement of the starting material **12c** under the reaction conditions (Scheme 9).

The structure of the vinylphosphonate **17** was confirmed by NMR studies using nuclear Overhauser effect. Irradiation of the vinyl proton caused 21% increase of the methyl protons and irradiation of the methyl group of *Z*-**17** (δ_P 16.8) gave a 17% enhancement of the vinyl proton. This indicates that these two moieties are on the same side of the olefinic bond. The *Z*-geometry of the vinylphosphonate **17** obtained as well as the fact of *cis*-geometry of selenoxide elimination, imply *anti*-approach during nucleophilic addition to the α-phosphorylvinyl selenoxides **12**. Unfortunately, the reaction of the *Z*-isomer of **12** with the potassium salt of 2-nitropropane gave a mixture of the same products and additionally some amount of the *E*-isomer of starting material, suggesting the presence of the equilibrium either between *E* and *Z* isomers of starting vinyl selenoxides **12** or *threo* and *erythro* Michael adducts. Taking into account the easy selenoxide elimination (Michael addition product was not detected), the *Z*→*E* isomerization of **12** through allylic selenoxide probably occurs much faster than nucleophilic addition of a bulky nitropropane and only the more stable *trans* isomer undergoes Michael addition selectively affording the product *Z*-**17**.

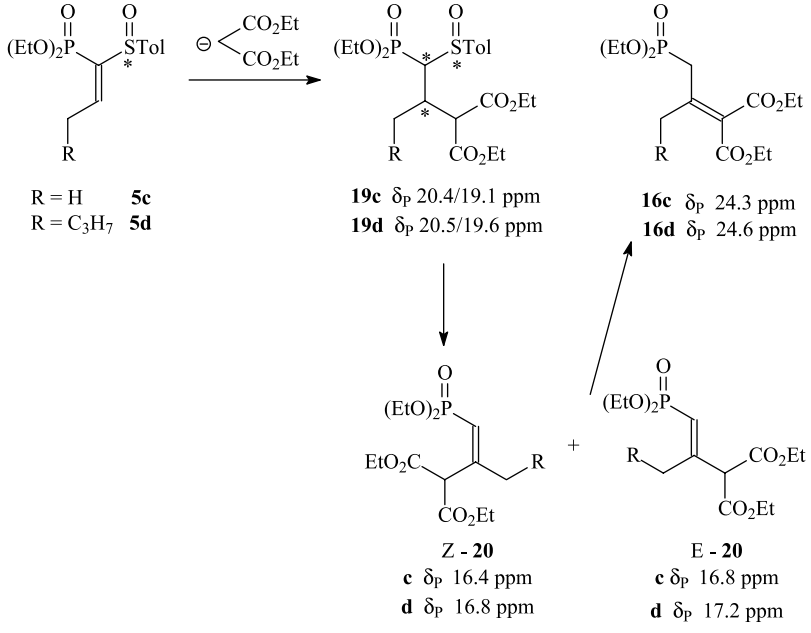


Scheme 9.

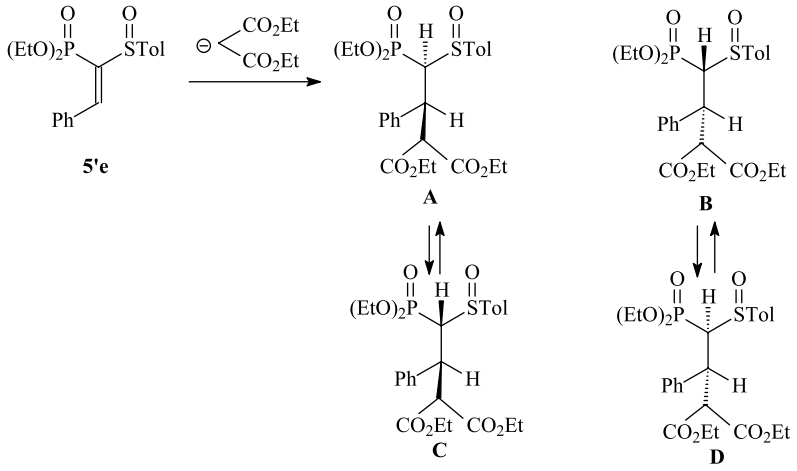
Nucleophilic addition to β-substituted α-phosphorylvinyl sulfoxides is more complex since the reaction should create two centres of chirality under stereochemical control of the sulfinyl group. Having in hand three chiral sulfoxide substrates (**5c**,**d**,**5′e**[15]) we decided to extend the studies on the nucleophilic addition and its stereoselectivity. In the reaction of (*E*)-(*S*)-(1-diethoxyphosphoryl-2-methyl)vinyl *p*-tolyl sulfoxide **5c** with the lithium salt of diethyl malonate, generated by LiH in THF solution, the desired adduct **19c** was formed exclusively as a 5:3 mixture of two (from four) possible diastereomers. The product **19** upon storage at room temperature slowly undergoes sulfoxide elimination yielding the vinylphosphonate **20**. However, in this case the ^{31}P NMR spectra indicated the presence of both *E* and *Z* isomers of **20** formed. Taking into account configurational requirement for *syn*-elimination of sulfenic acid, the presence of two isomers of **20** suggests that they were formed from **19** of *threo* and *erythro* configuration. Those products could be formed when nucleophilic addition to sulfoxide **5** partly occurs in *anti* and partly in *syn*-manner, what is rather unlikely because *anti*-addition was suggested in our model reaction with selenoxide **12c**. Another explanation presumes stereoselective attack of nucleophile leading to only one diastereomer of **19** and partial epimerization on the α-carbon atom under the reaction conditions (Scheme 10).

Addition of the lithium salt of diethyl malonate to *E*-(+)-(1-dimethoxyphosphoryl-2-phenyl)vinyl *p*-tolyl sulfoxide **5′e** affords a mixture of four diastereomers **19′e** δ_P: 21.2/20.9/22.4/21.5 ppm in a 22:15:3:2 ratio. Oxidation of the major pair of diastereomers (21.2/20.9) as well as the minor one (22.4/21.5) separately in order to destroy chirality at sulfur, gave in both cases a mixture of the sulfone **21′e** (18.3/17.9), that is the diastereomers of *threo* and *erythro* configuration. Also in this case we can presume *anti* addition of the malonate nucleophile leading to diastereomers **A** and **B** and then formation of diastereomers **C** and **D** caused by epimerisation on the α-carbon atom. According to our recent studies of asymmetric cyclopropanation of α-phosphorylvinyl sufoxides with sulfur ylides,[13] the major factor controlling the stereoselectivity of this reaction is the conformation of the sulfoxide, where sulfinyl and phosphoryl groups adopt an *anti* orientation, the former being syncoplanar with the carbon–carbon double bond. Nucleophilic addition of the sulfur ylide occurs exclusively from the less-hindered diastereotopic face occupied by the electron lone pair at sulfur. Although we do not have any configurational assignment of addition products **19′e**, it seems likely, that major diastereomer **19′e** (δ_P: 21.2 ppm) has configuration **A**, formed by the same facial nucleophilic attack of the lithium salt of diethyl malonate (Schemes 11 and 12).

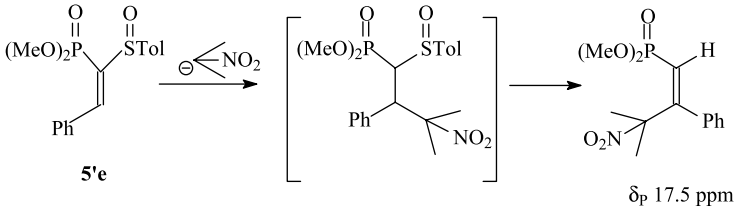
Nucleophilic addition of the sodium salt of 2-nitropropane to *E*-(+)-(1-dimethoxyphosphoryl-2-phenyl)vinyl *p*-tolyl



Scheme 10.



Scheme 11.



Scheme 12.

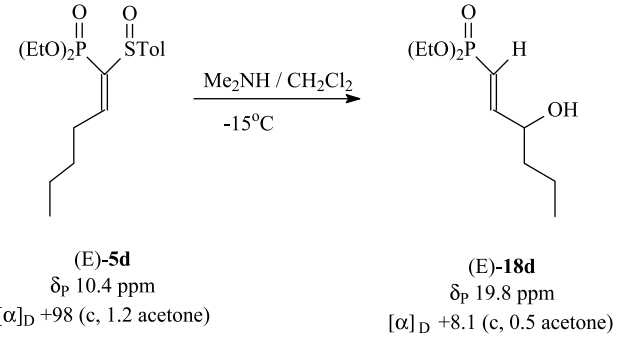
sulfoxide **5′e** was performed in a similar way to selenoxides. It was found that elimination of sulfenic acid from the primary adduct is so fast, that it can not be detected in ^{31}P NMR spectra. Also in this case only one vinylphosphonate was obtained (δ_P 17.5), because instantaneous sulfenic acid elimination from the adduct makes epimerization impossible.

2.3. Allylic alcohol synthesis

The synthesis of different types of γ-hydroxy α,β-unsaturated derivatives from sulfoxides and aldehydes was described as a one step procedure[16] (SPAC reaction, an abbreviation from sulfoxide piperidine aldehyde condensation) based on a sequence of reactions: Knoevenagel condensation, prototropic shift and allylic sulfoxide–sulfenate rearrangement. Usually, the SPAC reaction was carried in CH_3CN in the presence of piperidine at 0 to 60 °C. Using chiral sulfoxides, these reactions gave rise to asymmetric induction ranging from 10–70% ee.[17] In the case of sulfoxides **5c** and **5d**, the presence of a γ-hydrogen in the aliphatic chain creates the possibility of α,β to β,γ isomerization and sigmatropic rearrangement leading to allylic alcohol **18**. This reaction, confirmed already for the analogous vinyl selenoxide **12c**, also occurs in the case of vinyl sulfoxide **5d** affording γ-hydroxyhexenylphosphonate **18d** in excellent yield.

The first experiment leading to allylic alcohol was conducted using optically active α-phosphorylvinyl sulfoxide **5d** by addition of Me_2NH at 0 °C in CH_2Cl_2 solution. γ-Hydroxy- phosphonate **18d**, obtained in this way, exhibited optical rotation $[\alpha]_D+1.5$ (*c*, 0.8 acetone). According to the ^{1}H NMR spectra in the presence of (+)-(*R*)-*t*-butylphenylphosphinothioic acid this value corresponds only to 5% ee. Trying to improve the asymmetric induction, we decreased the temperature to −15 °C. In this case the reaction was complete after 3 days, but we were able to raise the optical purity of the product **18** to 25% $[\alpha]_D+8.1$ (*c*, 0.5 acetone). Because the typical procedure for SPAC reaction applied to α-phosphoryl sulfoxides requires rather vigorous conditions[18] (heating at 40 °C for 12–24 h), our modification using α,β-unsaturated α-phosphoryl sulfoxides seems to give the possibility to obtain optically active γ-hydroxyphosphonates (Scheme 13).

We have developed a general methodology to prepare α-heterosubstituted vinylphosphonates. The crucial step of



Scheme 13.

the synthesis is selenenylation of phosphonates either by phenylselenyl bromide, or elemental selenium, although some limitations of the letter reagent were defined. Applying our procedure we synthesized for the first time, in optically active and racemic form, α,β-unsaturated α-phosphoryl sulfoxides, important reagents in asymmetric synthesis. Our studies on the nucleophilic addition to α-phosphorylvinyl sulfoxides, due to epimerization of addition products, allowed us to present only considerations of the most probable stereochemistry of the process.

3. Experimental

3.1. General

^{1}H, ^{13}C, ^{77}Se and ^{31}P NMR spectra were recorded on a Bruker MSL 300 and Bruker AC 200 Spectrometer, using deuterochloroform as solvent. Mass spectra were recorded on Finnigan MAT95. IR spectra were recorded on Ati Mattson FTIR Spectrometer. The optical rotations were measured on a Perkin–Elmer 241 MC photopolarimeter in acetone solution. The microanalyses were performed on Elemental Analyzer EA 1108.

TLC was carried out on silica gel plates (Merck F_{254}) and silica gel 60 (70-230 ASTM) was used for chromatography. THF was freshly distilled over potassium/benzophenone.

3.2. General procedure for preparation of α-phosphoryl selenides 2

To a stirred solution of phosphonate **1** (20 mmol) in 100 mL of dry THF, a solution of *n*-BuLi (10 mL, 2.2 M in hexane, 22 mmol) was added at −78 °C. After 10 min, selenium powder (20 mmol) was added and the reaction mixture was warmed to appropriate temperature (−30 °C—**2a**; −20 °C—**2b**,**c**; −40 °C—**2d**) and kept at this temperature until the selenium disappeared. Then, the reaction mixture was cooled to −78 °C and methyl iodide (20 mmol) was added. The reaction solution was warmed up and treated with 30 mL of aqueous NH_4Cl. The organic layer was separated and collected with the 50 mL of chloroform extract of the water layer. The combined organic solution was dried over $MgSO_4$ and the solvent was evaporated to give the yellow oil. Purification was performed using column chromatography on silica gel (eluent: benzene–acetone 10:1).

3.2.1. Diethyl (α-methylselenenyl)ethylphosphonate 2a. A pale yellow oil. Yield: 4.42 g (85%); $n_D=1.4860$; IR (neat) 1241, 1028; ^{31}P NMR (81 MHz, $CDCl_3$) δ 28.7 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.3 (t, 6H, CH_3CH_2OP, $J=7.1$ Hz); 1.54 (dd, 3H, CH_3CH, $J_{P-H}=17.4$ Hz, $J_{H-H}=7.5$ Hz); 2.15 (s, 3H, $SeCH_3$); 2.76 (dq, 1H, CH_3CH, $J_{P-H}=13.2$ Hz, $J_{H-H}=7.5$ Hz); 4.14 (dq, 4H, CH_3CH_2OP, $J_{P-H}=8.0$ Hz, $J_{H-H}=7.1$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 4.5; 15.7; 16.3 (d, $J_{P-C}=6$ Hz); 25.5 (d, $J_{P-C}=52.2$ Hz); 62.5 (d, $J_{P-C}=6.9$ Hz); ^{77}Se NMR (57 MHz, $CDCl_3$) δ 536 ppm; HRMS (70 eV) $C_7H_{17}O_3PSe$ requires 260.0087 Found: 260.0075.

3.2.2. Diethyl (α-methylselenenyl)hexylphosphonate 2b. A slightly yellow oil. Yield: 5.30 g (84%); $n_D=1.4732$; IR (neat) 1240, 1024; ^{31}P NMR (81 MHz, $CDCl_3$) δ 28.18 ppm; ^{1}H NMR (200 MHz) δ: 0.84–0.93 (m, 3H, CH_3CH_2); 1.14–1.43 (m,10H: 6H of t, CH_3CH_2OP+4H of m, $(CH_2)_2CH_3$)); 1.45–2.01 (m, 4H, $-(CH_2)_2-$); 2.12 (s, 1H, $SeCH_3$); 2.57 (dt, H, C–*H*, $J_{P-H}=15$ Hz, $J_{H-H}=10$ Hz); 4.16 (dq, 4H, CH_3CH_2OP, $J_{P-H}=8.8$ Hz, $J_{H-H}=7.1$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 4.5; 13.9; 16.3 (d, $J=6.0$ Hz); 22.3; 27.5 (d, $J=12.0$ Hz); 28.3; 31.1; 33.9; 65.5 (d, $J=13.8$ Hz); ^{77}Se NMR (57 MHz, $CDCl_3$) δ 485 ppm; HRMS (70 eV) $C_{11}H_{25}O_3PSe$ requires 316.0700 Found: 316.0697.

3.2.3. Diethyl (α-methylselenenyl-α-phenyl)methylphosphonate 2c. A pale yellow oil. Yield: 5.35 g (83%); $n_D=1.5380$; ^{31}P NMR (81 MHz, $CDCl_3$) δ 23.98 ppm; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.23 ppm (2xt, 6H, CH_3CH_2O, $J=7.1$ Hz); 2.05 (s, 3H, $SeCH_3$); 4.02 (d, 1H, C*H*Ph, $J_{P-H}=17.4$ Hz); 3.23–4.23 (m, 4H, CH_3CH_2OP); 7.27–7.47 (m, 5H, Ar–*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 6.2 (d, $J=3.3$ Hz); 16.2 (t, $J=6.9$ Hz); 36.9 (d, $J_{C-P}=149$ Hz); 63.1 (d, $J=16.5$ Hz); 127.5; 128.5; 129.2; 135.7; ^{77}Se NMR ($CDCl_3$, 57 MHz) δ 606 ppm; HRMS (70 eV) $C_{12}H_{19}O_3PSe$ requires 322.0237 Found 322.02369.

3.2.4. Diethyl (α-methylselenenyl-α-methylsulfenyl)-methylphosphonate 2d. A slightly yellow oil. Yield: 4.26 g (73%); $n_D=1.4742$; ^{31}P NMR (81 MHz, $CDCl_3$) δ 21.08 ppm; ^{1}H NMR (200 MHz) δ: 1.34 (t, 6H, CH_3CH_2OP, $J_{H-H}=7.1$ Hz); 2.20 (d, 3H, SCH_3, $J_{P-H}=0.7$ Hz); 2.27 (d, 3H, $SeCH_3$, $J_{P-H}=1.7$ Hz); 3.74 (d, 1H, C*H*, $J_{P-H}=15$ Hz); 4.19 (dq, 4H, OCH_2CH_3, $J_{P-H}=8.8$ Hz, $J_{H-H}=7.1$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$); δ 5.6; 16.0 (d, $J=11.8$ Hz); 16.4 (d, $J_{P-C}=5.6$ Hz); 36.2 (d, $J_{P-C}=154.7$ Hz); 63.5 (d, $J_{P-C}=6.9$ Hz); HRMS (70 eV) $C_7H_{17}O_3PSSe$ requires 291.9801. Found 291.9797.

3.2.5. Diethyl (α-methylselenenyl-α-methylsulfenyl)-ethylphosphonate 2e. A slightly yellow oil. Yield: 4.41 g (72%); $n_D=1.4215$; ^{31}P NMR (81 MHz, $CDCl_3$): δ 23.7 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (2×t, 6H, CH_3CH_2OP, $J_{H-H}=7.1$ Hz); 1.77 (3H, d, CH_3, $J_{P-H}=5.3$ Hz); 2.16 (d, 3H, $SeCH_3$, $J_{P-H}=1.5$ Hz); 2.23 (d, 3H, SMe, $J_{P-H}=0.2$ Hz); 4.24 (dq, 4H, CH_3CH_2OP, $J_{P-H}=8.8$ Hz, $J_{H-H}=7.1$ Hz,); ^{13}C NMR (50 MHz, $CDCl_3$): δ 5.2; 13.7 (d, $J=3.3$ Hz); 16.3 (d, $J=5.9$ Hz); 23.3; 43.1 (d, $J=155$ Hz); 63.6 (t, $J=7.2$ Hz); ^{77}Se NMR (57 MHz, $CDCl_3$): δ 636 ppm; HRMS $C_8H_{19}O_3PSSe$ requires 305.9958. Found 305.9965.

3.3. Oxidation of α-phosphoryl selenides

To a solution of α-phosphoryl selenide (15 mmol) in 50 mL of dry pyridine, 30% solution of hydrogen peroxide (15 mmol) was added. The mixture was stirred vigorously at room temperature for 2 h. Then 50 mL of diethyl ether was added and the reaction mixture was washed with water (5×10 mL). The organic layer was dried and solvent evaporated affording vinylphosphonates which were purified by column chromatography (hexane–acetone 15:1).

3.3.1. Diethyl hexen-1-ylphosphonate 3b. A slightly yellow oil. Yield: 3.17 g (96%); $n_D=1.6828$ (isomer *E*); ^{31}P NMR (81 MHz, $CDCl_3$) δ 19.7 ppm (*E*)/18.1 ppm (*Z*)

21:1; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (t, 3H, CH_3, J_{H-H}=6.8 Hz); 1.31 (t, 6H, OCH_2CH_3, J_{H-H}=7.1 Hz); 1.23–1.45 (m, 4H, CH_2); 2.14–2.24 (m, 2H, CH_2); 4.05 (dq, 4H, CH_3CH_2OP, J_{P-H}=8.0 Hz, J=7.1 Hz); (for isomer *Z*) 5.55 (tdd, 1H, J_{H-H}=1.6, 7.0, 13.0 Hz, J_{P-H}=17.0 Hz); 6.55 (tdd, 1H, J_{H-H}=7.0, 11.0 Hz, J_{P-H}=46.3 Hz); (for the isomer *E*) 5.63 (tdd, 1H, =C–H_2, J_{P-H}=21.2 Hz, J_{H-H}= 1.5, 17.1 Hz,); 6.77 (tdd, 1H, =CH_2, J_{P-H}=22.0 Hz, J_{H-H}=6.6, 17.1 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz); δ 13.6; 16.1 (d, J=6.3 Hz); 21.9, 29.7, 33.6 (d, J=21.6 Hz); 61.3 (d, J=5.4 Hz); 116.5 (d, J=188 Hz); 153.8 (d, J=4.6 Hz); HRMS (70 eV) $C_{10}H_{21}O_3P$ requires 220.1228. Found 220.1214.

3.3.2. Diethyl (α-methylsulfenyl)vinylphosphonate 4a. A slightly yellow oil. Yield: 2.83 g (90%); n_D=1.4868; IR (neat) 1632, 1243, 1026; ^{31}P NMR (81 MHz) δ 14.5 ppm; ^{1}H NMR (200 MHz): δ 1.34 (2×t, 6H, J=7.1 Hz); 2.31 (s, 3H, SCH_3); 4.05 (dq, 4H, J_{H-H}=7.1 Hz, J_{P-H}=8.8 Hz); 5.55 (d, 1H, J_{P-H}=42.9 Hz); 6.18 (d, 1H, J_{P-H}=21.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 4.4 (d, J=6.2 Hz); 15.9 (d, J=6.9 Hz); 62.3 (d, J=4.8 Hz); 121.5 (d, J=7.6 Hz); 136.1 (d, J=19.7 Hz); HRMS (70 eV) $C_7H_{15}O_3PS$ requires 210.0479. Found 210.04715.

3.4. Oxidation of α-phosphorylvinyl methyl sulfide 4a to sulfoxide 5a

α-Phosphorylvinyl methyl sulfide **4a** (1 mmol) was dissolved in pyridine (50 mL) and 30% hydrogen peroxide (1 mmol) was added. The mixture was stirred for 6 days at room temperature and then Et_2O (50 mL) was added. The organic solution was washed with water (5×10 mL), dried and evaporated. Sulfoxide **5a** was purified by column chromatography on silica gel (hexane–acetone 20:1).

Yield: 0.266 g (85%); n_D=1.54443. A slightly yellow oil; IR (neat) 1595, 1254, 1027, 1043; ^{31}P NMR (81 MHz, $CDCl_3$): δ 10.22 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.34 (2×t, 6H, J=7.1 Hz); 2.79 (s, 3H, SOCH_3); 4.15 (4H, dq, J_{P-H}=8.8 Hz, J_{H-H}=7.1 Hz); 6.69 (d, 1H, J_{H-H}=19.3 Hz, *cis* C=CH); 6.77 (d, 1H, J=40.2 Hz, *trans* C=CH); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 11.1, 15.6 (d, J=6.2 Hz); 63.9 (d, J=89.4 Hz); 125.38 (d, J=100.7 Hz); 133.9 (d, J= 104 Hz); HRMS (70 eV) $C_7H_{15}O_4PS$ requires 226.0428. Found 226.0426.

3.5. Preparation of diethyl α-phosphorylvinyl methylsulfone 6a

Method A (by oxidation of the sulfoxide). The diethyl (α-methylsulfinyl)vinylphosphonate (1 mmol) was dissolved in acetone and two molar excess of hydrogen peroxide was added. The reaction mixture was heated in reflux for 2 h. On the next water (10 mL) was added and acetone was evaporated. The water layer was extracted with chloroform (3×15 mL). The combined organic layers were dried by magnesium sulfate and solvents were evaporated. The colourless oil was separated and purified by column chromatography (hexane–acetone 20:1). Yield 0.2 g (89%).

Method B (by Mannich reaction). Paraformaldehyde (2 mmol) was dissolved in 100 mL of benzene containing a mixture of 5 drops of piperidine and 10 drops of acetic acid and the solution was heated in reflux for 0.5 h. α-Phosphoryl sulfone (1 mmol) was added all at once and the solution was heated in reflux under Dean–Stark water separator for 54 h. The solvent was removed and product was purified by column chromatography (hexane–acetone 20:1).

3.5.1. Diethyl (α-methylsulfonyl)vinylphosphonate 6a. A pale yellow oil. Yield: 0.182 g (42%)—Method B; n_D= 1.5620; IR (neat) 1250, 1019; ^{31}P NMR (81 MHz, $CDCl_3$): δ 7.8 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.33 (t, 6H, CH_3CH$_2$OP, J=7.1 Hz); 3.16 (s, 3H, SO_2CH_3); 4.10–4.20 (m, 4H, CH_3CH_2OP); 6.98 (d, 1H, J_{P-H}=17.3 Hz, *cis* C=CH); 7.12 (d, 1H, J_{P-H}=39.3 Hz, *trans* C=CH); ^{13}C NMR ($CDCl_3$, 50 MHz); 16.6 (d, J=7.9 Hz); 18.7; 62.8; 131 (d, J=7.1 Hz); 139 (d, J=171 Hz). Anal. Calcd for $C_7H_{15}O_5PS$: C, 34.71%; H, 6.24%. Found: C, 34.83%; H, 6.32%.

3.5.2. α-Diethyl (1-*p*-tolylsulfonyl)vinylphosphonate 6b. A pale yellow oil. Yield: 0.232 g (73%); n_D=1.5718; IR (neat) 1590, 1261, 1024; ^{31}P NMR (81 MHz, $CDCl_3$): δ 7.6 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (t, 6H, CH_3CH$_2$OP, J=7.1 Hz); 2.43 (s, 3H, Ar–CH_3); 4.00–4.23 (m, 4H, CH_3CH_2OP); 6.94 (d, 1H, J_{P-H}=18.4 Hz, *cis* C=CH); 7.18 (d, 1H, J_{P-H}=37.5 Hz, *trans* C=CH); 7.32 and 7.81 (4H, aromatic); ^{13}C NMR ($CDCl_3$, 50 MHz); δ 16.0; 21.4; 63.1 (d, J=5.3 Hz); 126.3; 127.8; 128.6; 128.9; 132.9, (d, J=9.0 Hz); 143.2 (d, J=106 Hz). Anal. Calcd for $C_{13}H_{19}O_5PS$: C, 49.05%; H, 6.02%. Found: C, 49.11%; H, 6.13%.

3.6. Synthesis of optically active α-phosphorylvinyl sufoxides 5

Method A. To a stirred solution of optically active α-(diethylphosphoryl)-ethyl *p*-tolyl sulfoxide **7b** (1.52 g, 5 mmol) in 100 mL of dry THF a 2.2 M solution of *n*-butyllithium (5.5 mmol) in hexane was added dropwise at −78 °C. After 5 min, powder of selenium (0.4 g, 5 mmol) was added. The mixture was warmed slowly to −10 °C when dissolution of selenium was observed. A clear dark brown solution was treated then with methyl iodide (0.61 g, 5 mmol) and after 5 min the solution was quenched with 50 mL of aqueous solution of NH_4Cl. Organic solvents were evaporated and the remaining aqueous solution was extracted with chloroform (2×30 mL). The $CHCl_3$ extract was dried over anhydrous $MgSO_4$ and evaporated giving mixture of products ^{31}P NMR: 19.8 ppm (**8** major product), 24.3 ppm (9–10%). The mixture was dissolved in 50 mL of CH_2Cl_2 and 2 mL of H_2O_2/water (1:1) was added at 0 °C and the reaction was stirred vigorously at this temperature for 2 h. Then 30 mL of water was added. The extraction with CH_2Cl_2 afforded the crude product **5b** which was purified by column chromatography (hexane–acetone 10:1).

Method B. To a stirred THF solution (100 mL) of optically active α-phosphoryl sulfoxide **7** (10 mmol) 5 mL of 2.2 M *n*-BuLi in hexane solution (11 mmol) was added at −78 °C. After 5 min solution of phenylselenyl bromide (0.011 mol), prepared by addition of equimolar amount of bromine to 10 mL of THF solution of diphenyldiselenide, was added all

at once. The reaction mixture was stirred for 2–3 min and then poured to the cooled to 0 °C mixture of 20 mL of diethyl ether and 20 mL of aqueous solution of sodium carbonate. The organic layer was separated, dried over $MgSO_4$ and the solvent evaporated affording compound **10**. α-Phenylselenyl substituted α-phosphoryl sulfoxide **10** was then oxidized in CH_2Cl_2 solution (100 mL) using 3 mL of H_2O_2/water mixture in 1:1 ratio. α-Phosphorylvinyl sulfoxide **5** prepared in this way was purified by column chromatography.

3.6.1. α-Diethyl (1-*p*-tolylsulfinyl)vinylphosphonate 5b. A colourless oil. Yield: 2.11 g (70%); $[\alpha]_D=+157$ (*c*, 2.1, acetone); IR (neat) 1243,1021; ^{31}P NMR (81 MHz, $CDCl_3$) δ 9.8 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.18 (t, 1H, $J=7.2$ Hz); 2.39 (s, 3H, C*H*$_3$Ar); 3.89–4.22 (m, 4H, CH_3CH_2-OP); 6.72 (d, 1H, $J_{P-H}=18.8$ Hz, *cis* C=CH); 6.96 (d, 1H, $J_{P-H}=39.8$ Hz, *trans* C=CH); 7.28–7.57 (4H, aromatic). ^{13}C NMR (50 MHz, $CDCl_3$); δ 15.5 (2×d, $J=7.7$ Hz); 21.1; 62.3 (d, $J=5.7$ Hz); 126.0; 129.4; 132.1(d, $J=5.5$ Hz); 138.8; 142.3; 146.5 (d, $J=177.4$ Hz). Anal. Calcd for $C_{13}H_{19}O_4PS$: C, 51.65%; H, 6.33%. Found C, 51.78; H, 6.52%.

3.6.2. α-Diethyl (1-*p*-tolylsulfinyl)-propen-1-ylphosphonate 5c. A colourless oil. Yield (*E*+*Z*): 2.08 g (66%). Isomer *Z*: ^{31}P NMR (81 MHz, $CDCl_3$): δ 12.1 ppm; ^1H NMR (200 MHz, $CDCl_3$):δ 1.21 (t, 3H, $J=7.2$ Hz, C*H*$_3$CH$_2$O); 1.32 (t, 3H, $J=7.2$ Hz, C*H*$_3$CH$_2$O,); 2.29 (dd, 3H, $J=7.3$; 2.9 Hz, CH_3-=); 2.41 (s, 3H, C*H*$_3$Ar); 4.0–4.23 (m, 4H, CH_3CH_2O); 7.43 (dq, 1H, $J_{H-H}=7.4$ Hz, $J_{P-H}=23.3$ Hz); 7.25–7.68 (4H, aromatic). Isomer *E*: ^{31}P NMR (81 MHz, $CDCl_3$): δ 10.1 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.12; 1.16 (2×t, 6H, C*H*$_3$CH$_2$O); 2.22 (dd, 3H, $J_{P-H}=3.1$ Hz, $J_{H-H}=7.3$ Hz); 2.35 (s, 3H, CH_3Ar); 3.6–4.2 (m, 4H, CH_3CH_2O); 7.35 (dq, 1H, $J_{P-H}=41.3$ Hz); 7.20–7.58 (4H, aromatic). Anal. Calcd for $C_{14}H_{21}O_4PS$: C, 53.15%, H, 6.69%. Found C, 53.37%; H, 6.75%.

3.6.3. α-Diethyl (1-*p*-tolylsulfinyl)-1-hexen-1-ylphosphonate 5d. A colourless oil. Yield (*E*+*Z*): 2.28 g (63%). Isomer *E*: $[\alpha]_D=+98$ (*c*, 1.2 acetone); ^{31}P NMR (81 MHz, $CDCl_3$): δ 10.4 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 0.93 (t, 3H, $J_{H-H}=7.1$ Hz); 1.12; 1.16 (2×t, 6H, $J_{H-H}=7.1$ Hz); 1.20–1.57 (m, 4H,); 2.37 (s, 3H, CH_3Ar); 2.53 (m, 2H); 3.56–3.99 (m, 4H, CH_3CH_2O); 7.29 (dt, 1H, $J_{P-H}=41.4$ Hz, $J_{H-H}=7.9$ Hz); 7.22–7.57 (4H, aromatic) ^{13}C (50 MHz, $CDCl_3$): δ 13.4; 15.7; 21.0; 21.9; 29.6 (d, $J=6.2$ Hz); 30.3; 61.7 (d, $J=5.0$ Hz); 126.1; 129.2; 134.8(d, $J=181.3$ Hz); 140.5; 146.6; 150.5(d, $J=7.5$ Hz).

3.7. Preparation of α-phosphorylvinyl selenides 11

α-Phosphoryl α-phenylselenenyl sulfoxide **10** (1 mmol) obtained from α-phosphoryl sulfoxide according to the procedure described for preparation of α-phosphorylvinyl sulfoxides was dissolved in 10 mL of benzene and heated under reflux for 2 h. Evaporation of benzene afforded the crude product **11**.

3.7.1. α-Diethyl (1-phenylselenenyl)vinylphosphonate 11b. A yellow pale oil. Yield: 0.281 g (88%); IR (neat) 1610, 1245, 1024; ^{31}P NMR (81 MHz, $CDCl_3$): δ 14.9 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.32 (td, 6H, $J_{H-H}=7.0$ Hz, $J_{P-H}=0.6$ Hz, C*H*$_3$CH$_2$OP); 3.95–4.25 (m, 4H, CH_3CH_2-OP); 5.73 (d, 1H, $J_{P-H}=44$ Hz); 6.61 (d, 1H, $J_{P-H}=20.4$ Hz); 7.3–7.65 (m, 5H aromatic). ^{13}C (50 MHz, $CDCl_3$); δ 15.6 (d, $J=7.3$ Hz); 61.2 (d, $J=5.5$ Hz); 118.23 (d, $J=8.1$ Hz); 134.1 (d, $J=181.2$ Hz); 126.2; 127.3; 130.3; 131.7. Anal. Calcd for $C_{12}H_{17}O_3PSe$: C, 45.15%; H, 5.37%. Found C, 45.31%; H, 5.48%.

3.7.2. α-Diethyl (1-phenylselenenyl)propenylphosphonate 11c. A yellow pale oil. Yield: 0.276 g (83%); ratio of *E*/*Z* isomers 1:1 separated by column chromatography hexane–acetone 30:1. Isomer *E*: IR (neat) 1610, 1245, 1027; ^{31}P NMR (81 MHz, $CDCl_3$): δ 14.6 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.25 (t, 6H, $J_{H-H}=7.1$ Hz, C*H*$_3$CH$_2$OP); 2.12 (dd, 3H, $J_{H-H}=7.3$ Hz, $J_{P-H}=3.2$ Hz, C*H*$_3$C=); 3.8–4.2 (m, 4H, CH_3CH_2OP); 6.66 (dq, 1H, $J_{P-H}=46$ Hz, $J_{H-H}=7.3$, *H*C=); 7.2–7.6 (m, 5H, aromatic); ^{13}C NMR (50 MHz, $CDCl_3$) 15.8 (d, $J=6.5$ Hz); 18.5 (d, $J=17.0$ Hz); 62.0 (d, $J=5.8$ Hz); 120.5 (d, $J=17.0$ Hz); 126.4; 130.2; 130.6; 154.2 (d, $J=13.8$ Hz); ^{77}Se NMR (57 MHz, $CDCl_3$) δ 283.6, $J=19.2$ Hz. Isomer *Z*: ^{31}P NMR (81 MHz, $CDCl_3$) δ 15.7 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.26 (dt, 6H, $J_{H-H}=7.1$ Hz, $J_{P-H}=0.5$ Hz, C*H*$_3$CH$_2$O); 2.10 (dd, 3H, $J_{P-H}=3.0$ Hz, $J_{H-H}=6.9$ Hz, C*H*$_3$C=); 3.9–4.25 (m, 4H, CH_3CH_2OP); 7.52 (dq, 1H, $J_{P-H}=19.1$ Hz, $J_{H-H}=6.9$ Hz, *H*C=); 7.2–7.5 (m, 5H, aromatic). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.1 (d, $J=6.7$ Hz); 18.4 (d, $J=6.4$ Hz); 62.1 (d, $J=5.4$ Hz); 120.5 (d, $J=189.5$ Hz); 126.4; 128.6; 130.2; 130.6; 154.2 (d, $J=13.8$ Hz). Anal. Calcd for $C_{13}H_{19}O_3PSe$: C, 46.86%; H, 5.75%. Found: C, 46.93%; H, 5.91%.

3.7.3. α-Diethyl (1-phenylselenenyl)hexenylphosphonate 11d. A yellow pale oil. Yield: 0.322 g (86%); ratio of *E*/*Z* isomers 1:1. Separated isomer *E*: ^{31}P NMR (81 MHz, $CDCl_3$): δ 14.7 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 0.8–1.6 (m, 7H); 1.25 (6H, t, $J_{H-H}=7.0$ Hz, C*H*$_3$CH$_2$OP); 2.57 (tdd, 2H, $J_{H-H}=7.8$, 7.2 Hz, $J_{P-H}=2.6$ Hz, C*H*$_2$C=); 4.0 (4H, m, CH_3CH_2OP); 6.58 (dt, 1H, $J_{P-H}=46$ Hz, $J_{H-H}=7.8$ Hz, *H*C=); 7.25–7.56 (5H, aromatic). ^{13}C NMR (50 MHz, $CDCl_3$): 13.2; 15.8 (d, $J=6.1$ Hz); 21.8; 30.2; 34.4 (d, $J=22.1$ Hz); 62.1 (d, $J=5.7$ Hz); 117.1 (d, $J=185$ Hz); 147.2 (d, $J=5.7$ Hz); 126.1; 126.9; 130.7; 131.2. Anal. Calcd for $C_{16}H_{25}O_3PSe$: C, 51.21%; H, 6.71%. Found: C, 51.45%, H, 6.95%.

3.8. Oxidation of selenides 11 to selenoxides 12

Method A. 2 mmol of the selenide **11** was dissolved in acetone (10 mL) and aqueous solution of sodium metaperiodate (2 mmol) was added dropwise at 0 °C. The reaction mixture was kept overnight in refrigerator and on the next day 20 mL of water added and extracted with $CHCl_3$ (3×15 mL).

Method B. To a solution of 2 mmol of the selenide **11** in CH_2Cl_2 0.25 mL of 30% H_2O_2 was added and reaction mixture was stirred vigorously for 15 min. After then 10 mL of water was added and reaction was extracted with (3×10 mL) CH_2Cl_2.

3.8.1. 1-(Diethoxyphosphoryl)vinyl phenyl selenoxide

12b. A slightly yellow oil. Yield: 0.67 g (~100%); IR (neat) 1243, 1025, 843; ^{31}P NMR (81 MHz, $CDCl_3$) δ 10.8 ppm; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.15 (t, 6H, J_{H-H}=7.1 Hz, CH_3CH$_2$OP); 3.7–4.2 (m, 4H, CH$_3$CH_2OP); 6.77 (dd, 1H, J_{P-H}=18.4 Hz, J_{H-H}=1 Hz, *cis* C=CH); 7.12 (dd, 1H, J_{H-H}=1 Hz, J_{P-H}=41.1 Hz, *trans* C=CH); 7.4–7.82 (m, 5H, aromatic).

3.8.2. 1-(Diethylphosphoryl)propenyl phenyl selenoxide *E*-12c. A slightly yellow oil. Yield: 0.649 g (93%); IR (neat) 1247, 1021, 845; ^{31}P NMR (81 MHz, $CDCl_3$): δ 11.7 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.22 (t, 6H, J_{H-H}=7.1 Hz, CH_3CH$_2$OP); 2.15 (dd, 3H, J_{H-H}=7.3 Hz, J_{P-H}=3.0 Hz, CH_3C=); 3.4–4.2 (m, 4H, CH$_3$CH_2OP); 7.45 (dq, 1H, J_{P-H}=41.9 Hz, J_{H-H}=7.3 Hz, *trans* C=CH); 7.37–7.85 (m, 5H, aromatic).

Compound **Z-12c**. Yield: 0.677 g (97%); ^{31}P NMR (81 MHz, $CDCl_3$) δ 12.8 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.04 (td, 6H, J_{H-H}=7.0 Hz, J_{P-H}=0.5 Hz, CH_3CH$_2$OP); 2.22 (dd, 3H, J_{P-H}=2.8 Hz, J_{H-H}=7.3 Hz, CH_3C=); 4.15 (dq, 4H, J_{P-H}=8.5 Hz, J_{H-H}=7.0 Hz, CH$_3$CH_2OP); 7.46 (dq, 1H, J_{H-H}=7.3 Hz, J_{P-H}=21.2 Hz, C=CH); 7.22–7.61 (m, 5H, aromatic).

3.8.3. 1-(Diethylphosphoryl)hexenyl phenyl selenoxide *E*-12d. A slightly yellow oil. Yield: 0.75 g (96%); ^{31}P NMR (81 MHz, $CDCl_3$) 12.0 ppm ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.78–1.62 (m, 7H); 1.24 (t, 3H, J_{H-H}=7.0 Hz, CH_3CH$_2$OP); 2.55 (m, 2H, CH_2C=); 3.4–4.25 (m, 4H, CH$_3$CH_2OP); 7.38 (dt, J_{P-H}=42 Hz, J_{H-H}=7.9 Hz, CH=); 7.4–7.8 (m, 5H).

3.9. Preparation of alkynylphosphonates 13

The benzene solution (10 mL) of 1 mmol of the selenoxide **12** was heated under reflux for 3 h. After evaporation of solvent, alkynylphosphonate **13** was purified by distillation on Kugel Rohr.

3.9.1. Diethylphosphorylacetylene 13b. A slightly yellow oil. Yield: 0.15 g (93%); bp 80–85 °C/10 mm Hg. ^{31}P NMR (81 MHz, $CDCl_3$): δ 7.7 ppm; ^{1}H NMR (200 MHz, $CDCl_3$)): δ 1.38 (td, 6H, J_{H-H}=7.1 Hz, J_{P-H}=0.7 Hz, CH_3CH$_2$OP); 2.89 (d, 1H, J_{P-H}=13.2 Hz), 4.09 (dq, 4H, J_{P-H}=8.1 Hz, J_{H-H}=7.1 Hz CH$_3$CH_2OP ^{13}C NMR (50 MHz, $CDCl_3$): 15.8 (d, ^3J=6.9 Hz); 62.1 (d, ^2J=6.3 Hz); 70.2 (d, ^1J=307 Hz); 101.0 (d, ^2J=57.1 Hz). HRMS (70 eV) $C_6H_{11}O_3P$ requires 162.04458. Found: 162.0459.

3.9.2. Diethyl phosphorylpropyne 13c. A slightly yellow oil. Yield: 0.142 g (81%); bp 88–92 °C/10 mm Hg ^{31}P NMR (81 MHz, $CDCl_3$) δ 5.7 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (td, 6H, J_{H-H}=7.1 Hz, J_{P-H}=0.8 Hz, CH_3CH$_2$OP); 2.00 (d, 3H, J_{P-H}=4.7 Hz, CH_3C), 4.13 (dq, 4H, J_{P-H}=7.8 Hz, J_{H-H}=7.1 Hz, CH$_3$CH_2OP). ^{13}C NMR (50 MHz, $CDCl_3$): 4.4 (d, ^3J=4.7 Hz); 16.0 (d, ^3J=7.1 Hz); 61.7 (^2J=5.9 Hz); 69.9 (d, ^1J=305 Hz); 98.9 (d, ^2J=54.7 Hz). HRMS (70 eV) $C_7H_{13}O_3P$ requires 176.06023. Found 176.0592.

3.9.3. Diethyl phosphorylhexyne 13d. A slightly yellow oil. Yield: 0.207 g (95%); bp 50–55 °C/2 mm Hg ^{31}P NMR (81 MHz, $CDCl_3$): δ 5.4 ppm; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.92 (t, 3H, J=7.1 Hz); 1.22–1.60 (m, 2H); 1.37 (dt, 6H, J_{H-H}=7.0 Hz, J_{P-H}=0.7 Hz, CH_3CH$_2$OP); 2.35 (dt, 2H, J_{H-H}=7.0 Hz, J_{P-H}=4.4 Hz); 4.15 (dq, 4H, J_{P-H}=8.6 Hz, J_{H-H}=7.0 Hz, CH$_3$CH_2OP). HRMS (70 eV) $C_{10}H_{19}O_3P$ requires 218.1072. Found 218.1032.

3.10. Nucleophilic addition to α-phosphorylvinyl *p*-tolyl sulfoxide 5c

3.10.1. α-Diethyl (1-*p*-tolylsulfinyl)-(2-dimethylamino)-ethylphosphonate 7e. Cooled dimethylamine (0.5 mL) was added to 0.3 g (1 mmol) of α-phosphorylvinyl sulfoxide at 0 °C. The reaction mixture was stirred overnight. An excess of Me_2NH was removed affording **7e** as a mixture of diastereomers 2:1 obtained in quantitative yield. ^{31}P NMR (81 MHz, $CDCl_3$) 21.6/19.8 ppm ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 (t, 3H, J=7.1 Hz, CH_3CH$_2$OP); 2.20 (s, 3H, CH_3N); 2.22 (s, 3H, CH_3N); 2.40 (s, 3H, CH_3Ar); 2.74–2.87 (m, 2H, NCH$_2$); 3.08 (td, 1H, J_{P-H}=17.5 Hz, J_{H-H}=6.0 Hz—major); 3.35 (td, 1H, J_{P-H}=17.9 Hz, J_{H-H}=6.2 Hz—minor); 3.97–4.22 (m, 4H, CH$_3$CH_2OP); 7.26–7.34 and 7.51–7.62 (m, 4H, aromatic).

3.10.2. α-Diethyl (1-*p*-tolylsulfinyl)-(2-methoxy)-ethylphosphonate 7f. To a stirred methanol solution (25 mL) of 0.3 g (1 mmol) of (*S*)-(1-diethoxyphosphoryl)vinyl *p*-tolyl sulfoxide 0.057 g (1.2 mmol) of NaH (50%) was added at room temperature. After 2 h of stirring the reaction was quenched with aqueous solution of NH_4Cl, solution was extracted with chloroform (3×30 mL). The $CHCl_3$ extract was dried over anhydrous $MgSO_4$ and after evaporation afforded mixture of diastereomers: ^{31}P NMR (81 MHz, $CDCl_3$) 19.5/19.4 ppm in 2:1 ratio. Purification by column chromatography (hexane–acetone 10:1). Yield: 0.25 g (75%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (t, 6H, J=7.1 Hz, CH_3CH$_2$OP); 2.40 (s, 3H, CH_3Ar); 3.20 (td, 1H, J_{P-H}=17.2 Hz, J_{H-H}=3.6 Hz); 3.34 (s, 3H, CH_3O); 3.87–4.25 (6H, m, CH$_3$CH_2OP+CH$_3$OCH_2); 7.23–7.36 and 7.57–7.69 (4H, aromatic). ^{13}C NMR (50 MHz, $CDCl_3$): 16.0 (d, ^3J=4.8 Hz); 21.3; 58.9; 62.6 (d, J=6.4 Hz); 64.5 (d, J=138.4 Hz); 65.1 Hz; 125.4; 126.0; 129.4; 141.9. Anal. Calcd for $C_{14}H_{23}O_5PS$: C, 49.93%; H, 6.91%. Found: C, 49.82%, H, 6.95%.

3.10.3. α-Diethyl (1-*p*-tolylsulfinyl)-(2,2-dimethyl)-(2-nitro)-ethylphosphonate 7g. To a stirred THF solution (50 mL) of 0.3 g (1 mmol) of (*S*)-(1-diethoxyphosphoryl)-vinyl *p*-tolyl sulfoxide **5c** sodium salt of 2-nitropropane, generated by addition 0.057 g (1.2 mmol) of NaH (50%) to 0.12 g of 2-nitropropane in THF solution (15 mL), was added at 0 °C. The reaction mixture was stirred for 2 h (0 °C to room temperature) and was quenched with aqueous solution of NH_4Cl. Organic solvents were evaporated and the remaining aqueous solution was extracted with chloroform (2×30 mL). The $CHCl_3$ extract was dried over anhydrous $MgSO_4$ and evaporated giving mixture of diastereomers ^{31}P NMR (81 MHz, $CDCl_3$) 21.7/18.5 ppm in 2:1 ratio. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.22 (s, 3H); 1.32 (t, 6H, J=7.0 Hz, CH_3CH$_2$OP); 1.47 (s, 3H); 2.40 (s, 3H, CH_3Ar); 2.32–2.6 (m, 2H); 3.03 (major) (ddd, 1H, J_{P-H}=17.2 Hz, J_{H-H}=5.8, 3.4 Hz, PC*H*S); 3.35 (minor) (ddd, 1H, J_{P-H}=20.3 Hz, J_{H-H}=6.9, 3.6 Hz); 3.95–4.15

(4H, m, CH_3CH_2OP); 7.27–7.36 and 7.47–7.64 (4H, aromatic).

3.10.4. α-Diethyl (1-*p*-tolylsulfinyl)-(2,2-dicarboethoxy)-ethylphosphonate 7h. To a stirred THF solution (50 mL) of 0.3 g (1 mmol) of (*S*)-(1-diethoxyphosphoryl)vinyl *p*-tolyl sulfoxide **5c** lithium salt of diethyl malonate, generated by addition 0.016 g (2 mmol) of LiH to 0.19 g of diethyl malonate in THF solution, was added at 0 °C. The reaction mixture was stirred for 2 h (0 °C to room temperature) and was quenched with aqueous solution of NH_4Cl. Organic solvents were evaporated and the remaining aqueous solution was extracted with chloroform (2×30 mL). The $CHCl_3$ extract was dried over anhydrous $MgSO_4$ and evaporated giving mixture of diastereomers: ^{31}P NMR (81 MHz, $CDCl_3$) 21.6/18.9 ppm in 2:1 ratio, purified by column chromatography (hexane–acetone 10:1). Yield: 0.41 g (88%); ^1H NMR (200 MHz, $CDCl_3$): δ 1.1–1.38 (m, 12H, $CH_3CH_2OP+CH_3CH_2OC$); 1.38–1.79 (m, 2H); 2.37 (s, 3H, CH_3Ar); 3.16 (major) (td, 1H, $J_{P-H}=17.4$ Hz, $J_{H-H}=7.2$ Hz); 3.47 (minor) (td, 1H, $J_{H-H}=7.4$ Hz, $J_{P-H}=14.4$ Hz); 3.63 (major) (t, 1H, $J_{H-H}=7.1$ Hz); 3.87 (minor) (t, 1H, $J_{H-H}=7.1$ Hz); 3.87–4.21 (m, 8H, $CH_3CH_2OP+CH_3CH_2OC$); 7.25–7.36 (m, 2H, aromatic) and 7.47 (major) and 7.64 (minor) (4H, aromatic). Anal. Calcd for $C_{14}H_{31}O_8PS$: C, 51.94%; H, 6.76%. Found: C, 51.79.%, H, 6.95%.

3.11. Reaction of vinyl selenide 11c with Me_2NH

Cooled dimethylamine was added to α-phosphorylvinyl selenide **11c** (0.23 g, 1 mmol) at 0 °C. The reaction mixture was stirred overnight. An excess of Me_2NH was removed and residue was a mixture of diastereomers of **14** obtained in quantitative yield, ^{31}P NMR (81 MHz, $CDCl_3$) 26.6/26.7 ppm.

From *Z*-**11**—ratio 2:1 from *E*-**11**—ratio 10:1. ^1H NMR (200 MHz, $CDCl_3$): δ 1.25–1.34 (9H, m, $CH_3CH_2OP+CH_3C$); 2.22 and 2.25 (2×s, 6H, CH_3N); 3.07–3.17 (m, 1H, CHN); 3.22 (dd, 1H, $J_{P-H}=17.9$ Hz, $J_{H-H}=3.8$ Hz, PCHSe); 4.02–4.29 (m, 4H, CH_3CH_2OP); 7.23–7.69 (m, 5H, aromatic).

3.12. Oxidation of phosphonate 14

To a solution of adduct **14** (mixture of diastereomers) in CH_2Cl_2 mixture of 30% H_2O_2 and water (1:1) was added at (a) −20 °C, (b) room temperature. The reaction was stirred for 0.5–1 h at appropriate temperature yielding mixture of products where β-ketophosphonate **15** (a) or α-phosphorylvinyl selenoxide *E*-**12** (b) as major ones. In both cases *E*-**12** and **15** were separated by chromatography.

3.13. Nucleophilic addition to α-phosphorylpropenyl phenyl selenoxide 12c

3.13.1. Diethyl malonate. To a solution of 3 mmol of diethyl malonate in 20 mL THF, 0.16 g (3.3 mmol) of sodium hydride 50% in oil was added at room temperature. After 30 min the reaction mixture was cooled down to −78 °C and vinyl selenoxide *E*-**12c** (3 mmol) was added. The stirred reaction mixture was warmed up to room temperature, quenched with aqueous NH_4Cl (20 mL) and the product extracted with $CHCl_3$ (3×10 mL). The organic solution was dried over $MgSO_4$, solvent evaporated and the product **16** purified by column chromatography (hexane–acetone 18:1). Yield: 0.628 g (65%); IR(neat) 1729, 1252, 1044; ^{31}P NMR (81 MHz, $CDCl_3$) δ 24.3 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.26 (t, 6H, $J=7.1$ Hz, CH_3CH_2OP); 1.29 (t, 3H, $J_{H-H}=7.0$ Hz, CH_3); 1.31 (t, 3H, $J_{H-H}=7.0$ Hz; 2.36 (s, 3H, $CH_3C=$); 3.37 (d, 2H, $J=24.9$ Hz, $PCH_2C=$); 3.93–4.32 (m, 4H, CH_3CH_2OP).

2-Nitropropane. To a solution of 2-nitropropane (1 mmol) in 30 mL THF potassium *t*-butoxide (1 mmol) was added at room temperature and the mixture was stirred for 30 min. Then the phosphoryl selenoxide *E*-**12c** (1 mmol) was added and this mixture was stirred at room temperature for the next 0.5 h. The reaction was quenched with aqueous NH_4Cl (10 mL) and the product extracted with $CHCl_3$ (3×10 mL). The organic solution was dried over $MgSO_4$, solvent evaporated giving mixture of two products **17** and **18** in about 1:1 ratio, defined by ^{31}P NMR spectra. Both products were separated by column chromatography (benzene/acetone).

3.13.2. Diethyl (2,3-dimethyl)(3-nitro) buten-1 phosphonate 17. A pale yellow oil. Yield: 0.11 g (42%); IR (neat) 1241, 1050, 1025; ^{31}P NMR (81 MHz, $CDCl_3$): δ 16.8 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 1.33 (t, 6H, $J_{H-H}=7.0$ Hz, CH_3CH_2OP); 1.74 (s, 6H, CH_3CN); 2.08 (dd, 3H, $J_{H-H}=1$ Hz, $J_{P-H}=3.3$ Hz, $CH_3C=$); 4.10 (dq, 4H, $J_{P-H}=8.1$ Hz, $J_{H-H}=7.0$ Hz, CH_3CH_2OP); 5.73 (dq, 1H, $J_{P-H}=13.4$, $J_{H-H}=1$ Hz).

3.13.3. Diethyl 1-propen-3-ol phosphonate 18c. Yield: 0.087 g (45%); ^{31}P NMR (81 MHz $CDCl_3$) δ 19.5 ppm; ^1H NMR (200 MHz $CDCl_3$): δ 1.28 (t, 6H, $J_{H-H}=7.1$ Hz, CH_3CH_2OP); 2.93 (m, 1H, OH); 4.03 (dq, 4H, $J_{H-H}=7.1$ Hz, $J_{P-H}=7.7$ Hz CH_3CH_2OP); 4.22 (m, 2H, CH_2OH); 5.95 (tdd, 1H, $J_{H-H}=2.1$, 17.2 Hz, $J_{P-H}=21.3$ Hz, PCH=); 6.8 (tdd, 1H, $J_{H-H}=3.6$, 17.2 Hz, $J_{P-H}=22.6$ Hz, CH=).

Overhauser effect of **17**. Irradiation of the vinyl proton (δ 5.73 ppm) caused 21% increasing of signal 2.08 (dd, CH_3). Irradiation of the methyl protons (δ 2.08 ppm) caused a 17% enhancement of signal 5.73 (dq, =CH).

3.13.4. Diethyl 1-hexen-3-ol phopsphonate 18d. Yield: 0.18 g (76%); $[\alpha]_D=+8.1$ (*c* 0.5, acetone), ^{31}P NMR (81 MHz, $CDCl_3$); δ 19.8 ppm; ^1H NMR (200 MHz, $CDCl_3$): δ 0.91 (t, 3H, $J=7.2$ Hz, CH_3CH_2); 1.30 (t, 6H, $J=7.0$ Hz, CH_3CH_2OP); 1.2–1.6 (m, 4H); 2.63 (m, 1H, OH); 4.05 (dq, 4H, $J_{H-H}=7.0$ Hz, $J_{P-H}=8.2$ Hz, CH_3CH_2-OP); 4.25 (m, 1H, CHOH); 5.90 (ddd, 1H, $J_{H-H}=17.1$, 1.7 Hz, $J_{P-H}=21.0$ Hz, PCH); 6.77 (ddd, 1H, $J_{H-H}=17.1$, 4.2 Hz, $J_{P-H}=22.4$ Hz, CH=).

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One-pot synthesis of fluorine containing 3-cyano/ethoxycarbonyl-2-methyl-benzo[*b*]furans☆

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Abstract—Fluoro-substituted 3-cyano-2-methyl-benzo[*b*]furans and ethyl 2-methyl-benzo[*b*]furan-3-carboxylates are conveniently prepared in a single step in good yield by the microwave induced tandem intramolecular Wittig and Claisen rearrangement reactions of the corresponding [(aryloxyacetyl) (cyano) methylene] triphenylphosphorane and [(aryloxyacetyl) (ethoxycarbonyl) methylene] triphenylphosphoranes, respectively.

1. Introduction

Benzo[*b*]furan derivatives are an important class of organic compounds, which are known to be present in many natural products and possess physiological activity. Their application in agrochemicals,[1,2] pharmaceuticals,[1,3] cosmetics,[4,5] polymers and dyes[1] prompted development of various synthetic methods.[6] Claisen rearrangement of aryl propargylic ethers offers[7] the most elegant route for the synthesis of 2-alkyl-3-substituted benzo[*b*]furans. Previous research in our laboratory[8] established the formation of 2-alkyl-3-cyano-benzo[*b*]furans and 4-cyanobenzopyrans during thermolysis of [(aryl-oxyacetyl) (cyano) methylene] triphenylphosphoranes. In this method, decomposition of phosphoranes is conducted at elevated temperatures (250–300 °C) and the product is continuously distilled out of the reaction vessel under high vacuum. The aryl propargylic ether, allenyl phenol and the dienone intermediates are thermally unstable and decompose due to prolonged heating and result in the formation of phenols and polymeric mass, thereby reducing the isolated yield of the benzopyrans/benzofurans. We have overcome this problem by subjecting the solid phosphoranes to microwave irradiation for shorter duration. To our knowledge, fluorine-containing 2-alkyl-3-cyano/ethoxycarbonyl-benzofurans have not been reported. In the present investigation, we report for the first time the microwave assisted, facile synthesis of 3-cyano-2-methyl-benzo[*b*]furan and ethyl 2-methyl-benzo[*b*]furan-3-carboxylate derivatives from the corresponding [(fluoro-aryloxyacetyl) alkylidene] triphenylphosphoranes.

2. Results and discussion

[(Aryloxyacetyl) (cyano/ethoxycarbonyl) methylene] triphenylphosphoranes **8/9** were prepared in good yield by the transylidation reaction[9] of [(cyano/ethoxycarbonyl) methylene] triphenylphosphoranes **6/7** with corresponding aryloxyacetyl chloride **5**. The aryloxyacetyl chlorides **5** are prepared starting from the corresponding phenol **1** via ethyl aryloxyacetate **3** and aryloxyacetic acid **4**. The sequence of reactions is depicted in Scheme 1. Ethyl 3-chloro-4-fluoro-phenoxyacetate **3d**, 3-chloro-4-fluoro-phenoxy-acetic acid **4d**, 2-chloro-4-fluoro-phenoxyacetyl chloride **5b**, 3-chloro-4-fluoro-phenoxyacetyl chloride **5d** and all the ylides **8/9** reported in this study are new compounds and were fully characterized.

The [(aryloxyacetyl) alkylidene] triphenylphosphoranes **8/9** on microwave irradiation for 6–8 min resulted in the exclusive formation of 2-methyl-benzo[*b*]furan derivatives **13/14** (32–82%) along with triphenylphosphine oxide **12** (Table 1). The formation of 2-methyl-benzo[*b*]furan derivatives **13/14** is seen as a result of tandem intramolecular Wittig, Claisen rearrangement reactions[8]

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Keywords: Transylidation; Aryloxyacetyl alkylidenetriphenylphosphorane; Intramolecular Wittig reaction; Claisen rearrangement; 3-Cyano-2-methyl-benzofurans.

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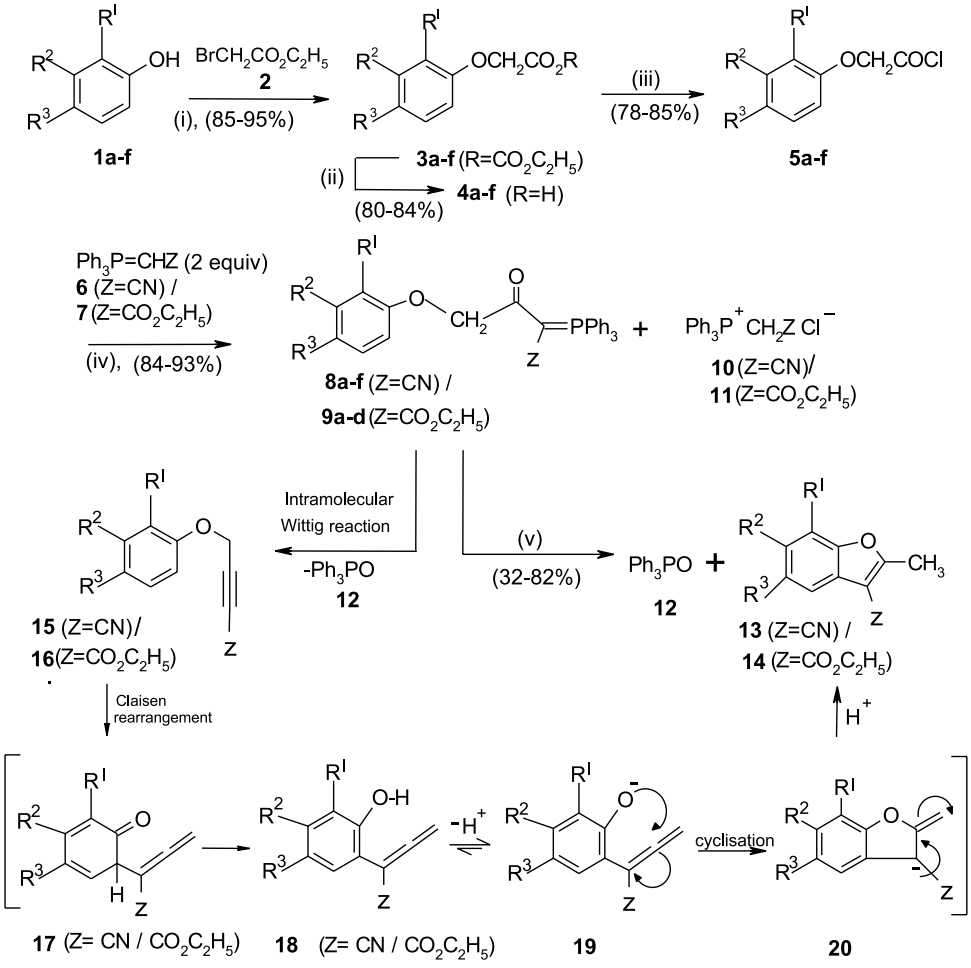


Scheme 1. K_2CO_3/acetone/NaI, △/3 h; (ii) 10% aq KOH, △/2 h; (iii) $SOCl_2$, hexane, △/1 h; (iv) DCM, rt/3 h; (v) MW/6–8 min. (a) $R^1=R^2=H$, $R^3=F$; (b) $R^1=Cl$, $R^2=H$, $R^3=F$; (c) $R^1=R^3=F$, $R^2=H$; (d) $R^1=H$, $R^2=Cl$, $R^3=F$; (e) $R^1=R^3=H$, $R^2=CF_3$; (f) $R^1=R^3=H$, $R^2=CH_3$.

followed by ring closure of the allenyl phenol intermediate **18**. The reaction pathway is shown in Scheme 1.

The formation of benzo[*b*]furan derivatives **13/14** is believed to have been facilitated by the formation of phenolate anion **19** which cyclizes[7] in preference to the 1,5-H shift.

The presence of polar substituents, polar medium in the form of triphenylphosphine oxide, **12** and the microwave energy seem to favor dissociation of phenol **18** in to the phenolate anion **19**.

The ^{1}H NMR spectra of all the benzofurans showed the presence of methyl proton signals as a singlet in the range δ 2.7–2.8 ppm. The IR spectra of the new 3-cyano-2-methyl-benzofuran analogues **13** prepared in this study showed the presence of CN absorption in the region 2230–2233 cm^{-1}.

Table 1. Synthesis of 2-methyl-benzo[*b*]furans **13** and **14**

S. No.	Ylide	R^1	R^2	R^3	Time (min)		Product	Yield (%)	
					MW	Thermal		MW	Thermal
1	**8a**	H	H	F	8	35	**13a**	73	5[a]
2	**8b**	Cl	H	F	8	—	**13b**	82	—
3	**8c**	F	H	F	8	—	**13c**	76	—
4	**8d**	H	Cl	F	7	—	**13d**	76	—
5	**8e**	H	CF_3	H	6	28	**13e**	32[b]	0[c]
6	**8f**	H	CH_3	H	7	—	**13g**	70	—
7	**9a**	H	H	F	7	30	**14a**	71	56
8	**9b**	Cl	H	F	7	—	**14b**	79	—
9	**9c**	F	H	F	6	—	**14c**	75	—
10	**9d**	H	Cl	F	7	—	**14d**	78	—

[a] In addition to compound **13a**, phenoxy acetylene **15a** was isolated in 51% yield.
[b] In addition to compound **13e**, phenoxy acetylene **15e** was isolated in 9% yield.
[c] Phenol **1e** was isolated in 73% yield.

The ester group present in the newly prepared ethyl 2-methyl-benzo[*b*]furan-3-carboxylates **14** showed C=O absorptions in the region 1705–1708 cm^{-1} in IR spectra.

The decomposition of ylides **8a–c** and **9a–c** (R^2=H) is expected to yield only one regioisomer of the corresponding 2-methyl-benzofurans **13a–c** and **14a–c** (Scheme 1). The ylides **8d–f** and **9d** (R^1=H; R^2=Cl, CF_3 or CH_3) could result in the formation of two regio-isomers of the respective 2-methyl-benzofurans due to the possibility of Claisen rearrangement of the initially formed aryl propargylic ethers **15d–f** and **16d** occurring in either direction (Schemes 1 and 2). In this study we have obtained a single 2-methyl-benzofuran regioisomer for each of these ylides.

The exclusive formation of 6-chloro-3-cyano-5-fluoro-2-methyl-benzofuran **13d** and 6-chloro-3-ethoxycarbonyl-5-fluoro-2-methyl-benzofuran **14d** is explained by the presence of a bulky Cl group at *meta* position in the aryl propargylic ether intermediates **15d** and **16d** which hinders its *ortho* position and favors Claisen rearrangement to take place away from it. Support for the structure of **13d** came from ^{1}H NMR which showed two doublets for 1H each at δ 7.40 (J=8.1 Hz) and 7.56 ppm (J=6.0 Hz) with different coupling constants attributable to $^3J_{H-F}$ and $^4J_{H-F}$ for the C_4–*H* and C_7–*H*, respectively. ^{1}H NMR signals for C_4–*H* and C_7–*H* in **14d** appeared at δ 7.67 (d, 1H, J=8.2 Hz) and 7.46 ppm (d, 1H, J=6.0 Hz). The absence of any H–H couplings and the observed H–F couplings (three bond and four bond) further support the structure **14d** for this regioisomer. Similarly, the presence of a powerful electron withdrawing CF_3 group at *meta* position in **15e** does not favor Claisen rearrangement towards its *ortho* position. Consequently, the decomposition of **8d** resulted in the formation of 4-[3^1-(trifluoromethyl) phenoxy]-but-2-ynenitrile **15e** along with 3-cyano-2-methyl-6-trifluoromethyl-benzofuran **13e** in reduced yield due to the rearrangement occurring at *para* position to CF_3 group. The product **13e** showed in its ^{1}H NMR spectrum a singlet for 1H at δ 7.64 for C_7–*H* and two doublets for 1H each at 7.47 and 7.68 ppm with a $^3J_{H-H}$ of 7.5 Hz assignable to C_4–*H* and C_5–*H*, respectively. The presence of a singlet and the absence of a triplet eliminates the presence of other possible regioisomer. The methyl substituent present in **15f** favors the rearrangement to its *ortho* position resulting in the formation of 3-cyano-2,4-dimethyl-benzofuran **13g** (Scheme 2). The ^{1}H NMR of the product **13g** showed a triplet for ^{1}H and δ 7.16 and two doublets at 7.03 and 7.28 ppm with $^3J_{H-H}$ of 7.5 Hz supporting the 3-cyano-2,4-dimethyl-benzofuran **13g** structure for this regioisomer.

The versatility of the exclusive formation of 2-methyl-benzo[*b*]furans **13/14** in excellent yield from [(aryloxyacetyl) alkylidene] triphenylphosphoranes **8/9** by microwave assisted tandem intramolecular-Wittig, Claisen rearrangement and cyclization reactions is demonstrated by the presence of substituents at various positions in the aryl moiety. However, the presence of electron withdrawing substituents in the aryl moiety had a detrimental effect on the yield of 2-methyl-benzofuran **13/14**. The presence of a *meta* CF_3 group in the aryl moiety resulted in the formation of 4-[(3^1-trifluoromethyl) phenoxy] but-2-ynenitrile, **15e** along with **13e**. All the benzofuran derivatives **13/14** prepared in this study are new compounds and were characterized by NMR and IR spectroscopy and mass spectrometry.

Thermolysis of [(4-fluorophenoxyacetyl) (cyano) methylene] triphenylphosphorane **8a** at 250–270 °C gave 4-(4^1-fluorophenoxy)-but-ynenitrile **15a** as the major compound with traces of 3-cyano-5-fluoro-2-methyl-benzofuran **13a** along with triphenylphosphine oxide **12**. Under similar conditions, [(4-fluorophenoxyacetyl) (cyano) methylene] triphenylphosphorane **9a** resulted in the formation of 3-ethoxycarbonyl-5-fluoro-2-methyl-benzofuran **14a** in 56% yield. However, in the thermolysis of [(3-trifluoromethylphenoxyacetyl) (cyano) methylene] triphenylphosphorane **8e** at 240–270 °C, 3-trifluoromethylphenol **1e** and triphenylphosphine oxide **12** are the only isolable products.

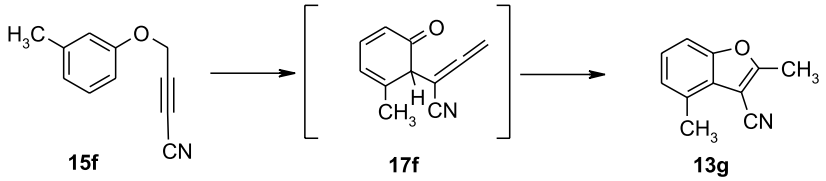
3. Conclusions

In this investigation, we have developed a solvent free microwave assisted one-pot synthesis of several new fluoro-substituted benzo[*b*]furans from the corresponding [(aryloxyacetyl) alkylidene] triphenylphosphoranes. The reaction combines intramolecular Wittig reaction of phosphoranes and Claisen rearrangement of the resulting aryl propargylic ethers followed by ring closure resulting in the exclusive formation of the corresponding benzo[*b*]furan derivatives and triphenylphosphine oxide in good yield. This method involves simple and general sequence, and offers a convenient one-pot synthesis of any 3-cyano/ethoxycarbonyl-2-methyl-benzo[*b*]furans in the laboratory.

4. Experimental

4.1. General

Melting points were determined in open glass capillaries on a Fisher Johnes melting point apparatus and are uncorrected IR spectra were recorded on FT-IR Schimadzu Perkin–Elmer 1310 infrared spectrophotometer. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded



Scheme 2.

on Varian Gemini spectrometer in $CDCl_3$ solvent using TMS as internal standard. Mass spectra were recorded on a VG-micro mass 7070H instrument at 70 eV. Elemental analyses were carried out on EI Elemental Vario EL (Germany) apparatus. Microwave irradiations were carried out using sealed tube (Aldrich, Ace pressure tube, 10.2 cm, 15 mL) in a domestic microwave oven (BPL BMO 700T).

4.2. General method for the preparation of ethyl aryloxyacetylacetate, 3a–f

Equimolar quantities of the respective fluoro-phenol **1** (10 mmol), ethyl bromoacetate **2** (1.67 g, 10 mmol) and catalytic amount of sodium iodide (10 mg) were refluxed in dry acetone (10 mL) in the presence of excess anhydrous potassium carbonate (2.76 g, 10 mmol) for 3 h. Acetone was removed from the reaction mixture and the residue was washed with water (25 mL). The resulting sufficiently pure solid ethyl aryloxyacetylacetate **3a–f** was filtered and dried.

4.2.1. Ethyl 3-chloro-4-fluoro-phenoxyacetate (3d). 1.46 g, 92% as a white solid; mp 48 °C; [found: C, 51.63; H, 4.31. $C_{10}H_{10}ClFO_3$ requires C, 51.62; H, 4.33%]; ν_{max} (KBr) 2926, 1738, 1155 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.41 (3H, t, $J=7.1$ Hz, CH_3), 4.26 (2H, q, $J=7.1$ Hz, CH_2), 4.53 (2H, s, OCH_2), 6.71–6.78 (1H, m), 6.90–6.95 (1H, m) and 6.98–7.04 (1H, m); *m/z* (EI-MS) 232 (M^+, 78), 234 (M+2, 26), 161 (33), 159 (100), 141 (16), 129 (71%).

4.3. General method for the preparation of aryloxyacetic acid (4a–f)

The ester **3** (10 mmol) was hydrolyzed in refluxing 10% aq potassium hydroxide (0.56 g, 10 mmol) solution for 1 h. The reaction mixture was cooled and neutralized with dilute HCl (2.5 mL). The title compound fluorophenoxyacetic acid **4a–f** obtained as solid, was filtered, washed with water (25 mL) and dried.

4.3.1. 3-Chloro-4-fluoro-phenoxyacetic acid (4d). 1.62 g, 80% as a white solid; mp 104–105 °C; [found: C, 47.01; H, 2.96. $C_8H_6ClFO_3$ requires C, 46.96; H, 2.96%]; ν_{max} (KBr) 3320, 2925, 1715, 1199 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.88 (2H, s, OCH_2), 6.73–6.77 (1H, m), 6.85–6.88 (1H, m), 7.18–7.21 (1H, m); *m/z* (EI-MS) 204 (M^+, 100), 187 (75%).

4.4. General method for the preparation of aryloxyacetyl chloride (5a–f)

The fluorophenoxyacetic acid **4** (10 mmol) was refluxed with freshly distilled thionyl chloride (2.36 g, 20 mmol) in hexane (10 mL) for 1–2 h. The hexane and excess thionyl chloride was removed by distillation at atmospheric pressure. Pure aryloxyacetyl chloride **5a–f** was obtained by distillation under reduced pressure.

4.4.1. 2-Chloro-4-fluoro-phenoxyacetyl chloride (5b). 1.78 g, 81% as a colourless liquid; bp 90–91 °C/12 mm; [found: C, 43.12; H, 2.27. $C_8H_5Cl_2FO_2$ requires C, 43.08; H, 2.26%]; ν_{max} ($CHCl_3$) 3040, 2925, 1795, 1210, 1088 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.89 (2H, s, $-OCH_2$), 6.83–6.88 (2H, m), 7.07–7.11 (1H, m); *m/z* (EI-MS) 222 (M^+, 18), 159 (100), 85 (54%).

4.4.2. 3-Chloro-4-fluoro-phenoxyacetyl chloride (5d). 1.84 g, 83% as a colourless liquid; bp 110–112 °C/13 mm; [found: C, 43.06; H, 2.26. $C_8H_5Cl_2FO_2$ requires C, 43.08; H, 2.26%]; ν_{max} ($CHCl_3$) 3036, 2920, 1793, 1088 cm^{-1}; δ_H 4.89 (2H, s, CH_2), 6.74 (1H, ddd, $^3J_{H-H}=8.1$ Hz, $^4J_{H-F}=3.1$ Hz, $^4J_{H-H}=2.6$ Hz), 6.87 (1H, dd, $^4J_{H-F}=3.2$ Hz, $^4J_{H-H}=2.6$ Hz), 7.04–7.14 (1H, m); *m/z* (EI-MS) 222 (M^+, 22), 161 (100), 129 (64%).

4.5. General method for the synthesis of [(aryloxyacetyl) alkylidene] triphenylphosphoranes (8a–f and 9a–d)

Phosphorane **6/7** (10 mmol), dry dichloromethane (10 mL) were taken in a two necked round bottom flask, cooled to 10–15 °C, added dropwise a solution of aryloxyacetyl chloride **5** (5 mmol) in dichloromethane (5 mL) in about 15 min and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with DCM (10 mL) and washed with water (3×20 mL), the organic layer separated and dried over anhydrous sodium sulphate. The solvent was removed on rotavapor and the crude product was purified by recrystallization (1:4 mixture of chloroform/hexane).

4.5.1. [(4-Fluorophenoxyacetyl) (cyano) methylene] triphenylphosphorane (8a). 2.08 g, 92.3% as a pale yellow solid; mp 155 °C; [found: C, 74.31; H, 4.85; N, 3.15. $C_{28}H_{21}FNO_2P$: C, 74.16; H, 4.66; N, 3.08%]; ν_{max} (KBr) 3060, 2895, 2172, 1622, 1190, 1105 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.92 (2H, s, $-COCH_2-$), 6.82–6.93 (4H, m), 7.40–7.75 (15H, unresolved); δ_C (50 MHz, $CDCl_3$) 47.0 (d, $^1J_{C-P}=126.2$ Hz, ylide carbon), 70.9 (d, $^3J_{C-P}=9.8$ Hz, CH_2), 115.5 (d, $^2J_{C-F}=23.1$ Hz, sp^2-carbon *ortho* to –F), 115.9 (d, $^3J_{C-F}=7.9$ Hz, sp^2-carbon *meta* to –F), 120.8 (d, $^2J_{C-P}=14.6$ Hz, *C*N), 122.5 (d, $^1J_{C-P}=93.7$ Hz, phenyl carbon attached to –P), 129.2 (d, $^3J_{C-P}=13.0$ Hz, phenyl carbon *meta* to –P), 133.3 (d, $^4J_{C-P}=2.3$ Hz, phenyl carbon *para* to –P), 133.6 (d, $^2J_{C-P}=10.4$ Hz, phenyl carbon *ortho* to –P), 154.4 (s, sp^2-carbon attached to –O and *para* to –F), 157.4 (d, $^1J_{C-F}=238.3$ Hz, sp^2-carbon attached to –F) and 190.2 ppm (d, $^2J_{C-P}=3.8$ Hz, *C*=O); *m/z*: 453 (MH^+, 2), 327 (42), 328 (100), 314 (18), 278 (9), 277 (33), 183 (25), 91 (63%).

4.5.2. [(2-Chloro-4-fluorophenoxyacetyl) (cyano) methylene] triphenylphosphorane (8b). 2.04 g, 84% as a yellow solid; mp 143 °C; [found: C, 69.13; H, 4.25; N, 2.91. $C_{28}H_{20}ClFNO_2P$ requires C, 68.92; H, 4.13; N, 2.17%]; ν_{max} (KBr) 3049, 2985, 2175, 1618, 1094 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 5.03 (2H, s, $-COCH_2-$), 6.83–6.92 (2H, m), 7.11–7.18 (1H, m) 7.42–7.68 (15H, unresolved); *m/z* (LSIMS) 488 (MH^+, 100), 489 (M+2, 33), 328 (96), 314 (28), 279 (76%).

4.5.3. [(2,4-Difluorophenoxyacetyl) (cyano) methylene] triphenylphosphorane (8c). 2.15 g, 91.6% as a pale yellow solid; mp 105 °C; [found: C, 71.39; H, 4.26; N, 3.01. $C_{28}H_{20}F_2NO_2P$ requires C, 71.33; H, 4.27; N, 2.97%]; ν_{max} (KBr) 3056, 2989, 2184, 1615, 1094 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.98 (2H, s, $-COCH_2-$), 6.62–6.96 (3H, m) 7.35–7.72 (15H, unresolved); *m/z* (LSIMS) 472 (MH^+, 98), 328 (100), 314 (22), 279 (44%).

4.5.4. [(3-Chloro-4-fluorophenoxyacetyl) (cyano) methylene] triphenylphosphorane (8d). 2.11 g, 87% as a pale yellow solid; mp 138 °C; [found: C, 68.91; H, 4.23; N, 2.88. $C_{28}H_{20}ClFNO_2P$ requires C, 68.92; H, 4.13; N, 2.17%]; ν_{max} (KBr) 3050, 2989, 2172, 1606, 1492, 1263, 1188, 1090 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.93 (2H, s, –COCH_2–), 6.78–6.87 (1H, m), 6.86–7.02 (2H, m) 7.40–7.80 (15H, unresolved); *m/z* (LSIMS) 488 (MH^+, 77), 328 (100), 279 (31%).

4.5.5. [(3-Trifluoromethylphenoxyacetyl) (cyano) methylene] triphenylphosphorane (8e). 2.12 g, 84.3% as a yellow solid; mp 149 °C; [found: C, 69.30; H, 4.28; N, 2.65. $C_{29}H_{21}F_3NO_2P$ requires C, 69.24; H, 4.20; N, 2.78%]; ν_{max} (KBr) 3062, 2986, 2174, 1606, 1120 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 5.08 (2H, s, –COCH_2–), 7.08–7.14 (2H, m), 7.14–7.19 (1H, m), 7.29–7.36 (1H, m) 7.46–7.71 (15H, unresolved); *m/z* (LSIMS) 504 (MH^+, 92), 328 (100), 314 (22), 279 (18%).

4.5.6. [(3-Methylphenoxyacetyl) (cyano) methylene] triphenylphosphorane (8f). 2.06 g, 92% as a pale yellow solid; mp 138–140 °C; [found: C, 77.81; H, 5.45; N, 3.12. $C_{29}H_{24}NO_2P$ requires C, 77.49; H, 5.38; N, 3.11%]; ν_{max} (KBr) 3055, 2980, 2169, 1612, 1162, 1099 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.31 (3H, s, CH_3), 4.92 (2H, s, –COCH_2–), 6.62–6.75 (3H, m), 7.03–7.14 (1H, m) 7.4–7.73 (15H, unresolved); *m/z* (LSIMS) 450 (MH^+, 100), 328 (93), 314 (43), 279 (81%).

4.5.7. [(4-Fluorophenoxyacetyl) (ethoxycarbonyl) methylene] triphenylphosphorane (9a). 2.29 g, 91.6% as a white solid; mp 96 °C; [found: C, 72.12; H, 5.24. $C_{30}H_{26}FO_4P$ requires C, 71.99; H, 5.23%]; ν_{max} (KBr) 3051, 2984, 1660, 1185 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.69 (3H, t, J=7.1 Hz, –COO–CH_2CH_3), 3.75 (2H, q, J=7.1 Hz, –COO–CH_2CH_3), 5.16 (2H, s, –COCH_2–), 6.72–6.88 (4H, m), 7.34–7.69 (15H, unresolved); *m/z* (LSIMS) 501 (MH^+, 41), 455 (23), 375 (100%).

4.5.8. [(2-Chloro-4-fluorophenoxyacetyl) (ethoxy-carbonyl) methylene] triphenylphosphorane (9b). 2.42 g, 90.6% as a white solid; mp 117 °C; [found: C, 67.41; H, 4.68. $C_{30}H_{25}ClFO_4P$ requires C, 67.35; H, 4.71%]; ν_{max} (KBr) 3056, 2978, 1654, 1190, 1102 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.69 (3H, t, J=7.1 Hz, –COO–CH_2CH_3), 3.72 (2H, q, J=7.1 Hz, –COOCH_2CH_3), 5.21 (2H, s, –COCH_2–), 6.52–6.58 (1H, m), 6.60–6.67 (1H, m), 6.93–7.0 (1H, m), 7.27–7.63 (15H, unresolved); *m/z* (LSIMS) 535 (MH^+, 37), 489 (18), 375 (100), 279 (21%).

4.5.9. [(2,4-Difluorophenoxyacetyl) (ethoxycarbonyl) methylene] triphenylphosphorane (9c). 2.40 g, 92.8% as a white solid; mp 122 °C; [found: C, 69.50; H, 4.88. $C_{30}H_{25}F_2O_4P$ requires C, 69.48; H, 4.88%]; ν_{max} (KBr) 3054, 2986, 1653, 1583, 1508, 1437, 1301, 1188, 1113 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.69 (3H, t, J=7.1 Hz, –COO–CH_2CH_3), 3.72 (2H, q, J=7.1 Hz, –COO–CH_2CH_3), 5.23 (2H, s, –COCH_2–), 6.45–6.79 (3H, m) 7.65–7.70 (15H, unresolved); *m/z* (LSIMS) 519 (MH^+, 47), 375 (100), 279 (29%).

4.5.10. [(3-Chloro-4-fluorophenoxyacetyl) (ethoxycarbonyl) methylene] triphenylphosphorane (9d). 2.33 g, 87.4% as a pale yellow solid; mp 116–118 °C; [found: C, 66.89; H, 4.65. $C_{30}H_{25}ClFO_4P$ requires C, 67.35; H, 4.71%]; ν_{max} (KBr) 3053, 2981, 1654, 1585, 1490, 1295, 1200, 1099 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 0.69 (3H, t, J=7.1 Hz, –COO–CH_2CH_3), 3.73 (2H, q, J=7.1 Hz, –COO–CH_2CH_3), 5.14 (2H, s, –COCH_2–), 6.64–6.71 (1H, m), 6.74–6.91 (2H, m) 7.37–7.72 (15H, unresolved); *m/z* (LSIMS) 535 (MH^+, 48), 375 (100), 279 (34%).

4.6. General method for the synthesis of 2-methyl-benzofuran derivatives (13a–e, 13g and 14a–d)

The procedure for the synthesis of substituted 2-methyl-benzofurans is explained by taking the example of **13a**. [(4-Fluorophenoxyacetyl) (cyano) methylene] triphenyl-phosphorane, **8a** 2.0 g (4.41 mmol) was taken in a sealed tube and subjected to controlled[10] microwave irradiation for 8 min. The dark brown reaction mixture was cooled to room temperature, dissolved in DCM (10 mL) and purified by column chromatography on silica gel (100–200 mesh) using hexane as eluent. Concentration of the initial fractions afforded 0.56 g (73%) of 3-cyano-5-fluoro-2-methyl-benzo-furan, **13a**. The later fractions eluted with a 1:1 mixture of hexane and ethyl acetate contained triphenylphosphine oxide, **12**.

4.6.1. 3-Cyano-5-fluoro-2-methyl-benzofuran (13a). 0.56 g, 73% as a white solid; mp 102 °C; [found: C, 68.59; H, 3.48; N, 7.98. $C_{10}H_6FNO$ requires C, 68.57; H, 3.45; N, 7.99%]; ν_{max} (KBr) 3058, 2231 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.71 (3H, s, CH_3), 7.08 (1H, ddd, $^3J_{H-H}$=9.0 Hz, $^3J_{H-F}$=8.7 Hz, $^4J_{H-H}$=2.7 Hz, C_6–*H*), 7.28 (1H, dd, $^3J_{H-F}$=8.0 Hz, $^4J_{H-H}$=2.7 Hz, C_4–*H*) 7.4 (1H, dd, $^3J_{H-H}$=8.9 Hz, $^4J_{H-F}$=3.7 Hz, C_7–*H*); δ_C (50 MHz, $CDCl_3$) 14.0 (s, *C*H_3), 91.7 (s, C_3), 105.6 (d, $^2J_{C-F}$=26.4 Hz, C_4), 112.4 (d, $^3J_{C-F}$=9.5 Hz, C_7), 112.7 (s, *C*N), 113.4 (d, $^2J_{C-F}$=26.4 Hz, C_6), 127.1 (d, $^3J_{C-F}$=7.6 Hz, C_9), 149.9 (s, C_8), 160.0 (d, $^1J_{C-F}$=242.3 Hz, C_5), 166.5 (s, C_2); *m/z* (EI-MS) 175 (m^+, 100), 174 (100), 147 (4), 141 (6), 120 (9), 75 (6), 43 (8%).

4.6.2. 7-Chloro-3-cyano-5-fluoro-2-methyl-benzofuran (13b). 0.75 g, 82% as a white solid; mp 124 °C); [found: C, 57.43; H, 2.45; N, 6.67. $C_{10}H_5ClFNO$ requires C, 57.30; H, 2.40; N, 6.68%]; ν_{max} (KBr) 3050, 2925, 2233, 1478, 1175, 1100 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.71 (3H, s, CH_3), 7.14 (1H, dd, $^3J_{H-F}$=8.5 Hz, $^4J_{H-H}$=2.6 Hz, C_6–*H*) 7.23 (1H, dd, $^3J_{H-F}$=8.6 Hz, $^4J_{H-H}$=2.6 Hz, C_4–*H*); δ_C (50 MHz, $CDCl_3$) 13.9 (s, *C*H_3), 92.6 (s, C_3), 104.3 (d, $^2J_{C-F}$=26.2 Hz, C_4), 112.0 (s, *C*N), 114.1 (d, $^2J_{C-F}$=28.9 Hz, C_6), 117.6 (d, $^3J_{C-F}$=12.0 Hz, C_7), 127.7 (d, $^3J_{C-F}$=11.7 Hz, C_9), 146.2 (s, C_8), 159.4 (d, $^1J_{C-F}$=245.9 Hz, C_5) and 167.0 (s, C_2); *m/z* (EI-MS) 209 (M^+, 100), 211 (M+2, 33), 208 (99), 175 (21), 147 (17%).

4.6.3. 3-Cyano-5,7-difluoro-2-methyl-benzofuran (13c). 0.64 g, 76% as a white solid; mp 83 °C; [found: C, 61.20; H, 2.65; N, 7.27. $C_{10}H_5F_2NO$ requires C, 62.18; H, 2.61; N, 7.25%]; ν_{max} (KBr) 3065, 2233, 1112, 1108 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.71 (3H, s, CH_3), 6.88 (1H, ddd, $^3J_{H-F}$=9.7 Hz, $^3J_{H-F}$=9.7 Hz, $^4J_{H-H}$=2.3 Hz, C_6–*H*) 7.14

(1H, dd, $^{3}J_{H-F}=8.2$ Hz, $^{4}J_{H-H}=2.3$ Hz, C_4–*H*); *m*/*z* (EI-MS) 193 (M^+, 100), 192 (93%).

4.6.4. 6-Chloro-3-cyano-5-fluoro-2-methyl-benzofuran (13d). 0.69 g, 76% as a pale yellow solid; mp 99 °C; [found: C, 57.31; H, 2.32; N, 6.90. $C_{10}H_5ClFNO$ requires C, 57.30; H, 2.40; N, 6.68%]; ν_{max} (KBr) 3061, 2922, 2231, 1480, 1171, 1105 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.70 (3H, s, C*H*$_3$), 7.40 (1H, d, $^{3}J_{H-F}=8.1$ Hz, C_4–*H*) 7.56 (1H, d, $^{4}J_{H-F}=6.0$ Hz, C_7–*H*); *m*/*z* (EI-MS) 209 (M^+, 98), 211 (M+2, 28), 208 (55), 174 (100%).

4.6.5. 3-Cyano-2-methyl-6-trifluoromethyl-benzofuran (13e). 0.31 g, 32% as a colourless liquid; [found: C, 58.70; H, 2.69; N, 6.36. $C_{11}H_6F_3NO$ requires C, 58.67; H, 2.68; N, 6.22%]; ν_{max} ($CHCl_3$) 3059, 2953, 2230, 1089 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.88 (3H, s, C*H*$_3$), 7.47 (1H, d, *J*=7.5 Hz, C_4–*H*), 7.64 (1H, s, C_7–*H*) 7.68 (1H, d, *J*=7.5 Hz, C_5–*H*); *m*/*z* (EI-MS) 225 (M^+, 100), 224 (96%).

4.6.6. 4-[3[1]-(Trifluoromethyl) phenoxy]-but-2-ynenitrile (15e). 0.09 g, 9% as a colourless liquid; [found: C, 58.75; H, 2.61; N, 6.33. $C_{11}H_6F_3NO$ requires C, 58.67; H, 2.68; N, 6.22%]; ν_{max} ($CHCl_3$) 3054, 2950, 2245, 2310 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.83 (2H, s, –OC*H*$_2$), 7.11 (1H, d, 7.5 Hz, C_6–*H*); 7.31 (1H, d, *J*=7.5 Hz, C_4–*H*), 7.44 (1H, t, *J*=7.5 Hz, C_5–*H*) 7.62 (1H, s, C_2–*H*); *m*/*z* (EI-MS) 225 (M^+, 100%).

4.6.7. 3-Cyano-2,4-dimethyl-benzofuran (13g). 0.52 g, 70% as a pale yellow solid; mp 52 °C; [found: C, 77.32; H, 5.21; N, 8.26. $C_{11}H_9NO$ requires C, 77.17; H, 5.30; N, 8.18%]; ν_{max} (KBr) 3058, 2221, 1486, 1101 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.63 (3H, s, C*H*$_3$), 2.67 (3H, s, C*H*$_3$), 7.03 (1H, d, *J*=7.5 Hz, C_7–*H*), 7.16 (1H, t, *J*=7.5 Hz, C_6–*H*), 7.28 (1H, d, *J*=7.5 Hz, C_5–*H*); δ_C (50 MHz, $CDCl_3$) 13.7 (s, *C*H$_3$ on C_2), 17.7 (s, *C*H$_3$ on C_4), 90.6 (s, C_3), 109.0 (s, C_7), 111.6 (s, *C*N), 125.3 (s, C_5), 125.4 (s, C_6), 125.7 (s, C_9), 131.1 (s, C_4), 153.7 (s, C_8), 164.9 (s, C_3); *m*/*z* (EI-MS) 171 (M^+, 98), 170 (100), 113 (46%).

4.6.8. 3-Ethoxycarbonyl-5-fluoro-2-methyl-benzofuran (14a). 0.69 g, 71% as a colourless liquid; [found: C, 64.93; H, 4.96. $C_{12}H_{11}FO_3$ requires C, 64.86; H, 4.98%]; ν_{max} (KBr) 3060, 1708, 1490, 1300, 1144, 1075 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.43 (3H, t, *J*=7.1 Hz, –OCH$_2$C*H*$_3$), 2.76 (3H, s, C*H*$_3$ on C_2), 4.38 (2H, q, *J*=7.1 Hz, –OC*H*$_2$CH$_3$), 6.95 (1H, ddd, $^{3}J_{H-H}=9.0$ Hz, $^{3}J_{H-F}=8.7$ Hz, $^{4}J_{H-H}=2.7$ Hz, C_6–*H*), 7.3 (1H, dd, $^{3}J_{H-H}=8.9$ Hz, $^{4}J_{H-F}=3.7$ Hz, C_7–*H*) 7.5 (1H, dd, $^{3}J_{H-F}=8.0$ Hz, $^{4}J_{H-H}=2.7$ Hz, C_4–*H*); δ_C (50 MHz, $CDCl_3$) 12.4 (s, OCH$_2$*C*H$_3$), 13.9 (s, *C*H$_3$ on C_2), 58.3 (s, O*C*H$_2$), 105.8 (d, $^{2}J_{C-F}=26.4$ Hz, C_4), 106.5 (s, C_3), 109.5 (d, $^{3}J_{C-F}=9.6$ Hz, C_7), 109.9 (d, $^{2}J_{C-F}=26.6$ Hz, C_6), 125.4 (d, $^{3}J_{C-F}=11.2$ Hz, C_9), 147.8 (s, C_8), 157.9 (d, $^{1}J_{C-F}=238.8$ Hz, C_5), 161.9 (s, –*C*OO–), 168.0 (s, C_2); *m*/*z* (EI-MS) 222 (M^+, 65), 193 (66), 177 (100%).

4.6.9. 7-Chloro-3-ethoxycarbonyl-5-fluoro-2-methyl-benzofuran (14b). 0.89 g, 79% as a pale yellow solid; mp 83 °C; [found: C, 56.16; H, 3.95. $C_{12}H_{10}ClFO_3$ requires C, 56.16; H, 3.93%]; ν_{max} (KBr) 3068, 1705, 1093 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.41 (3H, t, *J*=7.1 Hz, –OCH$_2$C*H*$_3$), 2.82 (3H, s, C*H*$_3$ on C_2), 4.41 (2H, q, *J*=7.1 Hz, –OC*H*$_2$CH$_3$), 7.06 (1H, dd, $^{3}J_{H-F}=8.6$ Hz, $^{4}J_{H-H}=2.6$ Hz, C_6–*H*), 7.52 (1H, dd, $^{3}J_{H-F}=8.6$ Hz, $^{4}J_{H-H}=2.6$ Hz, C_4–*H*); *m*/*z* (EI-MS) 256 (M^+, 78), 227 (63), 211 (100), 183 (16%).

4.6.10. 3-Ethoxycarbonyl-5,7-difluoro-2-methyl-benzofuran (14c). 0.79 g, 75% as a white solid; mp 72 °C; [found: C, 60.03; H, 4.21. $C_{12}H_{10}F_2O_3$ requires C, 60.0; H, 4.19%]; ν_{max} (KBr) 3060, 1706, 1075 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.45 (3H, t, –OCH$_2$C*H*$_3$, *J*=7.1 Hz), 2.81 (3H, s, C*H*$_3$ on C_2), 4.41 (2H, q, *J*=7.1 Hz, –OC*H*$_2$CH$_3$), 6.8 (1H, ddd, $^{3}J_{H-F}=9.7$ Hz, $^{3}J_{H-F}=9.7$ Hz, $^{4}J_{H-H}=2.3$ Hz, C_6–*H*) 7.4 (1H, dd, $^{3}J_{H-F}=8.2$ Hz, $^{4}J_{H-H}=2.3$ Hz, C_4–*H*); *m*/*z* (EI-MS) 240 (M^+, 78), 211 (53), 195 (100), 120 (41%).

4.6.11. 6-Chloro-3-ethoxycarbonyl-5-fluoro-2-methyl-benzofuran (14d). 0.88 g, 78% as a white solid; mp 70 °C; [found: C, 56.19; H, 4.05. $C_{12}H_{10}ClFO_3$ requires C, 56.16; H, 3.93%]; ν_{max} (KBr) 3062, 1705, 1093 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 1.46 (3H, t, –OCH$_2$C*H*$_3$, *J*=7.1 Hz), 2.79 (3H, s, C*H*$_3$ on C_2), 4.40 (2H, q, *J*=7.1 Hz, –OC*H*$_2$CH$_3$), 7.46 (1H, d, $^{4}J_{H-F}=6.0$ Hz, C_7–*H*) 7.67 (1H, d, $^{3}J_{H-F}=8.2$ Hz, C_4–*H*); *m*/*z* (EI-MS) 256 (M^+, 88), 227 (100), 211 (69%).

4.7. General method for the thermolysis of [(aryloxyacetyl) alkylidene] triphenylphosphoranes (8a, 8e and 9a)

The [(aryloxyacetyl) alkylidene] triphenylphosphorane (4.41 mmol) was taken in a short path vacuum distillation apparatus with wide ground glass joints and was heated for 20–30 min at 2–5 Torr, by immersing in a Wood's metal bath, to an external bath temperature ranging from 240 to 275 °C. The distillate collected in the receiver, cooled in dry ice acetone, was dissolved in dichloromethane (10 mL) and subjected to column chromatography as per the procedure in Section 4.6. Thermolysis results of the individual phosphoranes **8a**, **8e** and **9a** are given in Table 1.

4.7.1. 4-(4[1]-Fluorophenoxy)-but-2-ynenitrile (15a). 0.39 g, 51% as a colourless liquid; [found: C, 68.56; H, 3.47; N, 8.01. $C_{10}H_6FNO$ requires C, 68.57; H, 3.45; N, 7.99%]; ν_{max} (KBr) 2243, 2309, 3054 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 4.75 (2H, s, C*H*$_2$), 6.86 (2H, ddd, $^{3}J_{H-H}=7.5$ Hz, $^{3}J_{H-F}=6.8$ Hz, $^{4}J_{H-H}=2.3$ Hz, C_3–*H*), 7.01 (2H, ddd, $^{3}J_{H-H}=7.5$ Hz, $^{4}J_{H-F}=5.6$ Hz, $^{4}J_{H-H}=2.3$ Hz, C_2–*H*); δ_C (50 MHz, $CDCl_3$), 56.4 (s, O*C*H$_2$), 61.3 (s, sp-carbon attached to –CN), 79.4 (s, sp-carbon attached to –CH$_2$), 104.2 (s, *C*N), 116.3 (d, $^{2}J_{C-F}=23.3$ Hz, sp^2-carbon *ortho* to –F), 116.5 (d, $^{3}J_{C-F}=7.9$ Hz, sp^2-carbon *meta* to –F), 153.0 (s, sp^2-carbon attached to –O and *para* to –F), 158.4 (d, $^{1}J_{C-F}=238$ Hz, sp^2-carbon attached to –F); *m*/*z* (EI-MS) 175 (M^+, 100%).

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10. 600 W microwave power was used for decomposition of **13a–f**. For the remaining ylides **14a–d** 450 W microwave power was used.

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Efficient intramolecular Diels–Alder reactions of ester-tethered 1,7,9-decatrienoates catalyzed by bis-aluminated trifluoromethanesulfonamide

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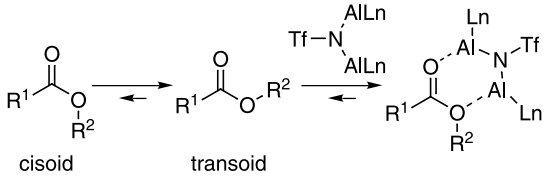
Abstract—Bis-aluminated trifluoromethanesulfonamide generated in situ by mixing $TfNH_2$ (1 mol) and methylaluminum reagent (2 mol) is an effective catalyst for the IMDA reaction of ester-tethered 1,7,9-decatrienoates.

1. Introduction

The Diels–Alder reaction is one of the most powerful means for the stereocontrolled construction of functionalized cyclohexene frameworks.[1] In particular, the intramolecular Diels–Alder (IMDA) reaction provides an efficient method for bicyclic and polycyclic compounds and it has been applied to the synthesis of a variety of complex molecules including natural products.[2,3] In the IMDA reactions, the reactivity of the substrate and the stereochemical outcome of the product are strongly influenced by the nature of the tether linking diene and dienophile parts. For example, incorporation of an ester linkage as the tether often has an adverse effect on the IMDA reaction resulting in requiring higher reaction temperature and longer reaction time but low yields of the cyclized products or in some cases even failure to obtain any cyclized products.[2–4] Such a reduced reactivity of ester tethered substrates, in particular 1,6,8-nona- and 1,7,9-decatriene systems, is attributed to a preference for the *transoid* geometry due primarily to dipole repulsion between carbonyl-oxygen and ethereal oxygen and a relatively high rotational barrier to *cisoid* form, in which the diene and the dienophile are in close proximity (Scheme 1).[5] Furthermore, poor overlap of the non-bonding electrons of the ethereal oxygen and carbonyl group in the transition state is considered to be a major factor responsible for this low reactivity.[4a,6] Towards to these issues, the use of polar solvent in the case of ester-tethered substrates[7] or the modification of tether moiety in the substrates from ester to acetal[8] or hydroxamate[9] have been reported, although limitation of substrates is one of the disadvantages in these reactions. It has been also documented that Lewis acid mediated reaction, which is often effective for intermolecular versions, does not always work well in the IMDA reactions of the ester tethered substrates.[5d,10]



Scheme 1.

$$CF_3SO_2NH_2 + 2MeAlLn \rightarrow TfN(AlLn)_2 + 2CH_4 \quad (1)$$

Recently we reported that the IMDA reaction of 1,7,9-decatrienoates can be efficiently promoted by a bidentate Lewis acid, in particular bis-aluminated trifluoromethanesulfonamide generated in situ by mixing $TfNH_2$ (1 mol) and methylaluminum reagent (2 mol) (Eq. 1).[11] We assumed that coordination of both oxygen atoms of the ester group to a bidentate Lewis acid (possibly in equilibrium with other complex forms such as a double coordination form toward carbonyl oxygen) would control the geometry of ester moiety to be a *cisoid* structure and would strongly enhance the reactivity of the dienophile as compared with the use of a monodentate Lewis acid (Scheme 1).[12] Further study using a variety type of substrates revealed the efficiency of these

Keywords: Bis-aluminated trifluoromethanesulfonamide; Intramolecular Diels–Alder reaction; 1,7,9-Decatrienoate; Bidentate Lewis acid.
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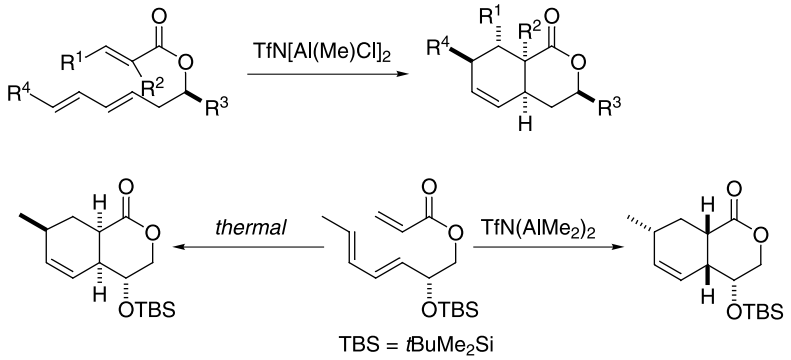


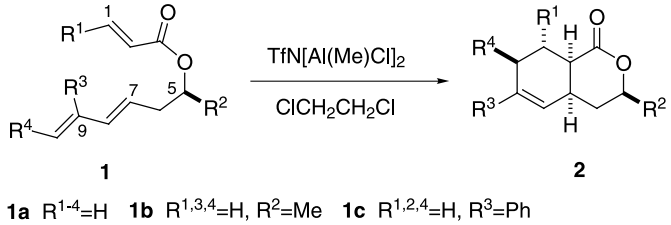
Scheme 2.

bidentate Lewis acids in the IMDA reaction of ester tethered substrates. In this paper, we report a detail of the IMDA reactions promoted by bis-aluminated trifluoromethanesulfonamide, which is applicable to 1,7,9-decatrienoate derivatives having various substituent patterns (Scheme 2).

2. Results and discussion

The IMDA reactions of 1,7,9-decatrienoates **1a**–**1e** catalyzed by $TfN[Al(Me)Cl]_2$ are shown in Table 1. In the presence of 1.1 equiv of $TfN[Al(Me)Cl]_2$, the reactions of **1a**–**1d** proceeded at 0 °C or room temperature within a short period (1–8 h) to give the cycloadducts **2a**–**2d** in good yields (entries 1, 2, 4, 6 and 7). Even on using a catalytic amount of Lewis acid (0.3 equiv) **2a**, **2b** and **2d** were obtained in good yields, although higher reaction temperature and longer reaction time were required (entries 3, 5 and 8). In all cases, each adduct **2a**–**2d** was obtained as a single *cis*-fused isomer (*endo* adduct) with illustrated relative configuration. In particular, the *trans* relationship between the Me group and the hydrogen on the ring-junction in **2b** indicated that the reaction proceeds via

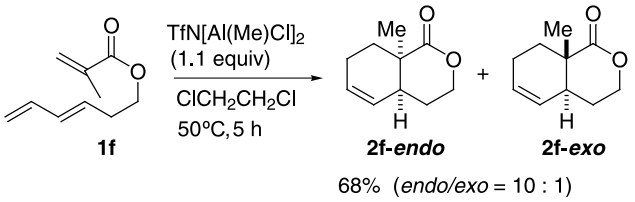
Table 1. $TfN[Al(Me)Cl]_2$ catalyzed IMDA reaction of 1,7,9-decatrienoates **1a**–**1e**



1a R^{1-4}=H **1b** $R^{1,3,4}$=H, R^2=Me **1c** $R^{1,2,4}$=H, R^3=Ph
1d R^{1-3}=H, R^4=Et **1e** R^1=Ph, R^{2-4}=H

Entry	1	Lewis acid (equiv)	Solvent	Temp. (°C)	Time (h)	2		Yield (%)[a]
1	**1a**	1.1	CH_2Cl_2	rt	1	**2a**		88
2	**1a**	1.1	Toluene	0	2	**2a**		90
3	**1a**	0.3	$ClCH_2CH_2Cl$	50	6	**2a**		85
4	**1b**	1.1	CH_2Cl_2	rt	1	**2b**		91
5	**1b**	0.3	$ClCH_2CH_2Cl$	50	7	**2b**		79
6	**1c**	1.1	CH_2Cl_2	0	8	**2c**		78
7	**1d**	1.1	CH_2Cl_2	rt	2	**2d**		82
8	**1d**	0.3	$ClCH_2CH_2Cl$	80	24	**2d**		77
9	**1e**	1.1	$ClCH_2CH_2Cl$	80	5	**2e**		78
10	**1e**	0.3	$ClCH_2CH_2Cl$	80	52	**2e**		71

[a] Isolated yield.

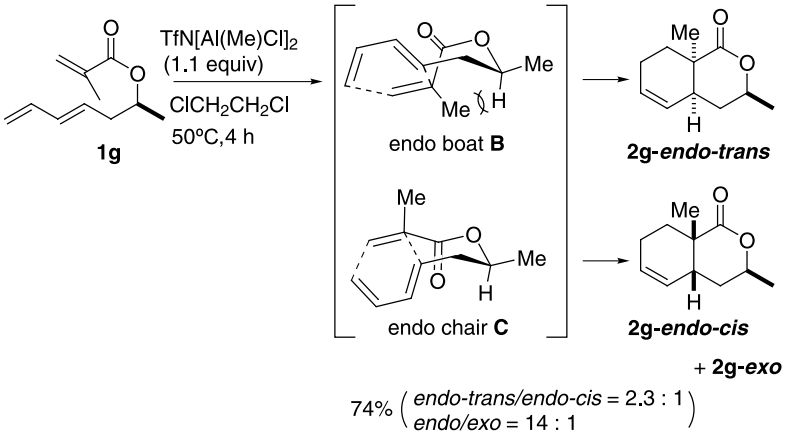


Scheme 3.

endo-boatlike transition state **A**. This conformational preference is in accord with the previous results in the thermal IMDA reactions of 1,7,9-decatrien-3-ones[2b] and 1,7,9-decatrienoates.[6,13] In the presence of $TfN[Al(Me)Cl]_2$, reaction of β-substituted acrylate **1e** also proceeded smoothly at 80 °C to give **2e** in good yield with an excellent *endo* selectivity (entries 9, 10).

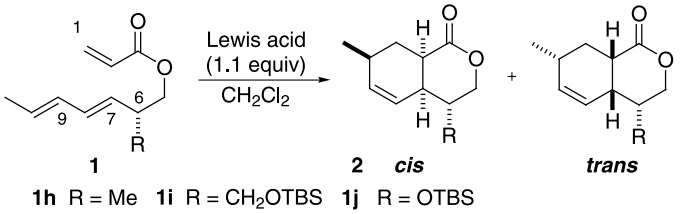
As far as we know, no successful example of the IMDA reaction of 1,7,9-decatriene systems tethered by an α-substituted α,β-unsaturated ester moiety such as **1f**, **1g** has been reported. Treatment of methacrylate derivative **1f** with 1.1 equiv of $TfN[Al(Me)Cl]_2$ at 50 °C gave the cycloadducts **2f** in 68% yield as an *endo*/*exo* mixture in a ratio of 10:1 (Scheme 3).

The bidentate Lewis acid also effected the Diels–Alder reaction of methacrylate derivative **1g** having a chiral center at 5-position to give a mixture of the cycloadducts **2g** in 74% yield, which consisted of two *endo* isomers (*trans*/*cis* = 2.3:1) and one *exo* isomer in a ratio of *endo*/*exo* = 14:1 (Scheme 4). The major isomer **2g**-*endo-trans* has a *trans* relationship between the Me group originally attached on the chiral center and the hydrogen on ring-junction. As in the case of the acrylates **1a**–**1e** shown in Table 1, this major isomer **2g**-*endo-trans* would be derived through *endo*-boatlike transition state **B**, in which α-Me substituent has a psuedo–flagpole interaction with axial hydrogen attached on the ethereal carbon. Therefore, *endo*-chairlike transition state **C** would be competitively contribute for the formation of the minor *cis*-isomer **2g**-*endo-cis*.[6b] Compared to an excellent chiral induction in the case of acrylate **1b** derived from the same secondary alcohol (see Table 1, entry 4), introduction of α-methyl group leading to the methacrylate structure **1g** caused a remarkable decrease in stereoselectivity.



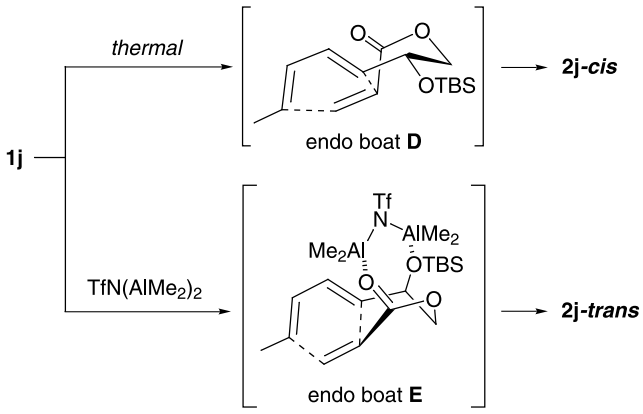
Scheme 4.

Table 2. IMDA reaction of 6-substituted 1,7,9-decatrienoates**1h**–**1j**



Entry	1	Lewis acid	Temp. (°C)	Time (h)	2	Yield (%)[a]	*cis*/*trans*[b]
1	**1h**	$TfN[Al(Me)Cl]_2$	rt	7	**2h**	83	7.3:1
2[c]	**1h**	—	140	12	**2h**	49	5.2:1
3	**1i**	$TfN(AlMe_2)_2$	0	5	**2i**	70	*cis* only
4[c]	**1i**	—	140	12	**2i**	64	*cis* only
5	**1j**	$TfN(AlMe_2)_2$	0	3	**2j**	74	1:7.2
6[c]	**1j**	—	140	12	**2j**	74	3.9:1

[a] Isolated yield.
[b] Based on isolated yield.
[c] Solvent: 1,2-dichlorobenzene.

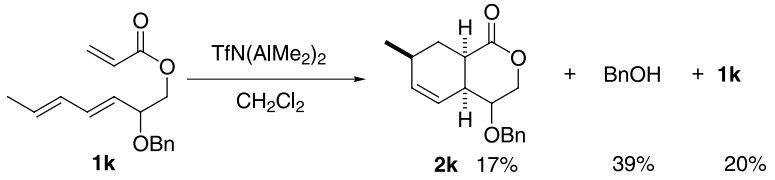


Scheme 5.

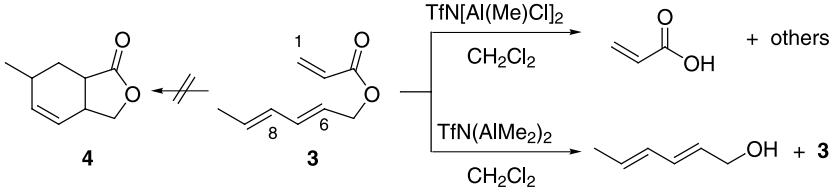
Next, we examined the substituent effect on the chiral induction during the IMDA reaction using the substrates having a substituent at 6-position. As the substrates, 1,7,9-decatrienoates having methyl **1h**, *t*-butyldimethylsiloxymethyl **1i** and *t*-butyldimethylsiloxy **1j** were used and the reactions were conducted under the thermal conditions or in the presence of the present bidentate Lewis acid. Results are shown in Table 2. In the presence of $TfN[Al(Me)Cl]_2$, the IMDA reaction of Me-substituted substrate **1h** proceeded at room temperature to give the *endo* adduct **2h** in 83% yield (entry 1). The major isomer of **2h** has *cis* configuration between the methyl group and the hydrogen on ring-junction (*cis*/*trans*=7.3:1). Under the thermal conditions, upon heating 140 °C for 12 h, the IMDA reaction of **1h** proceeded in *endo* selective manner to give the adduct **2h** in 49% yield with preferable formation of *cis* isomer (*cis*/*trans*=5.2:1, entry 2). A complete *endo* and *cis* selectivity was found in the reaction of siloxymethyl-substituted substrate **1i** under the thermal conditions (140 °C, 12 h) and $TfN(AlMe_2)_2$ catalyzed conditions (0 °C, 5 h) giving rise to the product **2i** as a single isomer (entries 3, 4). On the other hand, in the case of siloxy derivative **1j** the stereochemical outcome (*cis*/*trans* selectivity) was found different depending on the reaction conditions. Thus, the thermal reaction (140 °C, 12 h) of **1j** provided the *endo* adduct **2j** with *cis* favorable manner (74% yield, *cis*/*trans*=3.9:1, entry 6), while on using $TfN(AlMe_2)_2$ as a catalyst the reaction proceeded at 0 °C to give the same adduct **2j** in 74% yield, but the *trans* isomer was a major product (*cis*/*trans*=1:7.2, entry 5).

Observed *cis*/*trans* selectivity under the thermal or Lewis acid mediated conditions could be explained by considering the transition state shown in Scheme 5. In the case of thermal reaction of **1j**, the major isomer **2j**-*cis* would be derived via *endo*-boatlike transition state model **D**, in which sterically bulky TBSO substituent occupies a psuedo-equatorial position. Such a preferable conformation was also proposed in the IMDA reaction of *N*-sulfinylcarbamate derivatives having benzyloxy group at the similar position in the diene parts.[14] Likewise, the observed *cis*-selectivities of methyl-substituted substrate **1h** and silyloxymthyl-substituted substrate **1i** under both thermal and Lewis acid mediated conditions are also explained by this model **D**, in which methyl and siloxymethyl group, instead of TBSO group, occupy a psuedo-equatorial position. On the other hand, the Lewis acid mediated reaction of siloxy-substituted substract **1j** would proceed via *endo*-boatlike transition state **E**, in which the bidentate Leiws acid $TfN(AlMe_2)_2$ coordinated by oxygens of ester moiety possibly has an interaction with the oxygen atom of TBSO substituent when it occupies a psuedo-axial position.[15,16]

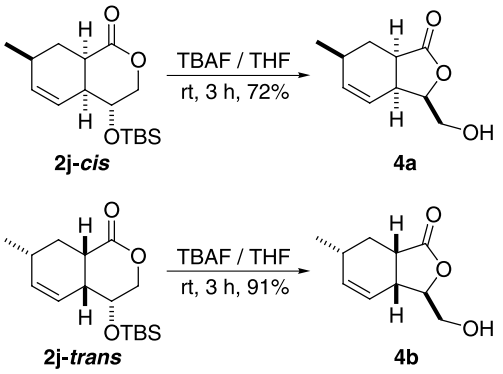
It is noted that a significant oxophilic nature of the bis-aluminated triflic amide brought about some limitation of the substrate structure. For example, the $TfN(AlMe_2)_2$ catalyzed reaction of the benzyloxy derivative **1k** instead of siloxy derivative **1j** provided the IMDA product **2k** in low yield (17%) due to the facile cleavage of allylic ether moiety to give benzyl alcohol in 39% yield, even though the starting material **1k** was recovered in 20% yield at the same time (Scheme 6). Similar problem was also observed in allylic ester type substrate, 1,6,8-nonatrienoate derivative **3** (Scheme 7). Thus, either $TfN[Al(Me)Cl]_2$ or $TfN(AlMe_2)_2$ did not effect the IMDA reaction of **3** due mainly to the cleavage of ester part. Therefore, it is difficult to prepare oxabicyclo[4.3.0]nonene derivative **4** from 2,4-alkadienyl acrylate **3** under the present Lewis acid catalyzed



Scheme 6.



Scheme 7.



Scheme 8.

conditions. Oxabicyclo[4.3.0]nonene derivative **4** could be prepared through the ring contraction of lactone moiety of the cycloadduct **2j** (Scheme 8).[17] As shown in Scheme 8, desilylation by tetrabutylammonium fluoride (TBAF) led to the formation of thermodynamically stable 5-membered lactone **4a** from **2i**-*cis* and **4b** from **2i**-*trans*, respectively.

3. Conclusion

We have shown that the IMDA reaction of 1,7,9-decatrienoate derivatives having ester moiety as the tether are efficiently catalyzed by bis-aluminated trifluoromethanesulfonamide, $TfN[Al(Me)Cl]_2$ or $TfN(AlMe_2)_2$. In place of the IMDA reactions of 1,6,8-nonatriene systems, we have developed two-step procedure, which involves the IMDA reactions of 1,7,9-decatrienoate having a silyloxy group at 6 position followed by ring contraction of lactone function of the cycloadduct. Further studies on the structure of the complex forms, and application to the synthesis of natural products are under way.

4. Experimental

4.1. General

Trifluoromethanesulfonamide, trimethylaluminum (1.0 M in hexane) and dimethylaluminum chloride (1.0 M in hexane) are available commercially. All reactions were conducted under an argon atmosphere. ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz in $CDCl_3$, and the chemical shifts are given in ppm using $CHCl_3$ (7.26 ppm) in $CDCl_3$ for ^1H NMR and $CDCl_3$ (77.01 ppm) for ^{13}C NMR as internal standard, respectively. Mass spectra and HRMS were recorded by EI or ESI methods. Column chromatography was performed on silica gel (70–230 mesh). Medium-pressure liquid chromatography (MPLC) was performed on a 30 cm×2.2 cm i.d. prepacked column (silica gel, 50 μm) with a UV or RI detector.

4.2. General procedure for the preparation of 1,7,9-decatrienoates 1: (3*E*)-3,5-hexadienyl acrylate (1a)

After a mixture of 3,5-hexadien-1-ol (1 g, 10.2 mmol), acryloyl chloride (1.0 mL, 12.4 mmol) and triethylamine (2.0 mL, 14.3 mmol) in CH_2Cl_2 (15 mL) was stirred for 2 h at 0 °C and then for 2 h at room temperature, the reaction mixture was extracted with ether after addition of H_2O. The organic layer was washed with brine, dried over $MgSO_4$, and purified by silica gel column chromatography (hexane/Et_2O=50:1) to give **1a** (1.35 g, 87% yield). ^1H NMR spectrum of **1a** was identical with that reported in the literature.[4b]

By a similar procedure for the preparation of **1a**, the substrates **1f**, **1h** were prepared from the corresponding dienyl alcohol and acid chloride.

The physical data of **1b**, **1d**, **1e**, **1g** were reported previously.[11]

4.2.1. (3*E*)-3,5-Hexadienyl 2-methylacrylate (1f). 76% yield. Colorless oil. IR (neat) ν cm^{-1}; 1720. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.93 (3H, s), 2.46 (2H, dt, J=6.7, 6.7 Hz), 4.19 (2H, t, J=6.7 Hz), 5.01 (1H, d, J=10.2 Hz), 5.13 (1H, d, J=16.8 Hz), 5.54 (1H, bs), 5.68 (1H, dt, J=15.1, 6.7 Hz), 6.09 (1H, bs), 6.13 (1H, dd, J=15.1, 10.2 Hz), 6.31 (1H, ddd, J=16.8, 10.2, 10.2 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 18.3, 31.9, 63.7, 116.0, 125.4, 129.7, 133.4, 136.4, 136.8, 167.4. EI-MS *m/z*: 166 (M^+). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 71.95; H, 8.45.

4.2.2. (2*R*,3*E*,5*E*)-2-Methyl-3,5-heptadienyl acrylate (1h). 79% yield. Colorless oil. IR (neat) ν cm^{-1}; 1730. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.06 (3H, d, J=6.8 Hz), 1.73 (3H, d, J=7.5 Hz), 2.57 (1H, m), 3.99 (1H, dd, J=10.7, 6.8 Hz), 4.06 (1H, dd, J=10.7, 6.7 Hz), 5.41–5.52 (1H, m), 5.58–5.69 (1H, m), 5.81 (1H, dd, J=10.4, 1.4 Hz), 5.96–6.10 (1H, m), 6.12 (1H, dd, J=17.3, 1.4 Hz), 6.39 (1H, dd, J=17.3, 1.4 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 17.0, 18.1, 36.1, 68.7, 128.4, 128.6, 13.6, 130.8, 131.5, 132.5, 166.5. EI-MS *m/z*: 180 (M^+). Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.36; H, 8.73.

4.2.3. (3*E*)-5-Phenyl-3,5-hexadienyl acrylate (1c). After a suspension of methyltriphenylphosphonium bromide (14.3 g, 40.0 mmol) in ether (80 mL) was treated with *n*-BuLi (14.8 mL, 2.44 M in hexane solution) for 3 h at room temparature, (*E*)-5-{*tert*-butyl(dimethyl)silyloxy}-1-phenyl-2-penten-1-one[18] (6.4 g, 22.0 mmol) in ether (20 mL) was added at 0 °C. The reaction mixture was stirred for 3 h at room temperture. After usual work-up (extracetd with AcOEt, dried over $MgSO_4$, and concentrated under reduced pressure), a solution of the residue in THF (4.0 mL) was treated with tetrabutylammonium fluoride (3.0 mL, 1.0 M THF solution) at 0 °C for 8 h. The usual workup and the subsequent purification by column chromatography on silica gel (hexane/AcOEt=9:1) gave (3*E*)-5-phenyl-3,5-hexadien-1-ol (316 mg, 91% yield) as colorless oil. IR (neat) ν cm^{-1}; 3335. ^1H NMR (400 MHz, $CDCl_3$) δ; 2.40 (2H, dt, J=7.2, 6.4 Hz), 3.67 (1H, t, J=6.4 Hz), 5.11 (1H, bs), 5.23 (1H, bs), 5.63 (1H, dt, J=15.7, 7.2 Hz), 6.42 (1H, d, J=15.7 Hz), 7.28–7.37 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 36.2, 61.9, 115.7, 127.4, 128.1, 128.2, 129.6, 134.3, 140.3, 147.7. ESI-MS *m/z*: 175 (M^++H). HRMS calcd for $C_{12}H_{15}O$: 175.123. Found: 175.1114.

After a mixture of the above dienyl alcohol (228 mg, 1.0 mmol), acryloyl chloride (0.22 mL, 1.1 mmol) and triethylamine (0.18 mL, 1.3 mmol) in CH_2Cl_2 (2.0 mL) was stirred for 5 h at −78 °C, usual work-up followed by the purification by silica gel column chromatography (hexane/Et_2O=50:1) gave **1c** (155 mg, 68% yield) as colorless oil. IR (neat) ν cm^{-1}; 1725. ^1H NMR (400 MHz, $CDCl_3$) δ; 2.38–2.55 (2H, m), 4.22 (1H, t, J=6.8 Hz), 5.12 (1H, bs), 5.22 (1H, bs), 5.54–5.69 (1H, m), 5.79 (1H, d, J=10.4 Hz), 6.12 (1H, dd, J=17.4, 10.4 Hz), 6.31–6.45 (2H, m), 7.13–7.47 (5H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 32.1, 63.6, 115.6, 127.4, 128.1, 128.2, 128.5, 128.8, 130.6, 134.0, 140.2, 147.7, 166.1. ESI-MS m/z: 229 (M^++H). HRMS calcd for $C_{15}H_{17}O_2$: 229.1229. Found: 229.1228.

4.2.4. (2*R,3*E*)-2-({[*tert*-Butyl(dimethyl)silyl]oxy}-methyl)-3,5-hexadienyl acrylate (1i).** To a solution of LDA (11.0 mL, 1.0 M in THF–hexane solution) and HMPA (1.8 mL), ethyl sorbate (1.50 mL, 10.0 mmol) in THF (5.0 mL) was added over 10 min at −78 °C. After being stirred for 2 h at −78 °C, the reaction mixture was treated with ethyl chloroformate (4.8 mL, 30.0 mmol) for 12 h at the same temperature. Usual work-up (Et_2O for extraction) followed by the purification by silica gel column chromatography (hexane/ether=25:1) gave diethyl 2-[(1*E*)-1,3-butadienyl]malonate (2.0 g, 93% yield) as colorless oil. ^1H NMR spectrum of this compound was identifical with that reported in the literature.[19] Diethyl 2-[(1*E*)-1,3-butadienyl]-malonate (1.6 g, 7.5 mmol) in ether (5 mL) was added to a solution of $LiAlH_4$ (0.57 g, 15.0 mmol) in ether (10 mL) at 0 °C. After being stirred for 6 h at 0 °C and then usual work-up, the residue in THF (5 mL) was added to a suspension of NaH (0.3 g, 60% activity, 7.5 mmol) in THF (15 mL) at 0 °C. After being stirred for 30 min at room temperature, the reaction mixture was treated with *tert*-butyldimethylchlorosilane (1.13 g, 7.5 mmol) for 2 h at room temperature. The reaction mixture was quenched by H_2O and then extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and purified by silica gel column chromatography (hexane/AcOEt=10:1) to give (2*R**,3*E*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3,5-hexadien-1-ol (1.1 g, 60% yield) as colorless oil. IR (neat) ν cm^{-1}; 3375. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.07 (6H, s), 0.90 (9H, s), 2.50–2.58 (2H, m), 3.64–3.81 (4H, m), 5.04 (1H, bd, J=9.9 Hz), 5.35 (1H, bd, J=16.7 Hz), 5.55 (1H, dd, J=15.3, 8.2 Hz), 6.16 (1H, dd, J=15.3, 10.2 Hz), 6.29 (1H, ddd, J=16.7, 10.2, 9.9 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; −5.6, −5.5, 18.2, 25.8, 46.4, 65.7, 66.1, 116.5, 131.4, 133.3, 136.8. ESI-MS m/z: 243 (M^++H). HRMS calcd for $C_{13}H_{27}O_2Si$: 243.1780, Found: 243.1785. Anal. Calcd for $C_{13}H_{26}O_2Si$: C, 64.41; H, 10.81. Found: C, 64.42; H, 10.82.

In a similar manner for the preparation of **1a**, reaction of the above dienyl alcohol (486 mg, 2.0 mmol) with acryloyl chloride (0.18 mL, 2.2 mmol) and triethylamine (0.33 mL, 2.4 mmol), and the subsequent purification by silica gel column chromatography (hexane/Et_2O=25:1) gave **1i** (498 mg, 84% yield) as colorless oil. IR (neat) ν cm^{-1}; 1730. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.03 (6H, s), 0.88 (9H, s), 2.58–2.68 (1H, m), 3.62 (1H, dd, J=9.9, 6.5 Hz), 3.67 (1H, dd, J=9.9, 5.2 Hz), 4.18–4.29 (1H, m), 5.03 (1H, bd, J=10.1 Hz), 5.14 (1H, bd, J=16.6 Hz), 5.62 (1H, dd, J=15.3, 8.2 Hz), 5.81 (1H, dd, J=10.4, 1.3 Hz), 6.11 (1H, dd, J=17.3, 10.4 Hz), 6.15 (1H, dd, J=15.3, 10.3 Hz), 6.30 (1H, ddd, J=16.6, 10.3, 10.1 Hz), 6.38 (1H, dd, J=17.3, 1.3 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; −5.5, 18.2, 25.8, 44.2, 63.1, 64.3, 116.4, 128.5, 130.5, 131.6, 133.3, 136.9, 166.1. ESI-MS m/z: 297 (M^++H). HRMS calcd for $C_{16}H_{29}O_3Si$: 297.1886, Found: 297.1870. Anal. Calcd for $C_{16}H_{28}O_3Si$: C, 64.82; H, 9.52. Found: C, 64.67; H, 9.48.

4.2.5. (2*R,3*E*,5*E*)-2-{[*tert*-Butyl(dimethyl)silyl]oxy}-3,5-heptadienyl acrylate (1j).** To the Grignard reagent prepared from Mg (432 mg, 18.0 mmol) and chloromethyl-isopropyloxydimethylchlorosilane (3.0 mL, 18.0 mmol) in THF (12.5 mL),[20] sorbic aldehyde (1.1 mL, 10.0 mmol) in THF (10 mL) was added at 0 °C. After being stirred for 2 h at 0 °C, the reaction mixture was quenched by saturated NH_4Cl and filitered through celite pad. After the filitate was extracted with ether, the organic layer was washed with brine, dried over $MgSO_4$, and evapolated. A mixture of the residue, H_2O_2 (9.0 mL) and $NaHCO_3$ (840 mg, 10.0 mmol) in THF–MeOH (1:1, 30 mL) was refluxed overnight, and then evaporated under reduced pressure. Extractive work-up followed by evaporation gave the crude mixture of 3,5-heptadiene-1,2-diol, which was treated with diisopropyl-ethylamine (2.1 mL, 12.0 mmol) and acrylory chloride (0.89 mL, 11.0 mmol) in THF (20 mL) for 5 h at −78 °C. Usual work-up and purification by column chromatography on silica gel (hexane/AcOEt=6:1) provided (2*R**,3*E*,5*E*)-2-hydroxy-3,5-hexadienyl acrylate (658 mg, 35% yield over 3 steps) as colorless oil. IR (neat) ν cm^{-1}; 1726, 3446. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.76 (3H, d, J=6.6 Hz), 4.09 (1H, dd, J=11.4, 7.6 Hz), 4.25 (1H, dd, J=11.4, 3.5 Hz), 4.39–4.48 (1H, m), 5.54 (1H, dd, J=15.3, 6.5 Hz), 5.74 (1H, dq, J=14.9, 6.6 Hz), 5.86 (1H, dd, J=10.4, 1.3 Hz), 6.04 (1H, dd, J=14.9, 10.5 Hz), 6.15 (1H, dd, J=17.3, 10.4 Hz), 6.30 (1H, dd, J=15.3, 10.5 Hz), 6.43 (1H, dd, J=17.3, 1.3 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 18.1, 68.1, 70.6, 127.5, 128.0, 130.5, 131.1, 131.4, 133.0, 166.2. ESI-MS m/z: 205 (M^++Na). HRMS: Calcd for $C_{10}H_{14}O_3Na$: 205.0841. Found: 205.0837.

After a mixture of the above alcohol (660 mg, 3.6 mmol), diisopropylethylamine (0.70 mL, 4.0 mmol) and *tert*-butyl-dimethylsilyl triflate (0.92 mL, 4.0 mmol) in Et_2O (7.5 mL) was stirred for 5 h at −78 °C, extractive work-up and purification by silica gel column chromatography (hexane/AcOEt=50:1) gave **1j** (593 mg, 56% yield) as colorless oil. IR (neat) ν cm^{-1}; 1730. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.04 (3H, s), 0.06 (3H, s), 0.90 (9H, s), 1.75 (3H, d, J=6.7 Hz), 4.03 (1H, dd, J=11.0, 7.5 Hz), 4.09 (1H, dd, J=11.0, 4.4 Hz), 4.36–4.44 (1H, m), 5.49 (1H, dd, J=15.2, 6.2 Hz), 5.65–5.75 (1H, m), 5.82 (1H, dd, J=17.3, 10.5 Hz), 5.99–6.08 (1H, m), 6.14 (1H, dd, J=17.3, 10.5 Hz), 6.22 (1H, dd, J=15.2, 10.5 Hz), 6.41 (1H, dd, J=17.3, 1.4 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; −4.9, −4.6, 18.0, 18.2, 25.7, 68.3, 71.1, 128.4, 129.2, 130.1, 130.7, 130.7, 131.8, 165.9. ESI-MS m/z: 319 (M^++Na). HRMS: Calcd for $C_{16}H_{29}O_3SiNa$: 319.1705, Found: 319.1704. Anal. Calcd for $C_{16}H_{28}O_3Si$: C, 64.82; H, 9.52. Found: C, 64.68; H, 9.52.

4.3. General procedure of Lewis acid mediated IMDA reactions of 1,7,9-decatrienoate derivatives: (4a*S**, 8a*R**)-6-phenyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (2c)

After a solution of triflic amide (82 mg, 0.55 mmol) in CH_2Cl_2 (4.5 mL) was treated with dimethylaluminum chloride (1.0 M in hexane, 1.1 mL, 1.1 mmol) for 30 min at room temperature, **1c** (114 mg, 0.50 mmol) in CH_2Cl_2 (2.5 mL) was added at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was quenched by 1 M HCl and extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$. Purification by column chromatography on silica gel (hexane/AcOEt=5:1) gave the product **2c** (89.0 mg, 78% yield) as white solid. Mp 82–83 °C. IR (neat) ν cm^{-1}; 1720. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.79–1.97 (2H, m), 2.11–2.21 (1H, m), 2.35–2.47 (2H, m), 2.52–2.63 (1H, m), 2.88 (1H, ddd, 6.2, 6.2, 3.9H), 2.91–2.98 (1H, m), 4.29–4.36 (2H, m), 5.95 (1H, bs), 7.23–7.29 (2H, m), 7.30–7.40 (3H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 24.1, 24.8, 28.5, 32.5, 40.0, 67.3, 124.8, 125.1, 127.4, 128.4, 139.5, 141.2, 173.4. ESI-MS *m/z*: 229 (M^+ + H). HRMS calcd for $C_{15}H_{17}O_2$: 229.1229, Found: 229.1248. Anal. Calcd for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found C, 78.88; H, 6.98.

The cycloadducts **2a**, **2b**, **2d**, **2e**, **2g** were reported previously.[11]

4.3.1. (4a*S**,8a*R**)- and (4a*S**,8a*S**)-8a-Methyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (2f-*endo* and 2f-*exo*).

The reaction of **1f** (83 mg, 0.50 mmol) with $TfN[Al(Me)Cl]_2$ (0.55 mmol) and the subsequent purification by column chromatography on silica gel (hexane/AcOEt=10:1) gave the product **2f** as *endo*/*exo* mixture (56.2 mg, 78% yield, *endo*/*exo*=9.5:1). **2g**-*endo*: IR (neat) ν cm^{-1}; 1722. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.33 (3H, s), 1.45 (1H, ddd, J=13.2, 7.3, 5.7 Hz), 1.63 (1H, dddd, J=8.1, 8.1, 5.7, 4.0 Hz), 2.00–2.07 (1H, m), 2.16 (1H, ddd, J=13.2, 4.8, 4.8 Hz), 2.13–2.27 (1H, m), 2.35 (1H, ddd, J=11.4, 5.7, 2.6 Hz), 4.23–4.28 (1H, m), 5.48 (1H, ddd, J=9.9, 4.7, 2.6 Hz), 5.58 (1H, ddd, J=9.9, 6.0, 3.7 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 22.4, 26.0, 26.3, 32.1, 38.8, 41.7, 67.4, 127.7, 129.8, 176.5. EI-MS *m/z*: 166 (M^+). HRMS calcd for $C_{10}H_{14}O_2$: 166.0994 (M^+), Found: 166.0981. **2g**-*exo*: IR (neat) ν cm^{-1}; 1722. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.16 (3H, s), 1.73–1.81 (1H, m), 1.86–1.99 (2H, m), 2.08–2.28 (3H, m), 2.47–2.52 (1H, m), 4.36 (1H, ddd, J=11.6, 11.6, 6.5 Hz), 4.49 (1H, ddd, J=11.6, 7.5, 1.9 Hz), 5.34–5.40 (1H, m), 5.64–5.71 (1H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 16.1, 22.6, 24.2, 29.8, 37.0, 39.8, 68.6, 126.5, 127.4, 176.6. EI-MS *m/z*: 166 (M^+). HRMS calcd for $C_{10}H_{14}O_2$: 166.0994 (M^+), Found: 166.0990.

4.3.2. (4*R*,4a*R*,7*S*,8a*R*)- and (4*R*,4a*S*,7*R*,8a*S*)-4,7-Dimethyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (2h-*cis* and 2h-*trans*).

A *cis*/*trans* mixture of **2h** obtained from the reaction of **1h** (90.5 mg, 0.50 mmol) with $TfN[Al(Me)Cl]_2$ (0.55 mmol) was separated by MPLC (hexane/AcOEt=10:1, flow rate 7.0 mL/min) to give **2h**-*cis* (69.4 mg, 71% yield) and **2h**-*trans* (9.5 mg, 10% yield) in the order of elution. **2h**-*cis*: IR (neat) ν cm^{-1}; 1732. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.00 (6H, d, J=6.6 Hz), 1.31–1.46 (1H, m), 1.68–1.78 (1H, m), 1.84–1.98 (1H, m), 2.09–2.16 (2H, m), 2.23–2.36 (1H, m), 2.80 (1H, ddd, J=9.8, 6.1, 3.4 Hz), 3.87 (1H, dd, J=11.2, 11.2 Hz), 4.24 (1H, dd, J=11.2, 4.5 Hz), 5.66–5.68 (2H, bs). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 13.4, 21.1, 30.9, 31.8, 33.0, 38.6, 39.9, 125.1, 135.4, 174.5. EI-MS *m/z*: 180 (M^+). HRMS calcd for $C_{11}H_{17}O_2$: 181.1229, Found: 181.1128. **2h**-*trans*: IR (neat) ν cm^{-1}; 1728. ^1H NMR (400 MHz, $CDCl_3$) δ; 1.02 (3H, d, J=7.2 Hz), 1.03 (3H, d, J=7.1 Hz), 1.77 (1H, ddd, J=14.1, 8.1, 6.3 Hz), 2.07–2.21 (2H, m), 2.23–2.33 (1H, m), 2.58–2.65 (1H, m), 2.79 (1H, ddd, J=7.9, 7.9, 4.9 Hz), 4.15 (1H, dd, J=10.9, 3.7 Hz), 4.24 (1H, ddd, J=10.2, 2.5, 2.5 Hz), 5.53 (1H, ddd, J=10.2, 2.7, 2.7 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 13.0, 21.4, 29.2, 31.6, 32.4, 36.0, 38.6, 72.6, 123.8, 137.0, 174.0. EI-MS *m/z*: 180 (M^+) HRMS calcd for $C_{11}H_{17}O_2$: 181.1229, Found: 181.1131.

4.3.3. (4*R**,4a*R**,8a*R**)-4-({[*tert*-Butyl(dimethyl)silyl]-oxy}methyl)-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (2i).

The reaction of **1i** (148 mg, 0.50 mmol) with $TfN(AlMe_2)_2$ (0.55 mmol) and the subsequent purification by column chromatography on silica gel (hexane/AcOEt=5:1) gave the product **2i** (103.8 mg, 70% yield). IR (neat) ν cm^{-1}; 1732. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.06 (6H, s), 0.90 (9H, s), 1.69–1.79 (1H, m), 1.85–1.92 (1H, m), 1.98–2.07 (2H, m), 2.15–2.31 (1H, m), 2.62–2.70 (1H, m), 2.76–2.85 (1H, m), 3.66–3.78 (2H, m), 4.18 (1H, dd, J=11.2, 8.7 Hz), 4.22 (1H, dd, J=11.2, 4.4 Hz), 5.54 (1H, bd, J=10.1 Hz), 5.79–5.85 (1H, m). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; −5.5, 18.2, 22.0, 22.8, 25.8, 32.9, 36.8, 42.5, 61.8, 68.8, 127.6, 174.2. ESI-MS *m/z*: 297 (M^++H). HRMS: Calcd for $C_{16}H_{29}O_3Si$: 297.1886, Found: 297.1884. Anal. Calcd for $C_{16}H_{28}O_3Si$: C, 64.82; H, 9.52. Found: C, 64.96; H, 9.44.

4.3.4. (4*R**,4a*S**,7*S**,8a*R**)- and (4*R**,4a*R**,7*R**,8a*S**)-4-{[*tert*-Butyl(dimethyl)silyl]-oxy}-7-methyl-3,4,4a,7,8,8a-hexahydo-1H-isochromen-1-one (2j-*trans* and 2j-*cis*).

A *cis*/*trans* mixture of **2j** obtained from the reaction of **1j** (148 mg, 0.50 mmol) with $TfN(AlMe_2)_2$ (0.55 mmol) was separated by MPLC (hexane/AcOEt=10:1, flow rate 7.0 mL/min) to give **2j**-*cis* (13.0 mg, 9% yield) and **2j**-*trans* (96.5 mg, 65% yield) in the order of elution. **2j**-*trans*: mp 78.0–79.5 °C. IR (KBr) ν cm^{-1}; 1736. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.05 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 1.00 (3H, d, J=7.1 Hz), 1.78 (1H, ddd, J=12.2, 12.0, 10.0 Hz), 2.17 (1H, ddd, J=12.2, 4.7, 4.2 Hz), 2.19–2.28 (1H, m), 2.55–2.60 (1H, m), 2.78 (1H, ddd, J=12.0, 7.8, 4.2 Hz), 4.02 (1H, dd, J=4.5, 2.3 Hz), 4.30 (2H, d, J=2.3 Hz), 5.50 (1H, ddd, J=10.0, 3.1, 3.1 Hz), 5.78 (1H, bd, J=10.0 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; −4.9, −4.6, 18.0, 21.0, 25.7, 30.0, 33.5, 37.6, 38.1, 66.7, 73.6, 124.6, 136.8, 173.5. ESI-MS *m/z*: 297 (M^++H). HRMS: Calcd for $C_{16}H_{29}O_3Si$: 297.1886, Found: 297.1884. **2j**-*cis*: mp 84.5–85.5 °C. IR (KBr) ν cm^{-1}; 1736. ^1H NMR (400 MHz, $CDCl_3$) δ; 0.08 (3H, s), 0.09 (3H, s), 0.89 (9H, s), 1.02 (3H, d, J=7.2 Hz), 1.60 (1H, ddd, J=13.2, 10.5, 8.1 Hz), 2.09 (1H, ddd, J=13.2, 4.9, 4.8 Hz), 2.25–2.27 (1H, m), 2.50–2.51 (1H, m), 2.93 (1H, ddd, J=10.5, 6.8, 4.8 Hz), 3.83 (1H, ddd, J=7.8, 7.5, 3.7 Hz), 4.00 (1H, dd, J=11.0, 3.7 Hz), 4.26 (1H, dd, J=11.0, 3.7 Hz), 5.64 (1H, ddd, J=10.1, 3.5, 2.5 Hz), 5.76 (1H, ddd, J=10.1, 2.1,

2.1 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; −4.8, −4.6, 18.0, 21.3, 25.7, 30.0, 32.5, 37.7, 39.5, 68.0, 71.6, 124.6, 135.8, 173.5. ESI-MS *m*/*z*: 297 ($M^+ + H$). HRMS: Calcd for $C_{16}H_{29}O_3Si$: 297.1886, Found: 297.1903. Anal. Calcd for $C_{16}H_{28}O_3Si$: C, 64.82; H, 9.52. Found: C, 64.56; H, 9.22.

4.4. Procedure for thermal IMDA reaction of 1,7,9-decatrienoates 1j

After a solution of **1j** (148 mg, 0.50 mmol) in 1,2-dichlorobenzene (9 mL) was stirred for 12 h at 140 °C, reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane/AcOEt=10:1) to give **2j** as a *cis*/*trans* mixture, which was separated by MPLC (hexane/AcOEt=10:1, flow rate 7.0 mL/min) to give **2j**-*cis* (87.4 mg, 59% yield) and **2j**-*trans* (22.4 mg, 15% yield) in the order of elution.

4.5. General procedure for conversion to oxabicyclo[4.3.0]nonene derivatives: (3*R**,3a*S**, 6*S**,7a*R**)-3-(hydroxymethyl)-6-methyl-3a,6,7,7a-tetrahydro-1-benzofuran-1(3*H*)-one (4a)

A mixture of **2j**-*cis* (104 mg, 0.25 mmol) and tetrabuthylammonium fluoride (0.75 mL, 1.0 M solution in THF) in THF (1 mL) was stirred for 3 h at room temperature, and then usual work-up followed by the purification by column chromatography on silica gel (hexane/AcOEt=1:1) gave **4a** (32.8 mg, 72% yield) as white solid. Mp 44–46 °C. IR (neat) ν cm^{-1}; 1771, 3447. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 1.03 (3H, d, J=7.2 Hz), 1.42 (1H, ddd, J=13.3, 10.3, 8.3 Hz), 2.05 (1H, ddd, J=13.3, 5.7, 5.7 Hz), 2.15–2.24 (1H, m), 2.79 (1H, ddd, J=10.3, 9.9, 5.7 Hz), 3.16–3.25 (1H, m), 3.51 (2H, d, J=5.8 Hz), 4.68 (1H, dt, J=8.7, 5.8 Hz), 5.54 (1H, ddd, J=10.1, 2.9, 2.8 Hz), 5.81 (1H, ddd, J=10.1, 2.3, 2.3 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.2, 28.3, 29.5, 35.3, 37.9, 63.5, 81.7, 119.9, 137.0, 179.0. ESI-MS *m*/*z*: 183 ($M^+ + H$). HRMS: Calcd for $C_{10}H_{15}O_3$: 183.1021. Found: 183.1012.

4.5.1. (3*R,3a*R**,6*R**,7a*S**)-3-(Hydroxymethyl)-6-methyl-3a,6,7,7a-tetrahydro-1-benzo-furan-1(3*H*)-one (4b).** 91% yield. White solid. Mp 57–58 °C. IR (neat) ν cm^{-1}; 1772, 3287. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 1.07 (3H, d, J=7.1 Hz), 1.24–1.37 (1H, m), 2.09 (1H, ddd, J=12.9, 4.8, 4.7 Hz), 2.20–2.31 (1H, m), 2.80 (1H, dd, J=12.6, 4.4 Hz), 2.89–2.97 (1H, m), 3.74 (1H, dd, J=12.6, 4.4 Hz), 3.99 (1H, dd, J=12.6, 2.4 Hz), 4.25 (1H, ddd, J=9.3, 4.4, 2.4 Hz), 5.58 (1H, ddd, J=10.0, 3.9, 2.6 Hz), 5.77 (1H, bd, J=10.0 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ; 21.6, 29.2, 29.7, 35.7, 39.7, 62.5, 84.5, 121.9, 136.6, 187.5. ESI-MS *m*/*z*: 183 ($M^+ + H$). HRMS: Calcd for $C_{10}H_{15}O_3$: 183.1021. Found: 183.1015.

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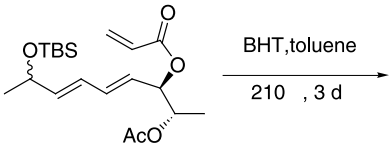

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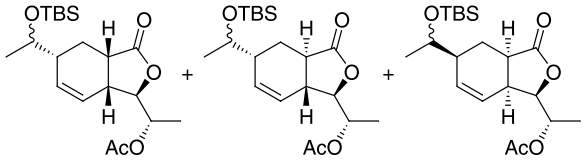

6 ***endo-cis*** ***exo-cis*** ***endo-trans***

endo-cis:exo-cis:endo-trans = 2.5 : 2.5 : 1

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Solvent effects on the oxidative free radical reactions of 2-amino-1,4-naphthoquinones

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Abstract—Solvent effects on the manganese (III) initiated oxidative free radical reactions of 2-amino-1,4-naphthoquinones are described. This free radical reaction provides a novel method for the synthesis of benzo[*f*]indole-4,9-diones, benzo[*f*]indole-2,4,9-triones, benzo[*b*]carbazole-6,11-diones and benzo[*b*]acridine-6,11-diones. High chemoselectivity was observed in different solvents.

1. Introduction

Carbon–carbon bond forming reactions mediated by radical have received considerable attention in organic synthesis during the last two decades.[1] Naturally occurring quinones such as mitosenes, kinamycins, murrayaquinones, etc. represent an important class of biologically significant natural products.[2] A common building block to these compounds is the indoloquinone unit. The development of new synthetic methodologies for the synthesis of indolequinone ring system is therefore important.[3,4] The oxidative free radical reaction mediated by metal salts has been developed into a versatile protocol for the formation of highly functionalized products from simple precursors.[1d–f,5–7] Among these, manganese (III) acetate and cerium (IV) ammonium nitrate have been used most efficiently. The solvent effects play an important role in this oxidative free radical reaction.[8] The free radical reaction of 1,4-naphthoquinones has been reported.[6c–j,9] In this report, we wish to describe the solvent effects on the oxidative free radical reaction between 2-amino-1,4-naphthoquinones and carbonyl compounds.

2. Results and discussion

2.1. The oxidative free radical reactions of 2-(alkylamino)-1,4-naphthoquinones

We reported previously that the manganese (III) acetate mediated reaction between 2-(alkylamino)-1,4-naphthoquinones **1** and β-keto ester **2** (R^2=OR) in acetic acid gave **3** and **4** (Eq. 1).[6h] The product distributions are highly dependent on the substituents of β-keto ester **2**. Indoles **3** and **4** were formed presumably via the reaction route outlined in Scheme 1. Initiation occurs with the manganese (III) acetate oxidation of **2** to produce radical **5**. This radical intermediate **5** undergoes intermolecular addition to the quinone ring followed by oxidation to give **6**, which undergoes either condensation to generate **3** (path a) or oxidation to produce radical **7** (path b). Radical **7** undergoes intramolecular cyclization followed by oxidation to produce **9**, which subsequently undergoes alkyl group (R^1) migration to produce **4**. On the contrary, when **1** and **2** were treated with cerium (IV) sulfate in methanol, indole **3** was obtained as the only product.[6j] This different reaction behavior of intermediate **6** can be ascribed to the presence of cerium salt, which acts as a Lewis acid and the condensation rate of **6** was enhanced.[10] Based on these results, we believe that the acidity of the reaction medium would affect the production distributions of this reaction. To test this hypothesis, this oxidative free radical reaction was performed in various solvents. When a solution of 2-(methylamino)-1,4-naphthoquinone (**1a**) in formic acid was treated with ethyl butyrylacetate (**2a**) and manganese (III) acetate at 0 °C for 30 min, **3a** was obtained exclusively in 85% yield (Table 1, entry 1). Other β-keto ester **2** behaved similarly giving only the corresponding condensation product **3** (entries 2–5). It is well known that Brønsted acid can also catalyze the condensation reaction of carbonyl compounds. These results demonstrate that the higher acidity of formic acid enhances the condensation rate of **6** and path a is the only reaction route. We next performed this reaction in less acidic or neutral solvents. Treatment of **1a** and **2a** with manganese (III) acetate in CF_3CH_2OH at 80 °C for 16 h resulted in the formation of **3a** (12%) and **4a** (63%). Results of this reaction between **1a** and **2a** in different solvents are summarized in

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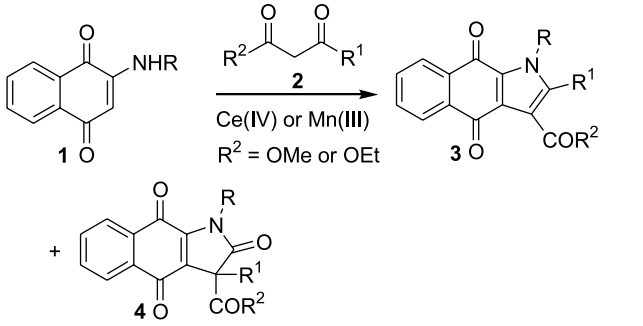
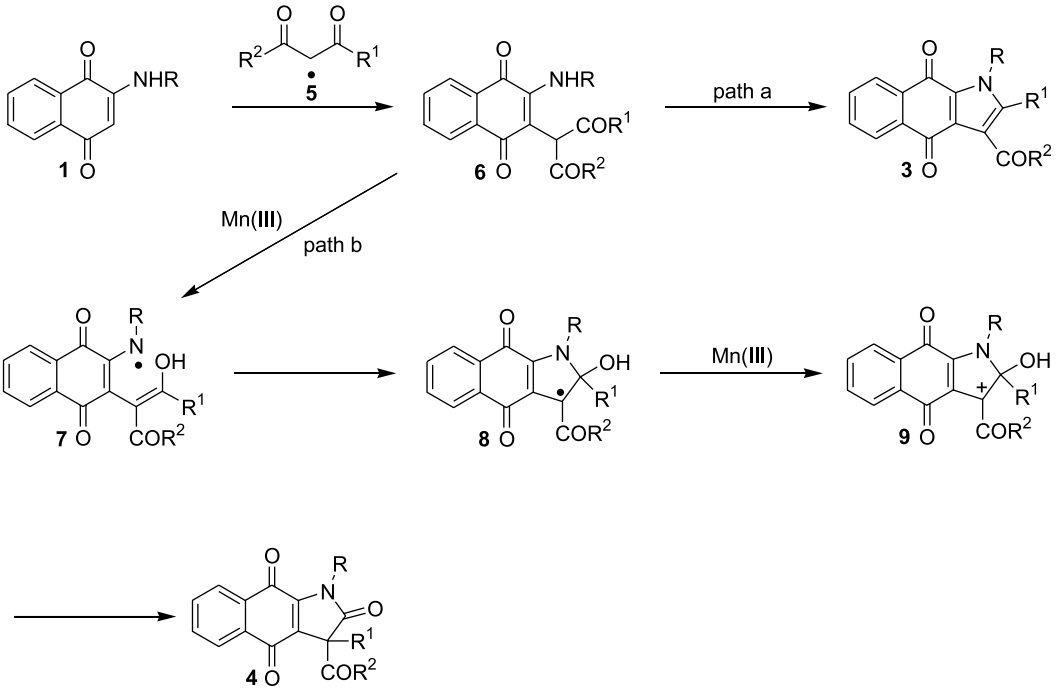
Table 1 (entries 7–10). In all cases, it gave higher **4a/3a** ratio than those performed in acetic acid (entry 6). This could account for the rate of condensation (path a) decreasing as the acidity of reaction medium decreases and the oxidation of **6** to produce radical **7** became the major route (path b). The scope of this reaction was explored using a variety of β-keto esters and the results are also illustrated in Table 1 (entries 11–14). In all cases, **4** is the major product.



(1)

Manganese (III) acetate mediated free radical reaction between 2-(alkylamino)-1,4-naphthoquinone **1** and simple ketone **10** in acetic acid produced **11** as the only product (Eq. 2).[6i] Indole **11** was formed presumably via a similar reaction route as shown in Scheme 1 (path a). Due to the instability of **1** in acidic medium, we expected that the radical reaction between **1** and **10** in neutral solvents would give **11** in better result. Indeed, when **1a** and acetone (**10a**) were reacted with manganese (III) acetate in acetonitrile at 80 °C for 39 h, **11a** was isolated in a better reaction yield (85%, Table 2, entry 1) than that performed in acetic acid (73%). The results of this reaction with a variety of simple ketones in different solvents are summarized in Table 2 (entries 1–11). In all cases, indole **11** was obtained in a better reaction yield than those performed in acetic acid. This reaction can also be performed with corresponding ketones as solvent and **11** was obtained in a similar (better) result. The regioselectivity of this reaction was also studied. With butanone (**10e**: R^1=H, R^2=Me), **11e** and **12a** were obtained in 37 and 57% yields, respectively (entry 12). These two products are derived from



Scheme 1.

Table 1. Free radical reactions between 2-(methylamino)-1,4-naphthoquinone (**1a**) and β-keto esters

Entry	β-Keto ester	Solvent time	Reaction	Product (yield (%))	
1	**2a**: R^1=*n*-Pr, R^2=OEt	HCO_2H	30 min	**3a** (85)	
2	**2b**: R^1=*i*-Pr, R^2=OMe	HCO_2H	30 min	**3b** (75)	
3	**2c**: R^1=Et, R^2=OMe	HCO_2H	30 min	**3c** (76)	
4	**2d**: R^1=$ClCH_2$, R^2=OEt	HCO_2H	30 min	**3d** (66)	
5	**2e**: R^1=$MeOCH_2$, R^2=OMe	HCO_2H	30 min	**3e** (63)	
6	**2a**: R^1=*n*-Pr, R^2=OEt	HOAc	16 h	**3a** (54)	**4a** (21)[a]
7	**2a**: R^1=*n*-Pr, R^2=OEt	CF_3CH_2OH	16 h	**3a** (12)	**4a** (63)
8	**2a**: R^1=*n*-Pr, R^2=OEt	CH_3CN	16 h	**3a** (13)	**4a** (48)
9	**2a**: R^1=*n*-Pr, R^2=OEt	C_6H_6	16 h	**3a** (8)	**4a** (56)
10	**2a**: R^1=*n*-Pr, R^2=OEt	$CHCl_3$	16 h	**3a** (8)	**4a** (52)
11	**2b**: R^1=*i*-Pr, R^2=OMe	CF_3CH_2OH	16 h	**3b** (8)	**4b** (79)
12	**2c**: R^1=Et, R^2=OMe	CF_3CH_2OH	16 h	**3c** (11)	**4c** (62)
13	**2d**: R^1=$ClCH_2$, R^2=OEt	CF_3CH_2OH	16 h	**3d** (6)	**4d** (72)
14	**2e**: R^1=$MeOCH_2$, R^2=OMe	CF_3CH_2OH	16 h		**4e** (73)

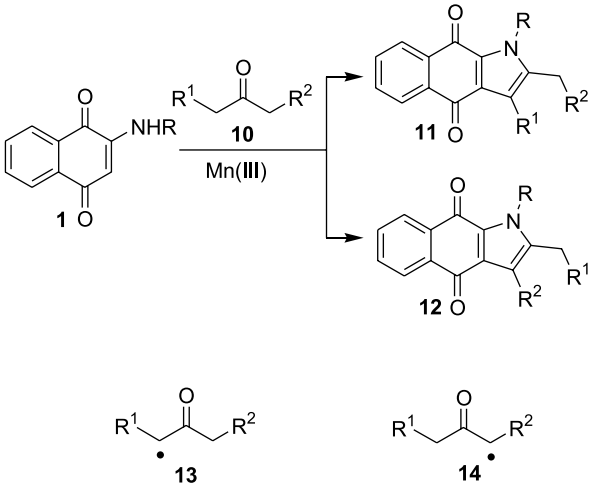
[a] The result has been reported previously.[6h]

Table 2. Free radical reactions between 2-(methylamino)-1,4-naphthoquinone (**1a**) and simple ketones

Entry	Ketone	Solvent	Reaction time	Product (yield (%))	
1	**10a**: R^1=H, R^2=H	CH_3CN	39 h	**11a** (85)	
2	**10a**: R^1=H, R^2=H	C_6H_6	42 h	**11a** (86)	
3	**10a**: R^1=H, R^2=H	$CHCl_3$	16 h	**11a** (80)	
4	**10a**: R^1=H, R^2=H	HCO_2H	30 min	**11a** (0)	
5	**10a**: R^1=H, R^2=H		16 h	**11a** (90)[a]	
6	**10b**: R^1=Me, R^2=Me	CH_3CN	41 h	**11b** (72)	
7	**10b**: R^1=Me, R^2=Me		16 h	**11b** (91)[a]	
8	**10c**: R^1 + R^2=$CH_2CH_2CH_2$	CH_3CN	16 h	**11c** (90)	
9	**10c**: R^1 + R^2=$CH_2CH_2CH_2$		24 h	**11c** (87)[a]	
10	**10d**: R^1 + R^2=CH_2CH_2	CH_3CN	22 h	**11d** (38)	
11	**10d**: R^1 + R^2=CH_2CH_2		26 h	**11d** (38)[a]	
12	**10e**: R^1=H, R^2=Me	CH_3CN	21 h	**11e** (37)	**12a** (57)
13	**10f**: R^1=H, R^2=*i*-Pr	CH_3CN	41 h	**11f** (70)	**12b** (17)

[a] The reaction was performed in corresponding ketone.

the intermolecular addition of radical **13a** and **14a**. The regioselectivity increases as the size of R^2 increases (entry 13).



(2)

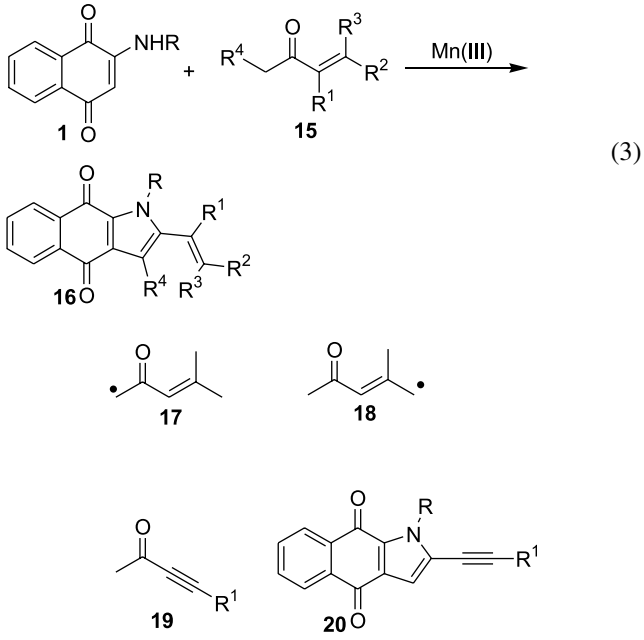
Unsaturated α′-keto radical can be generated regioselectively from the manganese (III) oxidation of α,β-unsaturated ketones.[7] We next studied the free radical reaction of 2-(alkylamino)-1,4-naphthoquinone **1** with α,β-unsaturated ketone **15** (Eq. 3). Treatment of 2-(methylamino)-1,4-naphthoquinone (**1a**) with *trans*-4-phenyl-3-buten-2-one (**15a**) (4 equiv) and manganese (III) acetate (5 equiv) in acetonitrile at 80 °C for 43 h gave indole **16a** in 31% yield (Table 3, entry 1). Using 10 equiv of **15a**, the desired indole **16a** was afforded in 58% yield (entry 2). We also performed this reaction in various solvents. In benzene, the yield of **16a** is 52% (35 h, 80 °C). In CF_3CH_2OH, the reaction rate is much slower. After heated at 80 °C for 86 h, the yield of **16a** is 35% based on 71% conversion of **1a**. In acetic acid, it proceeded in a much faster reaction rate (26 h, 45 °C), however, **16a** was obtained in a much poor yield (13%) and an uncharacterized product was also obtained. The results are summarized in Table 3 (entries 1–5). Best yields are obtained in acetonitrile. Indole **16a** was generated via a similar reaction route as shown in Scheme 1 (path a). The scope of this oxidative annulation process with other 4-aryl-3-buten-2-one **15** are also illustrated in Table 3 (entries 6–10). To study the steric effect on the reactivity of enone **15**, we also examined this reaction with **15f** and **15g**. On the reaction of **15f** with **1a**, indole **16f** was produced though in a slower reaction rate. After heated for 71 h, **16f** was obtained in 65% yield (entry 11). With **15g**, indole **16g** was also produced effectively via this oxidative annulation process (entry 12). These observations demonstrate that the bulkiness of substituent R^4 has little effect on this reaction. In order to test the regioselectivity of this reaction, butenone **15i** was allowed to react with **1a** and **16i** was obtained as the only product (entry 14). This

Table 3. Free radical reactions of 2-(methylamino)-1,4-naphthoquinone (**1a**) and α,β-unsaturated ketones

Entry	α,β-Unsaturated ketone	Solvent	Reaction time (h)	Product (yield(%))
1	**15a**: R^1=H, R^2=C_6H_5, R^3=H, R^4=H	CH_3CN	43	**16a** (31)[a]
2	**15a**: R^1=H, R^2=C_6H_5, R^3=H, R^4=H	CH_3CN	41	**16a** (58)
3	**15a**: R^1=H, R^2=C_6H_5, R^3=H, R^4=H	C_6H_6	35	**16a** (52)
4	**15a**: R^1=H, R^2=C_6H_5, R^3=H, R^4=H	HOAc	26	**16a** (13)
5	**15a**: R^1=H, R^2=C_6H_5, R^3=H, R^4=H	CF_3CH_2OH	86	**16a** (35)[b]
6	**15b**: R^1=H, R^2=4-Cl(C_6H_4), R^3=H, R^4=H	CH_3CN	45	**16b** (63)
7	**15b**: R^1=H, R^2=4-Cl(C_6H_4), R^3=H, R^4=H	C_6H_6	24	**16b** (39)
8	**15c**: R^1=H, R^2=4-MeO_2C(C_6H_4), R^3=H, R^4=H	CH_3CN	46	**16c** (64)
9	**15d**: R^1=H, R^2=4-MeO(C_6H_4), R^3=H, R^4=H	CH_3CN	66	**16d** (37)
10	**15e**: R^1=H, R^2=2-thienyl, R^3=H, R^4=H	CH_3CN	45	**16e** (59)
11	**15f**: R^1=H, R^2=C_6H_5, R^3=H, R^4=Me	CH_3CN	71	**16f** (65)
12	**15g**: R^1=H, R^2=C_6H_5, R^3=H, R^4=*i*-Pr	CH_3CN	64	**16g** (68)
13	**15h**: R^1=H, R^2=CO_2Me, R^3=H, R^4=H	CH_3CN	17	**16h** (59)
14	**15i**: R^1=H, R^2=Me, R^3=Me, R^4=H	CH_3CN	28	**16i** (50)
15	**15j**: R^1=Me, R^2=Me, R^3=H, R^4=H	CH_3CN	68	**16j** (41)
16	**15k**: R^1+R^2=$CH_2CH_2CH_2CH_2$, R^3=H, R^4=H	CH_3CN	45	**16k** (32)
17	**19a**: R^1=Ph	CH_3CN	40	**20a** (76)
18	**19b**: R^1=Et	CH_3CN	17	**20b** (66)

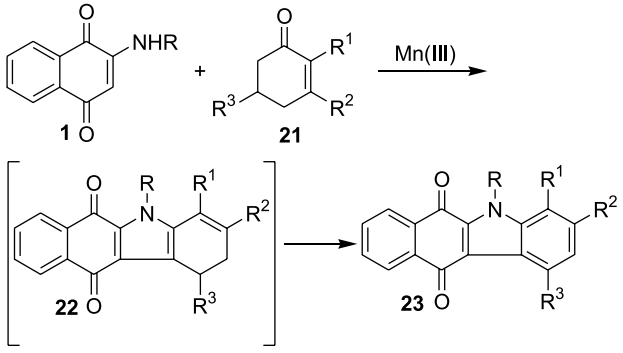
[a] The reaction was conducted with 4 equiv of **15a**.
[b] Based on 71% conversion of **1a**.

product **16i** was formed via the intermolecular addition of an α'-keto radical **17** to the quinone ring. No product derived from the addition of a γ-keto radical **18** to the quinone ring can be detected. Similarly, reaction of enones **15j** and **15k** with manganese (III) acetate gave annulation products **16j** and **16k**, respectively via the addition of a similar α'-keto radical (entries 15 and 16). Notably, butynone **19** behaved similarly, giving the corresponding annulation product **20** effectively (entries 17 and 18).



(3)

We also investigated this manganese (III) mediated radical reaction with 2-cyclohexenone **21** (Eq. 4). Reaction of 2-(methylamino)-1,4-naphthoquinone (**1a**) with 3-ethoxy-2-cyclohexenone (**21a**) and manganese (III) acetate in acetonitrile at 80 °C for 36 h provides **23a** in 66% yield (Table 4, entry 1). Carbazole **23a** was produced presumably from the dehydrogenation of **22a** (R^1=H, R^2=OEt, R^3=H), which was formed via a similar reaction route outlined in Scheme 1 (path a). With other 3-ethoxy-2-cyclohexenone **21**, the corresponding carbazole **23** was afforded effectively under identical conditions (entries 3–6). As shown in Table 4, benzene proved superior to acetonitrile as a reaction solvent. With 3-methyl-2-cyclohexenone (**21d**), in contrast to **22a**, the dehydrogenation of **22f** (R^1=H, R^2=Me, R^3=H) proceeded in a much slower reaction rate. After heating in benzene for 16 h, carbazoles **22f** and **23f** were obtained in 45 and 43% yields, respectively (entry 7). The different behavior between **22a** and **22f** suggests that the strong electron donating ethoxy group enhances the dehydrogenation rate of **22a**. Since the separation of **22f** and **23f** was problematic, the reaction mixture of **22f** and **23f** was heated further for another 99 h with another 2 equiv of manganese (III) acetate and **23f** was afforded in 66% yield (entry 8). The reaction yield of **23f** can be improved significantly to 79% by heating the crude product mixture of **22f** and **23f** directly with DDQ for 1 h (entry 9). Other 3-substituted cyclohexenones (**21e**, **21f**) behaved similarly, giving the corresponding product mixture of **22** and **23**, and again this crude product mixture could be converted to **23** effectively by heating further with DDQ (entries 10 and 11). With 3-unsubstituted 2-cyclohexenone **21**, carbazole **23** was formed in poor yield and no **22** could be detected (entries 12–15). When cyclohexenones **24** and **26**, bearing geminal dimethyl group, were allowed to react with **1**, dihydrocarbazoles **25** and **27** were obtained (entries 16 and 17).

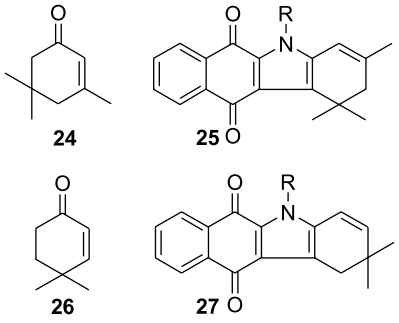


(4)

Table 4. Free radical reactions of 2-(alkylamino)-1,4-naphthoquinone **1** and 2-cyclohexenones

Entry	Quinone	2-Cyclohexenone	Solvent	Reaction time (h)	Product (yield(%))	
1	**1a**: R=Me	**21a**: R^1=H, R^2=OEt, R^3=H	CH_3CN	36	**23a** (66)	
2	**1b**: R=Et	**21a**: R^1=H, R^2=OEt, R^3=H	C_6H_6	26	**23b** (87)	
3	**1a**: R=Me	**21b**: R^1=H, R^2=OEt, R^3=Me	CH_3CN	43	**23c** (47)	
4	**1b**: R=Et	**21b**: R^1=H, R^2=OEt, R^3=Me	C_6H_6	24	**23d** (62)	
5	**1a**: R=Me	**21c**: R^1=H, R^2=OEt, R^3=Ph	CH_3CN	49	**23e** (66)	
6	**1a**: R=Me	**21c**: R^1=H, R^2=OEt, R^3=Ph	C_6H_6	39	**23e** (72)	
7	**1b**: R=Et	**21d**: R^1=H, R^2=Me, R^3=H	C_6H_6	16	**22f** (45)	**23f** (43)
8	**1b**: R=Et	**21d**: R^1=H, R^2=Me, R^3=H	C_6H_6	16	**23f** (66)[a]	
9	**1b**: R=Et	**21d**: R^1=H, R^2=Me, R^3=H	C_6H_6	16	**23f** (79)[b]	
10	**1a**: R=Me	**21e**: R^1=H, R^2=Me, R^3=Me	C_6H_6	19	**23g** (70)[b]	
11	**1a**: R=Me	**21f**: R^1=Cl, R^2=Me, R^3=H	C_6H_6	72	**23h** (56)[b]	
12	**1a**: R=Me	**21g**: R^1=H, R^2=H, R^3=H	CH_3CN	53	**23i** (19)	
13	**1b**: R=Et	**21g**: R^1=H, R^2=H, R^3=H	C_6H_6	39	**23j** (0)	
14	**1a**: R=Me	**21h**: R^1=Cl, R^2=H, R^3=H	CH_3CN	68	**23k** (20)	
15	**1a**: R=Me	**21h**: R^1=Cl, R^2=H, R^3=H	C_6H_6	34	**23k** (23)	
16	**1a**: R=Me	**24**	C_6H_6	23	**25** (76)	
17	**1b**: R=Et	**26**	C_6H_6	17	**27** (57)	

[a] The reaction mixture was reacted further with another 2 equiv of $Mn(OAc)_3$ for 99 h.
[b] The crude product was heated further with 1 equiv of DDQ for 1 h.

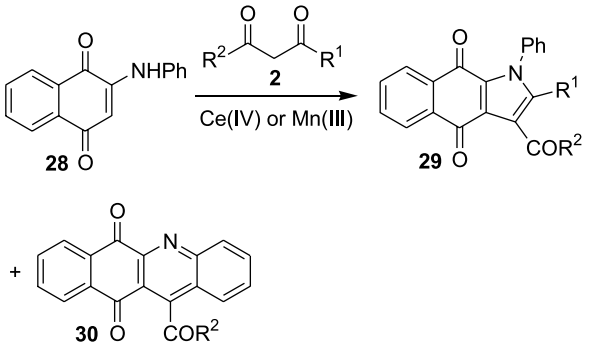


2.2. The oxidative free radical reactions of 2-(anilino)-1,4-naphthoquinones

Manganese (III) mediated free radical reaction between 2-(anilino)-1,4-naphthoquinone (**28**) and β-dicarbonyl compound **2** in acetic acid produced **29** and **30** (Eq. 5).[6h] In all cases, acridine **30** is the major product. A possible mechanism for this reaction is shown in Scheme 2. Oxidation of the β-dicarbonyl compound **2** by manganese (III) acetate oxidation produces radical **5**. This radical intermediate **5** undergoes intermolecular addition to the quinone ring followed by oxidation to give **31**, which undergoes either condensation to produce **29** (path a) or oxidation to generate radical **32** (path b). This radical **32** undergoes further intramolecular cyclization followed by aromatization to give **33**. Quinone **33** undergoes retro Claisen condensation followed by aromatization to produce **30**. On the contrary, in the reaction between **28** and **2** mediated by cerium (IV) sulfate, indole **29** was obtained as the only product.[6j] This is presumably due to the Lewis acidity of cerium salt, which enhances the condensation rate of **31** (path a). Based on these results, we expected that the chemoselectivity of this reaction would be affected by the acidity of the solvent. In agreement with this expectation, when 2-(anilino)-1,4-naphthoquinone (**28**) was reacted with ethyl butyrylacetate (**2a**) and manganese (III) acetate in 80% aqueous formic acid at 0 °C, **29a** was obtained as the only product in 66% yield and no trace of **30a** could be isolated (Table 5, entry 1). This can be ascribed to the higher acidity of formic acid, which promotes the condensation of **31**. These reaction conditions were then applied to other β-dicarbonyl compounds and the corresponding **29** was isolated as the only product. Steric hindrance plays an important role in the final outcome of this reaction. In most cases, the reaction yield decreases as the size of R^1 and R^2 increases (entries 1–6) and the condensation reaction occurs only on the less hindered carbonyl group of the 1,3-diones (entries 7–9). We next studied this reaction in less acidic or neutral solvents. Treatment of **28** with **2a** and manganese (III) acetate in CF_3CH_2OH at 80 °C resulted in the formation of **30a** (64%) and no trace of **29a** could be found (entry 10). This again is presumably due to the rate of condensation (path a) decreases as the acidity of reaction medium decreases and the oxidation of **31** (→**32**) occurred (path b). In attempt to investigate the range of solvents compatible with this reaction, this manganese (III) mediated reaction between **28** and **2a** was performed in various solvents. As shown in Table 5 (entry 10–13), it gave best results in CF_3CH_2OH. This investigation was extended to a number of other β-dicarbonyl compounds and the results are also summarized in Table 5 (entries 14–17). It shows the same selectivity, in all cases, acridine **30** was obtained as the only product.

Table 5. Free radical reactions between 2-(anilino)-1,4-naphthoquinone (**28**) and β-dicarbonyl compounds

Entry	β-Dicarbonyl compound	Solvent	Reaction time	Product (yield(%))
1	**2a**: R^1=*n*-Pr, R^2=OEt	HCO_2H	30 min	**29a** (66)
2	**2b**: R^1=*i*-Pr, R^2=Ome	HCO_2H	3.5 h	**29b** (15)
3	**2f**: R^1=Me, R^2=OEt	HCO_2H	30 min	**29c** (71)
4	**2g**: R^1=Me, R^2=Me	HCO_2H	30 min	**29d** (76)
5	**2h**: R^1=Et, R^2=Et	HCO_2H	30 min	**29e** (77)
6	**2i**: R^1=*i*-Pr, R^2=*i*-Pr	HCO_2H	5 h	**29f** (0)
7	**2j**: R^1=Me, R^2=Ph	HCO_2H	30 min	**29g** (68)
8	**2k**: R^1=Me, R^2=*i*-Bu	HCO_2H	1 h	**29h** (50)
9	**2l**: R^1=Me, R^2=*t*-Bu	HCO_2H	3.5 h	**29i** (17)
10	**2a**: R^1=*n*-Pr, R^2=OEt	CF_3CH_2OH	48 h	**30a** (64)
11	**2a**: R^1=*n*-Pr, R^2=OEt	CH_3CN	42 h	**30a** (46)
12	**2a**: R^1=*n*-Pr, R^2=OEt	C_6H_6	47 h	**30a** (48)
13	**2a**: R^1=*n*-Pr, R^2=OEt	$CHCl_3$	42 h	**30a** (46)
14	**2f**: R^1=Me, R^2=OEt	CF_3CH_2OH	39 h	**30a** (66)
15	**2g**: R^1=Me, R^2=Me	CF_3CH_2OH	23 h	**30b** (69)
16	**2h**: R^1=Et, R^2=Et	CF_3CH_2OH	23 h	**30c** (56)
17	**2i**: R^1=*i*-Pr, R^2=*i*-Pr	CF_3CH_2OH	23 h	**30d** (57)

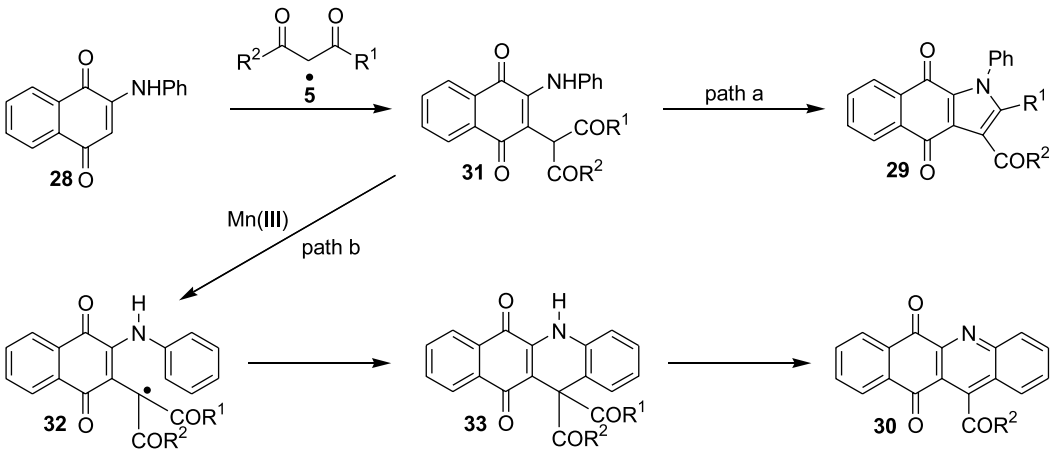


(5)

In conclusion, carbon radical can be generated from the manganese (III) acetate oxidation of carbonyl compounds and it undergoes efficient addition to the C–C double bond of 2-amino-1,4-naphthoquinones. This free radical reaction provides a novel method for the synthesis of benzo[*f*]indole-4,9-diones, benzo[*f*]indole-2,4,9-triones, benzo[*b*]carbazole-6,11-diones and benzo[*b*]acridine-6,11-diones. With β-dicarbonyl compounds, by changing the solvent, these products can be generated in high chemoselectivities. With simple ketones and α,β-unsaturated ketones, these reactions gave better results in neutral solvents.

3. Experimental

Melting points are uncorrected. Infrared spectra were taken with a Hitachi 260-30 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 or AVANCE 300 spectrometer. Chemical shifts are reported in ppm relative to TMS as internal reference. Elemental analyses were performed with Heraeus CHN-Rapid Analyzer. Analytical thin-layer chromatography was performed with precoated silica gel 60 F-254 plates (0.25 mm thick) from EM Laboratories and visualized by UV. The reaction mixture was purified by column chromatography over EM



Scheme 2.

Laboratories silica gel (70–230 mesh). The starting 2-amino-1,4-naphthoquinone **1**,[11] **28**,[4c] enones **21b**,[12a] **21c**,[12a] **21f**[12b] and **21h**[12b] were synthesized according to literature procedure. The spectra data of **3a**,[6j] **3b**,[6j] **3d**,[6h] **3e**,[6j] **4a**,[6h] **4d**,[6h] **11b–f**,[6i] **12a**,[6i] **12b**,[6i] **29a**,[6j] **29c**,[6j] **29d**,[6j] **29g**[6j] and **30a**[6h] have been reported.

3.1. Typical experimental procedure for the reaction between 2-(methylamino)-1,4-naphthoquinone (1a) and β-keto esters in formic acid

A mixture of 152 mg (0.81 mmol) of 2-(methylamino)-1,4-naphthoquinone (**1a**), 508 mg (3.22 mmol) of ethyl butyrylacetate (**2a**) and 1.29 g (4.81 mmol) of $Mn(OAc)_3$ in 10 mL of formic acid was stirred at 0 °C for 30 min. The reaction mixture was diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, 50 mL of saturated aqueous sodium bicarbonate, dried (Na_2SO_4), The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica (20 g) using dichloromethane–hexane (2:1) as eluent, followed by crystallization (ethyl acetate–hexane) to give 224 mg (85%) of **3a**.

3.2. Typical experimental procedure for the reaction between 2-(methylamino)-1,4-naphthoquinone (1a) and β-keto esters in less acidic or neutral solvent

A mixture of 150 mg (0.80 mmol) of 2-(methylamino)-1,4-naphthoquinone (**1a**), 520 mg (3.29 mmol) of ethyl butyrylacetate (**2a**) and 1.29 g (4.81 mmol) of $Mn(OAc)_3$ in 10 mL of CF_3CH_2OH was heated at 80 °C for 16 h. The reaction mixture was diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over 20 g of silica gel (eluted with 1:15 ethyl acetate–hexane and then 1:12 ethyl acetate–hexane) followed by crystallization (ethyl acetate–hexane) to give 174 mg (63%) of **4a** and 32 mg (12%) of **3a**.

3.2.1. 2-Ethyl-4,9-dihydro-3-(methoxycarbonyl)-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 3c. Yellow needles; mp 119–120 °C; IR ($CHCl_3$) 1715, 1660, 1600, 1505, 1275 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J=7.6 Hz, 3H, CH_3), 2.89 (q, J=7.6 Hz, 2H, CH_2), 3.96 (s, 3H, OCH_3), 4.07 (s, 3H, NCH_3), 7.63–7.70 (m, 2H, ArH), 8.08–8.17 (m, 2H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.3 (q), 18.2 (t), 32.8 (q), 52.1 (q), 112.8 (s), 125.6 (s), 126.1 (d), 126.7 (d), 130.5 (s), 132.9 (d), 133.1 (s), 133.3 (d), 133.8 (s), 147.5 (s), 165.0 (s), 176.4 (s), 179.5 (s). Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.64; H, 5.15; N, 4.71.

3.2.2. 2,3,4,9-Tetrahydro-3-isopropyl-3-(methoxycarbonyl)-1-methyl-2,4,9-trioxo-1*H*-benzo[*f*]indole 4b. Orange crystals; mp 153–154 °C; IR ($CHCl_3$) 2975, 1760, 1675, 1595, 1275, 1240 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (d, J=6.9 Hz, 3H, CH_3), 1.03 (d, J=6.9 Hz, 3H, CH_3), 3.06 (septet, J=6.9 Hz, 1H, CH), 3.55 (s, 3H, NCH_3), 3.71 (s, 3H, OCH_3), 7.73 (td, J=7.3, 1.4 Hz, 1H, ArH), 7.78 (td, J=7.3, 1.4 Hz, 1H, ArH), 8.09 (dd, J=7.3, 1.4 Hz, 1H, ArH), 8.11 (dd, J=7.3, 1.4 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 17.0 (q), 18.8 (q), 28.9 (q), 33.6 (d), 53.1 (q), 64.9 (s), 125.2 (s), 126.3 (d), 126.5 (d), 131.5 (s), 132.3 (s), 133.1 (d), 134.6 (d), 147.6 (s), 166.4 (s), 174.4 (s), 178.3 (s), 178.5 (s). Anal. Calcd for $C_{18}H_{17}NO_5$: C, 66.05; H, 5.23; N, 4.28. Found: C, 65.99; H, 5.26; N, 4.29.

3.2.3. 3-Ethyl-2,3,4,9-tetrahydro-3-(methoxycarbonyl)-1-methyl-2,4,9-trioxo-1*H*-benzo[*f*]indole 4c. Yellow crystals; mp 155–156 °C; IR ($CHCl_3$) 1760, 1675, 1600, 1240, 1390 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.74 (t, J=7.5 Hz, 3H, CH_3), 2.40–2.58 (m, 2H, CH_2), 3.56 (s, 3H, NCH_3), 3.71 (s, 3H, OCH_3), 7.74 (td, J=7.4, 1.6 Hz, 1H, ArH), 7.79 (td, J=7.4, 1.6 Hz, 1H, ArH), 8.09 (dd, J=7.4, 1.6 Hz, 1H, ArH), 8.13 (dd, J=7.4, 1.6 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 8.3 (q), 26.3 (t), 29.0 (q), 53.3 (q), 61.0 (s), 125.6 (s), 126.3 (d), 126.5 (d), 131.7 (s), 132.1 (s), 133.2 (d), 134.6 (d), 147.4 (s), 166.7 (s), 174.8 (s), 178.3 (s), 178.4 (s). Anal. Calcd for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47. Found: C, 65.10; H, 4.85; N, 4.42.

3.2.4. 2,3,4,9-Tetrahydro-3-(methoxycarbonyl)-3-(methoxymethyl)-1-methyl-2,4,9-trioxo-1*H*-benzo[*f*]indole 4e. Yellow crystals; mp 207–208 °C; IR ($CHCl_3$) 3015, 1760, 1730, 1650, 1240 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.25 (s, 3H, OCH_3), 3.55 (s, 3H, NCH_3), 3.72 (s, 3H, OCH_3), 4.21 (d, J=8.6 Hz, 1H, OCH), 4.33 (d, J=8.6 Hz, 1H,

OCH), 7.70–7.82 (m, 2H, ArH), 8.05–8.15 (m, 2H, ArH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 29.2 (q), 53.4 (q), 59.4 (q), 60.8 (s), 72.3 (t), 124.5 (s), 126.1 (d), 126.5 (d), 131.8 (s), 132.2 (s), 133.2 (d), 134.5 (d), 147.9 (s), 164.8 (s), 173.8 (s), 178.4 (s), 178.6 (s). Anal. Calcd for $C_{17}H_{15}NO_6$: C, 62.00; H, 4.59; N, 4.25. Found: C, 62.29; H, 4.63; N, 4.20.

3.3. Typical experimental procedure for the reaction between 2-(methylamino)-1,4-naphthoquinone (1a) and simple ketones

A mixture of 118 mg (0.63 mmol) of 2-(methylamino)-1,4-naphthoquinone (**1a**), 375 mg (6.47 mmol) of acetone (**10a**) and 1.03 g (3.84 mmol) of $Mn(OAc)_3$ in 10 mL of CH_3CN was heated at 80 °C for 16 h, followed by the addition of 1.04 g (3.88 mmol) of $Mn(OAc)_3$. The reaction mixture heated for another 23 h and then diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, dried (Na_2SO_4), The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica (20 g) using dichloromethane–hexane (1:1) as eluent, followed by crystallization (ethyl acetate–hexane) to give 121 mg (85%) of **11a**.

3.3.1. 4,9-Dihydro-1,2-dimethyl-4,9-dioxo-1*H*-benzo[*f*]indole 11a. Yellow crystals; mp 236–237 °C; IR ($CHCl_3$) 1650, 1600, 1500, 1485, 1250 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3H, $ArCH_3$), 4.00 (s, 3H, NCH_3), 6.52 (s, 1H, ArH), 7.60–7.70 (m, 2H, ArH), 8.09–8.17 (m, 2H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 12.2 (q), 32.6 (q), 107.2 (d), 126.28 (d), 126.32 (d), 128.2 (s), 130.6 (s), 132.7 (d), 132.9 (d), 133.5 (s), 134.2 (s), 139.9 (s), 175.6 (s), 181.1 (s). Anal. Calcd for $C_{14}H_{11}NO_2$: C, 74.65; H, 4.92; N, 6.22. Found: C, 74.64; H, 4.94; N, 6.25.

3.4. Typical experimental procedure for the reaction between 2-(methylamino)-1,4-naphthoquinone (1a) and α,β-unsaturated ketones

A mixture of 101 mg (0.54 mmol) of 2-(methylamino)-1,4-naphthoquinone (**1a**), 782 mg (5.35 mmol) of *trans*-4-phenyl-3-buten-2-one (**15a**) and 717 mg (2.67 mmol) of $Mn(OAc)_3$ in 10 mL of CH_3CN was heated at 80 °C for 24 h, followed by the addition of 287 mg (1.07 mmol) of $Mn(OAc)_3$. The reaction mixture heated for another 17 h and then diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over 20 g of silica gel (eluted with 2:1 dichloromethane–hexane) followed by crystallization (ethyl acetate–hexane) to give 97 mg (58%) of **16a**.

3.5. Typical experimental procedure for the reaction between 2-(methylamino)-1,4-naphthoquinone (1a) and α,β-unsaturated ketones followed by DDQ oxidation

A mixture of 120 mg (0.60 mmol) of 2-(ethylamino)-1,4-naphthoquinone (**1b**), 658 mg (5.98 mmol) of 3-methyl-2-cyclohexenone (**21d**) and 801 mg (2.99 mmol) of manganese (III) acetate in 10 mL of benzene was heated at 80 °C for 16 h. The reaction mixture was diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product mixture of **22f** and **23f** was then heated at 80 °C with DDQ (136 mg, 0.60 mmol) in 10 mL of benzene for another 1 h. The reaction mixture was diluted with 100 mL of ethyl acetate, washed with three 50 mL portions of water and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica (20 g) using dichloromethane–hexane (2:1) as eluent, followed by crystallization (ethyl acetate–hexane) to give **23f** (135 mg, 79%).

3.5.1. 4,9-Dihydro-1-methyl-(*E*)-4,9-dioxo-2-styryl-1*H*-benzo[*f*]indole 16a. Red needles; mp 205–206 °C; IR ($CHCl_3$) 3010, 2955, 1645, 1595, 1470 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11 (s, 3H, NCH_3), 6.90 (d, J= 16.1 Hz, 1H, =CH), 6.97 (s, 1H, ArH), 7.14 (d, J=16.1 Hz, 1H, =CH), 7.28–7.35 (m, 1H, ArH), 7.35–7.42 (m, 2H, ArH), 7.45–7.54 (m, 2H, ArH), 7.54–7.68 (m, 2H, ArH), 8.06–8.17(m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 32.7 (q), 105.4 (d), 114.1 (d), 126.3 (2×d), 126.8 (2×d), 128.6 (s), 128.7 (d), 128.9 (2×d), 130.8 (s), 132.7 (d), 133.1 (d), 133.4 (s), 134.1 (d), 134.5 (s), 136.1 (s), 141.1 (s), 175.5 (s), 180.8 (s). Anal. Calcd for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.82; N, 4.47. Found: C, 80.28; H, 4.83; N, 4.49.

3.5.2. (*E*)-2-[2-(4-Chlorophenyl)vinyl]-4,9-dihydro-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 16b. Red crystals; mp 264–265 °C; IR ($CHCl_3$) 2930, 1730, 1650, 1595, 1495 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (s, 3H, NCH_3), 6.96 (d, J=16.1 Hz, 1H, =CH), 7.05 (s, 1H, ArH), 7.16 (d, J=16.1 Hz, 1H, =CH), 7.36 (d, J=8.5 Hz, 2H, ArH), 7.45 (d, J=8.5 Hz, 2H, ArH), 7.63–7.72 (m, 2H, ArH), 8.12–8.20 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 32.8 (q), 105.6 (d), 114.8 (d), 126.5 (2×d), 127.9 (2×d), 128.7 (s), 129.1 (2×d), 131.0 (s), 132.8 (d), 132.9 (d), 133.2 (d), 133.5 (s), 134.47 (s), 134.54 (s), 134.6 (s), 140.8 (s), 175.7 (s), 181.0 (s). Anal. Calcd for $C_{21}H_{14}ClNO_2$: C, 72.52; H, 4.06; N, 4.03. Found: C, 72.30; H, 4.14; N, 3.98.

3.5.3. 4,9-Dihydro-(*E*)-2-[2-(4-methoxycarbonylphenyl)-vinyl]-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 16c. Orange needles; mp 208–209 °C; IR ($CHCl_3$) 1720, 1670, 1645, 1410, 1285 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 3H, OCH_3), 4.20 (s, 3H, NCH_3), 7.089 (d, J=16.1 Hz, 1H, =CH), 7.093 (s, 1H, ArH), 7.23 (d, J=16.1 Hz, 1H, =CH), 7.57 (d, J=8.3 Hz, 2H, ArH), 7.62–7.73 (m, 2H, ArH), 8.05 (d, J=8.3 Hz, 2H, ArH), 8.14–8.20 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 32.8 (q), 52.2 (q), 106.0 (d), 116.6 (d), 126.5 (2×d), 126.6 (2×d), 128.7 (s), 129.9 (s), 130.1(s), 130.17 (2×d), 131.2 (s), 132.7 (d), 133.0 (d), 133.2 (d), 133.4 (s), 134.5 (s), 140.4 (s), 166.6 (s), 175.8 (s), 180.9 (s). Anal. Calcd for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.18; H, 4.64; N, 3.70.

3.5.4. 4,9-Dihydro-(*E*)-2-[2-(4-methoxyphenyl)vinyl]-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 16d. Red needles; mp 207–208 °C; IR ($CHCl_3$) 3010, 2960, 1645, 1510, 1465 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 3H, OCH_3), 4.16 (s, 3H, NCH_3), 6.83 (d, J=16.0 Hz, 1H,

=CH), 6.92 (d, J=8.7 Hz, 2H, ArH), 7.00 (s, 1H, ArH), 7.16 (d, J=16.0 Hz, 1H, =CH), 7.46 (d, J=8.7 Hz, 2H, ArH), 7.61–7.70 (m, 2H, ArH), 8.12–8.16 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 32.7 (q), 55.4 (q), 105.0 (d), 112.0 (d), 114.3 (2×d), 126.4 (2×d), 128.2 (2×d), 128.8 (s), 128.9 (s), 130.6 (s), 132.7 (d), 133.1 (d), 133.5 (s), 133.9 (d), 134.6 (s), 141.7 (s), 160.2 (s), 175.4 (s), 181.1 (s). Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.67; H, 5.04; N, 4.02.

3.5.5. 4,9-Dihydro-1-methyl-4,9-dioxo-(*E*)-2-(2-thieylvinyl)-1*H*-benzo[*f*]indole 16e. Red needles; mp 215–216 °C; IR ($CHCl_3$) 3015, 2955, 1645, 1595, 1410 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (s, 3H, NCH_3), 6.74 (d, J=15.9 Hz, 1H, =CH), 6.98 (s, 1H, ArH), 7.04 (dd, J=4.6, 3.6 Hz, 1H, ArH), 7.14 (d, J=3.6 Hz, 1H, ArH), 7.28 (d, J=4.6 Hz, 1H, ArH), 7.30 (d, J=15.9 Hz, 1H, =CH), 7.60–7.69 (m, 2H, ArH), 8.10–8.16 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 32.7 (q), 105.3 (d), 113.5 (d), 125.9 (d), 126.4 (2×d), 127.0 (d), 128.0 (2×d), 128.7 (s), 130.8 (s), 132.8 (d), 133.1 (d), 133.4 (s), 134.6 (s), 140.8 (s), 141.6 (s), 175.5 (s), 180.9 (s). Anal. Calcd for $C_{19}H_{13}NO_2S$: C, 71.45; H, 4.10; N, 4.39. Found: C, 71.41; H, 4.11; N, 4.44.

3.5.6. 4,9-Dihydro-1,3-dimethyl-4,9-dioxo-(*E*)-2-styryl-1*H*-benzo[*f*]indole 16f. Red crystals; mp 218–219 °C; IR ($CHCl_3$) 3015, 2950, 1645, 1595, 1495, 1465 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (s, 3H, $ArCH_3$), 4.14 (s, 3H, NCH_3), 6.93 (d, J=16.5 Hz, 1H, =CH), 6.99 (d, J=16.5 Hz, 1H, =CH), 7.30–7.37 (m, 1H, ArH), 7.37–7.44 (m, 2H, ArH), 7.49–7.53 (m, 2H, ArH), 7.60–7.68 (m, 2H, ArH), 8.10–8.17 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 11.7 (q), 33.5 (q), 115.1 (d), 121.4 (s), 125.7 (s), 126.1 (d), 126.2 (d), 126.6 (2×d), 128.7 (d), 128.9 (2×d), 130.0 (s), 132.76 (d), 132.82 (d), 134.0 (s), 134.3 (s), 135.7 (d), 136.5 (s), 138.1 (s), 175.7 (s), 182.2 (s). Anal. Calcd for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N, 4.28. Found: C, 80.68; H, 5.24; N, 4.29.

3.5.7. 4,9-Dihydro-3-isopropyl-1-methyl-4,9-dioxo-(*E*)-2-styryl-1*H*-benzo[*f*]indole 16g. Red needles; mp 165–166 °C; IR ($CHCl_3$) 3010, 1590, 1460, 1410, 1360 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ1.43 (d, J=7.1 Hz, 6H, 2×CH_3), 3.54 (septet, J=7.1 Hz, 1H, CH), 4.11 (s, 3H, NCH_3), 6.82 (d, J=17.7 Hz, 1H, =CH), 6.96 (d, J=17.7 Hz, 1H, =CH), 7.32–7.38 (m, 1H, ArH), 7.39–7.45 (m, 2H, ArH), 7.52–7.56 (m, 2H, ArH), 7.64–7.68 (m, 2H, ArH), 8.12–8.20 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 21.1 (2×q), 25.9 (d), 34.3 (q), 115.9 (d), 125.1 (s), 125.9 (d), 126.6 (d), 126.7 (2×d), 128.8 (d), 128.9 (2×d), 131.4 (s), 132.3 (s), 132.7 (d), 132.9 (d), 133.8 (s), 134.3 (s), 136.1 (s), 137.3 (d), 137.6 (s), 176.2 (s), 181.1 (s). Anal. Calcd for $C_{24}H_{21}NO_2$: C, 81.10; H, 5.96; N, 3.94. Found: C, 81.14; H, 6.00; N, 3.91.

3.5.8. 4,9-Dihydro-(*E*)-2-[2-(methoxycarbonyl)vinyl]-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 16h. Yellow crystals; mp 248–249 °C; IR ($CHCl_3$) 3015, 2955, 1730, 1655, 1440 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 4.19 (s, 3H, NCH_3), 6.51 (d, J=15.8 Hz, 1H, =CH), 7.16 (s, 1H, ArH), 7.65 (d, J=15.8 Hz, 1H, =CH), 7.68–7.73 (m, 2H, ArH), 8.12–8.20 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 32.9 (q), 52.0 (q), 108.5 (d), 121.4 (d), 126.64 (d),126.66 (d), 128.3 (s), 130.0 (d), 132.4 (s), 133.32 (d), 133.36 (d), 133.40 (s), 134.3 (s), 137.4 (s), 166.6 (s), 176.3 (s), 180.5 (s). Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.01; H, 4.51; N, 4.72.

3.5.9. 4,9-Dihydro-1-methyl-2-(2-methyl-1-propenyl)-4,9-dioxo-1*H*-benzo[*f*]indole 16i. Orange needles; mp 159–160 °C; IR ($CHCl_3$) 3010, 2925, 1650, 1595, 1470 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 3.99 (s, 3H, NCH_3), 6.01 (s, 1H, =CH), 6.66 (s, 1H, ArH), 7.60–7.68 (m, 2H, ArH), 8.09–8.15 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 20.4 (q), 27.1 (q), 32.8 (q), 108.0 (d), 112.4 (d), 126.2 (2×d), 128.1 (s), 129.7 (s), 132.6 (d), 132.9 (d), 133.5 (s), 134.4 (s), 140.3 (s), 143.2 (s), 175.6 (s), 181.1 (s). Anal. Calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.93; H, 5.80; N, 5.28.

3.5.10. 4,9-Dihydro-1-methyl-(*E*)-2-(1-methyl-1-propenyl)-4,9-dioxo-1*H*-benzo[*f*]indole 16j. Orange needles; mp 101–102 °C; IR ($CHCl_3$) 3010, 1655, 1595, 1475, 1445 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 (d, J=6.5 Hz, 3H, CH_3), 1.98 (d, J=1.3 Hz, 3H, CH_3), 3.99 (s, 3H, NCH_3), 5.78 (qq, J=6.5, 1.3 Hz, 1H, =CH), 6.58 (s, 1H, ArH), 7.61–7.70 (m, 2H, ArH), 8.10–8.18 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.2 (q), 16.9 (q), 34.5 (q), 106.7 (d), 126.0 (s), 126.30 (d), 126.34 (d), 128.2 (s), 130.1 (d), 130.7 (s), 132.7 (d), 133.0 (d), 133.6 (s), 134.4 (s), 147.2 (s), 175.9 (s), 181.2 (s). Anal. Calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.94; H, 5.77; N, 5.30.

3.5.11. 2-(1-Cyclohexenyl)-4,9-dihydro-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 16k. Yellow needles; mp 155–156 °C; IR ($CHCl_3$) 3010, 2940, 1650, 1455, 1340 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67–1.74 (m, 2H, CH_2), 1.74–1.83 (m, 2H, CH_2), 2.22–2.31 (m, 4H, CH_2), 4.00 (s, 3H, NCH_3), 5.96 (s, 1H, =CH), 6.58 (s, 1H, ArH), 7.61–7.67 (m, 2H, ArH), 8.09–8.15 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 21.6 (t), 22.5 (t), 25.5 (t), 29.0 (t), 34.5 (q), 106.5 (d), 126.2 (2×d), 128.06 (s), 128.09 (s), 130.7 (s), 131.9 (d), 132.6 (d), 132.9 (d), 133.5 (s), 134.3 (s), 145.8 (s), 175.7 (s), 181.1 (s). Anal. Calcd for $C_{19}H_{17}NO_2$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.29; H, 5.90; N, 4.82.

3.5.12. 4,9-Dihydro-1-methyl-4,9-dioxo-2-(phenylethynyl)-1*H*-benzo[*f*]indole 20a. Orange needles; mp 193–194 °C; IR ($CHCl_3$) 3010, 1660, 1595, 1480, 1465 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (s, 3H, NCH_3), 6.97 (s, 1H, ArH), 7.36–7.41 (m, 3H, ArH), 7.52–7.57 (m, 2H, ArH), 7.63–7.68 (m, 2H, ArH), 8.09–8.18 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 34.3 (q), 78.5 (s), 97.9 (s), 112.8 (d), 121.6 (s), 125.2 (s), 126.4 (d), 126.6 (d), 127.8 (s), 128.5 (2×d), 129.3 (d), 131.0 (s), 131.6 (2×d), 133.1 (2×d), 133.5 (s), 134.0 (s), 175.6 (s), 180.3 (s). Anal. Calcd for $C_{21}H_{13}NO_2$: C, 81.01; H, 4.21; N, 4.50. Found: C, 80.94; H, 4.27; N, 4.50.

3.5.13. 2-(Butyn-1-yl)-4,9-dihydro-1-methyl-4,9-dioxo-1*H*-benzo[*f*]indole 20b. Yellow needles; mp 192–193 °C; IR ($CHCl_3$) 3010, 2990, 1660, 1595, 1465 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (t, J=7.5 Hz, 3H, CH_3), 2.51 (q, J=7.5 Hz, 2H, CH_2), 4.05 (d, J=1.9 Hz, 3H, NCH_3), 6.80 (d, J=1.9 Hz,1H, ArH), 7.61–7.68 (m, 2H, ArH),

8.07–8.16 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 13.3 (t), 13.5 (q), 34.1 (q), 69.5 (s), 101.1 (s), 111.9 (d), 126.0 (s), 126.4 (d), 126.5 (d), 127.6 (s), 130.4 (s), 132.95 (d), 133.02 (d), 133.6 (s), 134.0 (s), 175.5 (s), 180.4 (s). Anal. Calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.22; H, 5.09; N, 5.30.

3.5.14. 5-Ethyl-1,2,6,11-tetrahydro-3-methyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 22f. Red crystals; mp 195–196 °C; IR($CHCl_3$) 2985, 2930, 1635, 1595, 1575, 1475 cm^{-1}; ^{1}H NMR(400 MHz, $CDCl_3$) δ 1.40 (t, J=7.2 Hz, 3H, CH_3), 1.97 (s, 3H, CH_3), 2.39 (t, J=8.7 Hz, 2H, CH_2), 3.08 (t, J=8.7 Hz, 2H, CH_2), 4.46 (q, J=7.2 Hz, 2H, NCH_2), 6.15 (s, 1H, =CH), 7.56–7.66 (m, 2H, ArH), 8.06–8.13 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 15.9 (q), 20.1 (t), 24.3 (q), 29.5 (t), 40.2 (t), 110.2 (d), 118.7 (s), 124.6 (s), 125.9 (2×d), 128.1 (s), 132.1 (d), 132.8 (d), 133.9 (s), 134.9 (s), 138.5 (s), 143.7 (s), 174.0 (s), 182.4 (s). Anal. Calcd for $C_{19}H_{17}NO_2$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.32; H, 5.89; N, 4.84.

3.5.15. 3-Ethoxy-6,11-dihydro-5-methyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23a. Orange needles; mp 233–234 °C; IR ($CHCl_3$) 2990, 1655, 1625, 1595, 1480 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (t, J=7.0 Hz, 3H, CH_3), 4.11 (q, J=7.0 Hz, 2H, OCH_2), 4.18 (s, 3H, NCH_3), 6.75 (d, J=2.1 Hz, 1H, ArH), 7.01 (dd, J=8.9, 2.1 Hz, 1H, ArH), 7.62–7.72 (m, 2H, ArH), 8.10–8.20 (m, 2H, ArH), 8.27 (d, J=8.9 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.8 (q), 31.9 (q), 63.9 (t), 93.2 (d), 116.0 (d), 118.0 (s), 119.3 (s), 124.6 (d), 126.1 (d), 126.3 (d), 132.7 (d), 133.4 (d), 133.7 (s), 134.0 (s), 134.5(s), 141.5 (s), 159.6 (s), 178.4 (s), 181.3 (s). Anal. Calcd for $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59. Found: C, 74.77; H, 4.91; N, 4.57.

3.5.16. 3-Ethoxy-5-ethyl-6,11-dihydro-6,11-dioxo-5*H*-benzo[*b*]carbazole 23b. Orange needles; mp 190–191 °C; IR ($CHCl_3$) 2990, 1655, 1625, 1505, 1475 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (t, J=7.2 Hz, 3H, CH_3), 1.44 (t, J=7.0 Hz, 3H, CH_3), 4.03 (q, J=7.0 Hz, 2H, OCH_2), 4.60 (q, J=7.2 Hz, 2H, NCH_2), 6.66 (d, J=2.1 Hz, 1H, ArH), 6.92 (dd, J=8.8, 2.1 Hz, 1H, ArH), 7.60 (td, J=7.2, 1.7 Hz, 1H, ArH), 7.64 (td, J=7.2, 1.7 Hz, 1H, ArH), 8.05 (dd, J=7.2, 1.7 Hz, 1H, ArH), 8.10 (dd, J=7.2, 1.7 Hz, 1H, ArH), 8.19 (d, J=8.8 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.7 (q), 14.9 (q), 40.1 (t), 63.7 (t), 93.1 (d), 115.6 (d), 118.0 (s), 119.2 (s), 124.5 (d), 125.9 (d), 126.1 (d), 132.6 (d), 133.2 (d), 133.6 (s), 133.7 (s), 133.8 (s), 140.3 (s), 159.3 (s), 177.8 (s), 181.2 (s). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 5.37; N, 4.39. Found: C, 75.19; H, 5.42; N, 4.37.

3.5.17. 3-Ethoxy-6,11-dihydro-1,5-dimethyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23c. Red needles; mp 227–228 °C; IR ($CHCl_3$) 2925, 1655, 1615, 1595, 1495 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (t, J=6.9 Hz, 3H, CH_3), 2.94 (s, 3H, $ArCH_3$), 4.08 (q, J=6.9 Hz, 2H, OCH_2), 4.16 (s, 3H, NCH_3), 6.53 (s, 1H, ArH), 6.75 (s, 1H, ArH), 7.59–7.69 (m, 2H, ArH), 8.04–8.17 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.8 (q), 23.7 (q), 32.1 (q), 63.7 (t), 90.3 (d), 117.2 (d), 118.0 (s), 120.7 (s), 125.7 (d), 126.5 (d), 132.4 (d), 133.0 (s), 133.4 (d), 134.4 (s), 134.9 (s), 136.8 (s), 142.3 (s), 159.2 (s), 178.7 (s), 180.1 (s). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 5.37; N, 4.39. Found: C, 75.07; H, 5.39; N, 4.40.

3.5.18. 3-Ethoxy-5-ethyl-6,11-dihydro-1-methyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23d. Red crystals; mp 168–169 °C; IR ($CHCl_3$) 2985, 2930, 1655, 1615, 1595, 1495 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (t, J=7.2 Hz, 3H, CH_3), 1.47 (t, J=7.0 Hz, 3H, CH_3), 2.95 (s, 3H, $ArCH_3$), 4.09 (q, J=7.0 Hz, 2H, OCH_2), 4.70 (q, J=7.2 Hz, 2H, NCH_2), 6.58 (d, J=2.0 Hz, 1H, ArH), 6.74–6.80 (m, 1H, ArH), 7.63 (td, J=7.3, 1.6 Hz, 1H, ArH), 7.67 (td, J=7.3, 1.6 Hz, 1H, ArH), 8.09 (dd, J=7.3, 1.6 Hz, 1H, ArH), 8.15 (dd, J=7.3, 1.6 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.7 (q), 14.8 (q), 23.7 (q), 40.2 (t), 63.7 (t), 90.3 (d), 117.0 (d), 118.3 (s), 120.9 (s), 125.7 (d), 126.5 (d), 132.4 (d), 133.0 (s), 133.4 (d), 134.36 (s), 134.38 (s), 136.9 (s), 141.2 (s), 159.2 (s), 178.3 (s), 180.2 (s). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.45; H, 5.77; N, 4.15.

3.5.19. 3-Ethoxy-6,11-dihydro-5-methyl-6,11-dioxo-1-phenyl-5*H*-benzo[*b*]carbazole 23e. Red crystals; mp 246–247 °C; IR ($CHCl_3$) 3010, 1655, 1615, 1495, 1480 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (t, J=7.0 Hz, 3H, CH_3), 4.12 (q, J=7.0 Hz, 2H, OCH_2), 4.24 (s, 3H, NCH_3), 6.77 (d, J=2.0 Hz, 1H, ArH), 6.93 (d, J=2.0 Hz, 1H, ArH), 7.35–7.40 (m, 2H, ArH), 7.43–7.48 (m, 3H, ArH), 7.57–7.62 (m, 2H, ArH), 7.95–7.99 (m, 1H, ArH), 8.06–8.11 (m, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.8 (q), 32.2 (q), 64.0 (t), 92.3 (d), 116.2 (s), 117.6 (d), 120.2 (s), 125.7 (d), 126.7 (d), 127.2 (d), 127.4 (2×d), 128.8 (2×d), 132.2 (d), 132.9 (s), 133.4 (d), 134.5 (s), 135.5 (s), 140.3 (s), 142.1 (s), 142.4 (s), 158.8 (s), 178.8 (s), 178.9 (s). Anal. Calcd for $C_{25}H_{19}NO_3$: C, 78.72; H, 5.02; N, 3.67. Found: C, 78.71; H, 5.11; N, 3.60.

3.5.20. 5-Ethyl-6,11-dihydro-3-methyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23f. Orange crystals; mp 223–224 °C; IR ($CHCl_3$) 2930, 1655, 1625, 1595, 1475 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (t, J=7.1 Hz, 3H, CH_3), 2.50 (s, 3H, $ArCH_3$), 4.72 (q, J=7.1 Hz, 2H, NCH_2), 7.20 (d, J=8.1 Hz, 1H, ArH), 7.21 (s, 1H, ArH), 7.63–7.72 (m, 2H, ArH), 8.13 (d, J=7.2 Hz, 1H, ArH), 8.18 (d, J=7.2 Hz, 1H, ArH), 8.29 (d, J=8.1 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 15.2 (q), 22.3 (q), 40.1 (t), 110.4 (d), 119.1(s), 122.0 (s), 123.5 (d), 126.2 (d), 126.3 (d), 126.6 (d), 132.7 (d), 133.6 (d), 133.7 (s), 134.12 (s), 134.13 (s), 137.8 (s), 139.5 (s), 178.7 (s), 181.2 (s). Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 78.86; H, 5.25; N, 4.84.

3.5.21. 6,11-Dihydro-1,3,5-trimethyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23g. Orange needles; mp 247–248 °C; IR ($CHCl_3$) 3010, 1655, 1620, 1595, 1495, 1480 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H, $ArCH_3$), 2.96 (s, 3H, $ArCH_3$), 4.18 (s, 3H, NCH_3), 6.94 (s, 1H, ArH), 7.00 (s, 1H, ArH), 7.60–7.73 (m, 2H, ArH), 8.10 (d, J=7.3 Hz, 1H, ArH), 8.17 (d, J=7.3 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 21.9 (q), 23.5 (q), 32.1 (q), 107.8 (d), 120.3 (s), 121.6 (s), 125.7 (d), 126.6 (d), 128.2 (d), 132.4 (d), 133.0 (s), 133.6 (d), 134.5 (s), 135.1 (s), 135.3 (s), 137.8 (s), 141.3 (s), 179.3 (s), 180.0 (s). Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 78.92; H, 5.28; N, 4.87.

3.5.22. 4-Chloro-6,11-dihydro-3,5-dimethyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23h. Orange needles; mp 269–270 °C; IR ($CHCl_3$) 3065, 2915, 1660, 1520, 1465, 1250 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.52 (s, 3H, $ArCH_3$), 4.70 (s, 3H, NCH_3), 7.25 (d, J=8.2 Hz, 1H, ArH), 7.65–7.79 (m, 2H, ArH), 8.12–8.25 (m, 2H, ArH), 8.31 (d, J=8.2 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 20.7 (q), 35.5 (q), 118.2 (s), 118.9 (s), 121.7 (d), 125.1 (s), 126.1 (d), 126.7 (d), 127.8 (d), 131.7 (s), 133.0 (d), 133.7 (d+s), 135.5 (s), 136.1 (s), 136.5 (s), 179.0 (s), 181.1 (s). Anal. Calcd for $C_{18}H_{12}ClNO_2$: C, 69.80; H, 3.90; N, 4.52. Found: C, 69.67; H, 3.97; N, 4.52.

3.5.23. 6,11-Dihydro-5-methyl-6,11-dioxo-5*H*-benzo[*b*]-carbazole 23i. Yellow needles; mp 213–214 °C; IR ($CHCl_3$) 3015, 2925, 1660, 1595, 1480 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.19 (s, 3H, NCH_3), 7.30–7.45 (m, 3H, ArH), 7.64 (td, J=7.4, 1.5 Hz, 1H, ArH), 7.69 (td, J=7.4, 1.5 Hz, 1H, ArH), 8.10 (dd, J=7.4, 1.5 Hz, 1H, ArH), 8.16 (dd, J=7.4, 1.5 Hz, 1H, ArH), 8.38 (d, J=8.0 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 31.9 (q), 110.7 (d), 118.6 (s), 123.7 (d), 123.8 (s), 124.4 (d), 126.1 (d), 126.3 (d), 127.1 (d), 132.7 (d), 133.4 (s), 133.6 (d), 134.0 (s), 135.0 (s), 139.8 (s), 179.0 (s), 180.9 (s). Anal. Calcd for $C_{17}H_{11}NO_2$: C, 78.15; H, 4.24; N, 5.36. Found: C, 78.16; H, 4.25; N, 5.36.

3.5.24. 4-Chloro-6,11-dihydro-5-methyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 23k. Yellow needles; mp 225–226 °C; IR ($CHCl_3$) 3015, 2925, 1665, 1600, 1470 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.62 (s, 3H, NCH_3), 7.21 (t, J=7.8 Hz, 1H, ArH), 7.34 (d, J=7.8 Hz, 1H, ArH), 7.63–7.74 (m, 2H, ArH), 8.07–8.18 (m, 2H, ArH), 8.37 (d, J=7.8 Hz, 1H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 35.1 (q), 118.5 (s), 118.7 (s), 122.5 (d), 124.8 (d), 126.1 (d), 126.6 (d), 126.8 (s), 129.0 (d), 132.9 (d), 133.5 (s), 133.6 (s), 133.8 (d), 135.2 (s), 135.7 (s), 178.9 (s), 180.7 (s). Anal. Calcd for $C_{17}H_{10}ClNO_2$: C, 69.05; H, 3.41; N, 4.74. Found: C, 68.82; H, 3.37; N, 4.71.

3.5.25. 1,2,6,11-Tetrahydro-1,1,3,5-tetramethyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 25. Red needles; mp 152–153 °C; IR ($CHCl_3$) 3010, 2915, 1640, 1590, 1410 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 6H, 2×CH_3), 1.98 (s, 3H, CH_3), 2.28 (s, 2H, CH_2), 4.03 (s, 3H, NCH_3), 6.19 (s, 1H, =CH), 7.59–7.64 (m, 2H, ArH), 8.07–8.13 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 24.2 (q), 27.7 (2×q), 32.5 (q), 33.1 (s), 47.5 (t), 109.4 (d), 124.7 (s), 125.6 (d), 126.4 (d), 127.6 (s), 130.0 (s), 132.4 (d), 132.6 (d), 134.1 (s), 134.2 (s), 138.7 (s), 142.6 (s), 175.1 (s), 181.3 (s). Anal. Calcd for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27; N, 4.59. Found: C, 78.70; H, 6.27; N, 4.62.

3.5.26. 5-Ethyl-1,2,6,11-tetrahydro-2,2-dimethyl-6,11-dioxo-5*H*-benzo[*b*]carbazole 27. Orange crystals; mp 142–143 °C; IR ($CHCl_3$) 2970, 2930, 1640, 1595, 1480 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.14 (s, 6H, 2×CH_3), 1.42 (t, J=7.2 Hz, 3H, CH_3), 3.01 (s, 2H, CH_2), 4.52 (q, J=7.2 Hz, 2H, NCH_2), 5.94 (d, J=10.0 Hz, 1H, =CH), 6.32 (d, J=10.0 Hz, 1H, =CH), 7.60–7.70 (m, 2H, ArH), 8.09–8.17 (m, 2H, ArH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 16.1 (q), 28.5 (q), 28.6 (q), 33.6 (s), 34.9 (t), 40.5 (t), 112.3 (d), 120.0 (s), 125.3 (s), 126.1 (2×d), 128.8 (s), 132.4 (d), 132.9 (d), 133.8 (s), 134.8 (s), 136.5 (s), 144.0 (d), 174.6 (s), 182.4 (s). Anal. Calcd for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27; N, 4.59. Found: C, 78.25; H, 6.28; N, 4.55.

3.6. Typical experimental procedure for the reaction between 2-(anilino)-1,4-naphthoquinone (28) and β-dicarbonyl compounds in formic acid

A mixture of 151 mg (0.61 mmol) of 2-(anilino)-1,4-naphthoquinone (**28**), 383 mg (2.42 mmol) of ethyl butyrylacetate (**2a**) and 646 mg (2.41 mmol) of $Mn(OAc)_3$ in 10 mL of 80% aqueous formic acid was stirred at 0 °C for 30 min. The reaction mixture was diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, 50 mL of saturated aqueous sodium bicarbonate, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over 20 g of silica gel (eluted with 2:1 dichloromethane–hexane) followed by crystallization (ethyl acetate–hexane) to give 155 mg (66%) of **29a**.

3.6.1. 4,9-Dihydro-2-isopropyl-3-(methoxycarbonyl)-4,9-dioxo-1-phenyl-1*H*-benzo[*f*]indole 29b. Yellow powders; mp 210–211 °C; IR ($CHCl_3$) 1730, 1665, 1595, 1290, 1240 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (d, J=7.1 Hz, 6H, 2×CH_3), 2.81 (septet, J=7.1 Hz, 1H, CH), 4.03 (s, 3H, OCH_3), 7.28–7.34 (m, 2H, ArH), 7.55–7.60 (m, 3H, ArH), 7.62 (td, J=7.2, 1.6 Hz, 1H, ArH), 7.66 (td, J=7.2, 1.6 Hz, 1H, ArH), 7.94–7.99 (m, 1H, ArH), 8.13 (dd, J=7.2, 1.6 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 21.4 (2×q), 26.1 (d), 52.7 (q), 113.2 (s), 125.9 (s), 126.3 (d), 126.5 (d), 127.5 (2×d), 129.5 (2×d), 129.7 (d), 130.0 (s), 133.2 (2×d), 133.3 (s), 133.4 (s), 136.8 (s), 148.4 (s), 166.4 (s), 174.6 (s), 180.1 (s). Anal. Calcd for $C_{23}H_{19}NO_4$: C, 73.98; H, 5.13; N, 3.75. Found: C, 74.04; H, 5.18; N, 3.74.

3.6.2. 2-Ethyl-4,9-dihydro-4,9-dioxo-1-phenyl-3-propionyl-1*H*-benzo[*f*]indole 29e. Yellow powders; mp 120–121 °C; IR ($CHCl_3$) 1660, 1600, 1495, 1465, 1270 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (t, J=7.5 Hz, 3H, CH_3), 1.26 (t, J=7.3 Hz, 3H, CH_3), 2.56 (q, J=7.5 Hz, 2H, CH_2), 3.16 (q, J=7.3 Hz, 2H, CH_2), 7.25–7.35 (m, 2H, ArH), 7.54–7.61 (m, 3H, ArH), 7.64 (td, J=7.6, 1.4 Hz, 1H, ArH), 7.68 (td, J=7.6, 1.4 Hz, 1H, ArH), 7.98 (dd, J=7.6, 1.4 Hz, 1H, ArH), 8.16 (dd, J=7.6, 1.4 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 8.6 (q), 14.4 (q), 18.3 (t), 37.0 (t), 122.2 (s), 125.2 (s), 126.2 (d), 126.7 (d), 127.3 (2×d), 129.5 (2×d), 129.6 (d), 130.8 (s), 133.2 (s), 133.3 (2×d), 133.5 (s), 136.8 (s), 147.2 (s), 174.9 (s), 181.0 (s), 202.8 (s). Anal. Calcd for $C_{23}H_{19}NO_3$: C, 77.29; H, 5.36; N, 3.92. Found: C, 77.02; H, 5.35; N, 3.97.

3.6.3. 4,9-Dihydro-3-isovaleryl-2-methyl-4,9-dioxo-1-phenyl-1*H*-benzo[*f*]indole 29h. Pale yellow powders; mp 151–152 °C; IR ($CHCl_3$) 2960, 1660, 1595, 1500, 1285 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (d, J=6.8 Hz, 6H, 2×CH_3), 2.16 (s, 3H, $ArCH_3$), 2.27 (septet, J=6.8 Hz, 1H, CH), 3.08 (d, J=6.8 Hz, 2H, CH_2), 7.27–7.32 (m, 2H, ArH), 7.55–7.60 (m, 3H, ArH), 7.64 (td, J=7.4, 1.5 Hz, 1H, ArH), 7.69 (td, J=7.4, 1.5 Hz, 1H, ArH), 7.99 (dd, J=7.4, 1.5 Hz, 1H, ArH), 8.18 (dd, J=7.4, 1.5 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.6 (q), 22.7

(2×q), 25.4 (d), 52.5 (t), 123.1 (s), 125.0 (s), 126.2 (d), 126.8 (d), 127.1 (2×d), 129.6 (3×d), 130.9 (s), 133.1 (s), 133.27 (d), 133.31 (d), 133.5 (s), 136.9 (s), 141.8 (s), 174.9 (s), 180.8 (s), 202.2 (s). Anal. Calcd for $C_{24}H_{21}NO_3$: C, 77.61; H, 5.70; N, 3.77. Found: C, 77.56; H, 5.77; N, 3.73.

3.6.4. 4,9-Dihydro-2-methyl-4,9-dioxo-1-phenyl-3-pivaloyl-1*H*-benzo[*f*]indole 29i. Yellow powders; mp 155–156 °C; IR ($CHCl_3$) 1660, 1595, 1505, 1430, 1280 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 9H, 3×CH_3), 2.03 (s, 3H, ArCH_3), 7.29–7.33 (m, 2H, ArH), 7.54–7.60 (m, 3H, ArH), 7.63 (td, J=7.4, 2.0 Hz, 1H, ArH), 7.66 (td, J=7.4, 2.0 Hz, 1H, ArH), 7.98–8.19 (m, 1H, ArH), 8.14–8.56 (m, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.6 (q), 27.1 (3×q), 45.9 (s), 122.4 (s), 126.2 (s), 126.4 (d), 126.5 (d), 127.2 (2×d), 129.5 (3×d), 130.4 (s), 133.1 (d), 133.3 (d), 133.6 (s), 136.3 (s), 136.9 (s), 174.6 (s), 180.6 (s), 211.6 (s). Anal. Calcd for $C_{24}H_{21}NO_3$: C, 77.61; H, 5.70; N, 3.77. Found: C, 77.62; H, 5.69; N, 3.77.

3.7. Typical experimental procedure for the reaction between 2-(anilino)-1,4-naphthoquinone (28) and β-dicarbonyl compounds in less acidic or neutral solvent

A mixture of 151 mg (0.61 mmol) of 2-(anilino)-1,4-naphthoquinone (**28**), 390 mg (2.47 mmol) of ethyl butyrylacetate (**2a**) and 969 g (3.61 mmol) of $Mn(OAc)_3$ in 10 mL of CF_3CH_2OH was heated at 80 °C for 24 h, followed by the addition of 971 mg (3.62 mmol) of $Mn(OAc)_3$. The reaction mixture was heated for another 24 h and then diluted with 100 mL of ethyl acetate, washed with 50 mL of saturated aqueous sodium bisulfite, three 50 mL portions of water, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over 20 g of silica gel (eluted with dichloromethane) followed by crystallization (chloroform–hexane) to give 129 mg (64%) of **30a**.

3.7.1. 12-Acetyl-6,11-dihydro-6,11-dioxo-benzo[*b*]acridine 30b. Pale yellow powders; mp 314–315 °C; IR ($CHCl_3$) 1690, 1600, 1335, 1310, 1255 cm^{-1}; ^1H NMR (400 MHz, CF_3COOD) δ 3.06 (s, 3H, CH_3), 8.11–8.22 (m, 2H, ArH), 8.30–8.41 (m, 2H, ArH), 8.53–8.64 (m, 3H, ArH), 8.88 (d, J=8.7 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, CF_3COOD) δ 30.9 (q), 123.0 (s), 123.1 (d), 127.8 (d), 127.9 (s), 129.5 (d), 129.6 (d), 131.8 (s), 133.0 (s), 135.0 (d), 137.4 (d), 138.8 (d), 140.4 (s), 141.4 (d), 141.6 (s), 159.9 (s), 176.2 (s), 179.5 (s), 205.6 (s). Anal. Calcd for $C_{19}H_{11}NO_3$: C, 75.74; H, 3.68; N, 4.65. Found: C, 75.52; H, 3.68; N, 4.67.

3.7.2. 6,11-Dihydro-6,11-dioxo-12-propionyl-benzo[*b*]-acridine 30c. Pale yellow powders; mp 218–219 °C; IR ($CHCl_3$) 1690, 1595, 1550, 1335, 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.49 (t, J=7.1 Hz, 3H, CH_3), 2.67–2.83 (m, 1H, CH_2), 3.06–3.23 (m, 1H, CH_2), 7.76–7.96 (m, 4H, ArH), 7.97–8.04 (m, 1H, ArH), 8.29–8.35 (m, 1H, ArH), 8.47–8.51 (m, 1H, ArH), 8.54 (d, J=8.8 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 7.8 (q), 37.6 (t), 122.7 (s), 125.0 (s), 126.0 (d), 127.8 (d), 128.3 (d), 130.7 (d), 132.1 (d), 133.2 (s), 133.5 (d), 134.0 (s), 134.9 (d), 135.1 (d), 147.7 (s), 150.1 (s), 151.6 (s), 181.2 (s), 182.6 (s), 206.0 (s). Anal. Calcd for $C_{20}H_{13}NO_3$: C, 76.18; H, 4.16; N, 4.44. Found: C, 76.23; H, 4.12; N, 4.42.

3.7.3. 6,11-Dihydro-12-isobutyryl-6,11-dioxo-benzo[*b*]-acridine 30d. Pale yellow powders; mp 229–230 °C; IR ($CHCl_3$) 1690, 1600, 1335, 1255, 1105 cm^{-1}; ^1H NMR (400 MHz, $C_2D_2Cl_4$) δ 1.12 (d, J=6.9 Hz, 3H, CH_3), 1.35 (d, J=6.9 Hz, 3H, CH_3), 3.00 (septet, J=6.9 Hz, 1H, CH), 7.71–7.88 (m, 4H, ArH), 7.96 (t, J=7.5 Hz, 1H, ArH), 8.24–8.29 (m, 1H, ArH), 8.34–8.40 (m, 1H, ArH), 8.43 (d, J=8.5 Hz, 1H, ArH); ^{13}C NMR (100.6 MHz, $C_2D_2Cl_4$) δ 18.1 (q), 19.0 (q), 42.4 (d), 123.5 (s), 125.5 (s), 127.0 (d), 127.9 (d), 128.3 (d), 130.8 (d), 131.9 (d), 133.2 (s), 133.9 (d), 135.2 (d), 135.4 (d), 147.7 (s), 149.9 (s), 151.3 (s), 181.4 (s), 182.8 (s), 209.4 (s). Anal. Calcd for $C_{21}H_{15}NO_3$: C, 76.58; H, 4.59; N, 4.25. Found: C, 76.49; H, 4.63; N, 4.11.

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Stereoselective synthesis of hyptolide and 6-*epi*-hyptolide

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Abstract—The first stereoselective syntheses of the naturally occurring, α,β-unsaturated lactone hyptolide **1** and of its nonnatural epimer at C-6 are described. Ethyl L-lactate was the chiral starting material. Key steps of these syntheses were a Brown's asymmetric allylation, a Carreira's asymmetric ethynylation and a ring closing metathesis.

1. Introduction

Lactone rings constitute a structural feature of a broad range of natural products.[1] Many of these lactones, most particularly those being α,β-unsaturated,[1a] display pharmacologically relevant properties. In recent times, we have been interested in the synthesis of natural lactones of such structural type. Among them, the conjugated δ-lactones hyptolide **1**,[2] spicigerolide **2**,[3] anamarine **3**,[4] synrotolide **4**[5] and synargentolide **5**[6] (Fig. 1) have been isolated from species of *Hyptis*, *Syncolostemon* and related genera of the family Lamiaceae. These compounds contain a polyoxygenated chain connected with the unsaturated lactone ring and have been found to show a range of pharmacological properties, such as cytotoxicity against human tumor cells, antimicrobial or antifungal activity, etc.[7] Other structurally similar lactones have been found to be antimicrobial.[8] While these properties make attractive synthetic goals out of the aforementioned lactones, efforts in this direction have been limited for many years to the syntheses of **2** and its nonnatural enantiomer.[9] Very recently, we have published stereoselective syntheses of **1**, **2**, **3** and of some nonnatural analogs of **2**, as well as a study of their cytotoxic activity.[10] In the present paper, we describe in full our stereoselective syntheses of **1** and its nonnatural analogue **6**, the latter being an epimer of **1** at the lactone-closing carbon atom C-6.

For the retrosynthetic analysis of **1** and **6**, we have relied upon the same general concept used in our previous

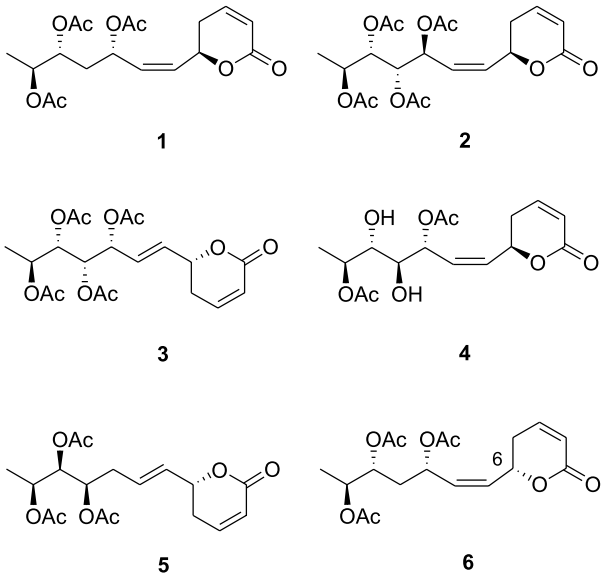

Figure 1. Five naturally occurring γ-pyrones (**1**–**5**) isolated from *Hyptis* spp. and related genera, and a nonnatural analogue (**6**).

syntheses, where asymmetric allylations and ring-closing metatheses played the key role in the construction of the 6-alkenyl-2-pyrone moiety.[10,11] As shown in Scheme 1a, this analysis points to a 6-deoxyaldohexose as the starting material in the cases of **2** and **3**. Their respective syntheses were thus designed according to this circumstance.[10b,c] For lactone **1**, however, the same analysis leads to a 3,6-dideoxyaldohexose (3,6-dideoxy-L-*ribo*hexose). Since neither this sugar nor derivatives thereof are commercially available, we developed a retrosynthetic concept where the asymmetric ethynylation of a 2,5-dideoxyaldopentose was a crucial feature.[10a] The latter compound was to be

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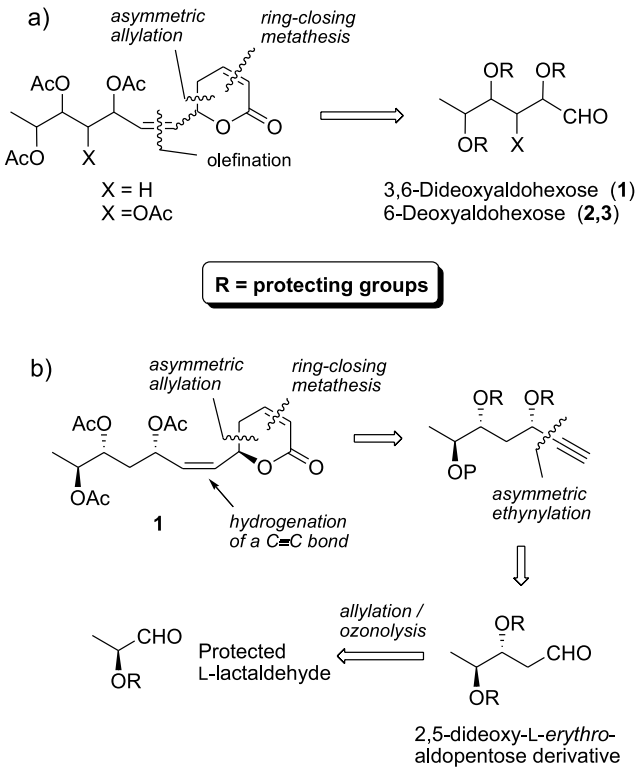


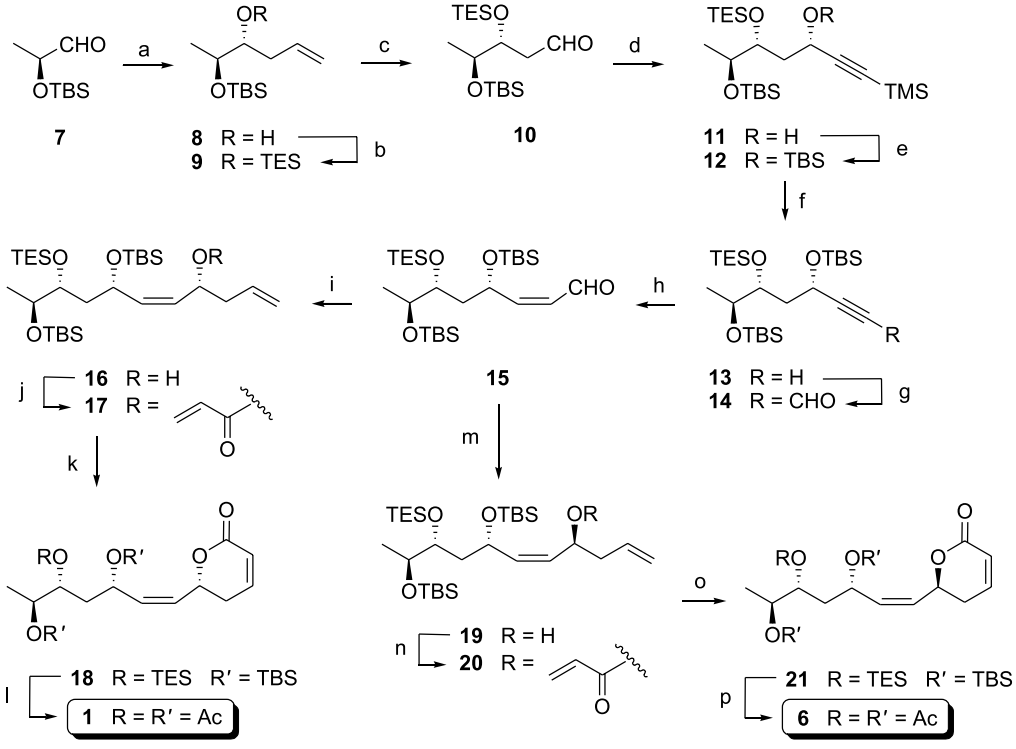
Scheme 1. Retrosynthetic analysis for lactones **1**–**3**.

made from a suitably protected L-lactaldehyde via an allylation/ozonolysis sequence (Scheme 1b).

2. Results and discussion

The synthesis started with the known chiral aldehyde **7** (Scheme 2), easily prepared in two steps from ethyl L-lactate.[12] Asymmetric allylation of **7** to homoallyl alcohol **8**[13] was performed with Brown's B-allyl diisopinocampheylborane,[14] prepared in turn from (+)-DIP-Cl (diisopinocampheylboron chloride) and allylmagnesium bromide. Protection of the hydroxyl group of **8** as a TES derivative[15,16] was followed by oxidative cleavage[17] of the olefinic bond to yield β-silyloxy aldehyde **10**. Several ethynyl metal reagents were tested on this aldehyde but no sufficiently stereoselective addition was achieved. Finally, Carreira's asymmetric protocol[18–20] solved the problem and provided propargyl alcohol **11** as a single diastereomer. Alcohol silylation followed by selective cleavage of the C-silyl group furnished the terminal acetylene **13**, which was C-formylated to **14** via the intermediate lithium acetylide.

Semihydrogenation of the C≡C bond in **14** was performed using Lindlar catalyst and provided the *Z* enal **15**, which was then subjected as above to Brown's asymmetric allylation. This afforded allyl alcohol **16** as a single diastereomer.



Scheme 2. Synthesis of hyptolide **1** and 6-*epi*-hyptolide **6**. Reaction conditions: (a) allylBIpc$_2$ [prepared from allylmagnesium bromide and (+)-DIP-Cl], Et_2O, −78 °C (82%, 92:8 diastereomeric mixture). (b) TESOTf, 2,6-lutidine, CH_2Cl_2, room temperature, 87%. (c) OsO_4 (cat.), NMO, *t*BuOH/THF/H_2O, then $NaIO_4$, aq THF, 78%. (d) TMSC≡CH, $Zn(OTf)_2$, Et_3N, (−)-*N*-methylephedrine, toluene, room temperature, 77%. (e) TBSOTf, 2,6-lutidine, 0 °C, CH_2Cl_2, 87%. (f) aq K_2CO_3/MeOH, room temperature, 86%. (g) BuLi, THF, 0 °C, then DMF, 70%. (h) H_2, Lindlar catalyst, 84%. (i) allylBIpc$_2$ [from (+)-DIP-Cl], Et_2O, −78 °C, (79%, single diastereomer). (j) acryloyl chloride, NEt_3, cat. DMAP, CH_2Cl_2, room temperature, 70%. (k) 10% PhCH=$RuCl_2(PCy_3)_2$, CH_2Cl_2, △, 82% (l) PPTS, aq MeOH, △, then Ac_2O, Et_3N, cat. DMAP, CH_2Cl_2, room temperature, 83%. (m) allylBIpc$_2$ [from (−)-DIP-Cl], Et_2O, −78 °C, (66%, 85:15 diastereomer mixture). (n) acryloyl chloride, NEt_3, cat. DMAP, CH_2Cl_2, room temperature, 72%. (o) 10% PhCH=$RuCl_2(PCy_3)_2$, CH_2Cl_2, △, 90%. (p) PPTS, aq MeOH, △, then Ac_2O, Et_3N, cat. DMAP, CH_2Cl_2, room temperature, 73%. Abbreviations: TMS, trimethylsilyl; TES, triethylsilyl; TBS, *t*-butyldimethylsilyl; Ipc, isopinocampheyl; NMO, *N*-methylmorpholine *N*-oxide; DMAP, 4-dimethylaminopyridine; PPTS, pyridinium *p*-toluenesulfonate.

Acylation of **16** with acryloyl chloride furnished acrylate **17**, which was then subjected to ring-closing metathesis in the presence of Grubbs' standard ruthenium catalyst $PhCH{=}RuCl_2(PCy_3)_2$.[21] The expected conjugated δ-lactone **18** was formed in good yield. Finally, cleavage of all silyl groups of **18** and acetylation of the three hydroxyl functions was achieved in 83% yield to afford **1**, identical in its physical and spectral properties to the natural compound.[2b] The same methodology was then used to obtain lactone **6**. Enal **15** was now subjected to asymmetric allylation with the chiral B-allyl diisopinocampheylborane prepared from (−)-DIP-Cl. This furnished alcohol **19**, subsequently converted into **6** through the same reaction sequence as for **1**.

3. Conclusions

In summary, a total synthesis of the natural lactone hyptolide **1** in enantiopure form has been achieved in a highly stereoselective way using ethyl L-lactate as the chiral starting material. Three C–C bonds were created by means of asymmetric reactions. Sizeable amounts of **1** have thus been made available for pharmacological studies. Furthermore, lactone **6**, a nonnatural diastereomer of **1** needed for further biological studies, has been prepared by means of a small modification in the synthetic route described above (use of the enantiomeric Ipc_2Ballyl reagent in the last allylation step).

4. Experimental

4.1. General

NMR spectra were measured at 400 or 500 MHz in $CDCl_3$ solution at 25 °C. The signals of the deuterated solvent ($CDCl_3$) were taken as the reference (the singlet at δ 7.25 for ^{1}H NMR and the triplet centered at 77.00 ppm for ^{13}C NMR data). Carbon atom types (C, CH, CH_2, CH_3) were determined with the DEPT pulse sequence. Mass spectra were run by the electron impact (EIMS, 70 eV), the CIMS (CH_4 as the gas carrier) or the fast atom bombardment mode (FABMS, *m*-nitrobenzyl alcohol matrix) on a VG AutoSpec mass spectrometer. IR data are given only for compounds with relevant functions (OH, C=O, C≡C–H) and were recorded as oily films on NaCl plates (oils) or as KBr pellets (solids). Optical rotations were measured at 25 °C. Reactions which required an inert atmosphere were carried out under N_2 with flame-dried glassware. Et_2O and THF were freshly distilled from sodium/benzophenone ketyl and transferred via syringe. Dichloromethane was freshly distilled from CaH_2. Tertiary amines were freshly distilled from KOH. Toluene was freshly distilled from sodium wire. Commercially available reagents were used as received. Unless detailed otherwise, 'work-up' means pouring the reaction mixture into brine, followed by extraction with the solvent indicated in parenthesis. If the reaction medium was acidic (basic), an additional washing with 5% aq $NaHCO_3$ (aq NH_4Cl) was performed. Drying over anhydrous Na_2SO_4 and elimination of the solvent under reduced pressure were followed by chromatography of the residue on a silica gel column (60–200 μm) with the indicated eluent. Where solutions were filtered through a Celite pad, the pad was additionally washed with the same solvent used, and the washings incorporated to the main organic layer. Reagent acronyms are explained in the caption of Scheme 2.

4.1.1. (2*S*,3*R*)-2-(*tert*-Butyldimethylsilyloxy)hex-5-en-3-ol (8). Allylmagnesium bromide (commercial 1 M solution in Et_2O, 10 mL, 10 mmol) was added dropwise under N_2 via syringe to a solution of (+)-DIP-Cl (3.85 g, 12 mmol) in dry Et_2O (50 mL) cooled in a dry ice–acetone bath. After replacing the latter by an ice bath, the mixture was stirred for 1 h. The solution was then allowed to stand, which caused precipitation of magnesium chloride. The supernatant solution was then carefully transferred to another flask via canula. After cooling at −78 °C, a solution of **7** (1.5 g, ca. 8 mmol) in dry Et_2O (25 mL) was added dropwise via syringe. The resulting solution was further stirred at the same temperature for 1 h. The reaction mixture was then quenched through addition of phosphate pH 7 buffer solution (50 mL), MeOH (50 mL) and 30% H_2O_2 (25 mL). After stirring for 30 min, the mixture was poured onto satd. aq $NaHCO_3$ and worked up (Et_2O). Column chromatography on silica gel (hexanes–EtOAc, 95:5) afforded **8** (1.51 g, 82%, 92:8 *anti*/*syn* diastereomer mixture) as a colourless oil with spectral properties identical to those reported for the racemic compound.[13] The mixture of diastereomers was used as such in the next step.

4.1.2. (2*S*,3*R*)-2-(*tert*-Butyldimethylsilyloxy)-3-(triethylsilyloxy)hex-5-ene (9). Alcohol **8** (1.38 g, ca. 6 mmol) was dissolved under N_2 in dry CH_2Cl_2 (25 mL), cooled to 0 °C and treated sequentially with 2,6-lutidine (1 mL, ca. 9 mmol) and TESOTf (1.7 mL, 7.5 mmol). The reaction mixture was then stirred for 1 h at the same temperature and worked up (extraction with CH_2Cl_2). Column chromatography on silica gel (hexanes–EtOAc, 95:5) afforded **9** (1.8 g, 87%): oil, $[\alpha]_D = +4.5$ (*c* 2; $CHCl_3$); ^{1}H NMR (500 MHz) δ 5.86 (1H, m), 5.10–5.00 (2H, m), 3.69 (1H, quint, $J=6$ Hz), 3.54 (1H, q, $J=6$ Hz), 2.25 (2H, t, $J=6$ Hz), 1.11 (3H, d, $J=6$ Hz), 0.97 (9H, t, $J=8$ Hz), 0.90 (9H, s), 0.62 (6H, q, $J=8$ Hz), 0.06 (6H, s); ^{13}C NMR (125 MHz) δ 18.2 (C), 135.7, 77.0, 71.5 (CH), 116.6, 38.5, 5.2 (×3) (CH_2), 26.0 (×3), 19.2, 7.0 (×3), −4.3, −4.6 (CH_3). HR EIMS, *m*/*z* (% rel. int.) 315.2127 (M^+ − Et((22), 303 (24), 287 (58), 73 (100); calcd for $C_{18}H_{40}O_2Si_2$–Et, $M=315.2175$.

4.1.3. (3*R*,4*S*)-4-(*tert*-Butyldimethylsilyloxy)-3-(triethylsilyloxy)pentanal (10). Compound **9** (1.72 g, 5 mmol) was dissolved in a mixture of THF (10 mL), *t*-BuOH (25 mL) and water (3 mL). Then, NMO (700 mg, ca. 6 mmol) and OsO_4 (4% aqueous solution, 1.25 mL, 0.2 mmol) were added. The mixture was then stirred at room temperature for 2 h. A solution of $NaIO_4$ (1.5 g, ca. 7 mmol) in water (6 mL) was added, and the stirring was continued at room temperature for 2 h. After this time, a satd. aqueous solution of Na_2SO_3 was added (30 mL), with subsequent stirring for 5 min. Work-up (extraction with CH_2Cl_2) and column chromatography on silica gel (hexanes–EtOAc, 95:5) provided aldehyde **10** (1.35 g, 78%): oil, $[\alpha]_D = +16.6$ (*c* 1.1; $CHCl_3$); IR ν_{max} (cm^{-1}) 1713 (C=O); ^{1}H NMR (500 MHz) δ 9.83 (1H, t, $J=2.5$ Hz), 3.96 (1H, q, $J=5$ Hz), 3.76 (1H, dq, $J=4.5$, 6.5 Hz), 2.56 (1H, ddd, $J=16$, 5, 2.5 Hz), 2.53 (1H, ddd, $J=16$, 5, 2.5 Hz), 1.12 (3H, d,

J=6.5 Hz), 0.95 (9H, t, J=8 Hz), 0.87 (9H, s), 0.62 (6H, q, J=8 Hz), 0.05 (6H, s); ^{13}C NMR (125 MHz) δ 18.1 (C), 202.0, 73.3, 72.5 (CH), 47.3, 5.0 (×3) (CH_2), 25.9 (×3), 20.3, 6.8 (×3), −4.5, −4.6 (CH_3). HR EIMS, *m/z* (% rel. int.) 317.1970 (M^+−Et((24), 289 (22), 159 (100), 131 (96), 73 (77); calcd for $C_{17}H_{38}O_3Si_2$−Et, M=317.1968.

4.1.4. (3*S*,5*R*,6*S*)-6-(*tert*-Butyldimethylsilyloxy)-5-(triethylsilyloxy)-1-(trimethylsilyl)hept-1-yn-3-ol (11). (−)-*N*-Methylephedrine (1.16 g, 6.5 mmol) and zinc triflate (2.18 g, 6 mmol) were suspended under N_2 in dry toluene (15 mL), followed by addition of triethyl amine (910 μL, 6.5 mmol). The resulting mixture was stirred at room temperature for 2 h. After addition of ethynyl trimethylsilane (920 μL, 6.5 mmol), the stirring was continued for 15 min. A solution of aldehyde **10** (1.04 g, 3 mmol) in dry toluene (15 mL) was then added via syringe. The reaction mixture was stirred at room temperature for 18 h. Work-up (extraction with Et_2O) and column chromatography on silica gel (hexanes–EtOAc, 95:5) provided alcohol **11** (1.03 g, 77%): oil, $[\alpha]_D$=+19.6 (*c* 7.3; $CHCl_3$); IR ν_{max} (cm^{-1}) 3400 (br, OH); ^{1}H NMR (500 MHz) δ 4.58 (1H, m), 3.80–3.75 (2H, m), 3.45 (1H, d, J=4 Hz, OH), 1.96 (1H, dt, J=14.5, 7 Hz), 1.79 (1H, dt, J=14.5, 5 Hz), 1.10 (3H, d, J=6.5 Hz), 0.95 (9H, t, J=8 Hz), 0.88 (9H, s), 0.62 (6H, q, J=8 Hz), 0.14 (9H, s), 0.07 (3H, s), 0.06 (3H, s); ^{13}C NMR (125 MHz) δ 107.1, 88.8, 18.2 (C), 74.6, 72.8, 59.8 (CH), 41.4, 5.0 (×3) (CH_2), 25.9 (×3), 19.0, 6.9 (×3), −0.2 (×3), −4.7, −4.8 (CH_3). HR CIMS, *m/z* (% rel. int.) 445.2980 (M+H^+((41), 427 (40), 303 (100), 295 (70), 181 (44), 159 (47); calcd for $C_{22}H_{49}O_3Si_3$, M=445.2989.

4.1.5. (3*S*,5*R*,6*S*)-3,6-Bis(*tert*-butyldimethylsilyloxy)-5-(triethylsilyloxy)-1-(trimethylsilyl)hept-1-yne (12). Alcohol **11** (890 mg, 2 mmol) was dissolved under N_2 in dry CH_2Cl_2 (10 mL), cooled to 0 °C and treated sequentially with 2,6-lutidine (350 μL, 3 mmol) and TBSOTf (575 μL, ca. 2.5 mmol). The reaction mixture was then stirred for 1 h at the same temperature and worked up (extraction with CH_2Cl_2). Column chromatography on silica gel (hexanes–EtOAc, 95:5) afforded **12** (973 mg, 87%): oil, $[\alpha]_D$ −3.2 (*c* 1.3; $CHCl_3$); ^{1}H NMR (500 MHz) δ 4.53 (1H, dd, J=9, 5.5 Hz), 3.85 (1H, m), 3.75 (1H, dq, J=2, 6.5 Hz), 1.80–1.70 (2H, m), 1.10 (3H, d, J=6.5 Hz), 0.99 (9H, t, J=8 Hz), 0.93 (9H, s), 0.92 (9H, s), 0.67 (6H, q, J=8 Hz), 0.17 (9H, s), 0.14 (3H, s), 0.12 (3H, s), 0.08 (3H, s), 0.06 (3H, s); ^{13}C NMR (125 MHz) δ 107.6, 89.5, 18.4, 18.2 (C), 74.5, 73.0, 62.1 (CH), 43.2, 5.3 (×3) (CH_2), 26.1 (×3), 26.0 (×3), 18.1, 7.0 (×3), −0.2 (×3), −4.6 (×3), −4.8 (CH_3). HR EIMS, *m/z* (% rel. int.) 558.3769 (M^+((1), 501 (28), 405 (66), 369 (100), 241 (37); calcd for $C_{28}H_{62}O_3Si_4$, M=558.3776.

4.1.6. (3*S*,5*R*,6*S*)-3,6-Bis(*tert*-butyldimethylsilyloxy)-5-(triethylsilyloxy)hept-1-yne (13). Compound **12** (950 mg, 1.7 mmol) was dissolved in MeOH (20 mL) and treated with K_2CO_3 (276 mg, 2 mmol). The reaction mixture was then stirred for 4 h at room temperature and worked up (extraction with EtOAc). Column chromatography on silica gel (hexanes–EtOAc, 95:5) furnished alkyne **13** (712 mg, 86%): oil, $[\alpha]_D$ −1.1 (*c* 1.45; $CHCl_3$); IR ν_{max} (cm^{-1}) 3312 (C≡C–*H*); ^{1}H NMR (500 MHz) δ 4.52 (1H, td, J=7, 2 Hz), 3.80–3.70 (2H, m), 2.41 (1H, d, J=2 Hz), 1.80 (2H, m), 1.10 (3H, d, J=6 Hz), 0.98 (9H, t, J=8 Hz), 0.90 (9H, s), 0.89 (9H, s), 0.65 (6H, q, J=8 Hz), 0.14 (3H, s), 0.12 (3H, s), 0.08 (3H, s), 0.06 (3H, s); ^{13}C NMR (125 MHz) δ 85.5, 18.2 (×2) (C), 74.2, 72.9, 72.8, 61.4 (CH), 43.0, 5.2 (×3) (CH_2), 26.0 (×3), 25.8 (×3), 18.7, 7.0 (×3), −4.6 (×2), −4.7, −4.9 (CH_3). HR EIMS, *m/z* (% rel. int.) 486.3397 (M^+((1), 457 (6), 429 (49), 405 (46), 377 (25), 327 (36), 297 (84), 169 (100); calcd for $C_{25}H_{54}O_3Si_3$, M=486.3380.

4.1.7. (4*S*,6*R*,7*S*)-4,7-Bis(*tert*-butyldimethylsilyloxy)-6-(triethylsilyloxy)oct-2-ynal (14). Alkyne **13** (681 mg, 1.4 mmol) was dissolved in THF (25 mL) and treated at −78 °C under N_2 with *n*BuLi (1.1 mL of a 1.6 M solution in hexanes, 1.76 mmol). The reaction mixture was then stirred for 2 h at 0°C, followed by addition of dry DMF (870 μL, 11.2 mmol). After stirring at the same temperature for 1 h and work-up (extraction with EtOAc), column chromatography on silica gel (hexanes–EtOAc, 95:5) yielded aldehyde **14** (505 mg, 70%): oil, $[\alpha]_D$=+0.5 (*c* 1.45; $CHCl_3$); IR ν_{max} (cm^{-1}) 2201 (C≡C), 1675 (C=O); ^{1}H NMR (500 MHz) δ 9.25 (1H, s), 4.74 (1H, t, J=7.5 Hz), 3.76–3.70 (2H, m), 1.90–1.85 (2H, m), 1.10 (3H, d, J=6 Hz), 0.98 (9H, t, J=8 Hz), 0.90 (9H, s), 0.89 (9H, s), 0.65 (6H, m), 0.14 (3H, s), 0.12 (3H, s), 0.08 (3H, s), 0.06 (3H, s); ^{13}C NMR (125 MHz) δ 97.7, 84.3, 18.2 (×2) (C), 176.2, 73.9, 72.9, 61.4 (CH), 42.1, 5.2 (×3) (CH_2), 26.0 (×3), 25.8 (×3), 19.1, 7.0 (×3), −4.5, −4.6, −4.7, −5.0 (CH_3). HR EIMS, *m/z* (% rel. int.) 514.3326 (M^+((1), 473 (12), 405 (19), 343 (25), 131 (64), 73 (100); calcd for $C_{26}H_{54}O_4Si_3$, M=514.3330.

4.1.8. (2*Z*,4*S*,6*R*,7*S*)-4,7-Bis(*tert*-butyldimethylsilyloxy)-6-(triethylsilyloxy)oct-2-enal (15). Commercial Lindlar catalyst (5% Pd on $CaCO_3$ poisoned with lead, 80 mg) was suspended in dry CH_2Cl_2 (5 mL) and stirred for 10 min under an atmosphere of H_2. Aldehyde **14** (490 mg, 0.95 mmol) was dissolved in CH_2Cl_2 (5 mL) and added via syringe to the catalyst suspension. The reaction mixture was then stirred under H_2 at room temperature until consumption of the starting material (about 1 h, TLC monitoring) and filtered through a pad of Celite. Removal of all volatiles in vacuo and column chromatography of the residue on silica gel (hexanes–EtOAc, 95:5) afforded enal **15** (413 mg, 84%): oil, $[\alpha]_D$=+14.5 (*c* 1.15; $CHCl_3$); IR ν_{max} (cm^{-1}) 1690 (C=O); ^{1}H NMR (500 MHz) δ 10.20 (1H, d, J=7.7 Hz), 6.56 (1H, dd, J=11.5, 8 Hz), 5.90 (1H, dd, J=11.5, 7.7 Hz), 5.11 (1H, q, J=8 Hz), 3.80 (1H, m), 3.71 (1H, m), 1.80 (1H, dt, J=14, 8 Hz), 1.80 (1H, dt, J=14, 8 Hz), 1.10 (3H, d, J=6 Hz), 0.97 (9H, t, J=8 Hz), 0.90 (18H, s), 0.65 (6H, m), 0.10 (3H, s), 0.08 (6H, s), 0.05 (3H, s); ^{13}C NMR (125 MHz) δ 18.2 (×2) (C), 191.5, 154.1, 128.3, 73.6, 72.6, 67.1 (CH), 43.3, 5.3 (x 3) (CH_2), 26.0 (×3), 25.8 (×3), 18.6, 7.0 (×3), −4.4 (×2), −4.5, −4.6 (CH_3). HR EIMS, *m/z* (% rel. int.) 516.3530 (M^+((1), 329 (24), 159 (25), 73 (100); calcd for $C_{26}H_{56}O_4Si_3$, M=516.3486.

4.1.9. (5*Z*,4*R*,7*S*,9*R*,10*S*)-7,10-Bis(*tert*-butyldimethylsilyloxy)-9-(triethylsilyloxy)undeca-1,5-dien-4-ol (16). Aldehyde **15** (207 mg, 0.4 mmol) was subjected to asymmetric allylation following the same reaction conditions used for the synthesis of **8**. This gave alcohol **16** as a single diastereomer (177 mg, 79%): oil, $[\alpha]_D$=+9 (*c* 1.45;

$CHCl_3$); IR ν_{max} (cm^{-1}) 3360 (br, OH); ^1H NMR (500 MHz) δ 5.82 (1H, m), 5.55 (1H, dd, $J=11$, 8 Hz), 5.42 (1H, dd, $J=11$, 7.5 Hz), 5.20–5.10 (2H, m), 4.56 (1H, m), 4.46 (1H, m), 3.84 (1H, m), 3.75 (1H, m), 2.50 (1H, br s, OH), 2.30 (2H, t, $J=7$ Hz), 1.70 (2H, m), 1.10 (3H, d, $J=6$ Hz), 0.98 (9H, t, $J=8$ Hz), 0.90 (18H, s), 0.64 (6H, m), 0.10 (3H, s), 0.09 (3H, s), 0.08 (3H, s), 0.07 (3H, s); ^{13}C NMR (125 MHz) δ 18.2 (x 2) (C), 134.7, 134.1, 131.5, 73.8, 72.2, 67.2, 67.1 (CH), 118.2, 43.4, 42.1, 5.2 ($\times3$) (CH_2), 26.0 ($\times3$), 25.8 ($\times3$), 18.0, 7.0 ($\times3$), -4.3, -4.4 ($\times2$), -4.7 (CH_3). HR EIMS, *m/z* (% rel. int.) 501.3269 (M^+–*t*Bu((1), 409 (16), 405 (13), 369 (36), 303 (31), 267 (56), 241 (100), 163 (57); calcd for $C_{29}H_{62}O_4Si_3$–*t*Bu, $M=501.3252$.

4.1.10. (2*Z*,1*R*,4*S*,6*R*,7*S*)-1-Allyl-4,7-bis(*tert*-butyldimethylsilyloxy)-6-(triethylsilyloxy)oct-2-en-1-yl acrylate (17). Alcohol **16** (168 mg, 0.3 mmol) was dissolved under N_2 in dry CH_2Cl_2 (5 mL), cooled to 0 °C and treated sequentially with triethylamine (100 μL, ca. 0.7 mmol), DMAP (2 mg) and acryloyl chloride (50 μL, ca. 0.6 mmol). The reaction mixture was stirred for 12 h at room temperature and then worked up (extraction with CH_2Cl_2). Column chromatography on silica gel (hexanes–EtOAc, 19:1) afforded acrylate **17** (129 mg, 70%): oil, $[\alpha]_D$ -3.1 (*c* 1.1; $CHCl_3$); IR ν_{max} (cm^{-1}) 1726 (C=O); ^1H NMR (500 MHz) δ 6.40 (1H, d, $J=17.2$ Hz), 6.10 (1H, dd, $J=17.2$, 10.5 Hz), 5.80 (1H, d, $J=10.5$ Hz), 5.75 (1H, m), 5.65 (1H, m), 5.60 (1H, dd, $J=11$, 8.5 Hz), 5.35 (1H, dd, $J=11$, 10 Hz), 5.15–5.05 (2H, m), 4.65 (1H, m), 3.95–3.90 (2H, m), 2.45–2.35 (2H, m), 1.65 (2H, m), 1.10 (3H, d, $J=6$ Hz), 0.98 (9H, t, $J=8$ Hz), 0.90 (9H, s), 0.89 (9H, s), 0.64 (6H, m), 0.10 (3H, s), 0.09 (3H, s), 0.07 (3H, s), 0.01 (3H, s); ^{13}C NMR (125 MHz) δ 165.1, 18.2, 18.1 (C), 138.0, 132.8, 130.5, 128.7, 125.7, 73.4, 71.7, 69.2, 66.7 (CH), 118.3, 43.4, 39.5, 5.2 (x 3) (CH_2), 26.0 ($\times3$), 25.9 ($\times3$), 17.4, 7.0 ($\times3$), -4.4 ($\times3$), -4.9 (CH_3). HR EIMS, *m/z* (% rel. int.) 555.3381 (M^+–*t*Bu((1), 409 (16), 405 (13), 369 (36), 303 (31), 267 (56), 241 (100), 163 (57); calcd for $C_{32}H_{64}O_5Si_3$–*t*Bu, $M=555.3357$.

4.1.11. (6*R*)-6-(1*Z*,3*S*,5*R*,6*S*)-3,6-Bis(*tert*-butyldimethylsilyloxy)-5-(triethylsilyloxy)hept-1-enyl)(-5,6-dihydropyran-2-one (18). Ester **17** (123 mg, 0.2 mmol) was dissolved under N_2 in dry, degassed CH_2Cl_2 (25 mL) and treated with ruthenium catalyst $PhCH{=}RuCl_2(PCy_3)_2$ (17 mg, 0.02 mmol) dissolved in the same solvent (2 mL). The mixture was heated at reflux until consumption of the starting material (ca. 3 h, TLC monitoring!). Solvent removal in vacuo and column chromatography of the residue on silica gel (hexanes–EtOAc, 80:20) furnished pyrone **18** (96 mg, 82%): oil, $[\alpha]_D$ -1.3 (*c* 1.2; $CHCl_3$); IR ν_{max} (cm^{-1}) 1742 (C=O); ^1H NMR (500 MHz) δ 6.86 (1H, m), 6.06 (1H, dd, $J=9.8$, 1.5 Hz), 5.70 (1H, dd, $J=11$, 8 Hz), 5.54 (1H, dd, $J=11$, 9.5 Hz), 5.31 (1H, td, $J=9.5$, 4.5 Hz), 4.56 (1H, td, $J=6.5$, 8 Hz), 3.83 (1H, m), 3.75 (1H, m), 2.45–2.30 (2H, br m), 1.67 (2H, m), 1.09 (3H, d, $J=6$ Hz), 0.97 (9H, t, $J=8$ Hz), 0.90 (9H, s), 0.89 (9H, s), 0.65 (6H, m), 0.08 (3H, s), 0.07 (3H, s), 0.06 (6H, s); ^{13}C NMR (125 MHz) δ 163.7, 18.2, 18.1 (C), 144.4, 138.3, 125.6, 121.8, 73.9, 73.6, 72.4, 66.8 (CH), 43.2, 30.0, 5.2 ($\times3$) (CH_2), 26.0 ($\times3$), 25.9 ($\times3$), 18.7, 7.0 ($\times3$), -4.2, -4.4 ($\times2$), -4.8 (CH_3). HR EIMS, *m/z* (% rel. int.) 527.3042 (M^+ –*t*Bu((4), 423 (16), 395 (33), 267 (100), 189 (23), 73 (34); calcd for $C_{30}H_{60}O_5Si_3$–*t*Bu, $M=527.3044$.

4.1.12. (6*R*)-6-((1*Z*,3*S*,5*R*,6*S*)-3,5,6-Triacetoxyhept-1-enyl)(-5,6-dihydropyran-2-one, hyptolide (1). Compound **18** (87 mg, 0.15 mmol) was dissolved in MeOH (5 mL) and treated with PPTS (4 mg, 0.015 mmol). After addition of water (0.2 mL), the reaction mixture was stirred overnight at reflux and worked up (extraction with EtOAc). The oily residue was dissolved under N_2 in dry CH_2Cl_2 (5 mL), followed by addition of triethylamine (170 μL, 1.2 mmol), DMAP (5 mg, 0.04 mmol) and acetic anhydride (95 μL, 1 mmol). After stirring at room temperature for 3 h, work-up (extraction with CH_2Cl_2) and column chromatography of the residue on silica gel (hexanes–EtOAc, 50:50) provided hyptolide **1** (45 mg, 83%): colorless solid, mp 82–86 °C; lit.[2b] mp 87 88 °C; $[\alpha]_D=+12.1$ (*c* 0.68; $CHCl_3$), lit.[2b] $[\alpha]_D=+11.2$ (*c* 0.6; $CHCl_3$); IR ν_{max} (cm^{-1}) 1735; ^1H NMR (400 MHz) δ 6.85 (ddd, $J=10$, 5.5, 3 Hz, 1H), 6.00 (ddd, $J=10$, 2.5, 1 Hz, 1H), 5.74 (dd, $J=10.2$, 8.5 Hz, 1H), 5.50 (m, 2H), 5.24 (ddd, $J=10.7$, 8.5, 4.7 Hz, 1H), 4.96 (dq, $J=3.5$, 6.5 Hz, 1H), 4.88 (dt, $J=9.3$, 3.5 Hz, 1H), 2.45–2.35 (m, 2H), 2.03 (s, 3H), 2.00 (m, 1H), 1.99 (s, 3H), 1.98 (s, 3H), 1.80 (m, 1H), 1.16 (d, $J=6.5$, 3H); ^{13}C NMR (100 MHz) δ 170.6, 170.3, 169.7, 163.4 (C), 144.6, 131.3, 130.8, 121.5, 73.8, 71.0, 70.5, 66.6 (CH), 34.8, 29.5 (CH_2), 21.1, 21.0, 19.9, 14.7 (CH_3). HR EIMS, *m/z* (% rel. int.) 369.1570 (M+H($^+$ (2), 239 (94), 206 (96), 188 (84), 145 (100), 91 (99); calcd for $C_{18}H_{25}O_8$, $M=369.1550$.

4.1.13. (2*Z*,1*S*,4*S*,6*R*,7*S*)-1-Allyl-4,7-Bis(*tert*-butyldimethylsilyloxy)-6-(triethylsilyloxy)oct-2-en-1-yl acrylate (20). Aldehyde **15** (207 mg, 0.4 mmol) was subjected to asymmetric allylation following the same reaction conditions used for the synthesis of **16** except for the use of (−)-DIP-Cl. This gave alcohol **19** as an 85:15 mixture of diastereomers with **17** (148 mg, 66%), which proved impossible to separate under standard chromatographic conditions at atmospheric pressure. The mixture was then subjected to acylation with acryloyl chloride as described above for the conversion of 16 into **17**. This yielded **20** (117 mg, 72%) as an 85:15 mixture with the C-6 epimer **17**. The following ^{13}C NMR signals are those of the major stereoisomer **20**: ^{13}C NMR (125 MHz) δ 164.9, 18.3, 18.2 (C), 137.9, 132.8, 128.5, 125.8, 73.4, 71.5, 69.3, 66.8 (CH), 130.6, 118.3, 43.3, 39.5, 5.2 ($\times3$) (CH_2), 26.0 ($\times3$), 25.9 ($\times3$), 17.0, 7.1 ($\times3$), -4.0, -4.1, -4.4 ($\times2$) (CH_3). Again, the mixture proved not separable and was used as such in the metathesis step.

4.1.14. (6*S*)-6-(1*Z*,3*S*,5*R*,6*S*)-3,6-Bis(*tert*-butyldimethylsilyloxy)-5-(triethylsilyloxy)hept-1-enyl)(-5,6-dihydropyran-2-one (21). The mixture of esters **20** and **17** from above was subjected to ring-closing metathesis under the same conditions as for the conversion of **17** into **18**. This yielded an 85:15 mixture of pyrones **21** and **18** (112 mg, 90%). The following ^{13}C NMR signals are those of the major stereoisomer **21**: ^{13}C NMR (125 MHz) δ 163.6, 18.2, 18.1 (C), 144.4, 137.5, 126.3, 121.6, 74.1, 73.7, 72.5, 67.4 (CH), 43.4, 30.1, 5.3 ($\times3$) (CH_2), 26.0 ($\times3$), 25.9 ($\times3$), 18.8, 7.1 ($\times3$), -4.1 -4.2, -4.4 ($\times2$) (CH_3). As above, the mixture proved not separable and was used as such in the last step.

4.1.15. (6*S*)-6-((1*Z*,3*S*,5*R*,6*S*)-3,5,6-Triacetoxyhept-1-enyl)(-5,6-dihydropyran-2-one, 6-*epi*-hyptolide (6). The mixture of pyrones **18** and **21** was subjected to the same protocol of desilylation/acetylation as in the synthesis of **1** to yield a 85:15 mixture of **6** and **1** (52 mg, 73%). The mixture was subjected to separation via HPLC under the following conditions: LiChroCART® 250/10 column filled with LiChrospher® Si 60 (10 μm); the separation was started with a hexane–EtOAc gradient from 70:30 to 60:40 within 9 min; then a second gradient from 60:40 to 50:50 within 6 min, followed by isocratic elution with the latter solvent mixture. Lactone **6** was eluted from the column after 29 min. Solvent removal yielded the compound as an oil, which could not be induced to crystallize: $[\alpha]_D = +11$ (*c* 0.6; $CHCl_3$); IR ν_{max} (cm^{-1}) 1732 (C=O); ^1H NMR (500 MHz) δ 6.90 (1H, ddd, $J=9.8$, 5.8, 2.5 Hz), 6.04 (1H, dd, $J=9.8$, 2.5 Hz), 5.70 (1H, dd, $J=10.8$, 8.8 Hz), 5.56 (1H, m), 5.49 (1H, dd, $J=10.8$, 10 Hz), 5.40 (1H, m), 5.08 (1H, quint, $J=4$ Hz), 5.03 (1H, m), 2.55 (1H, dt, $J=18.5$, 5 Hz), 2.37 (1H, ddt, $J=18.5$, 10.8, 2.5 Hz), 2.06 (3H, s), 2.05 (6H, s), 2.05 (1H, overlapped m), 1.90 (1H, m), 1.23 (3H, d, $J=6.5$ Hz); ^{13}C NMR (125 MHz) δ 170.3 (×2), 170.2, 163.5 (C), 144.8, 131.8, 130.4, 121.5, 73.7, 71.2, 70.4, 67.7 (CH), 34.4, 29.4 (CH_2), 21.1 (×2), 21.0, 14.9 (CH_3). HR FABMS, *m/z* 369.1563 ($M+H^+$(; calcd for $C_{18}H_{25}O_8$, $M=369.1549$. Anal. Calcd for $C_{18}H_{24}O_8$: C, 58.69; H, 6.57. Found, C, 58.79; H, 6.41.

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16. The use of two different silyl groups (e.g. TES and TBS) is not necessary in principle. However, we had initially planned the use of standard achiral ethynylating reagents (such as ethynylmagnesium bromide) in the reaction with **9**. In anticipation of unsatisfactory diastereoselectivities in these reactions (as it turned out to be the case), we envisaged the oxidation of the resulting alcohols (**10**+epimer) to a conjugated ynone, followed by stereoselective reduction. Since a free β-hydroxy carbonyl group might be convenient

for that purpose, we placed an easily cleavable silyl group (TES) at this position. However, when we finally resorted to the asymmetric ethynylation of **9**, we decided to maintain the already present TES group.

17. When aldehyde **10** was obtained via ozonolysis of **9** and subsequent ozonide reduction with PPh_3 or Me_2S, the asymmetric ethynylation to **11** did not work.
18. Frantz, D. E.; Fässler, R.; Carreira, E. M. *J. Am. Chem. Soc.* **2000**, *122*, 1806–1807. In addition to trimethylsilylacetylene, we also used 2-methyl-3-butyn-2-ol: Boyall, D.; López, F.; Sasaki, H.; Frantz, D.; Carreira, E. M. *Org. Lett.* **2000**, *2*, 4233–4236. However, whereas the addition step was successful, all attempts to cleave the acetone fragment solely led to decomposition.
19. Aside from the contributions of Carreira's group, we have found only few examples of the use of their asymmetric ethynylation methodology: (a) Dieter, R. K.; Yu, H. *Org. Lett.* **2001**, *3*, 3855–3858. (b) Maezaki, N.; Kojima, N.; Asai, M.; Tominaga, H.; Tanaka, T. *Org. Lett.* **2002**, *4*, 2977–2980. (c) Amador, M.; Ariza, X.; García, J.; Ortiz, J. *Tetrahedron Lett.* **2002**, *43*, 2691–2694. For a failure, see: (d) Gung, B. W.; Dickson, H. D.; Seggerson, S.; Bluhm, K. *Synth. Commun.* **2002**, *32*, 2733–2740.
20. Further related enantioselective ethynylation methodologies have been recently reported. See, for example: (a) Lu, G.; Li, X. S.; Zhou, Z. Y.; Chan, W. L.; Chan, A. S. C. *Tetrahedron: Asymmetry* **2001**, *12*, 2147–2152. (b) Jiang, B.; Chen, Z. L.; Xiong, W. N. *Chem. Commun.* **2002**, 1524–1525.
21. (a) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012–3043. (b) Trnka, T.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18–29.

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Erratum

Erratum to "Efficient solution phase parallel synthesis of norstatine analogs" [Tetrahedron 60 (2004) 9043]

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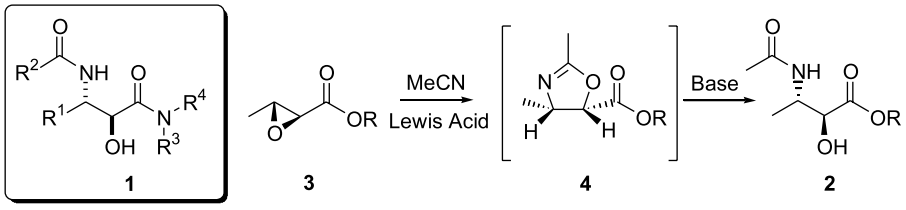
The publisher regrets that an error has occurred in structure 4, Figure 1, of the above paper. A corrected version is shown below:



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