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Contents

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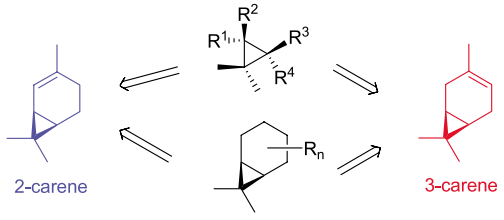
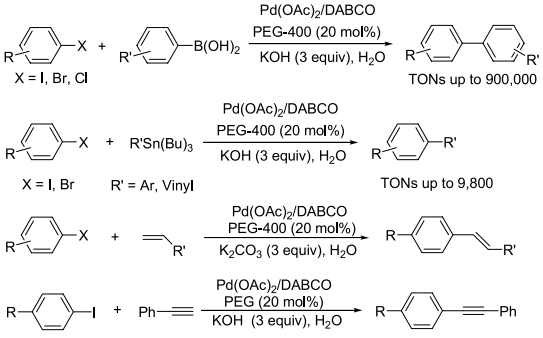
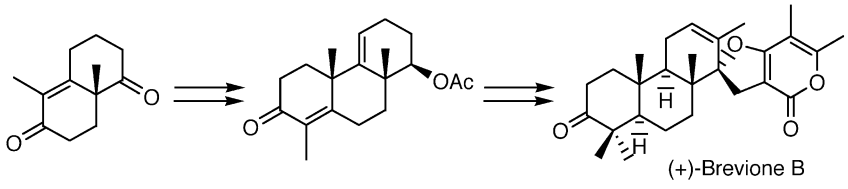
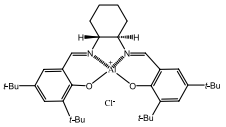


The review represents a systematic survey of the use of 2- and 3-carenes in the synthesis of chiral non-racemic organic compounds containing a 2,2-dimethyl-1,3-disubstituted cyclopropane fragment.

ARTICLES







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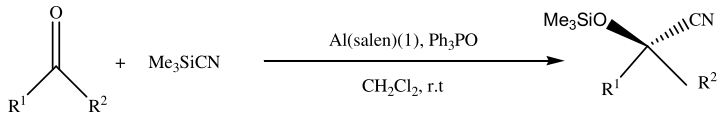

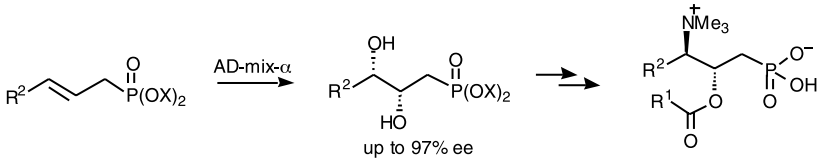

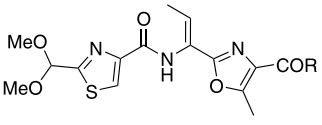

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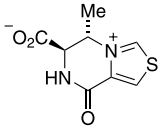
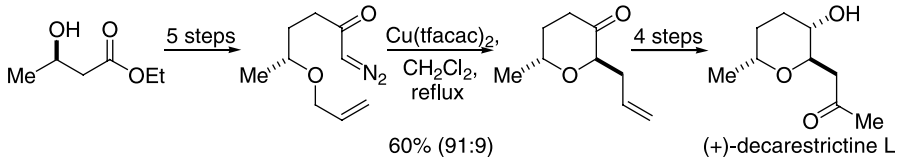
sulfomycinic amide, R = NH_2

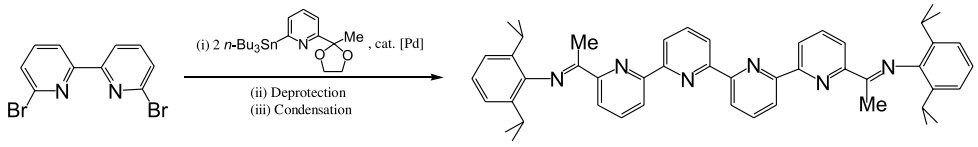


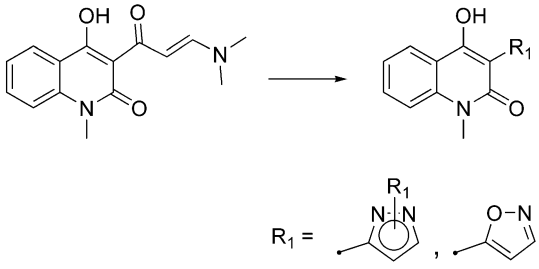
(±)-sulfomycinine

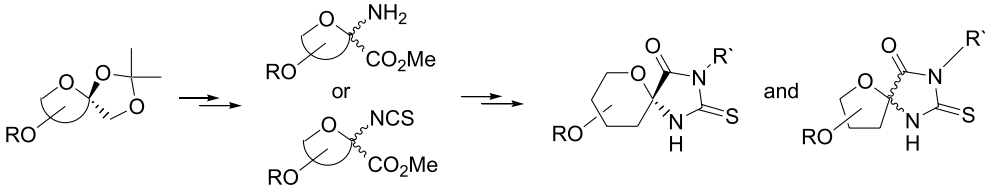


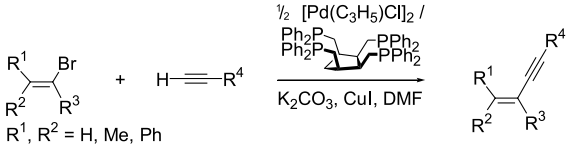
60% (91:9)

(+)-decarestrictine L

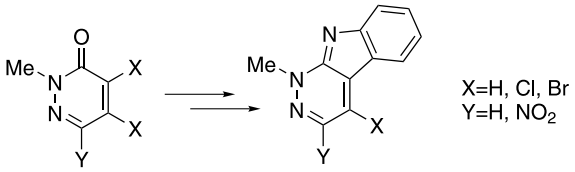


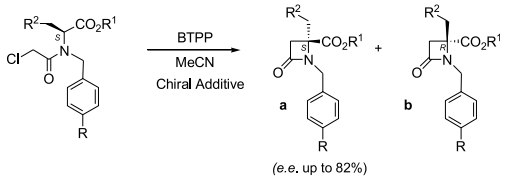


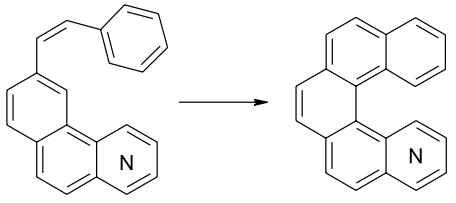




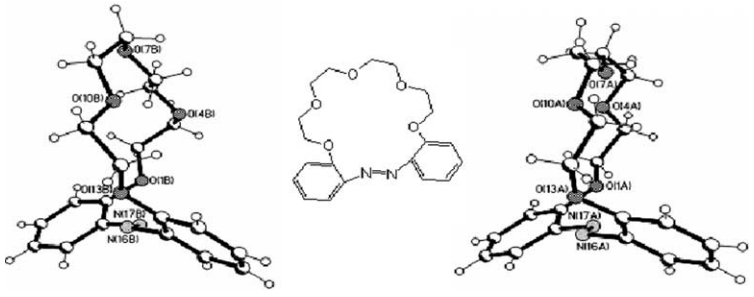
R^1, R^2 = H, Me, Ph
R^3 = H, Me, Et, CH_2CH_2OH, Ph
R^4 = Ph, $(CH_2)_7CH_3$, $C(=CH_2)CH_3$, CH_2OH, CH_2OCH_3, $(CH_2)_2OH$, $(CH_2)_3OH$, $CH(OH)(CH_3)$, $CH_2(OCH_2CH_3)_2$, $CH_2N(CH_2CH_2CH_3)_2$, $C(CH_3)=CHCH_2OH$

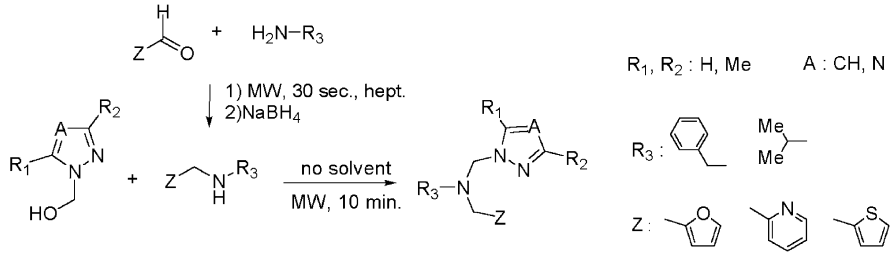


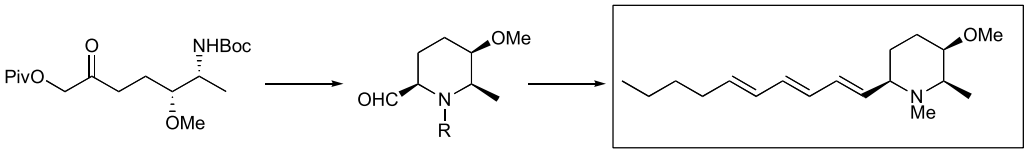


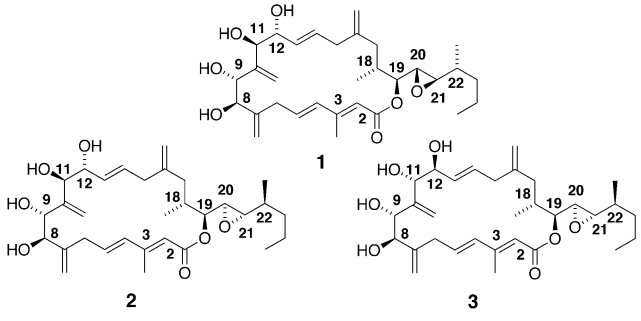


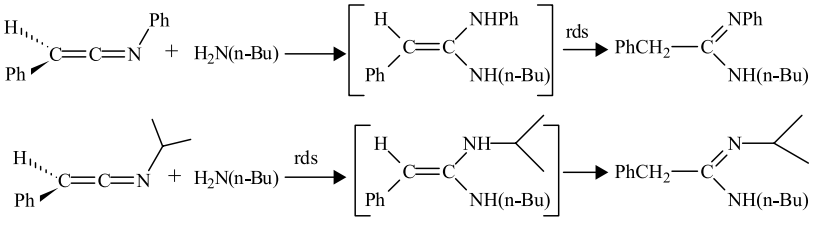
The synthesis of some of this fascinating class of molecule is reported, together with the use of NMR spectroscopy for the structure evaluation and a theoretical explanation of the results.

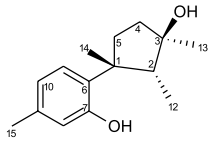












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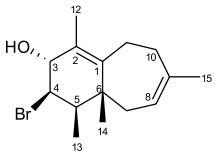

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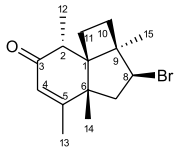

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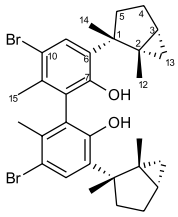

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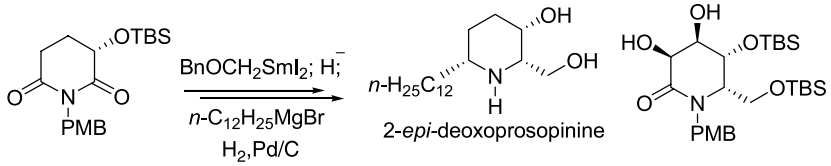

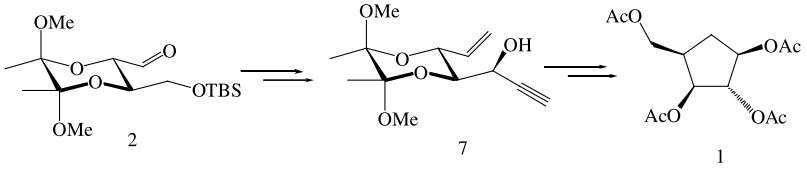

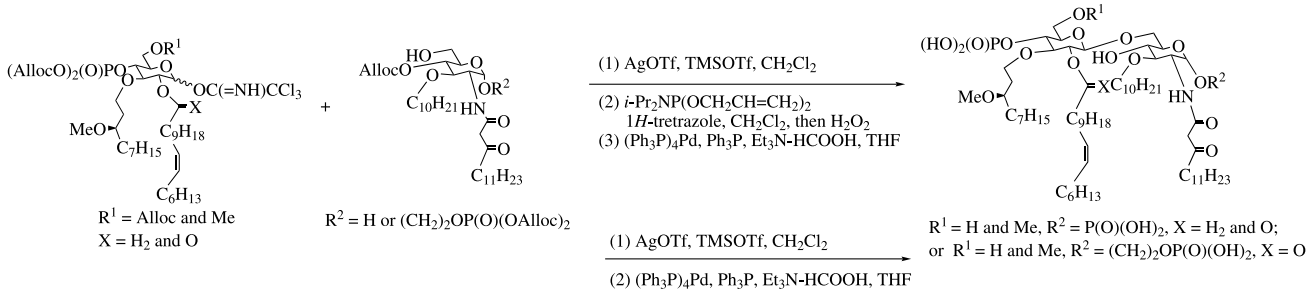

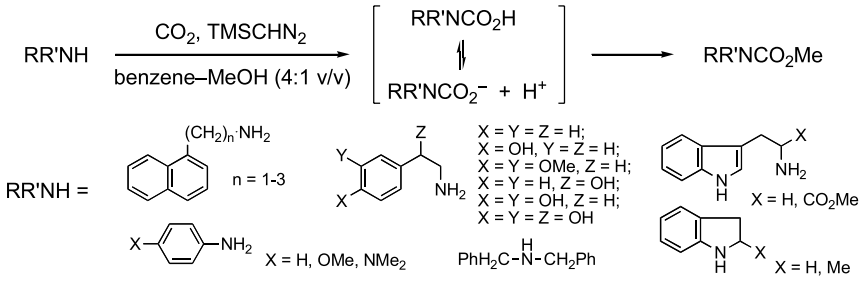

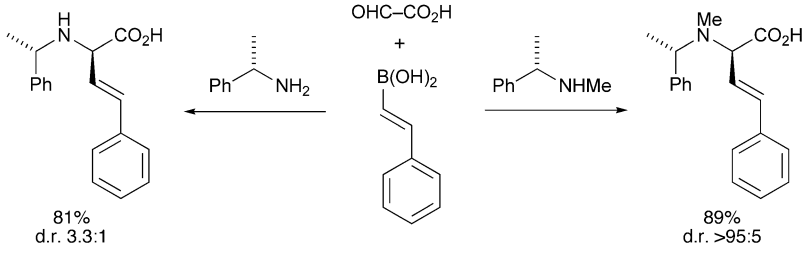

OTHER CONTENTS

*Corresponding author
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Use of monoterpenes, 3-carene and 2-carene, as synthons in the stereoselective synthesis of 2,2-dimethyl-1,3-disubstituted cyclopropanes

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Abstract—The current review represents a systematic survey of the use of 2- and 3-carenes in the synthesis of chiral non-racemic organic compounds containing a 2,2-dimethyl-1,3-disubstituted cyclopropane fragment. The synthetic approaches to the cyclopropane derivatives are classified on the basis of the retention of their parent carane bicyclic skeleton in the final product or cleavage of the six-membered ring along the synthetic route.

Contents

1. Introduction

The last two decades have witnessed an increased interest in stereo- and enantioselective synthesis. This development reflected a growing need for an efficient synthetic methodology to produce enantio-enriched biologically active compounds finding application as pharmaceuticals, agrochemicals, flavours and fragrances, etc. It is common knowledge that the configuration of a chiral compound often has a profound effect on its biological activity. In the pharmaceutical industry, the current trend aims at developing single-enantiomer drugs in the areas where racemates are still in use, which clearly calls for the methodology for

Keywords: Cyclopropane; Chiral; Carenes.
Abbreviations: AIBN, 2,2′-azobisisobutyronitrile; CSI, chlorosulfonyl isocyanate; DBU, 1,8-diazabicyclo[4.3.0]undec-7-ene; DIBAL, diisobutylaluminium hydride; DMAP, 4-dimethylaminopyridine; IBX, *o*-iodoxybenzoic acid; Ipy_2BF_4, bis(pyridine)iodonium(I) tetrafluoroborate; MCPBA, *m*-chloroperoxybenzoic acid; NBS, *N*-bromosuccinimide; PCC, pyridinium chlorochromate; PPA, polyphosphoric acid; TEMPO, 2,2,6,6-tetramethyl-1-pyperidinyloxy, free radical; TPAP, tetrapropylammonium perruthenate; *p*-TsOH, *p*-toluenesulfonic acid.
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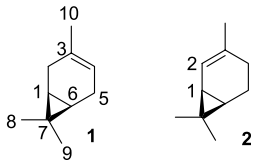


Figure 1.

the preparation of the required active isomers in the enantiomerically pure form. One of the ways of achieving this target relies on the use of the chiral pool of readily available and renewable natural products. For the preparation of compounds incorporating a 2,2-dimethyl-1,3-disubstituted cyclopropane structural unit, the derivatives of (+)-3-carene (**1**) and (+)-2-carene (**2**) (Fig. 1) represent an obvious starting point. The important feature of these monoterpenes is that they can be obtained from natural sources in both enantiomeric forms,[1–3] which together with their distinct bicyclic structure and high enantiopurity makes them attractive starting materials for the enantioselective synthesis of various natural and biologically active products.

Earlier exploitation of chirons **1** and **2** in organic synthesis was covered in a number of monographs[3,4] and overviews.[5–7] The current review is concentrated on the literature published in the last 15 years and some works not included in the earlier accounts.[3–7]

The review is divided into two parts according to the two main strategies employed in the utilisation of the carenes **1** and **2** in organic synthesis. In the first part, we will focus on the synthetic sequences that retain the bicyclic carane framework, while the second part will cover transformations leading to a deeper elaboration of the parent structure where only the native 2,2-dimethylcyclopropane fragment remains intact. Some protocols for the isomerisation of 3-carene to 2-carene are also discussed.

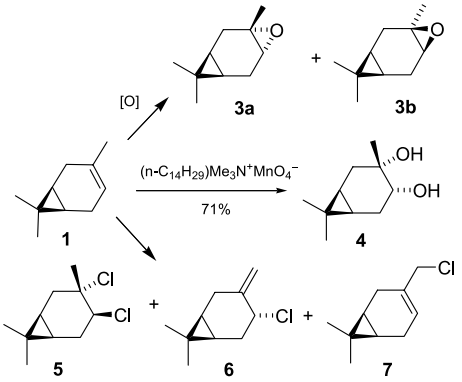
The skeletal rearrangements of monoterpenes of a carane series resulting in the opening of the cyclopropane ring to give derivatives of *m*- and *p*-menthane[8–13] were reviewed recently elsewhere[14,15] and will not be discussed here.

Since both sets of enantiomers of the carenes **1** and **2** can be equally used in organic synthesis, the current review will preserve the configuration of synthons employed in the original papers.

2. Syntheses with retention of the bicyclic carane skeleton

2.1. Synthetic schemes based on 3-carene

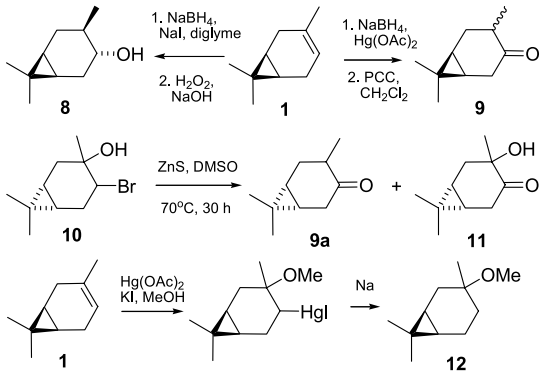
Functionalisation of the double bond in carenes **1** and **2** provides an obvious entry to further synthetic transformations. Epoxides **3a**,**b** (Scheme 1) serve as important intermediates in many synthetic schemes as they offer a wide variety of opportunities for subsequent development. They can be readily prepared from 3-carene by a number of methods including transition metal-catalysed oxidation with hydrogen peroxide or molecular oxygen and treatment with



Scheme 1.

sulfonic peracids or dimethyldioxirane.[16–30] High-yielding stereoselective protocols for the exclusive formation of α-oxide **3a**, employing hydrogen peroxide in the presence of hexafluoroacetone[31] and polymer-supported methyltrioxorhenium as catalyst,[32] were also reported. Vicinal cis-dihydroxylation of alkenes carried out with tetradecyltrimethylammonium permanganate in a two-phase solvent system produced diol **4** in good yields.[33] Stereo- and chemoselective trans-dichlorination of 3-carene is readily achieved in 84% yield by treatment with a 4:1 mixture of tetradecyltrimethylammonium permanganate–trimethylchlorosilane in dichloromethane (**1**→**5**, Scheme 1).[34] The dichloro derivative **5** was also obtained by allylic chlorination of (+)-3-carene **1** carried out with chlorinating reagents, such as *t*-butyl hypochlorite or *N*-chlorosuccinimide, in the presence of radical initiators. In this case, however, it was formed in minor quantities, while the monochloride **6** was the major product, accompanied by a variable amount of the allylic isomer **7**. Hypochlorite exhibited a better selectivity towards **6** (up to 78% in the mixture), although the overall yield in these reactions did not exceed 35%.[35]

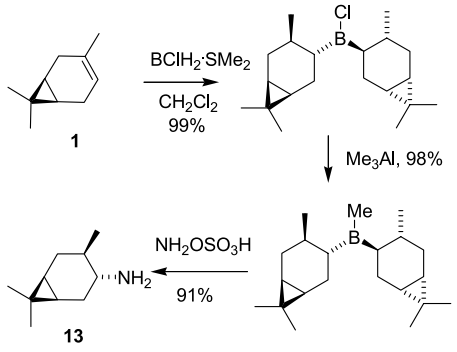
Hydroboration of 3-carene with sodium borohydride followed by oxidation with hydrogen peroxide furnished the secondary alcohol **8** in 70% yield[36] (Scheme 2). Use of a stronger oxidant such as PCC led directly to the formation of ketone **9** (yield 79%).[37] Ketone **9a** of the opposite enantiomeric series was prepared from bromohydrin **10**, as shown in Scheme 2, but its formation was accompanied by a substantial amount of hydroxyketone **11**.[38] Reversal of regiochemistry in the oxygenation of the double bond has



Scheme 2.

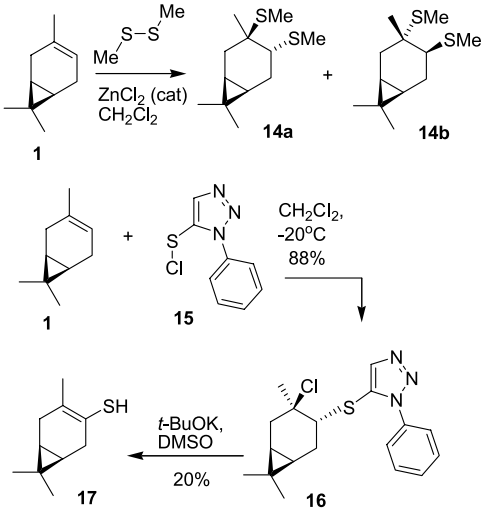
been achieved by methanolysis of 3-carene in the presence of $Hg(OAc)_2$ followed by reductive demercuration to furnish tertiary ether **12** in 80% overall yield.[39]

Enantiomerically pure primary and secondary amines, finding applications as resolving reagents, chiral auxiliaries and synthetic building blocks, are becoming increasingly important targets in asymmetric synthesis. A highly efficient method of stereoselective amination of 3-carene is shown in Scheme 3.[40] The synthesis involved converting the terpene into the *B*-chloroditerpenylborane by treatment with chloroborane-methyl sulfide followed by reaction with trimethylaluminium to form the *B*-methylditerpenylborane, and the latter was converted into the amine by treatment with hydroxylamine-*O*-sulphonic acid. Amine **13** was prepared on a 20 g scale in 88% overall yield.



Scheme 3.

Groups containing sulphur are generally regarded as valuable functionalities and find practical use in many applications. Thio-derivatives of a carane series, however, do not occur in nature and, therefore, have to be produced synthetically. Several methods for the introduction of a thio-group into the carene skeleton were reported (Scheme 4). A mixture of diastereomeric disulphides **14a**,**b** was formed upon addition of $(MeS)_2$ to 3-carene catalysed by $ZnCl_2$.[41] Regio- and stereoselective addition of sulphenyl chloride **15** to 3-carene produced compound **16**, which was transformed into thiol **17** in 17% overall yield.[42]



Scheme 4.

Isomeric carene epoxides **3a**,**b** served as a starting point in the synthesis of a series of 1,2-oxysulphides **18**–**21** (Scheme 5). In this way, isomeric pairs of alkylthiocaranol-carboxylic acids (**19a**,**b**), 4-(2-hydroxyethylthio)-caran-3-ols (**20a**,**b**) and caranthiolactones (**21a**,**b**) have been prepared.[43,44]

Treatment of α-epoxide **3a** with thiourea produced the corresponding β-epithiocarane **22** with inversion of configuration (Scheme 6). The thio-oxide **22** can be opened with alkyl- and arylmercaptans to furnish the carane derivatives **23** containing sulphide and mercapto functions. Alkylation of caranthiols **23** with alkyl halides under basic conditions led to the disulphides **24** with two different groups. Opening of the thio-oxide **22** with mercaptoethanol yielded compound **25** (32%).[25,26] These results were mirrored in the β-epoxide **3b** series, where slightly higher yields were produced. The reactions shown in Scheme 6 allow introduction of a wide range of RS functionality into the carane moiety, but the overall yields are usually low to moderate.

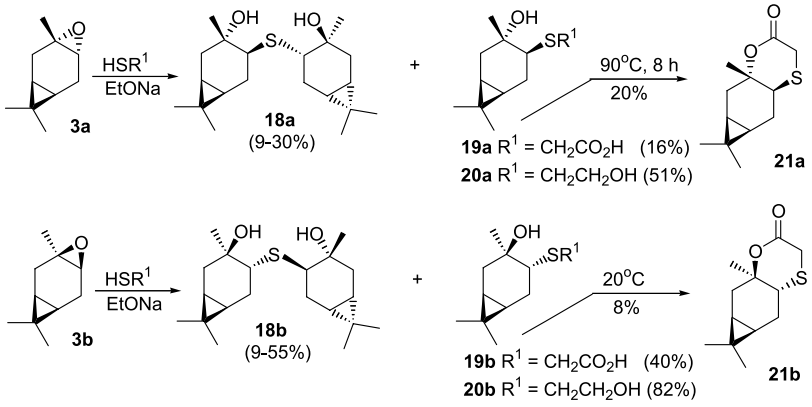
In the presence of a catalytic amount of askanite-bentonite clay, the reaction of α-epoxide **3a** with methacrolein produced a mixture of *cis*-diol derivatives **26** and **27**, accompanied by aldehyde **28**, formed as a result of ring contraction (Scheme 7).[27] At the same time, skeletal rearrangements dominated the reaction of the isomeric β-epoxide **3b** and only a minor amount of ketone **29** retaining the original framework was produced.[27] The yield of the ketone **29** can be improved by up to 37% employing $(TiO)SO_4$ in place of askanite-bentonite clay.[10]

The thiol-catalysed radical-chain redox rearrangement of cyclic benzylidene acetals derived from 1,2-diols of terpene origin has been investigated recently.[45] The rearrangement of the benzylidene acetal **30** derived from carane-3,4-diol **4** was carried out in the presence of triisopropylsilanethiol at 70 °C in hexane employing *t*-BuON=NO*t*-Bu as an initiator. It produced a 1:1 diastereoisomeric mixture of benzoate esters **31**, accompanied by minor quantities (up to 14%) of the bis-acetal **32** (Scheme 8).

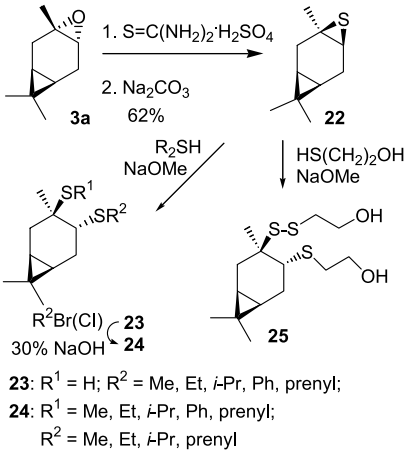
Opening of the epoxide **3a** with morpholine in the presence of a Lewis acid resulted in the formation of aminoalcohol **33** in 66% yield (Scheme 9). Asymmetric addition of lithium cyclopropylacetylenide **34** to ketimines **35** mediated by aminoalcohol **33** (3 equiv) produced compounds **36a**,**b**, representing second-generation analogues of non-nucleoside reverse transcriptase inhibitor, Efavirenz, which is widely prescribed in the treatment of HIV. In the key addition step, compound **36b** was obtained after a single crystallisation in 85% yield and 99.6% enantiomeric purity.[46,47]

According to another report, the chiral aminoalcohol **33** was employed as a catalyst for the asymmetric addition of diethylzinc to a wide range of aliphatic and aromatic aldehydes, leading to enantio-enriched alcohols in 33–98% ee.[48]

The related unsubstituted aminoalcohol **39** can be prepared in two steps from carene oxide **3a** via the azide **37**



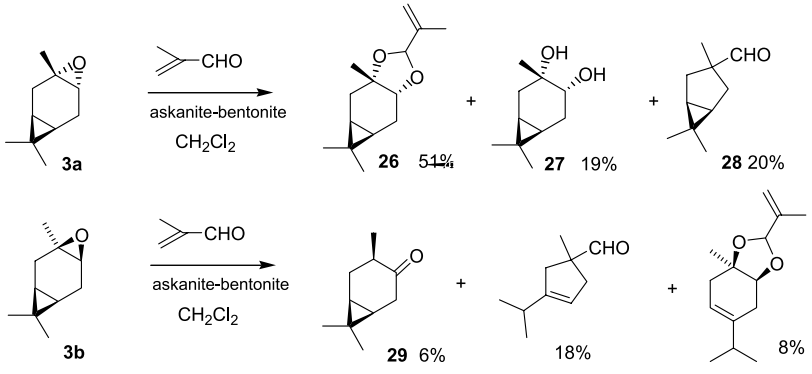
Scheme 5.



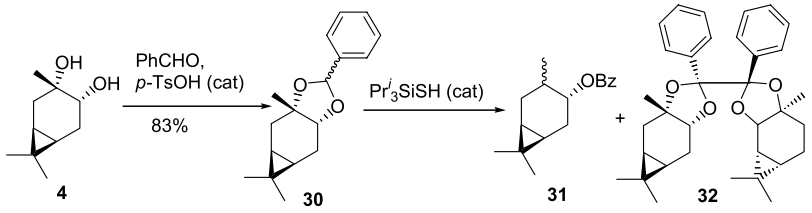
Scheme 6.

(Scheme 10). Regioselectivity of the epoxide opening with aqueous NaN_3 exhibited a strong dependency on the pH of the reaction medium. In an acidic solution at pH 4.2, the azide **38** was formed predominantly (ratio **37**/**38** 14:86), while, under basic conditions at pH 9.6, the regioselectivity changed in favour of the azide **37** 65:35.[49] Reduction of the azide **37** was carried out with $NaBH_4/CoCl_2 \cdot 6H_2O$ in water at 25 °C under heterogeneous catalytic conditions to furnish amine the **39** in both high yield and purity.[50]

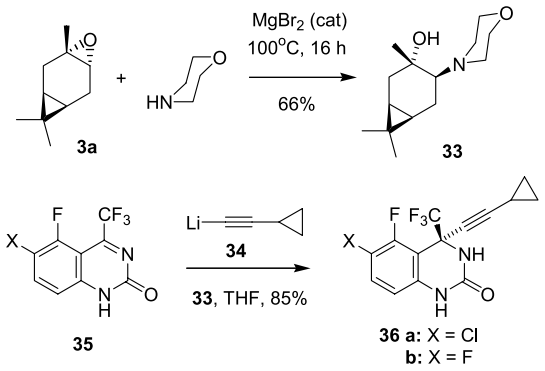
Methanolysis of the epoxide **3a** in the presence of Lewis or Brönsted acids proceeded regioselectively with the attack at the more substituted terminus (**3a**→**40**, Scheme 11).[21,51] The resulting alcohol **40** was converted into the acid **41** in 65% yield by treatment of the corresponding sodium alcoholate with monochloroacetic acid in toluene followed by [2+2] cycloaddition with imines **42** mediated by triphosgene to furnish a mixture of diastereomeric β-lactams **43** and **44**.[21]



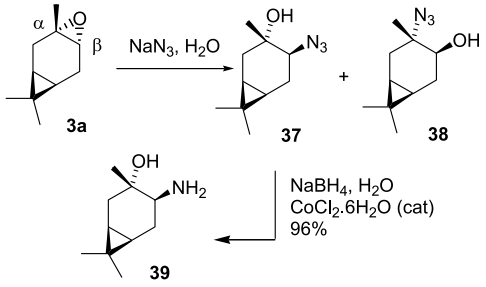
Scheme 7.



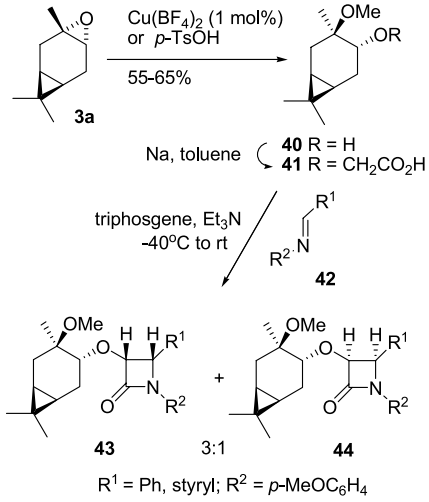
Scheme 8.



Scheme 9.



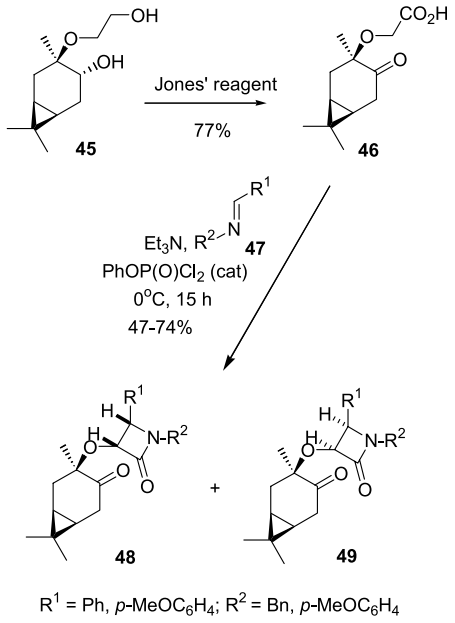
Scheme 10.



Scheme 11.

In a similar fashion, diol **45** obtained by opening epoxide **3a** with ethylene glycol was oxidised to ketoacid **46**, which was further reacted with imines **47** in the presence of phenyl dichlorophosphate to give a nearly 1:1 mixture of β-lactams **48** and **49** (Scheme 12).[52]

Treatment of 3-carene with NBS and ethylene glycol furnished compound **50**, which can be viewed as a bromo-analogue of alcohol **45** (Scheme 13). When the corresponding acid chloride **51** was reacted with imine **47**, however, the diastereoselectivity of formation of β-lactams remained low, giving a 3:2 mixture of **53** and **54**.[53] As an extension of this work, the diastereoselective synthesis of tetracyclic lactams **55** and **56**, was reported.[54] In this case, α,β-



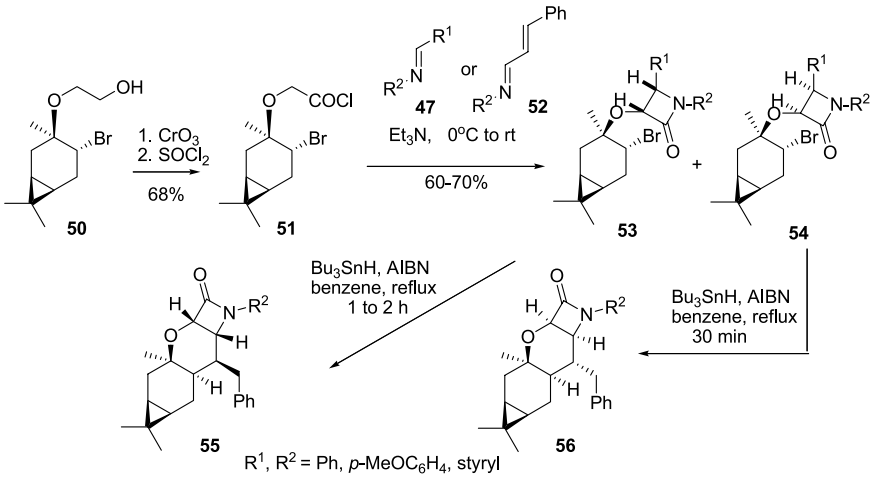
Scheme 12.

unsaturated imines **52** were employed in cycloadditions with the acid chloride **51**, and the lactams **53** and **54** were chromatographically separated prior to the intramolecular cyclisation. Notably, compounds **54** reacted faster than their isomers **53**.

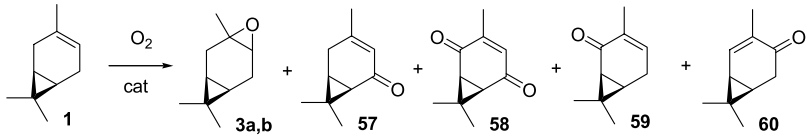
In its current state, auto-oxidation of terpenes to produce oxygenated derivatives can hardly be regarded as a valuable synthetic tool, due to the low conversion rates and poor selectivity. From a technological point of view, however, it represents an attractive methodology and still remains the subject of extensive studies, as O_2 is widely seen as a 'green' reagent. In the case of 3-carene, transition metal-catalysed oxidation, as a rule, leads to complex mixtures containing varying amounts of products **3a**,**b** and **57**–**60** (Scheme 14), resulting from epoxidation and/or allylic oxidation.[55,56] Some improvement in selectivity towards the formation of **57** accompanied by minor quantities of **3a**,**b** and **58** was reported recently.[20]

Selective transformation of saturated carane **61**, obtained from 3-carene by borane reduction, into ketone **62** can be achieved by Ru-catalysed oxidation with $NaIO_4$.[57] The ketone **62** was further selectively reduced to alcohol **63** (Scheme 15).

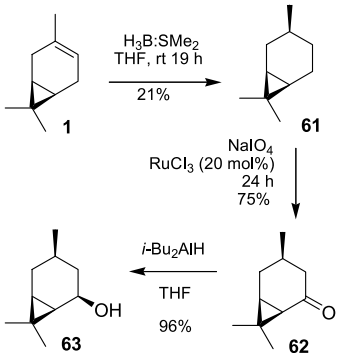
A different approach to caranone **62** involves the hydrogenation of carenone **57**, readily available by auto-oxidation of 3-carene.[20,58,59] Ketone **62** represents a key intermediate in the synthesis of C_2-symmetrical chiral bipyridine **70** (Scheme 16). In the first, unsuccessful approach, **62** was converted into oxime **64** followed by reductive acylation to form enamide **65**, which under Vilsmeier conditions, failed to produce the desired chloropyridine **66**, due to acid-induced fragmentation of the carene bicyclic framework. In the revised sequence, pyridone **69** was successfully constructed via annulation of enone **67** with the Kröhnke salt **68**. Subsequent chlorination with $POCl_3$ followed by Ni(0)-mediated dimerisation furnished the target bipyridine



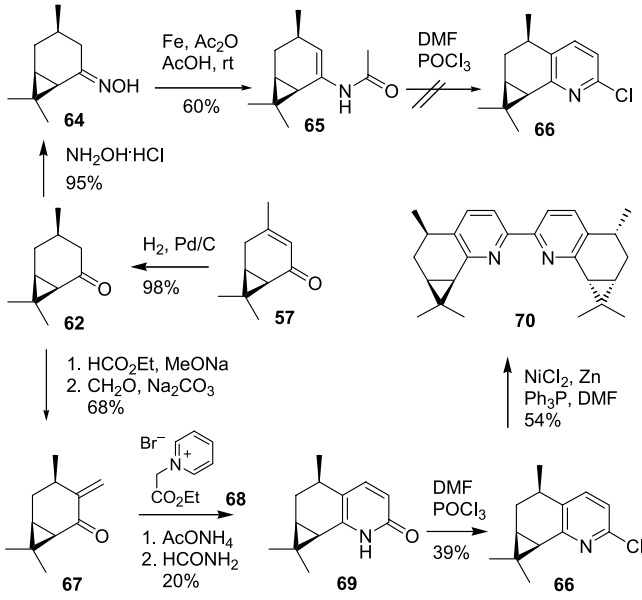
Scheme 13.



Scheme 14.



Scheme 15.



Scheme 16.

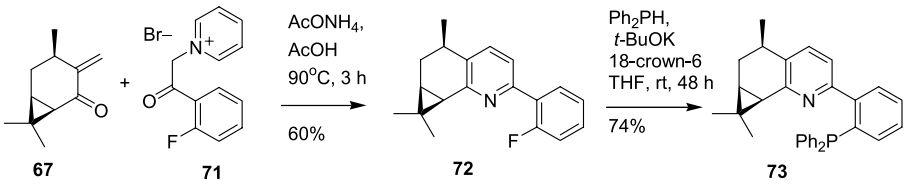
70, which showed good promise as a ligand in Cu-catalysed asymmetric allylic oxidation of cyclic alkenes with peroxyesters.[58]

An analogous strategy was employed in the preparation of the chiral phosphinopyridine ligand **73** (Scheme 17).[59] Here, Kröhnke annulation of enone **67** with **71** gave rise to pyridine **72** followed by aromatic nucleophilic substitution of fluoride with potassium diphenylphosphide to give **73**. The ligand **73** was employed in a Pd-catalysed asymmetric Heck addition of phenyl triflate to 2,3-dihydrofuran.

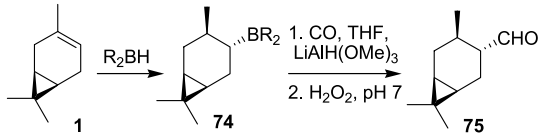
Carene derivatives functionalised at position 4 serve as important intermediates in the synthesis of natural products and compounds finding application in fragrance or pharmaceutical and agrochemical research. 4-Formylcaranone **75** was prepared in 40% overall yield from 3-carene via hydroboration followed by carbonylation of the resulting organoborane **74** (Scheme 18).[60]

4-Hydroxy-3(10)-carene **76**, readily accessible from 3-carene, was employed as a key intermediate in the synthesis of compounds **78**, **79** and **85**, tested as odorants,[61] and spirolactones **82**–**84**, exhibiting some promising insecticidal action.[62] The synthetic sequence is illustrated in Scheme 19. Ireland–Claisen rearrangement of allylic alcohol **76** produced γ,δ-unsaturated ester **77**, which was further transformed into alcohol **78**, acid **80** or epoxide **81**. Lactonisation of **80** and **81** gave rise to spiro-derivatives **83** and **84**. Treatment of the latter with DBU furnished **85**.

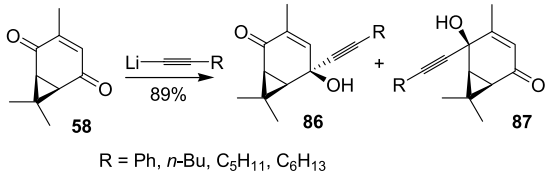
Addition of lithium acetylides to dione **58** proceeded stereoselectively from the face opposite to the bulky dimethylcyclopropane unit, but the regioselectivity of the addition was less impressive, leading to a mixture of isomers **86** and **87** (Scheme 20).[63]



Scheme 17.

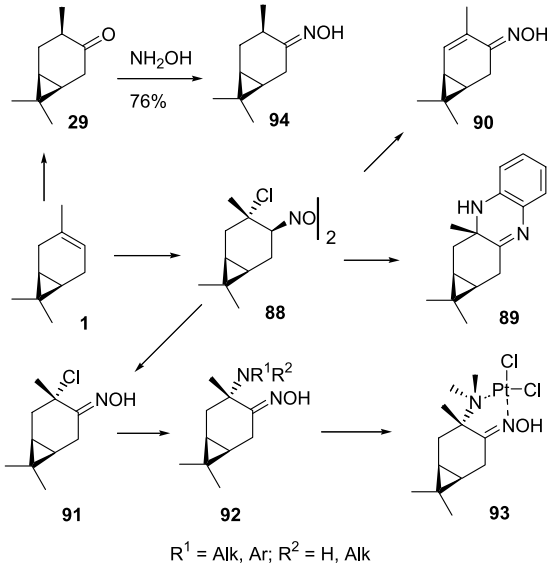


Scheme 18.



Scheme 20.

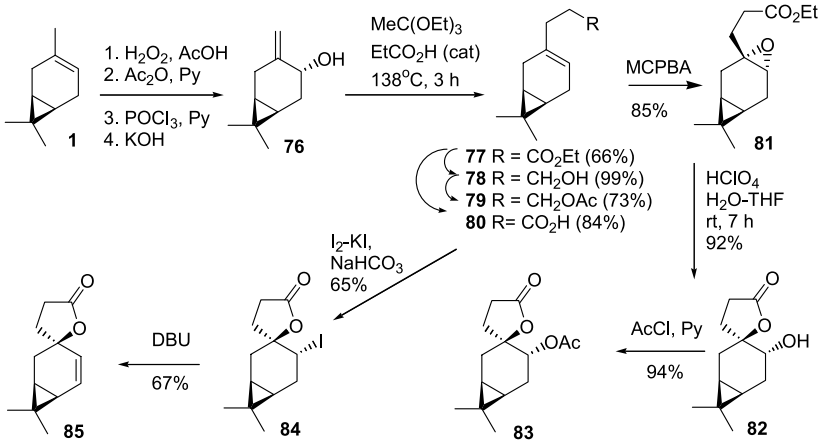
Addition of nitrosyl chloride represents another powerful strategy for the functionalisation of 3-carene.[64] The reaction proceeded via the formation of dimeric nitrosyl chloride **88**, which rearranged into α-chloroketoxime **91** (Scheme 21). Addition of *o*-phenylenediamine to dimer **88** furnished hexahydrophenazine **89** in 66% yield.[65] Less nucleophilic diamines, such as 3,4-diaminofurazane and 4,5-diaminobenzofurazane, led to elimination of HCl, resulting in the formation of unsaturated oxime **90**. Ketoximes **92**, obtained by nucleophilic substitution of the chloride in **91**, served as ligands in the preparation of a number of chiral transition metal complexes, for example, **93**.[66–68] The parent unsubstituted oxime **94** can be synthesised via caran-4-one **29**.[69]



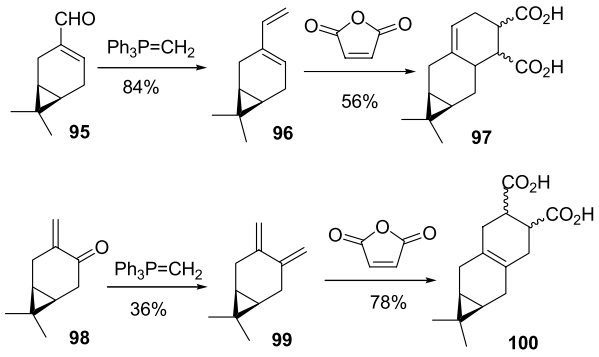
Scheme 21.

3-Caren-10-al **95** and 3(10)-caren-4-one **98**, both easily accessible from 3-carene via carene oxide **3**, were chosen as precursors in the synthesis of tetracyclic compounds **97** and **100**, respectively, (Scheme 22).[70] The sequence included Wittig olefination to furnish *cis*-dienes **96** and **99** followed by Diels–Alder cycloaddition to maleic anhydride. Of these two reactions, the latter proceeded in good yield, giving products **97** and **100** as mixtures of *syn* and *anti* isomers, while the olefination was accompanied by the formation of a number of by-products arising from enolisation, dimerisation and conjugate addition, which reduced the yield of the desired dienes.

Aerobic oxidation of 3-carene catalysed by complexes of Co(II) in the presence of sacrificial *iso*-butyraldehyde, produced epoxide **3**, which reacted in situ with trimethylsilyl isothiocyanate **101** to give the tricyclic oxazolidin-2-thione



Scheme 19.

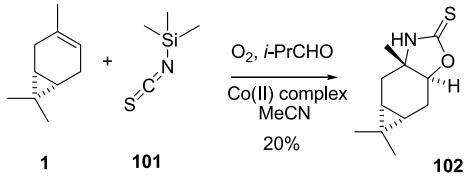


Scheme 22.

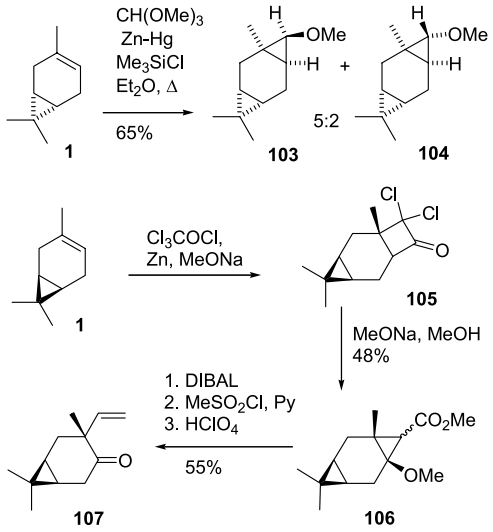
102 (Scheme 23).[71] As a point of interest, this reaction resulted in a rather rare trans ring junction, which was established by X-ray crystallography.

Dicyclopropane derivatives **103**, **104** and **106** (Scheme 24) represent other types of tricyclic systems based on the carane skeleton. Cyclopropanation of 3-carene proceeded readily by reaction of an orthoformate with the alkene in the presence of Me_3SiCl and zinc amalgam in refluxing ether to give a mixture of **103** and **104** in 65% overall yield.[72] The process is characterised by a distinct stereochemical preference for the formation of the more hindered *endo* isomer **103**.

A related dicyclopropane analogue **106** was synthesised in two steps via a stereoselective [2+2] addition of dichloroketene to 3-carene, under ultrasound irradiation, yielding the adduct **105**, followed by a sodium methoxide-mediated ring contraction to furnish the bifunctional



Scheme 23.



Scheme 24.

cyclopropane ester **106** as a mixture of *exo* and *endo* isomers.[73,74] The mixture was further converted in a high yield into α-vinyl ketone **107**, which can serve as a versatile intermediate for a variety of synthetic transformations. In the case of 2-carene, an analogous reaction sequence led to the opening of the cyclopropane ring.[75]

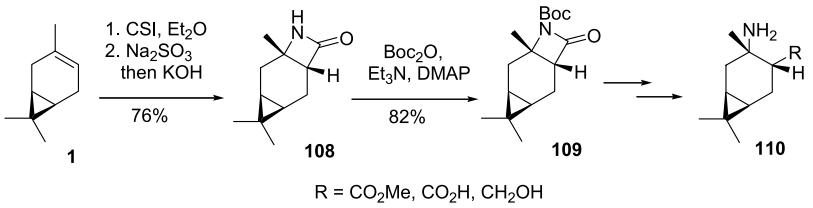
In a similar fashion, cycloaddition of chlorosulfonyl isocyanate to 3-carene proceeded regio- and stereoselectively, according to the Markovnikov rule, furnishing enantiomerically pure β-lactam **108** (Scheme 25).[76] After having activated the amide with a *tert*-butoxycarbonyl group, the resulting *N*-Boc-protected β-lactam **109** was easily converted into homochiral β-amino acid derivatives **110**, which may serve as chiral building blocks in the asymmetric synthesis of potential pharmacophores, modified analogues of natural peptides or employed as chiral auxiliaries in enantioselective synthesis.

4α-Acetyl-2-carene **111**, accessible in one step from 3-carene, was employed in a number of stereoselective transformations shown in Scheme 26. Reduction of **111** with sodium borohydride demonstrated a poor selectivity, giving a 2:3 mixture of diastereomeric alcohols **112** and **113**.[77] Crystalline alcohol **113** was subsequently employed in the preparation of ester **114**.[78] Reductive amination of ketone **111** with monoethanolamine led to the carene derivative **115**, which was further reacted with *p*-anisaldehyde to furnish a 1:1 mixture of isomeric 1,3-oxazalidines **116** in 76% overall yield. A sequence of reactions including isomerisation of **111** into **117** followed by Wittig olefination (**117**→**118**) and cycloaddition with tetracyanoethylene produced the tricyclic derivative **119**. Cyclopropanation of the unconjugated diene **121**, which can be obtained either by olefination of **111** or via the homoallylic alcohol **120**, with dichlorocarbene resulted in the formation of a mixture of mono- and di-adducts **122** and **123**.[79]

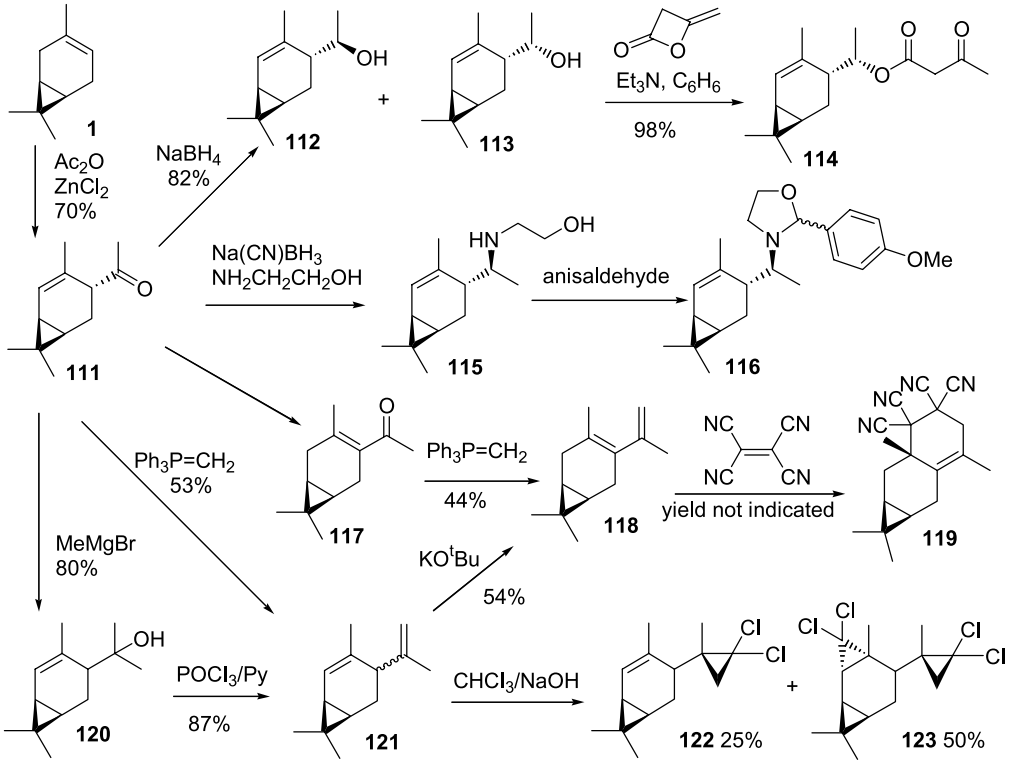
Silylation of ketone **111**, carried out under conditions of either thermodynamic or kinetic control, gave rise to diastereomeric enol ethers **124** or **125**, respectively, (Scheme 27).[80] Hydrolysis of silyl enol ether **124** furnished 4β-acetyl-2-carene **126**. Ozonolysis of the isomeric **125** followed by a reductive work up furnished 2-caren-4-one **60**.[80] This α,β-unsaturated ketone was employed in the preparation of diethyl oxophosphonates via a regioselective conjugate addition.[81] In a related process shown in Scheme 28, addition of dibenzylphosphine oxide to ketone **111** gave rise to the tricyclic compound **127** in 88% yield.[82]

3-Carene-derived acetonides of 1,3-diols such as **128** can serve as efficient chiral auxiliaries to direct a face-selective functionalisation of an adjacent unsaturated unit. Iodofunctionalisation of **128** (Scheme 29), using bis(pyridine)iodonium(I) tetrafluoroborate (Ipy_2BF_4) as a promoter, resulted in the clean formation of **129** as a single diastereoisomer.[83]

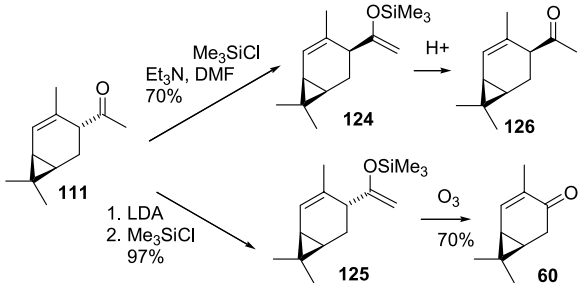
3-Carene **1** served as a starting point in the synthesis of deoxy analogues of phorbol **137** (Scheme 30), a well-established activator of protein kinase C. The synthetic route proceeded via **130** and the bicyclic ketones **131** and **132**, common intermediates in the synthesis of 13-deoxyphorbols, which were successfully converted into



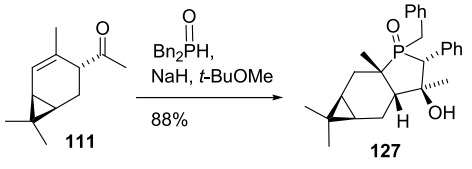
Scheme 25.



Scheme 26.

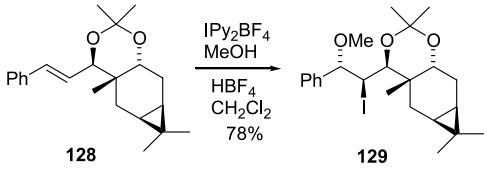


Scheme 27.



Scheme 28.

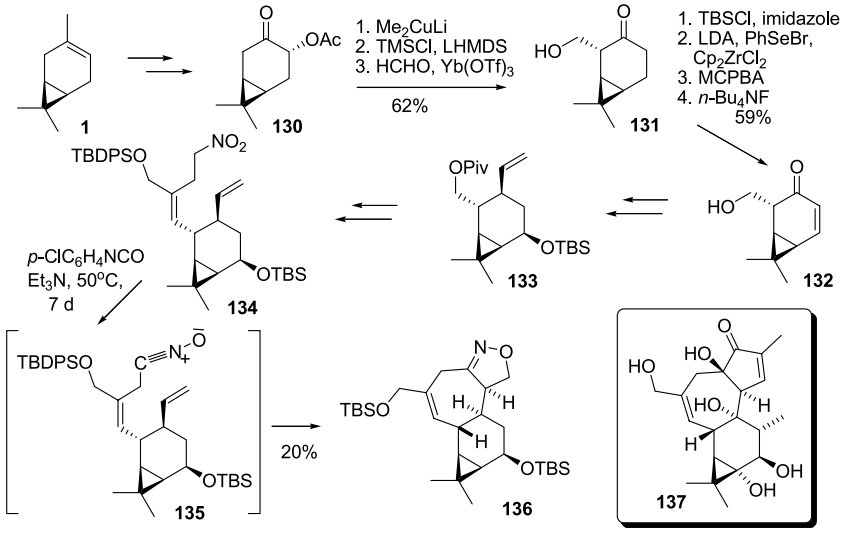
the vinyl derivative **133** followed by further transformations, leading to compound **134** with a nitro group in the side chain. In the key step, intramolecular cycloaddition via a possible intermediacy of **135** furnished the target



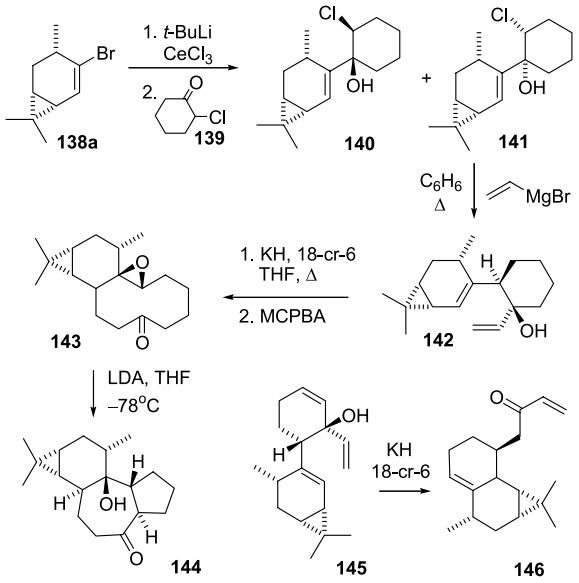
Scheme 29.

9-deoxyphorbol analogue **136**.[84] It should be mentioned, however, that the last step was very slow and conversion in 7 days reached only 20%, due to the unfavourable alignment of the reacting groups in the transition state.

In another synthetic approach to the phorbol-related tetracyclic framework, vinyl bromide (−)-**138a**, which can be prepared from 3-norcaranone, was utilised as the key synthetic intermediate (Scheme 31).[85] Addition of the nucleophile derived from **138a** to the racemic chloroketone **139** led to chlorohydrins **140** and **141** in a 1:1 ratio. Treatment of **141** with excess vinylmagnesium bromide yielded **142**. Anionic oxy-Cope rearrangement of **142** followed by epoxidation produced the α-isomer **143**, along with its β-isomer. Base-promoted cyclisation of **143** under kinetically controlled conditions resulted in the



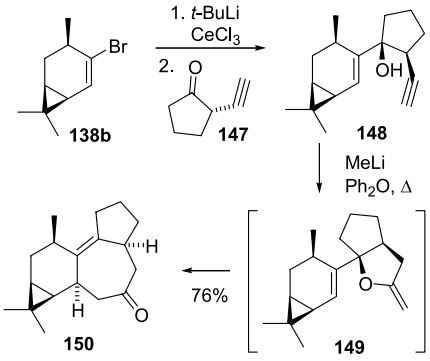
Scheme 30.



Scheme 31.

formation of the target **144**. In the course of this work, one of the first intramolecularly competitive anionic oxy-Cope rearrangements was investigated, as illustrated in Scheme 31, by conversion of **145** into **146**.[85,86]

A related approach to the tetracyclic phorbol skeleton, described recently,[87] made use of a tandem anionic 5-*exo-dig* cyclisation/Claisen rearrangement sequence (Scheme 32). In this case, enantiomeric vinyl bromide (+)-**138b**, prepared in five steps from (+)-3-carene, was analogously metallated by consecutive treatment with *t*-BuLi and anhydrous $CeCl_3$, followed by addition to the homochiral ketone **147** to furnish alkynol **148** in 82% yield. The alkynol **148**, on exposure to catalytic MeLi and heat for a period of 1 h, was readily converted into the target tricyclic compound **150** as a single stereoisomer in 76% yield. As the reaction progressed, the initial anionic 5-*exo-dig* cyclisation gave rise to the allyl vinyl ether **149**, which



Scheme 32.

was perfectly set up for a Claisen rearrangement, ultimately leading to **150**.

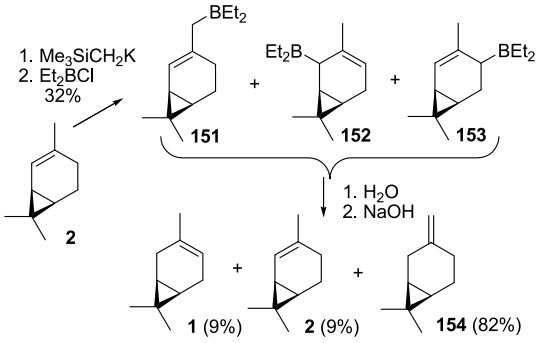
2.2. Isomerisation of 2- and 3-carene

2-Carene **2**, possessing the same bicyclic skeleton as the isomeric 3-carene **1**, has a higher reactivity than its 3-isomer, because the carbon–carbon double bond is conjugated with the cyclopropyl unit. A wider application of 2-carene in target organic synthesis is, however, hampered by its low availability, as the content of the 2-isomer in the carene oil fraction does not exceed 5–7%. Isomerisation of the inexpensive, abundant, 3-carene, can be viewed as an attractive method for producing 2-carene.

Investigation of the gas-phase isomerisation of 3-carene performed on the surface of basic zeolites revealed that the best in the series, sodium-loaded NaX zeolite, at 200 °C, can afford a 78% selectivity with respect to 2-carene at 36% conversion of 3-carene.[88] In related work, promising results were achieved with a silica-supported nickel catalyst modified by tin that provided 2-carene with 91% selectivity at 48% conversion of 3-carene.[89] In general, thermodynamically controlled isomerisation of 3-carene by strong bases at higher temperatures leads to a 40:60 equilibrium mixture of 2- and 3-carene. The equilibrium is approached from both

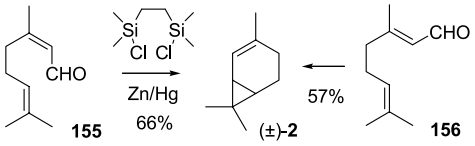
sides. From the point of view of synthetic utility, however, these methods are not ideal, as separation of the isomers represents a formidable task.

Isomerisation of 2-carene via a sequence that involved a two step formation of boranes **151–153** followed by hydrolysis afforded the rare 3(10)-carene **154** as the major product, along with minor quantities of the isomeric carenes **1** and **2** (Scheme 33).[90]



Scheme 33.

Synthesis of racemic 2-carene from linear terpenoids, such as neral **155** and geranial **156**, was based on intramolecular cyclopropanation employing 1,2-bis-(chlorodimethylsilyl) ethane and zinc (Scheme 34).[72b] Development of the asymmetric version of this reaction has not, however, been attempted.

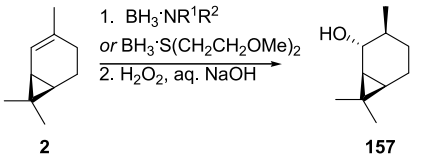


Scheme 34.

2.3. Synthetic schemes based on 2-carene

Functionalisation of 2-carene is usually performed in the same manner as for the isomeric 3-carene. It is, however, pertinent to note that, in 2-carene, the double bond is conjugated with the cyclopropane ring, which makes it more reactive compared to the 3-isomer, but also facilitates the opening of the dimethylcyclopropane unit. As a consequence, milder reaction conditions are generally employed in order to keep the bicyclic skeleton intact.

Synthesis of 2-caranol **157** (Scheme 35) proceeded in high yield and stereoselectivity via hydroboration of 2-carene followed by oxidation with alkaline hydrogen peroxide.[91,92]

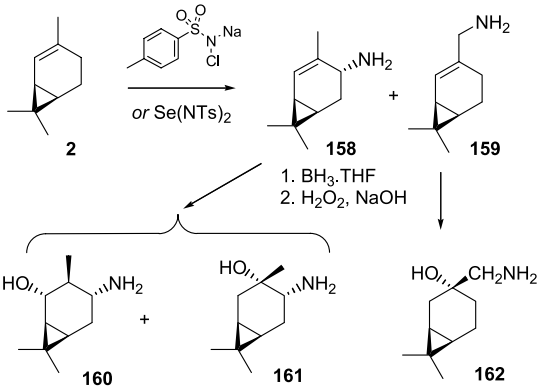


R^1 = *i*-Pr, *i*-Bu; R^2 = Et, CH_2CH_2OMe, *i*-Pr, *i*-Bu, *s*-Bu

Scheme 35.

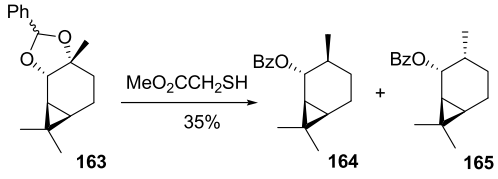
When catecholborane was employed in the hydroboration step, oxidation of the resulting *B*-alkylcatecholborane was achieved using molecular oxygen under neutral conditions, but this protocol led to substantial cleavage of the cyclopropane ring.[93]

Allylic amination of 2-carene with chloramine-T or selenium diimide (Scheme 36) after removal of the protecting group furnished a mixture of regioisomeric amines **158** and **159**, which were further converted into a set of aminoalcohols **160–162**, although the yields in the key steps were rather low.[94,95]



Scheme 36.

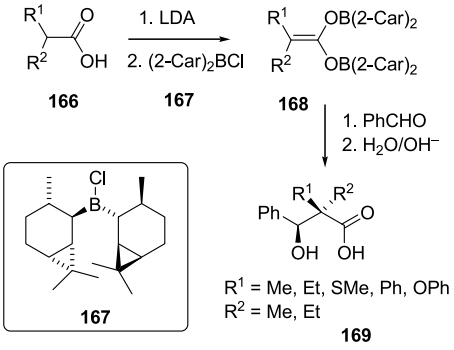
A mixture of benzoate esters **164** and **165** was obtained by the thiol-catalysed radical redox rearrangement of cyclic benzylidene acetal **163**, derived from the corresponding 1,2-diol (Scheme 37, for an analogous rearrangement in the 3-carene series, see Scheme 8). Due to the radical nature of the process, it was accompanied by an extensive opening of the cyclopropane ring, resulting in low yields of the bicyclic esters **164** and **165**.[45]



Scheme 37.

The boron enolates **168** of a number of mono- and disubstituted acetic acids **166** were prepared using (−)-di-2-isocaranylchloroborane **167** in THF at −78 °C. The addition reactions to benzaldehyde to give *syn* 2-substituted 3-hydroxy-3-phenylpropionic acids **169** proceeded highly diastereo- and enantioselectively in good yields (Scheme 38).[95] The related chiral auxiliaries derived from 3-carene gave rise to a preferential formation of the *anti* adducts, but with lower enantioselectivity.

The [4+2] cycloadditions of (+)-2-isocaranyl vinyl ether **170** with (*E*)-2-aryl-1-cyano-1-nitroalkenes **171**[96] and (*E*)-3-diazenylbut-2-enes such as **173**[97] carried out in water, to give **172** and **174**, respectively, were reported recently (Scheme 39). The reactions occurred in a heterogeneous



Scheme 38.

phase under mild conditions and were fast and highly stereoselective, although the diastereoselectivity was low.

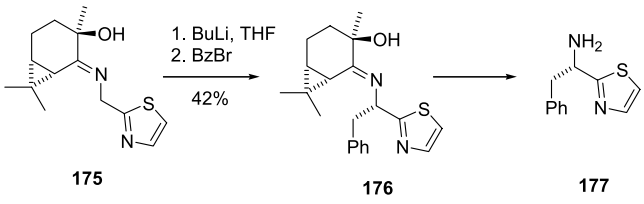
Chiral Schiff base **175**, which can be prepared in three easy steps from 2-carene was used in the asymmetric synthesis of (*S*)-dolaphenine **177** and other related analogues (Scheme 40), serving as building blocks in the synthesis of some natural products of marine origin. The key alkylation step yielding **176** was accomplished with good diastereoselectivity.[98]

Unsaturated ketone **178** represents a valuable synthon for the construction of chiral heterocycles. It can be prepared by a direct photochemical ene reaction of (+)-2-carene **2** with singlet oxygen (Scheme 41), giving a 1:3 mixture of the desired (−)-**178** and the *endo*-isomer **179**.[99] Alternatively, the *exo*-methylene ketone **178** can be synthesised from (+)-2-carene **2** via stereoselective epoxidation with MCPBA, followed by deprotonation of the resulting epoxide **180** with LDA to afford the allylic alcohol **181**. Oxidation of the latter, using a catalytic modification of the Dess–Martin protocol, afforded (−)-**178** in good yield.[58] Using the synthetic protocols described earlier for the preparation of **70** and **73** (Schemes 16 and 17), which rely on Kröhnke anulation as a key ring-forming step, a series of chiral pyridine derivatives **182**,[59] **183**,[59] **184**,[58] **185**[100] and **186**[99] were synthesised. Complexes of these ligands with transition metals were successfully applied to asymmetric catalysis and supramolecular chemistry.

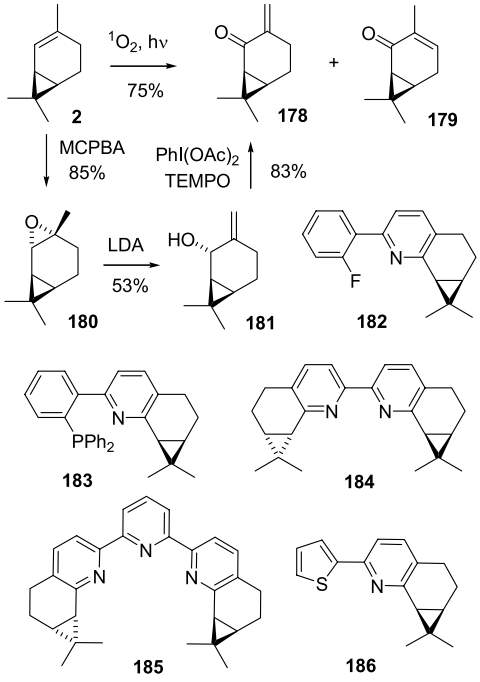
3. Syntheses where only the 2,2-dimethylcyclopropane fragment is retained

3.1. Synthetic schemes based on 3-carene

The six-membered ring of the carane framework can undergo a broad spectrum of skeletal rearrangements, resulting in non-symmetrical structures, which incorporate



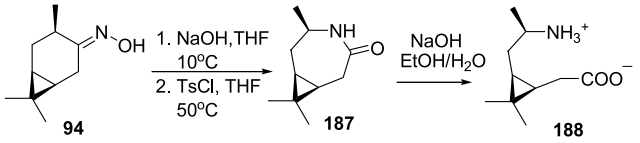
Scheme 40.



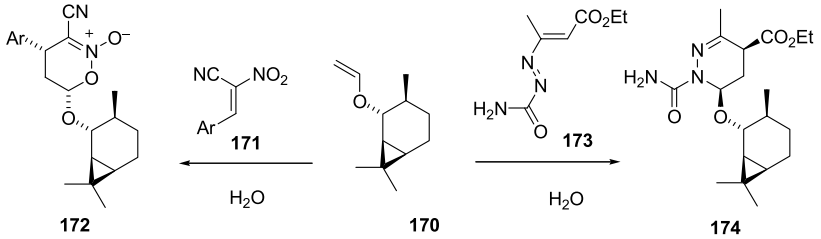
Scheme 41.

the dimethylcyclopropane ring. A good illustration is the Beckmann rearrangement of ketoxime **94** into lactam **187**, which is further hydrolysed into amino acid **188** (Scheme 42).[69]

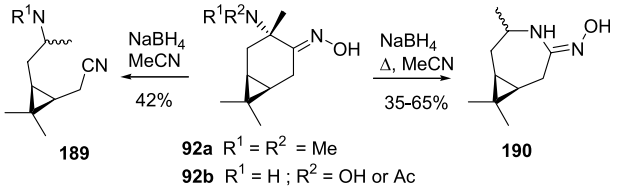
Treatment of dimethylamino oxime **92a** with $NaBH_4$ in acetonitrile (Scheme 43) triggered a reductive rearrangement, leading to a mixture of nitriles **189**,[101] while, in the case of α-hydroxylamino or α-acetylamino oximes **92b**, the



Scheme 42.



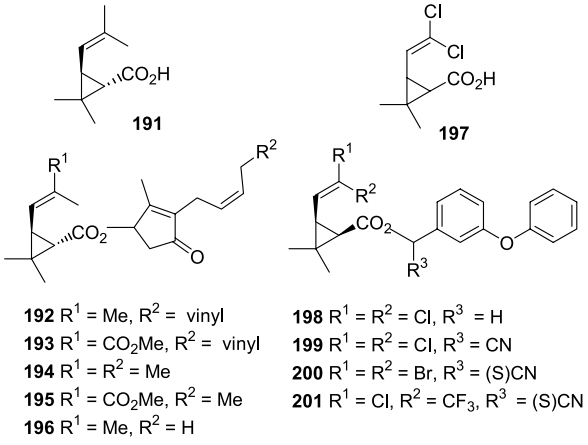
Scheme 39.



Scheme 43.

reaction produced a cyclic amido oxime **190**.[102] The mechanism by which the cyclic compound is formed is not clear, but the presence of acetonitrile was found to be crucial.

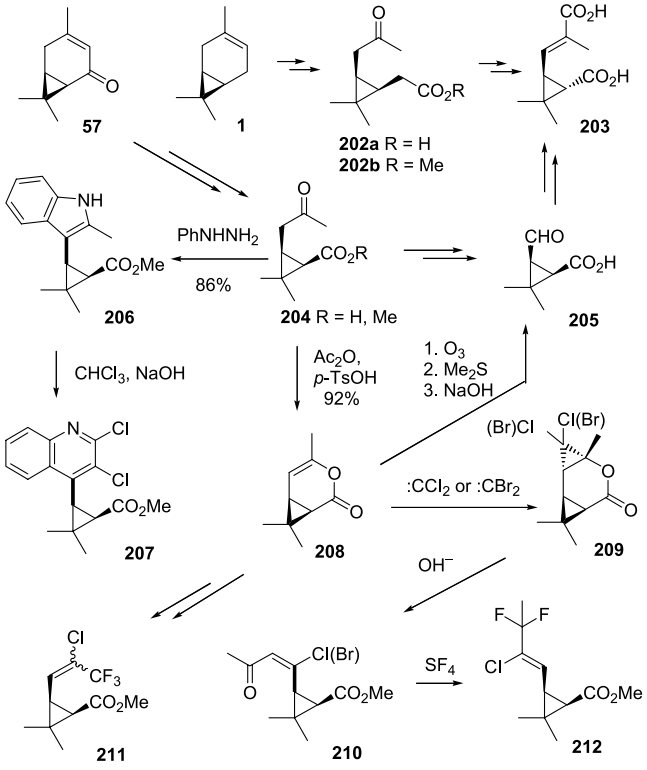
Chrysanthemic acid **191** and its esters **192–196** (Scheme 44),[103] isolated from various species of camomiles and chrysanthemums, signify an important class of compounds containing a dimethylcyclopropane unit,[104,105] Despite exhibiting distinct insecticidal properties, the esters **192–196** did not find any practical applications for a long time, due to their photo- and oxidative lability. The first synthesis, in 1973, of a stable pyrethroid, permethrin **198**, a 3-phenoxybenzyl ester of permethrinic acid **197**, sparked off a pursuit of new stable analogues of the pyrethroid family, leading to the discovery, inter alia, of cypermethrin **199**, deltamethrin **200** and cigalothrin **201**, that proved to be far superior in action to the previously known pyrethroids or insecticides of other classes.[5–7]



Scheme 44.

In many cases, the strategy for stereoselective synthesis of pyrethroid esters rests on the preparation of a chiral cyclopropyl carboxylic acid as the main structural feature. In a number of synthetic schemes utilising 3-carene congeners, compounds **202** and **204** played the role of the key intermediates.[5,6,106–108] They can be obtained by ozonolysis of 3-carene **1**[109] or ketone **57**,[110,111] respectively, followed by a reductive work-up (Scheme 45). The synthetic potential of **204** has been explored in the preparation of a series of pyrethroid acid derivatives, including heterocycles **206** and **207**.[5] Aldehyde **205** and unsaturated lactone **208** were employed in the synthesis of pyrethrinic acid **203**, tricyclic lactone **209** and some analogues of chrysanthemic acid **210–212**.[7,9,107,112–114]

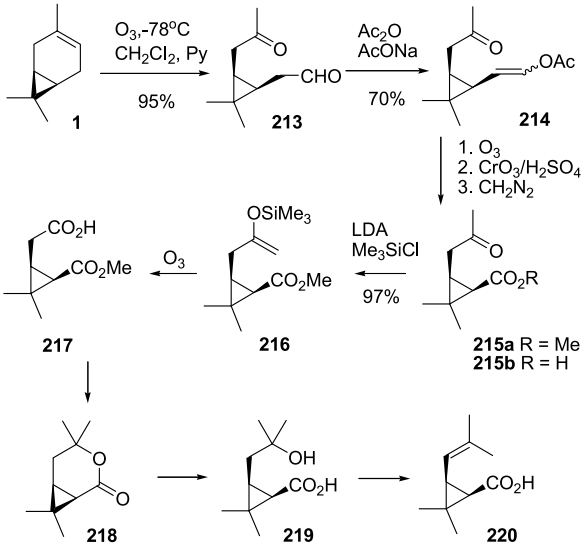
A synthetic route towards monomethyl ester **217**, belonging



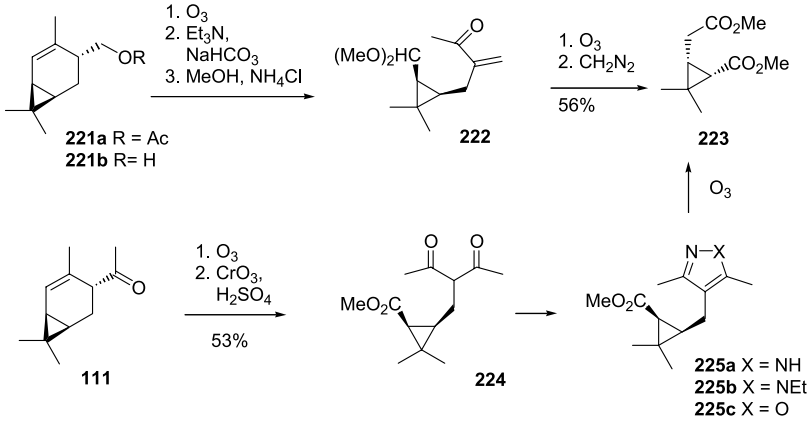
Scheme 45.

to a family of *cis*-homocaronic acids, is outlined in Scheme 46.[115] Enol ester **214**, obtained from (+)-3-carene **1** via ketoaldehyde **213**, was ozonised followed by oxidative work-up to furnish acid **215b**, which, after esterification with diazomethane, gave the ketoester **215a**. This was converted into the silyl enol ether **216** and, after another ozonolysis, yielded ester **217**, an important intermediate in the synthesis of (−)-*cis* chrysantemic acid **220**, which can be completed through (+)-lactone **218** and the corresponding acid **219**.[116]

(+)-3-Carene **1** was also employed as a synthetic entry towards derivatives of the enantiomeric *cis*-homocaronic



Scheme 46.



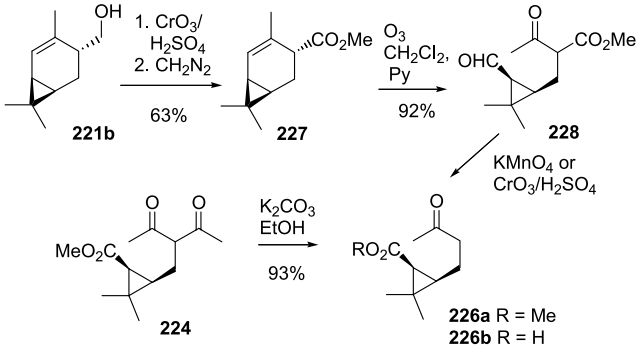
Scheme 47.

acid **223**, where compounds **221** and **111** served as the key intermediates (Scheme 47). Ozonolysis of **221a**,**b** and protection of the aldehyde function as the dimethyl acetal produced the α,β-unsaturated ketone **222**, which was ozonised once again. During the last step, concomitant oxidation of the dimethyl acetal group into the methyl ester took place. The product mixture was converted into the *cis* dimethyl ester **223** by treatment with diazomethane.[117]

In an alternative approach, the diketone **224**, obtained by ozonolysis of (+)-4α-acetyl-2-carene **111**, was converted in high yield into pyrazoles **225a**,**b** or oxazole **225c**. The heterocycles **225a–c** were treated with ozone followed by esterification, eventually furnishing the target *cis* diester **223** in good overall yield.[117–119]

The enantiomeric series of derivatives of *cis*-ketoacid **215**, shown in Scheme 46, can be accessed via 4-acetyl-2-carene **111** or 4α-hydroxymethyl-2-carene **221b**.[120] The synthetic sequence is illustrated in Scheme 48. Ozonolysis of the methyl ester **227**, obtained from **221b** following standard protocols, yielded aldehyde **228**, which, after oxidation accompanied by decarboxylation, furnished acid **226b**. The derivatives **226a**,**b** can also be synthesised in high yield from β-diketone **224** by treatment with an alcoholic solution of potassium carbonate.[119,121]

Ketonitrile **229**, readily attainable from dimeric nitrosyl chloride **88**, was used as the key intermediate in the synthesis of (1*R*)-*cis*-chrysanthemylamine **231** (Scheme 49). Addition of MeMgI to **229** and dehydration of the resulting



Scheme 48.

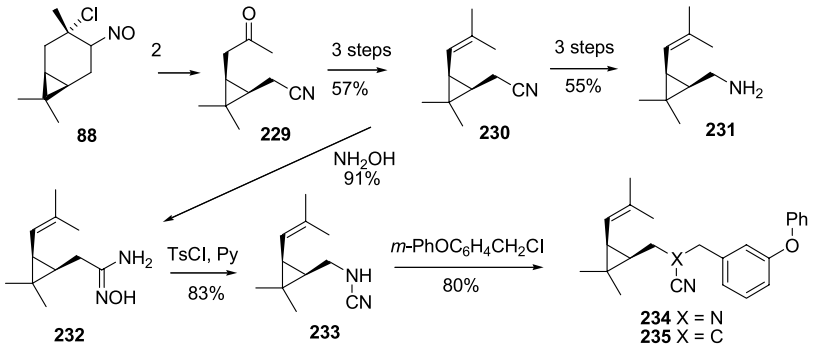
alcohol led to a mixture of unsaturated compounds, which were isomerised into **230**. The latter was further transformed into amine **231**.[122] A similar strategy was employed in the synthesis of compound **234**, an aza-analogue of the pyrethroid, cyphenothrin **235**.[123,124] Treatment of nitrile **230** with hydroxylamine produced amidoxime **232**, which was subjected to Thiemann rearrangement to furnish the cyanamide **233**. Alkylation of the latter yielded the target **234**.

Ketone **111** proved to be a popular starting point for the synthesis of a wide variety of novel pyrethroid analogues. The pyrethroid, deltamethrin **200**, a very potent insecticide finding a broad application in agriculture, contains carboxylic acid **238a**[125] as a major component. Derivatives in both enantiomeric series of **238a**, the methyl esters **238b** and **242**, can be accessed from **111** as shown in Scheme 50. Synthesis of the 1*R*,3*S* isomer involved ozonolysis of **111**, followed by olefination to give the dibromovinyl derivative **236**, which, after introducing a double bond to give **237**, was ozonised to furnish **238a** followed by esterification into target **238b**.[120,126] The ester **238b** can be readily isomerised into the thermodynamically more stable *trans*-isomer **239** by treatment with a strong base.

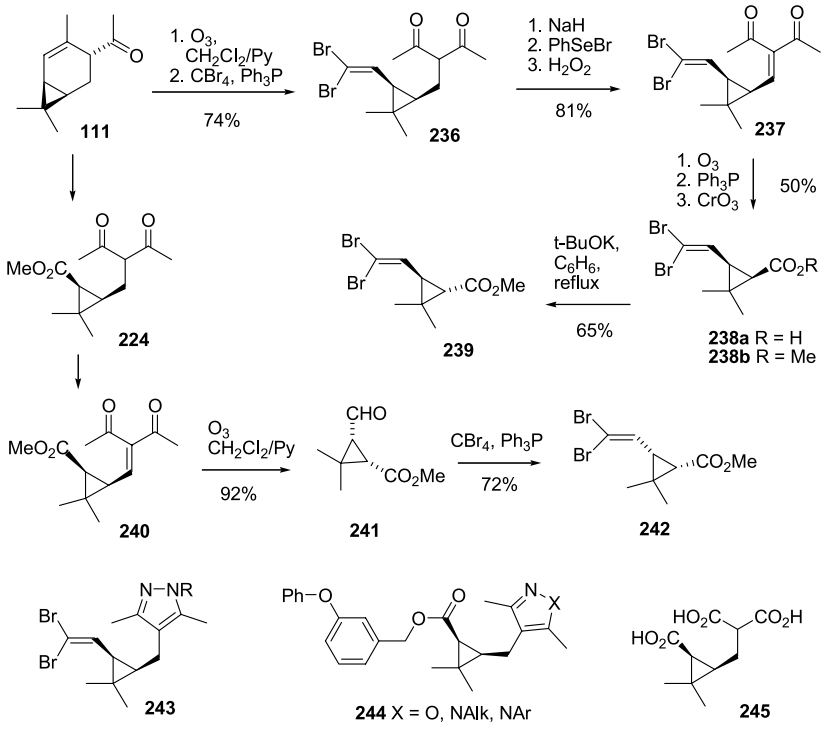
Synthesis of the ester **242** of the enantiomeric 1*S*,3*R* series employed similar chemical transformations applied in the reverse order, which proceeded via steps forming diketoesters **224** and **240** and aldehyde **241**.[120] In related sequences, compounds **236** and **224** were converted into the heterocyclic pyrethroids **243** and **244**, respectively,[127,128] while diketoester **224** also served as a precursor for the acid **245**, a building block in the synthesis of a family of insecticides.[129]

Non-racemic monoprotected dialdehyde **248**, which represents a valuable chiral synthon for further synthetic elaboration, can be easily prepared from enone **222** by the sequence of reactions shown in Scheme 51. Ozonolysis of **222** produced 1,2-diketone **246**, which was converted into enol acetate **247** followed by ozonolysis coupled with a reductive work-up to furnish the desymmetrised aldehyde **248**.[80,117]

Investigation of the reactivity of dihalocarbenes towards diacetetate **249** was reported recently (Scheme 52).[130] Dicyclopropane derivative **250** was obtained when



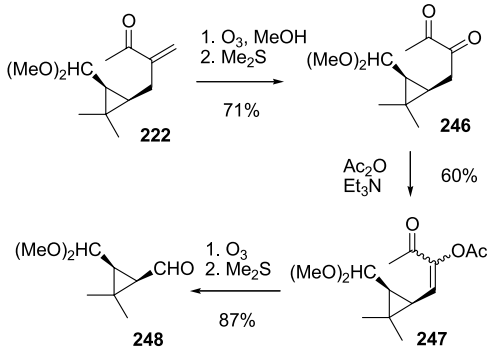
Scheme 49.



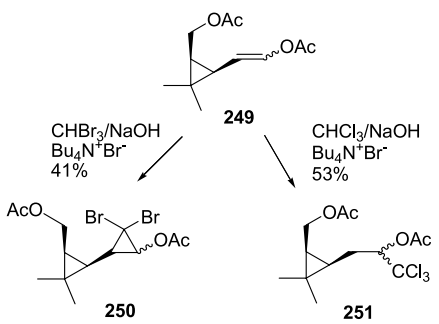
Scheme 50.

bromoform was employed as a source of carbene, whereas, in chloroform, trichloromethylation took place instead, giving **251**. It worth noting that, in both cases, stereoselectivity of the addition remained poor.

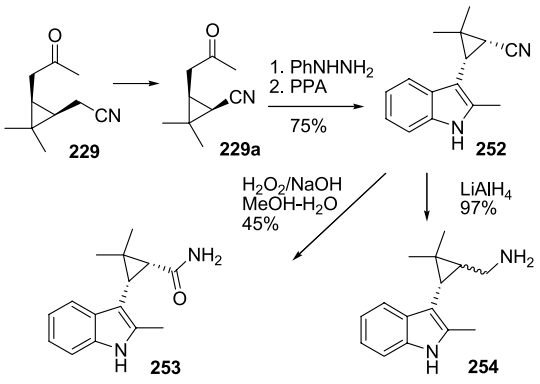
Fischer indolisation of ketonitrile **229a**, available in two easy steps from **229**, led to 3-indolylcyclopropane **252** as a single diastereoisomer (Scheme 53). Hydrolysis of the nitrile group in **252**, performed with H_2O_2/NaOH in aqueous methanol, produced amide **253** with retention of configuration, while reduction with $LiAlH_4$ led to a mixture of diastereomeric amines **254**.[131–133]



Scheme 51.

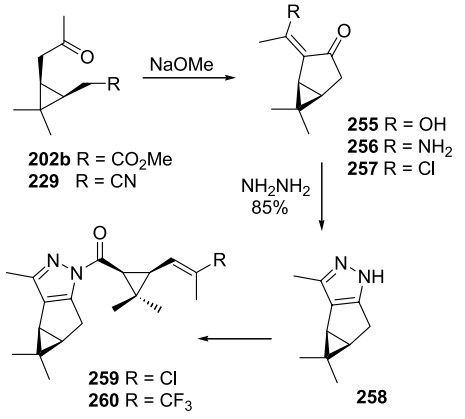


Scheme 52.



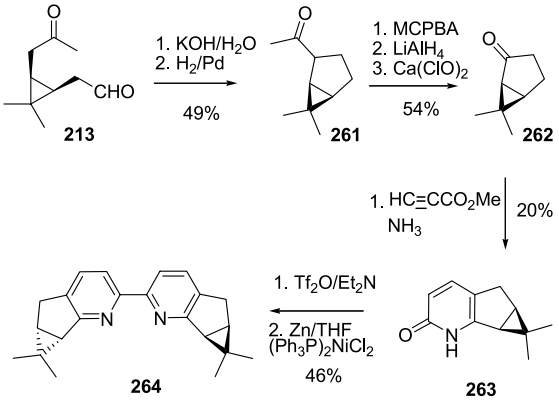
Scheme 53.

Ester **202b** and nitrile **229** can both be employed in the synthesis of pyrazole **258** via the respective intermediates **255** and **256** (Scheme 54).[134–138] Pyrazole **258** served as a resolving agent for racemic *cis*-permethric and *cis*-cyhalothric acids through formation of the corresponding adducts **259** and **260**, readily separable by crystallisation.[137] Chlorovinyl ketone **257** was also used for a chiral resolution of racemic primary and secondary amines, including some alkaloids.[138]



Scheme 54.

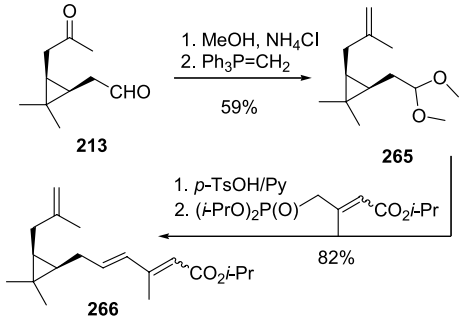
The synthesis of chiral bypyridine **264**, finding application in asymmetric catalysis, is outlined in Scheme 55.[58] Controlled aldol-type cyclisation of ketoaldehyde **213** followed by hydrogenation afforded ketone **261**. A sequence



Scheme 55.

of reactions including Baeyer–Villiger oxidation, reduction of the resulting acetate into an alcohol and oxidation of the latter provided ketone **262**. Pyridine annulation was accomplished with methyl propiolate and ammonia yielding pyridone **263**, which, after triflation, was dimerised to give the target bipyridine **264**.[58]

Derivatives of 3-carene were employed in the synthesis of optically active insect pheromones containing a 2,3-dimethylcyclopropane fragment,[139,140] as illustrated in Scheme 56, by preparation of the triene **266** by stepwise olefination of ketoaldehyde **213**, proceeding via the dimethyl acetal intermediate **265**.[141]



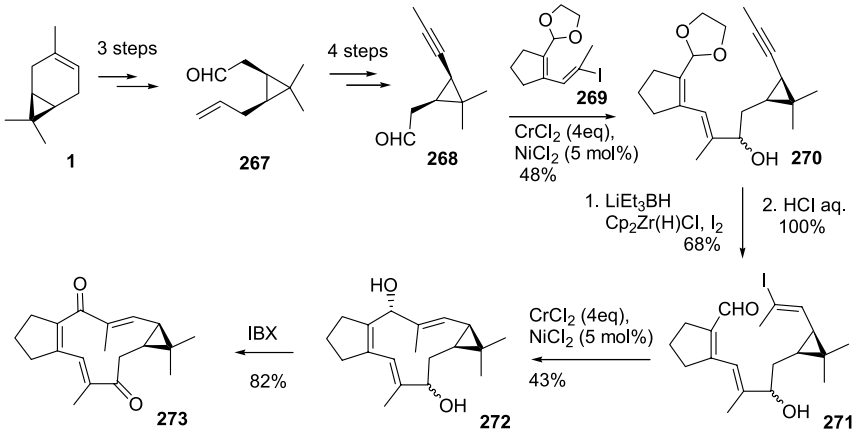
Scheme 56.

(+)-3-Carene **1** provided the stereochemical template required for the preparation of tricyclic diketone **273**, which served as an advanced intermediate in the synthetic route towards a series of diterpenoids isolated from *Euphorbiacea* and *Thymeleacea* (Scheme 57).[142] Aldehyde **267**, prepared from 3-carene, was converted into alkyne **268** and then coupled with vinyl iodide **269**, mediated by Cr(II)–Ni(II), to produce **270**. This was transformed into vinyl iodide **271** and subjected to the intramolecular Cr(II)–Ni(II) coupling, yielding an 11-membered macrocycle **272**, which, after oxidation with IBX, furnished the target product **273**.

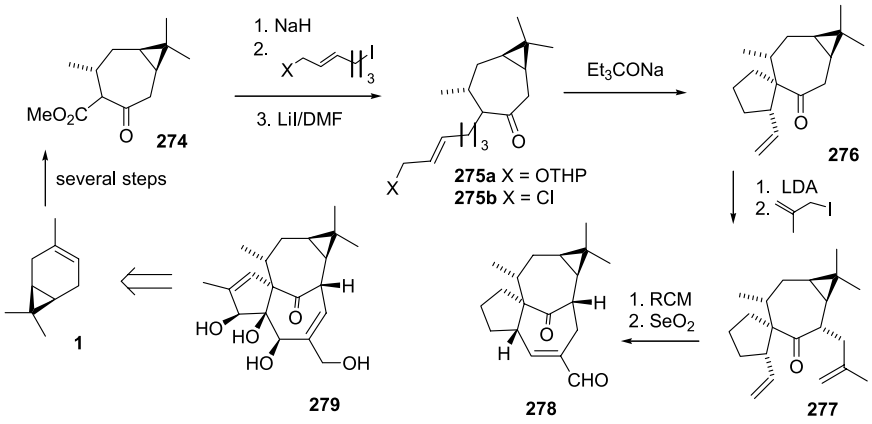
A formal synthesis of the optically active ingenol **279**, an attractive target, due to its anti-HIV activity, was accomplished utilising (+)-3-carene **1** as a source of the chiral dimethylcyclopropane fragment (Scheme 58).[143] Ketoester **274**, prepared in five steps from 3-carene **1**, was converted into the protected alcohol **275a**, which was then chlorinated to give **275b**. Spirocyclisation to **276** was achieved with the help of bulky Et_3CCONa followed by allylation under standard conditions to yield the ketone **277**. The key step in the synthesis, a ring-closing metathesis (RCM), was performed using a second-generation Grubbs catalyst, which, after allylic oxidation with SeO_2, furnished the aldehyde **278**, completing the formal synthesis of the target ingenol **279**.[144]

3.2. Synthetic schemes based on 2-carene

The less abundant, 2-carene, has found considerably fewer applications, compared to its 3-isomer. In one of the rare examples, (+)-2-carene **2** was employed independently by two research groups as a source of chirality in the asymmetric synthesis of sequiterpenes, (+)-nortaylorione **283** and (+)-taylorione **284**[145,146] (Scheme 59).

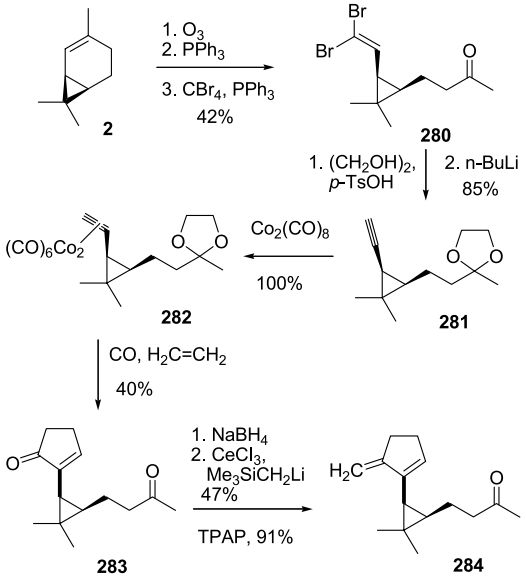


Scheme 57.



Scheme 58.

Dibromoalkene intermediate **280**, prepared in a one-pot reaction sequence from 2-carene, was converted into alkyne **281**, which, on complexation with $Co_2(CO)_8$ in toluene, furnished **282** in quantitative yield. Pauson–Khand cyclisation of the latter followed by hydrolysis of the ketal group produced (+)-nortaylorione **283**. Transformation of **283** into (+)-taylorione **284** was achieved in three steps using a modified Peterson olefination.



Scheme 59.

4. Conclusions

The unique features of 2- and 3-carenes, such as their chiral 2,2-dimethylcyclopropane fragment, reactive double bond and bicyclic skeleton, coupled with their ready availability and relatively low cost, make these monoterpenes useful chiral synthons. A broad spectrum of synthetic applications of the family of carene derivatives in both the 2- and 3-isomeric series demonstrates their great potential for asymmetric synthesis. New developments in this field can confidently be expected in the near future.

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Biographical sketch

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PEG-400 promoted $Pd(OAc)_2$/DABCO-catalyzed cross-coupling reactions in aqueous media

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Abstract—PEG-400 [poly(ethylene glycol-400)] was found to improve the $Pd(OAc)_2$/DABCO-catalyzed aqueous Suzuki–Miyaura and Stille cross-coupling reactions. In the presence of $Pd(OAc)_2$, DABCO, and PEG-400, a variety of aryl halides were coupled with arylboronic acids or organotin compounds efficiently to afford the corresponding cross-coupled products in moderate to excellent yields. The turnover numbers was up to 900,000 for the Suzuki–Miyaura reaction and up to 9800 for the Stille reaction. The catalyst system was also effective for Heck and Sonogashira cross-coupling reactions to some extent.

1. Introduction

Palladium-catalyzed cross-coupling reactions were one of the most powerful tools for the formation of C–C bond in organic synthesis.[1–5] The recent development of highly active palladium–ligand complexes has allowed chemists to extend such protocol to less reactive but inexpensive and readily available aryl chlorides.[2] Cross-coupling reactions are usually carried out in polar organic solvent under inert and anhydrous conditions due to the instability of most catalysts and coupling reagents. From an economic and environmental standpoint, however, it is desirable to avoid any use of hazardous and expensive organic solvents. To satisfy these concerns, water or aqueous solution represents a very attractive medium for organic reactions,[6] and considerable effort has been directed to the application of water or aqueous solutions in cross coupling reactions.[2–5] Very recently, we have demonstrated DABCO as an efficient ligand for the palladium-catalyzed Suzuki–Miyaura, Sonogashira and Stille cross-coupling reactions.[7] However, water was not an effective solvent for the $Pd(OAc)_2$/DABCO-catalyzed Suzuki–Miyaura reaction.[7a] In the earlier work, phase-transfer catalysts such as crown esters and TBAB could significantly promote the cross-coupling reaction.[3–5] Moreover, our recent results also showed that the use of PEG as medium would favor the Suzuki–Miyaura reaction.[7d] Thus, we expected that the addition of phase-transfer catalysts might help improve the $Pd(OAc)_2$/DABCO-mediated aqueous cross-coupling reactions.[7d,8] Indeed, we found that $Pd(OAc)_2$/DABCO-catalyzed Suzuki–Miyaura, Stille and Heck reactions proceeded efficiently in aqueous media when PEG-400 was used as the phase-transfer catalyst, but the same conditions were ineffective for the Sonogashira cross-coupling reactions. Here we report the details of those reactions (Eqs. 1–4 in Scheme 1).

2. Results and discussion

2.1. PEG-400 promoted $Pd(OAc)_2$ and DABCO-catalyzed aqueous Suzuki–Miyaura cross-coupling reaction

The effect of PEG on palladium-catalyzed aqueous Suzuki–Miyaura cross-coupling reaction of *p*-bromoanisole (**1a**) with phenylboronic acid (**2a**) was investigated, and the results are summarized in Table 1. Initially, three phase-transfer catalysts including PEG-400, 18-crown-6, and TBAB were tested, and PEG-400 was the most effective to improve the reaction (entries 1–4). Without the addition of any phase-transfer catalysts, the treatment of **1a** with **2a**, $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), and KOH (3 equiv) for 4 h using water as the medium afforded 87% yield of the corresponding coupled product **3** (entry 1), whereas the yield of **3** was increased to 100% when 20 mol% of PEG was added (entry 2). A series of bases, such as KOH, NaOH, K_3PO_4, NaOAc, K_2CO_3, and Cs_2CO_3, were then evaluated, where KOH produced the highest yield (entries 2 and 5–9). A set of solvents including H_2O, acetone, toluene, DMF, ethanol, and MeCN were also

Keywords: PEG-400; $Pd(OAc)_2$/DABCO; Cross-coupling reaction; Aryl halide; Arylboronic acids; Organotin compounds.
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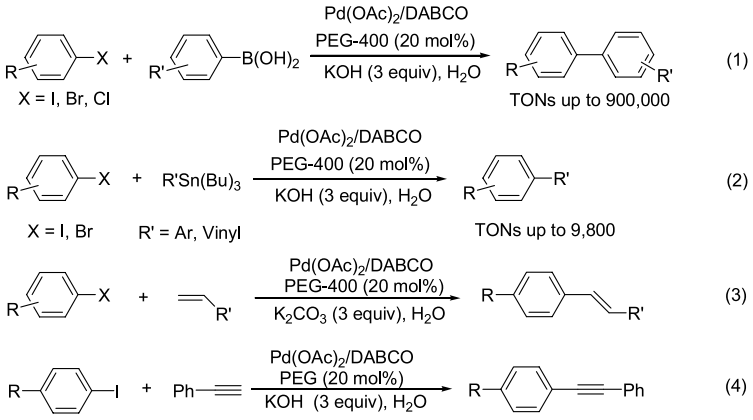
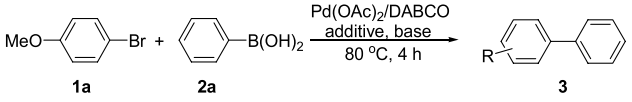


Scheme 1.

Table 1. Palladium-catalyzed Suzuki–Miyaura cross-coupling reaction of 4-bromoanisole (**1a**) with phenylboronic acid (**2a**)[a]



Entry	Additive	Base	Solvent	Yield (%)[b]
1	0	KOH	H_2O	87
2	PEG-400	KOH	H_2O	100
3	18-Crown-6	KOH	H_2O	90
4	TBAB	KOH	H_2O	91
5	PEG-400	NaOH	H_2O	84
6	PEG-400	K_3PO_4	H_2O	48
7	PEG-400	NaOAc	H_2O	79
8	PEG-400	K_2CO_3	H_2O	89
9	PEG-400	Cs_2CO_3	H_2O	91
10	PEG-400	KOH	Acetone	65
11	PEG-400	KOH	Toluene	Trace
12	PEG-400	KOH	DMF	Trace
13	PEG-400	KOH	CH_3CH_2OH	Trace
14	PEG-400	KOH	MeCN	Trace
15[c]	PEG-400	KOH	H_2O	87
16[d]	PEG-400	KOH	H_2O	100
17[e]	PEG-400	KOH	H_2O	79

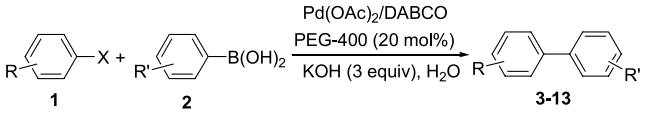
[a] Under otherwise indicated, the reaction conditions were as follows: **1a** (0.50 mmol), **2a** (0.60 mmol), $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), additive (20 mol%), base (3 equiv), and solvent (5 mL) at 80 °C under N_2 for 4 h.
[b] Isolated yield.
[c] Without DABCO.
[d] $Pd(OAc)_2$ (0.1 mol%) and DABCO (0.2 mol%) for 13 h.
[e] $Pd(OAc)_2$ (0.01 mol%) and DABCO (0.02 mol%) for 15 h.

examined, and H_2O gave the best results as solvent (entries 2 and 10–14). Only 65% yield of **3** was isolated with acetone, the reported effective solvent (entry 10).[7a] Finally, the effect of DABCO was evaluated, and the results indicated that DABCO has a fundamental influence on the reaction (entries 2 and 15). In the absence of DABCO, the yield of **3** was reduced to 87% (entry 15). It is interesting to note that the reaction can be carried out efficiently under lower Pd loadings (entries 16 and 17). In the presence of $Pd(OAc)_2$ (0.1 mol%), DABCO (0.2 mol%), PEG (20 mol%), KOH (3 equiv), and H_2O (5 mL), coupling of **1a** with **2a** gave the desired product **3** in 100% yield (entries 16). Further decreasing the Pd loading to 0.01 mol% led to a moderate yield (79% yield, TONs=7900; entry 17)

As shown in Table 2, the $Pd(OAc)_2$/DABCO/PEG/KOH system was effective for coupling of various aryl halides with arylboronic acids **2a–d** in aqueous media. High yields were still obtained for coupling of aryl iodides when Pd loadings were decreased to 0.0001% (entries 5 and 6). In the presence of 0.0001 mol% Pd, *p*-nitroiodobenzene (**1b**) was coupled with **2a** efficiently to give 90% yield of the corresponding product **7** (entry 5). For the coupling of deactivated aryl iodide **1c** with **2a**, 88% yield of the corresponding product **3** was also isolated after 4 h in the presence of 0.001 mol% Pd (entry 6). However, for the coupling reaction of aryl bromides, the efficiency of the $Pd(OAc)_2$/DABCO/PEG/KOH system was decreased. The suitable Pd loadings were 2–0.01 mol% (entries 1–3 and 7–18). For example, the coupling of bromide **1e** with arylboronic acids **2a** and **2d**, respectively, afforded good yields of the corresponding products **8** and **9** in the presence of 0.01 mol% Pd (entries 11 and 12). It is noteworthy that the use of ethanol as co-solvent is essential to improve the coupling reactions of aryl halides bearing electron-withdrawing groups (entries 4, 5, 7–12, and 19–21). In the absence of ethanol, low yields were observed for coupling of halides **1b**, **1d**, **1e**, **1i**, and **1j**, whereas moderate to good yields were obtained when these reactions were employed in ethanol–H_2O (1/4). It is worth mentioning that the system was effective for coupling of the activated aryl chlorides. In the presence of 2 mol% of $Pd(OAc)_2$, 4 mol% of DABCO, and 20 mol% of PEG, moderate yields were obtained for the reaction of **2a** with activated aryl chlorides **1i** and **1j**, respectively, in ethanol/water (entries 20 and 21). Attempts to couple **2a** with chlorides **1k** and **1l**, respectively, both afforded low yields (entries 22 and 23).

Compared with Zhang's results,[8] both DABCO and PEG play crucial roles in the reaction. In the presence of catalytic amounts of DABCO and PEG, the loadings of $Pd(OAc)_2$ might be reduced to 0.1–0.0001 mol% while high yields were still obtained (Tables 1 and 2). However, 1 mol% of $Pd(OAc)_2$ and 175 mol% of PEG were required to improve the reaction under DABCO-free conditions.[8] In the presence of 1 mol% of $Pd(OAc)_2$, moderate yields were observed when a catalytic amount of PEG-2000 was added. For example, <60% yield was obtained in the presence of 50 mol% of PEG-2000.[8] Similarly, without DABCO the yield was decreased under our conditions (entry 15 in Table 1). Furthermore, only a moderate yield was isolated

Table 2. Palladium-catalyzed Suzuki–Miyaura coupling reaction in aqueous media[a]



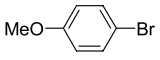
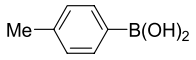
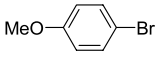
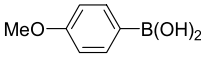
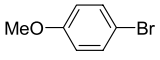
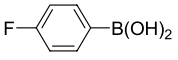
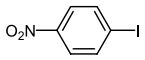
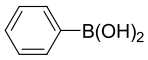
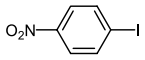
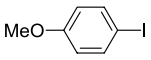
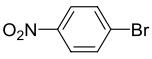
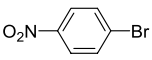
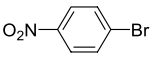
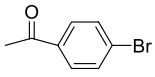
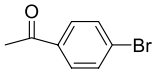
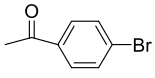
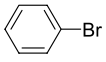
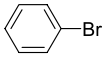
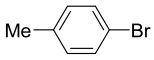
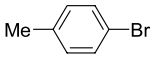
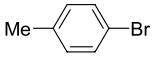
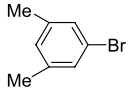
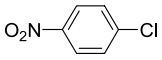
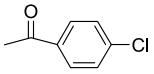
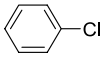
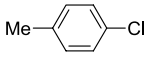
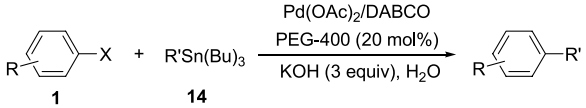
Entry	ArX	$ArB(OH)_2$	Pd (mol%)	Time (h)	Yield (%)[b]
1	 (**1a**)	 (**2b**)	0.1	16	83 (**4**)
2	 (**1a**)	 (**2c**)	0.1	16	76 (**5**)
3	 (**1a**)	 (**2d**)	0.1	16	90 (**6**)
4[c,d]	 (**1b**)	 (**2a**)	0.001	24	38 (**7**)
5[e]	 (**1b**)	(**2a**)	0.0001	24	90 (**7**)
6	 (**1c**)	(**2a**)	0.001	15	88 (**3**)
7[f]	 (**1d**)	(**2a**)	0.01	22	Trace (**7**)
8[e]	 (**1d**)	(**2a**)	0.01	22	29 (**7**)
9[e]	 (**1d**)	(**2a**)	2	14	93 (**7**)
10[g]	 (**1e**)	(**2a**)	0.01	36	Trace (**8**)
11[e]	 (**1e**)	(**2a**)	0.01	11	93 (**8**)
12[e]	 (**1e**)	(**2d**)	0.01	26	80 (**9**)
13	 (**1f**)	(**2a**)	0.1	13	96 (**10**)
14	 (**1f**)	(**2b**)	0.1	13	93 (**11**)
15	 (**1g**)	(**2a**)	0.1	10	84 (**11**)

Table 2 (*continued*)

Entry	ArX	$ArB(OH)_2$	Pd (mol%)	Time (h)	Yield (%)[b]
16[h]	 (**1g**)	(**2a**)	1	0.5	65 (**11**)
17	 (**1g**)	(**2b**)	0.1	10	80 (**12**)
18	 (**1h**)	(**2a**)	0.1	18	57 (**13**)
19[ij]	 (**1i**)	(**2a**)	2	24	Trace (**7**)
20[ej]	(**1i**)	(**2a**)	2	24	50 (**7**)
21[ej]	 (**1j**)	(**2a**)	2	24	52 (**8**)
22[j]	 (**1k**)	(**2a**)	2	22	35 (**3**)
23[j]	 (**1l**)	(**2a**)	2	24	24 (**11**)

[a] Under otherwise indicated, the reaction conditions were as follows: **1** (0.50 mmol), **2** (0.60 mmol), $Pd(OAc)_2$–DABCO (1/2), PEG-400 (20 mol%), KOH (3 equiv), and H_2O (5 mL) under N_2 at 80 °C.
[b] Isolated yield.
[c] Without DABCO.
[d] Compound **1b** (>55%) was recovered.
[e] CH_3CH_2OH–$_2O$ (1/4; 5 mL).
[f] Compound **1d** (>90%) was recovered.
[g] Compound (>90%) **1e** was recovered.
[h] **1** (1.0 mmol), **2** (1.50 mmol), $Pd(OAc)_2$ (1 mol%), PEG-400 (3.5 g), Na_2CO_3 (2 equiv), and H_2O (3 g) under N_2 at 50 °C.
[i] Compound **1i** (>95%) was recovered.
[j] At 120 °C.

Table 3. Palladium-catalyzed Stille coupling reaction in aqueous media[a]



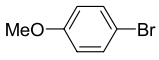
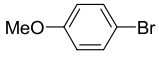
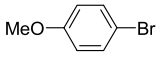
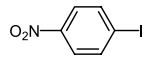
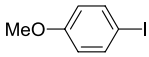
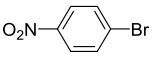
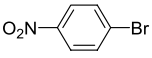
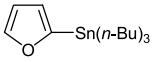
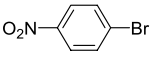
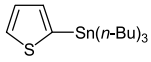
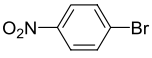
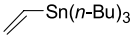
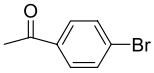
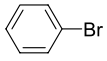
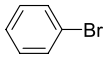
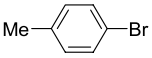
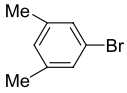
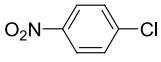
Entry	ArX	$R'Sn(Bu)_3$	Time (h)	Yield (%)[b]
1	 (**1a**)	$PhSn(n\text{-}Bu)_3$ (**14a**)	16	92 (**3**)
2[c]	 (**1a**)	(**14a**)	26	71 (**3**)
3[d]	 (**1a**)	(**14a**)	26	40 (**3**)

Table 3 (*continued*)

Entry	ArX	$R'Sn(Bu)_3$	Time (h)	Yield (%)[b]
4[d]	 (**1b**)	(**14a**)	10	98 (**7**)
5[d]	 (**1c**)	(**14a**)	12	93 (**3**)
6	 (**1d**)	(**14a**)	10	100 (**7**)
7	 (**1d**)	 (**14b**)	16	70 (**15**)
8	 (**1d**)	 (**14c**)	15	86 (**16**)
9	 (**1d**)	 (**14d**)	15	78 (**17**)
10	 (**1e**)	(**14a**)	10	100 (**8**)
11	 (**1f**)	(**14a**)	16	95 (**10**)
12	 (**1f**)	(**14b**)	15	87 (**18**)
13	 (**1g**)	(**14a**)	15	100 (**11**)
14	 (**1h**)	(**14a**)	10	93 (**13**)
15	 (**1i**)	(**14a**)	24	11 (**7**)

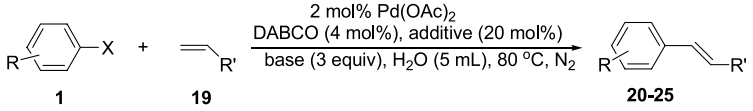
[a] Under otherwise indicated, the reaction conditions were as follows: **1** (0.50 mmol), **14** (0.60 mmol), $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), PEG-400 (20 mol%), KOH (3 equiv), and H_2O (5 mL) at 80 °C under N_2.
[b] Isolated yield.
[c] $Pd(OAc)_2$ (0.1 mol%) and DABCO (0.2 mol%).
[d] $Pd(OAc)_2$ (0.01 mol%) and DABCO (0.02 mol%).

when PEG-400 was used to replace PEG-2000 under Zhang's conditions (65% yield, entry 16 in Table 2).

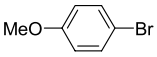
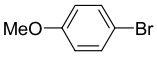
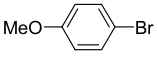
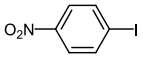
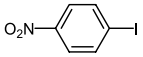
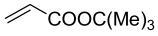
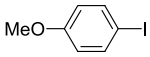
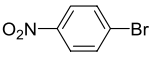
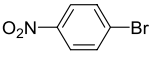
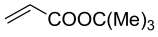
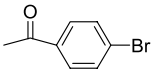
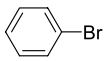
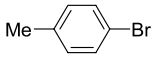
2.2. PEG-400 promoted $Pd(OAc)_2$ and DABCO-catalyzed aqueous stille cross-coupling reaction

The $Pd(OAc)_2$/DABCO/PEG/KOH system was further extended to effect the Stille cross-coupling reaction, and the results are summarized in Table 3. Initially, the efficiency of the $Pd(OAc)_2$/DABCO/PEG/KOH system for the reaction of the substrate **1a** with $PhSn(Bu)_3$ (**14a**) was evaluated in water. In the presence of 2 mol% of $Pd(OAc)_2$, 4 mol% of DABCO and 20 mol% of PEG, the coupling reaction of bromide **1a** with **14a** proceeded smoothly to afford 92% yield of the corresponding product **3** (entry 1). The catalyst loadings was decreased to 0.1 mol% for coupling of the substrate **1a** resulting in a moderate yield of **3** after prolonged stirring (entry 2). However, a low yield

Table 4. Palladium-catalyzed Heck coupling reaction in aqueous media[a]



1 **19** **20-25**

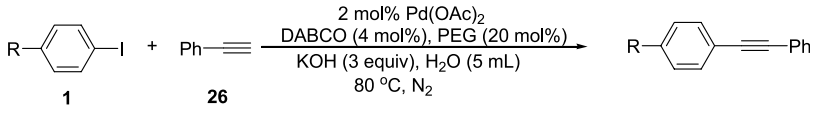
Entry	ArX	Olefine	Time (h)	Yield (%)[b]
1	 (**1a**)	 (**19a**)	36	10 (**20**)
2[c]	 (**1a**)	(**19a**)	36	55 (**20**)
3[d]	 (**1a**)	(**19a**)	17	78 (**20**)
4	 (**1b**)	(**19a**)	4	98 (**21**)
5	 (**1b**)	 (**19b**)	4	98 (**22**)
6	 (**1c**)	(**19a**)	4	90 (**20**)
7	 (**1d**)	(**19a**)	15	90 (**21**)
8	 (**1d**)	 (**19b**)	15	94 (**22**)
9	 (**1e**)	(**19b**)	15	87 (**23**)
10	 (**1f**)	(**19a**)	36	68 (**24**)
11	 (**1g**)	(**19a**)	36	60 (**25**)

[a] Under otherwise indicated, the reaction conditions were as follows: **1** (0.50 mmol), **19** (0.60 mmol), $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), PEG-400 (20 mol%), K_2CO_3 (3 equiv), and H_2O (5 mL) at 80 °C under N_2.
[b] Isolated yield.
[c] KOH (3 equiv) instead of K_2CO_3.
[d] TBAB (20 mol%) instead of PEG.

was isolated when the Pd loading was further reduced to 0.01 mol% (entry 3). Furthermore, the system was effective for coupling of aryl iodides **1b** and **1c** providing excellent yields in the presence of 0.01 mol% Pd (entries 4 and 5). The results also indicated that other aryl bromides **1d–h** were treated with **14a–d**, $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), PEG (20 mol%) and KOH (3 equiv) to afford the corresponding products in moderate to good yields (entries 6–14). For example, coupling of bromide **1d** with **14a–d**, respectively, gave the corresponding coupled products **7** and **15–17** in 100%, 70%, 86%, and 78% yields (entries 6–9). Unfortunately, coupling of activated aryl chloride **1i** with **14a** was unsuccessful (entry 15).

2.3. The $Pd(OAc)_2$/DABCO/PEG/KOH system for aqueous Heck and Sonogashira cross-coupling reactions

As demonstrated in Table 4, the $Pd(OAc)_2$/DABCO/PEG/



1 26

run	R	t (h)	Isolated yield (%)
1	NO_2 (**1b**)	20	80 (**27**)
2	OMe (**1c**)	24	35 (**28**)

Scheme 2.

KOH system was less effective for the Heck and Sonogashira cross-coupling reactions. In the presence of $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), PEG (20 mol%), and KOH (3 equiv), a low yield of the desired product **20** was isolated for the reaction of bromide **1a** with styrene **19** (entry 1 in Table 4). It is interesting to note that the yield of **20** was enhanced to 55% when K_2CO_3 was used as the base (entry 2). As indicated in the earlier report,[5a] TBAB might be a better promoter for the Heck reaction. Indeed, in the presence of $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), TBAB (20 mol%), and K_2CO_3 (3 equiv), the yield of **20** was increased to 78% (entry 3). However, our interesting is extended PEG as the additive to improve the cross-coupling reactions. Thus, treatment of a number of aryl iodides and activated aryl bromides with olefins were tested in the presence of $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), PEG (20 mol%), and K_2CO_3 (3 equiv), and moderate to excellent yields of the desired products were obtained (entries 4–11). For example, the reaction of bromide (**1d**) with **19a** or **19b** gave the corresponding (*E*)-olefins **21** and **22** in 90% and 94% yields, respectively (entries 7 and 8).

The Sonogashira cross-coupling reaction of 1-iodo-4-nitrobenzene (**1b**) with phenylacetylene (**26**) gave 80% yield of the corresponding product **27** in the presence of $Pd(OAc)_2$ (2 mol%), DABCO (4 mol%), PEG (20 mol%), and KOH (3 equiv) (run 1 in Scheme 2). However, only 35% yield of the corresponding alkyne **28** was isolated after 24 h when the reaction of *p*-iodoanisole (**1c**) with alkyne **26** was conducted (run 2). Other bases including K_2CO_3, Cs_2CO_3, Et_3N, and NaOAc were also evaluated but only resulted in low yield. Rather low yields were obtained for the Sonogashira cross-coupling products due to the formation of red oils, by-products which were also observed in our previous results.[9]

3. Conclusion

In summary, $Pd(OAc)_2$/DABCO-catalyzed cross-coupling reactions in aqueous media promoted by PEG-400 has been developed. In the presence of $Pd(OAc)_2$, DABCO, and PEG, coupling of a variety of aryl halides with arylboronic acids or organotin compounds was carried out efficiently in aqueous media to afford the corresponding cross-coupled products in moderate to excellent yields and high TONs. The TONs is up to 900,000 for the Suzuki–Miyaura reaction and up to 9800 for the Stille reaction. Moreover, the present reaction conditions were also effective for the Heck and Sonogashira cross-coupling reactions to some extent.

4. Experimental

4.1. Typical experimental procedure for the PEG promoted $Pd(OAc)_2$/DABCO-catalyzed cross-coupling reactions in aqueous media

A mixture of **1** (0.50 mmol), **2** (**14**, **19** or **26**, 0.60 mmol), $Pd(OAc)_2$ (the indicated amount), DABCO (the indicated amount), PEG-400 (20 mol%), KOH (3 equiv), and H_2O (5 mL) was stirred under N_2 at 80–120 °C until complete consumption of starting material as monitored by TLC and GC analysis. After the mixture was filtered and evaporated, the residue was purified by flash column chromatography (hexane or hexane/ethyl acetate) to afford the desired coupled products **3**–**13**, **15**–**18**, **20**–**25**, **27**, and **28**, which are all known compounds.[2–5,7]

Acknowledgements

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Synthesis and absolute configuration of brevione B, an allelochemical isolated from *Penicillium* sp.

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Abstract—Breviones A–E (**1**–**5**), allelochemicals isolated from *Penicillium brevicompactum* Dierckx, are structurally unique diterpenoid derivatives. The first synthesis of both enantiomers of brevione B (**2**) was accomplished by employing the double S_N2'-type tandem reaction as a key step, and the absolute configuration of the naturally occurring **2** was established.

1. Introduction

Allelopathy is commonly defined as any direct or indirect effect by one plant, including microorganisms, on another through the production of chemical compounds released into the environment. It includes both inhibitory and stimulative reciprocal biochemical interactions.[1a] Such chemical compounds are called allelochemicals or allelopathic agents. Nowadays, allelochemicals have received a considerable amount of attention due to the agricultural potential of these compounds as natural and environmentally-friendly herbicides.[1] In 2000, Macías and his co-workers isolated breviones A–E (**1**–**5**) from *Penicillium brevicompactum* Dierckx as allelochemicals.[2] These compounds are structurally unique natural products consisting of diterpene and polyketide subunits. Especially, the spiro-fused CDE ring portion of breviones A–D (**1**–**4**) is characteristic and unusual. We became interested in the biological activities and the unique structures of breviones, and undertook a project to synthesize them. Although we have already achieved the first synthesis of (±)-brevione B (**2**),[3] the absolute configurations of the naturally occurring **2** and other breviones have still remained unsolved. Thus, we initiated the synthesis of optically active brevione B (**2**) to determine its absolute stereochemistry. Herein, we report the first synthesis of both enantiomers of **2** in detail (Fig. 1).

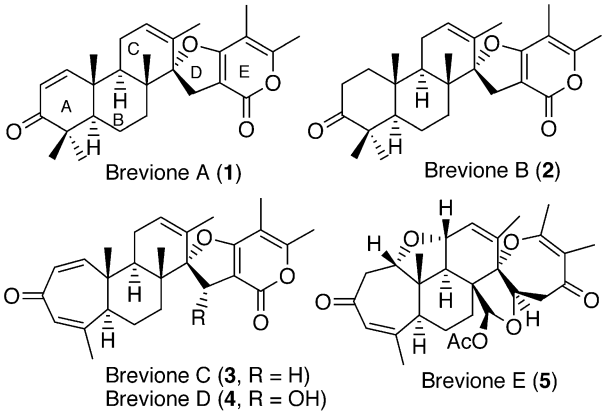

Figure 1. Structures of breviones.

2. Results and discussion

Because a crucial point in the synthesis of breviones should be the construction of the characteristic spiro-fused CDE ring framework, we first tried to establish our original methodology to resolve it. That was the direct coupling of the vinylepoxide **6** and the α-pyrone **7**[4] by the palladium-mediated double S_N2'-type tandem reaction as illustrated in Scheme 1.[5,6] The first synthesis of (±)-**2** was then achieved by employing the developed tandem reaction as a key step.[3,7] Thus, the remaining problem was how to prepare the optically active form. In other words, there was a need to carry out optical resolution or asymmetric reaction at an appropriate stage. Our synthetic plan for **2**, which is identical to that of our racemate synthesis, is shown in Scheme 1. The target compound **2** might be readily

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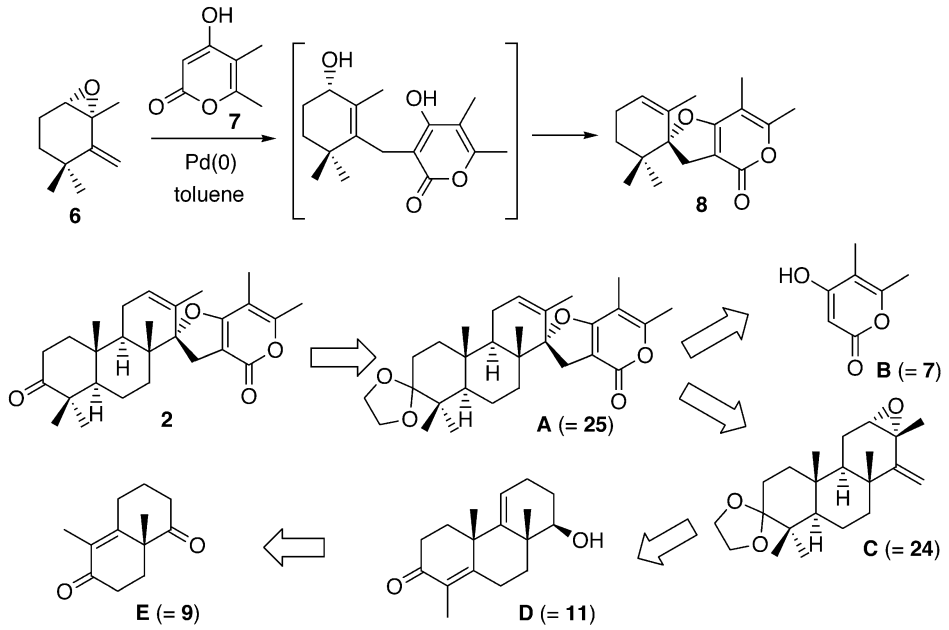
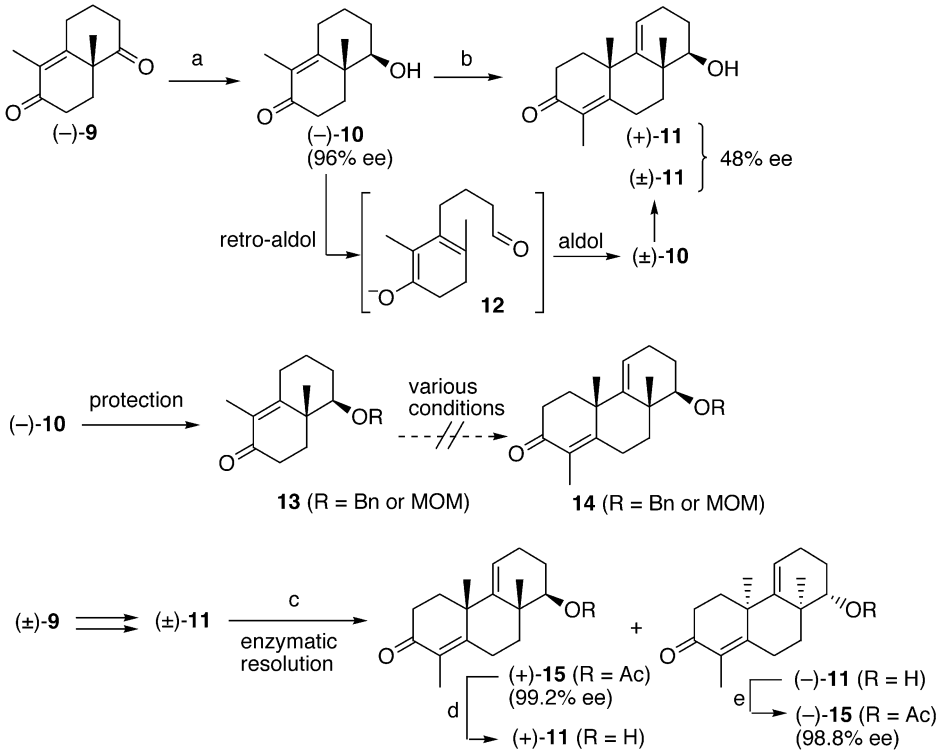


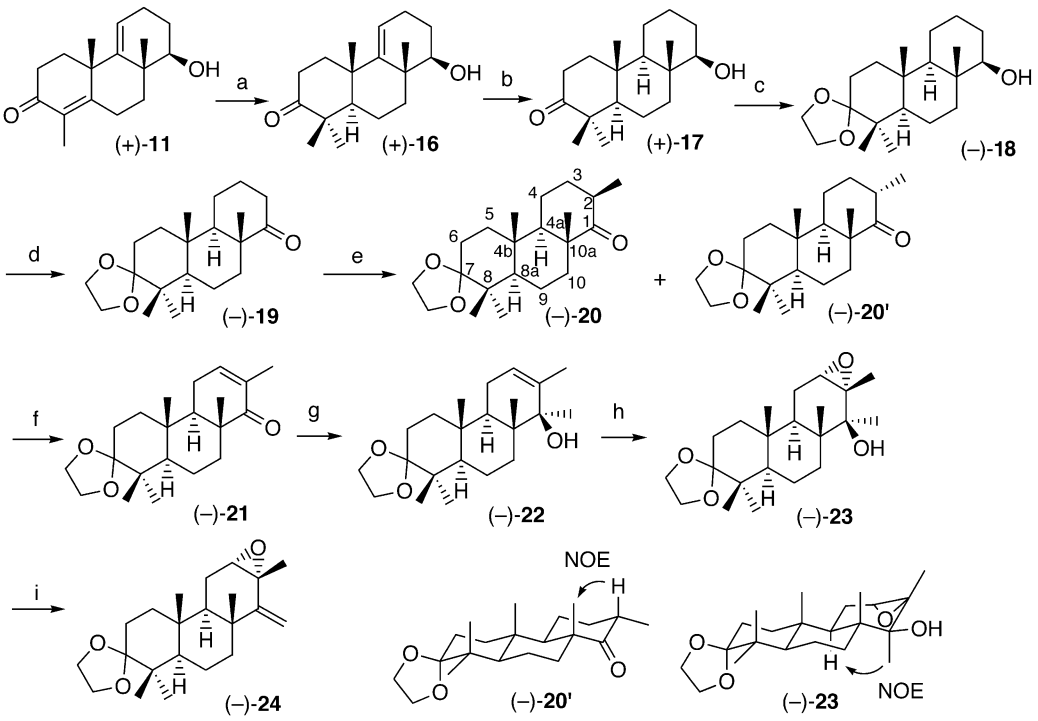
Scheme 1. Synthetic plan for **2**.

obtainable from **A**. For the preparation of **A**, the direct coupling of **B** and **C** by our original methodology should be possible. The key intermediate **C** might be prepared from the methylated Wieland–Miescher ketone **E** via the tricyclic ketone **D**. Therefore, it was quite obvious that the enantioselective synthesis of **2** should be possible just by starting from the known optically active **E**.[8]

Scheme 2 shows the first and unsuccessful route for the intermediate (+)-**11**. We synthesized the known optically active diketone (−)-**9** according to the reported procedure.[8] Chemoselective reduction of (−)-**9** with $NaBH_4$ gave (−)-**10**,[9] whose enantiomeric purity was estimated to be ca. 96% ee by HPLC analysis. Robinson annulation of (−)-**10** with ethyl vinyl ketone (EVK) in the presence of NaOMe in MeOH–toluene gave the adduct (+)-**11** (63%).[10] However, surprisingly, the enantiomeric purity of the yielded (+)-**11** was seriously decreased, <50% ee based on HPLC



Scheme 2. Synthesis of both enantiomers of **11**. Reagents and conditions: (a) $NaBH_4$, EtOH (92%); (b) EVK, NaOMe, MeOH, toluene (63% based on the consumed SM); (c) Chirazyme L-2, c.-f., C2, lyo. (100 wt%), vinyl acetate, Et_2O, room temperature, 40 h [50% for (−)-**11**, 49% for (+)-**15**]; (d) K_2CO_3, MeOH (94%); (e) Ac_2O, DMAP, pyridine.



Scheme 3. Synthesis of the key intermediate **24**. Reagents and conditions: (a) Li, NH_3, THF; MeI (92%); (b) H_2, Pd–C, EtOH (87%); (c) $HO(CH_2)_2OH$, *p*-TsOH, toluene (70%); (d) PDC, MS 4 Å, CH_2Cl_2 (88%); (e) LDA, THF, HMPA; MeI [95% for a mixture of (−)-**20**/(−)-**20′**]; (f) LDA, THF, HMPA, PhSeBr; aq H_2O_2, CH_2Cl_2 (89% based on the consumed SM); (g) MeLi, THF (98%); (h) *m*-CPBA, $NaHCO_3$, CH_2Cl_2 (80%); (i) $SOCl_2$, pyridine (72%).

analysis. This phenomenon was rationally explained by the retro-aldol reaction, which generated **12** as illustrated in Scheme 2, and the identical racemization was observed and reported by Honda and his co-workers.[10c] Thus, we then prepared the corresponding Bn or MOM ether (**13**) by protection of the hydroxyl group, because the protection was thought to prevent the problematic retro-aldol reaction. However, unfortunately, Robinson annulation of **13** with EVK was not successful at all even after an in-depth study of the reaction conditions.[11] Therefore, we dropped the first route and turned to the second option, enzymatic resolution. Enzymatic resolution of (±)-**11** was carried out in Et_2O in the presence of vinyl acetate as an acetyl donor. After screening of over 10 hydrolytic enzymes, Chirazyme L-2 (Roche Diagnostics Co.) was found to be the best.[12] The optimized conditions, Chirazyme L-2, c.-f., C2, lyo. (100 wt%), vinyl acetate, Et_2O at room temperature for 40 h, could resolve (±)-**11** almost perfectly to yield the alcohol (−)-**11** (50%) and the enantiomeric acetate (+)-**15** (49%). The enantiomeric purities of the resulting (−)-**11** and (+)-**15** were estimated by HPLC analysis to be 98.8 and 99.2% ee, respectively. The yielded optically active acetate (+)-**15** was then hydrolyzed to give (+)-**11**.

With both enantiomers of **11** in hand, we continued the synthesis of both enantiomers of **2**. One of the enantiomers (+)-**11** was subjected to reductive methylation to give (+)-**16** (92%).[10c] Hydrogenation of (+)-**16** in the presence of Pd–C as a catalyst afforded (+)-**17** (87%), whose carbonyl group was protected as ethylenedioxy acetal to furnish (−)-**18** (70%). The hydroxyl group of (−)-**18** was then oxidized to give (−)-**19** (88%). Methylation of the ketone (−)-**19** was performed by treatment with LDA and MeI to give methylated products, (−)-**20** and its 2-epimer (−)-**20′**, as a diastereomeric mixture (95% based on the consumed SM). The ratio of (−)-**20**:(−)-**20′** was ca. 7:1 based on ^{1}H NMR analysis, and it was noted that the ratio ranged from 5:1 to 8:1 depending on conditions. The major diastereomer was estimated to be (−)-**20** (2β-isomer), although we tentatively reported that the major diastereomer was **20′** (2α-isomer) in our racemate synthesis.[3] At that time we did not separate these diastereomers and did not consider the orientation of 2-Me, because both **20** and **20′** could be converted into the desired enone **21**. However, we herein could establish the relative configurations of (−)-**20** and (−)-**20′** as follows. The diastereomeric mixture of (−)-**20**/(−)-**20′** was treated with LDA and PhSeBr to furnish the corresponding selenide, which was immediately oxidized with H_2O_2 to give (−)-**21** (89% based on the consumed SM) and the recovered (−)-**20′** (8%). It was noteworthy that the recovered starting ketone was pure (−)-**20′**, not a mixture of (−)-**20**/(−)-**20′**. This was due to a base-mediated epimerization yielding the thermodynamically more stable (−)-**20′**. A very similar epimerization has already been reported.[13] The relative stereochemistry of (−)-**20′** was established by the observation of NOE between 2-H and 10a-Me as shown in Scheme 3.

The enone (−)-**21** was treated with MeLi to give (−)-**22** (98%). We tentatively assigned the installed 1-Me to be in α-orientation based on consideration of the steric effect, but could not confirm it by NOE studies due to poor resolution of the crucial signals. The allylic alcohol (−)-**22** was then epoxidized with *m*-CPBA to furnish (−)-**23** (80%) as a single diastereomer. We tried to establish the relative stereochemistry of (−)-**23** at this stage. Firstly, the α-orientation of 1-Me was established by the observation of NOE between 1-Me and 4a-H as shown in Scheme 3.

Table 1. Coupling of **7** and **24**

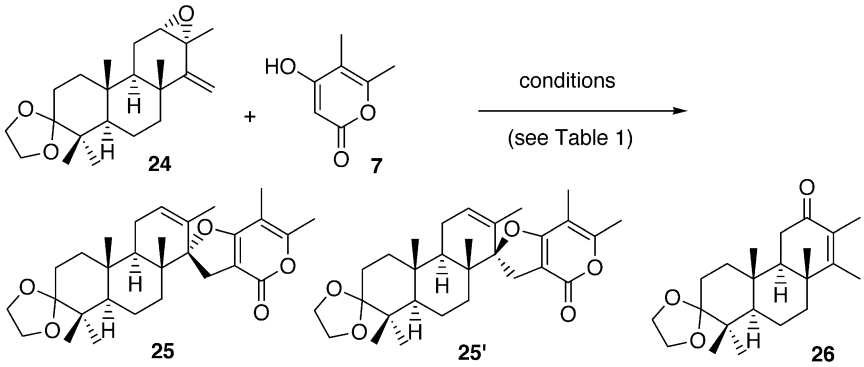
Entry	Base	Additive	Conditions	**25**+**25′** (%)[a]	**25**:**25′**	**24**; **26** (%)[a]
1	NaH	$BF_3 \cdot OEt_2$	THF, 0 °C–room temperature, 12 h	Decomp.[b]	—	—
2	NaH	$Yb(OTf)_3$	THF, 0 °C–room temperature, 12 h	NR	—	—
3	NaH	—	Toluene, 110 °C, 24 h	21	5:1	28; 29
4	NaH	HMPA	Toluene, 110 °C, 12 h	NR	—	—
5	NaH	—	THF, reflux, 12 h	NR	—	—
6	NaH	—	1,4-Dioxane, reflux,12 h	NR	—	—
7	*t*-BuOK	—	Toluene, 110 °C, 15 h	Trace[c]	—	—
8	Cs_2CO_3	—	Toluene, 110 °C, 15 h	<10%[c]	—	—
9	—	—	Toluene, 110 °C, 20 h	44	5:1	21; 15
10	—	—	1,4-Dioxane, reflux, 12 h	NR	—	—
11	—	—	Anisole, 150 °C, 5 h	51	7:1	Trace; 16

[a] Isolation yield.
[b] The major product was **26**, but it was not isolated.
[c] A large part of the starting material **24** was recovered.

Thus, the remaining was the orientation of the oxirane-ring. Although the α-orientation of it could not be confirmed by NOE studies, it was clearly supported by the fact that 3-H was observed as a broad triplet, $J=1.8$ Hz. Because conformational analyses based on Molecular Mechanics and also Semi-Empirical calculations (Spartan ′04 Ver. 1.0.3, Wevefunction, Inc.) suggest that dihedral angles between 3-H and 4-H_2 are both ca. 60° in **23**, while they should be ca. 90 and 30° in the corresponding β-epoxide. The reason for the diastereoselective epoxidation might be steric hindrance due to the angular methyl group (10a-Me). The alcohol (−)-**23** was then carefully dehydrated by treatment with $SOCl_2$ in pyridine to furnish the desired vinylepoxide (−)-**24** (72%).

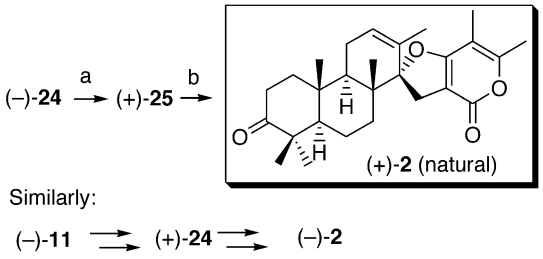
The next stage was the crucial step, the coupling of **24** and **7**. Of course, we first attempted our original methodology, the palladium-mediated tandem reaction. However, as we have already reported, the desired spiro-fused adducts **25**/**25′** could not be obtained at all by our palladium-catalyzed reaction.[3] The major by-product was the enone **26** probably derived from β-elimination of the π-allylpalladium complex. The reason for these failures might be steric hindrance, because **24** was estimated to be more sterically hindered than the model substrate **6**. Although these unexpected failures were surprising, we fortunately happened to find a possibility that the desired adducts **25**/**25′** were obtained by a simple base-mediated S_N2'-type epoxide-opening and following dehydrative *O*-alkylation.[3] Thus, we then examined reaction conditions without a palladium catalyst. The representative results are summarized in Table 1. First, addition of a Lewis acid, for enhancing the electrophilicity of vinylepoxide, was attempted (entries 1 and 2). Although these trials were fruitless, we found that the desired coupling proceeded in refluxing toluene without any Lewis acid to afford a mixture of **25** and its epimer **25′** in 21% yield (entry 3). The ratio of **25**:**25′** was estimated to be ca. 5:1 based on ^{1}H NMR analysis. We then tried to improve the isolation yield by changing the solvent or the base, but there was no improvement in yield (entries 4–8). However, delightfully, we were able to find that the desired coupling reaction proceeded smoothly in refluxing toluene without any base, furnishing a mixture of **25** and **25′** in 44% yield (**25**:**25′**=ca. 5:1) (entry 9). Furthermore, anisole was found to be the better solvent to give the adducts **25**/**25′** in 51% yield (**25**:**25′**=ca. 7:1) (entry 11).[14] Putting aside the detailed explanations, this reaction proceeded just by heating two substrates (**7** and **24**) in an aromatic solvent without any base and additive. These results were surprising to us, but the α-pyrone **7** might be construed as an activator of the epoxide due to its weak acidity in these cases. In any event, we were able to successfully construct the characteristic spiro-fused framework of breviones by the double S_N2'-type tandem reaction (Scheme 4).

The yielded mixture of (+)-**25**/**25′** was purified by careful chromatography and recrystallization to give the pure (+)-**25**.[15] Finally, the protecting group of (+)-**25** was removed by treatment with acetic acid to furnish (+)-brevione B (**2**), $[\alpha]_D^{25}$ +120 (*c* 0.204 in $CHCl_3$), {lit.,[2] $[\alpha]_D^{25}$ +65.9 (*c* 0.24 in $CHCl_3$)}. The various spectral data of synthetic (+)-**2** are in good accord with those of the natural product. It was also noteworthy that the synthesized (+)-**2** was crystals, mp



Scheme 4. Synthesis of **25**, the key step.

166–168 °C (from hexane–Et_2O), while the reported natural brevione B was an oil. Similarly, (+)-**24**, which was derived from (−)-**11**, was also converted into (−)-**2**, $[\alpha]_D^{23}$ −114 (*c* 0.254 in $CHCl_3$). Therefore, the absolute configuration of the naturally occurring brevione B (**2**) was determined as shown in Scheme 5.



Scheme 5. Synthesis of brevione B (**2**). Reagents and conditions: (a) **7**, anisole, 150 °C, 5 h [51% for a mixture of (+)-**25**/**25′**]; (b) aq AcOH, 40 °C, 36 h (85%).

3. Conclusion

In conclusion, the first synthesis of both enantiomers of brevione B (**2**) was accomplished by employing an enzymatic resolution and the double S_N2'-type tandem reaction as key steps. The absolute configuration of the naturally occurring brevione B was determined by our synthesis. Furthermore, it can be easily deduced that other breviones must have the same absolute configurations.[16]

4. Experimental

4.1. General

Melting points were uncorrected. IR spectra were measured on a Jasco IR A-102 spectrophotometer or a Shimadzu IR-408 spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz on a JEOL JNM-AL300 spectrometer. The peak for $CHCl_3$ in $CDCl_3$ (at δ 7.26) was used for the internal standard. Chemical shifts are reported in ppm and *J* values are given in Hz. ^{13}C NMR spectra were recorded at 75 MHz on a JEOL JNM-AL300 spectrometer. The peak for $CDCl_3$ (at δ 77.0) was used for the internal standard. Optical rotations were taken with a HORIBA SEPA-300 polarimeter. Mass spectra were measured with an Applied Biosystems QSTAR XL spectrometer. Analytical HPLC was carried out with a Shimadzu LC-10A system.

4.1.1. (−)-(4a*R*,5*R*)-5-Hydroxy-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3*H*)-one (−)-10. According to the reported procedure,[9] (−)-**9** (1.04 g, 5.41 mmol) was converted into (−)-**10** (970 mg, 92%): $[\alpha]_D^{28}$ −178 (*c* 0.146, $CHCl_3$), {lit.[9] $[\alpha]_D^{25}$ −164.6 (*c* 2.16, $CHCl_3$); lit.[10c] $[\alpha]_D^{26}$ −170 (*c* 1.2, $CHCl_3$)}. The IR and NMR data were in good accord with those of the reported.[9]

4.1.2. Determination of the enantiomeric purity of (−)-10. The enantiomeric purity of (−)-**10** was estimated by HPLC analysis: [column: Chiralcel® OD-H (4.6×250 mm); solvent, hexane/2-propanol=30:1; flow rate, 0.5 ml/min; detection at 254 nm]: t_R/min 41.1 [ca. 98%, (−)-**10**], 43.9 [ca. 2%, (+)-**10**]. The enantiomeric purity of (−)-**10** was estimated to be ca. 96% ee. It was noted that these peaks were not perfectly separated.

4.1.3. (+)-(4a*S*,8*R*,8a*R*)-8-Hydroxy-1,4a,8a-trimethyl-4,4a,6,7,8,8a,9,10-octahydrophenanthren-2(3*H*)-one (+)-11 [conversion of (−)-10 into (+)-11]. A mixture of (−)-**10** (846 mg, 4.35 mmol) and NaOMe (1.1 g, 20 mmol) in MeOH (16 ml) and toluene (4 ml) was stirred for 1 h at room temperature. After addition of EVK (1.3 ml, 13 mmol), this mixture was heated under reflux for 8 h. It was then poured into satd aq NH_4Cl and extracted with Et_2O. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the recovered **10** (308 mg, 36%) and (+)-**11** (456 mg, 63% based on the consumed SM): $[\alpha]_D^{29}$ +40 (*c* 0.22, $CHCl_3$), {lit.[10c] $[\alpha]_D^{25}$ +50 (*c* 2.0, $CHCl_3$; 56% ee). The IR and NMR data were in good accord with those of the reported.[10c]

4.1.4. Determination of the enantiomeric purity of (+)-11. In the conventional manner, (+)-**11** was converted into the corresponding benzoate, which was subjected to HPLC analysis: [column: Chiralcel® OD–H (4.6×250 mm); solvent, hexane/2-propanol=30:1; flow rate, 0.5 ml/min; detection at 254 nm]: t_R/min 27.1 [74%, (+)-**11** benzoate], 29.0 [26%, (−)-**11** benzoate]. The enantiomeric purity of (+)-**11** was estimated to be 48% ee.

4.1.5. Enzymatic resolution of (±)-11. To a solution of (±)-**11** (10.0 g, 38.4 mmol) in Et_2O (1 l), vinyl acetate (18 ml, 0.19 mol) and Chirazyme L-2, c.-f., C2, lyo. (10 g) were added. This mixture was stirred for 40 h at room temperature. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (−)-**11** (4.99 g, 50%) and (+)-**15** (5.74 g, 49%). The recovered immobilized enzyme was reusable in the matter of course.

Compound (−)-(4a*R*,8*S*,8a*S*)-**11**: mp 100–102 °C; $[\alpha]_D^{24}$ −108 (*c* 0.278, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 261.1831, calcd for $C_{17}H_{25}O_2$ $[M+H]^+$: 261.1849.

(+)-(4a*S*,8*R*,8a*R*)-8-Acetoxy-1,4a,8a-trimethyl-4,4a,6,7,8,8a,9,10-octahydrophenanthren-2(3*H*)-one (+)-**15**: mp 81–82 °C; $[\alpha]_D^{23}$ +57 (*c* 0.11, $CHCl_3$); IR (nujol) 1730 (s, C=O), 1660 (s, C=O), 1600 (m, C=C) cm^{-1}; ^{1}H NMR δ 1.30 (s, 3H, Me), 1.37 (s, 3H, Me), 1.71 (s, 3H, 1-Me), 1.71–2.05 (m, 6H), 2.04 (s, 3H, Ac), 2.22 (m, 2H), 2.30–2.59 (m, 4H), 4.68 (m, 1H, 8-H), 5.48 (t, *J*=3.6 Hz, 1H, 5-H); ^{13}C NMR δ 11.0, 21.2, 21.7, 22.4, 23.8, 24.3, 27.7, 33.63, 33.66, 34.7, 37.7, 40.9, 77.5, 120.1, 128.1, 147.3, 162.5, 170.7, 198.2; HRTOFMS (ESI, positive) *m*/*z* found 303.1976, calcd for $C_{19}H_{27}O_3$ $[M+H]^+$: 303.1954.

4.1.6. Determination of the enantiomeric purities of (−)-11 and (+)-15. The enantiomeric purity of (+)-**15** was estimated by HPLC analysis: [column: Chiralcel® OD–H (4.6 ×250 mm); solvent, hexane/2-propanol=30:1; flow rate, 0.5 ml/min; detection at 254 nm]: t_R/min 22.0 [0.4%, (−)-**15**], 24.3 [99.6%, (+)-**15**]. The enantiomeric purity of (+)-**15** was estimated to be 99.2% ee.

In the conventional manner, (−)-**11** was converted into the

corresponding acetate (−)-**15**, which was subjected to HPLC analysis: t_R/min 21.8 [98.9%, (−)-**15**], 24.3 [1.1%, (+)-**15**]. The enantiomeric purity of (−)-**15** was estimated to be 98.8% ee.

4.1.7. (+)-(4a*S*,8*R*,8a*R*)-11 [methanolysis of (+)-15]. To a solution of (+)-**15** (5.56 g, 18.4 mmol) in MeOH (50 ml), K_2CO_3 (0.51 g, 3.7 mmol) was added at 0 °C. After stirring for 6 h at room temperature, the reaction mixture was diluted with water and extracted with Et_2O. The extract was washed with brine, dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (+)-**11** (4.50 g, 94%) as a white crystalline solid: mp 101–102 °C; $[\alpha]_D^{22}$ +114 (*c* 0.128, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 261.1869, calcd for $C_{17}H_{25}O_2$ $[M+H]^+$: 261.1849.

4.1.8. (+)-(4a*S*,8*R*,8a*R*,10a*R*)-8-Hydroxy-1,1,4a,8a-tetramethyl-3,4,4a,6,7,8,8a,9,10,10a-decahydrophenanthren-2(1*H*)-one (+)-16. To liquid NH_3 (50 ml) was added Li (113 mg, 16.3 mmol) at −78 °C. After stirring for 30 min, a solution of (+)-**11** (847 mg, 3.25 mmol) in THF (10 ml) was added. This mixture was stirred for 2 h under reflux, and MeI (2.0 ml, 32 mmol) was added. The reaction mixture was stirred for 1 h under reflux and then quenched with MeOH. After removal NH_3, the resulting mixture was diluted with dil HCl and extracted with Et_2O. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (+)-**16** (827 mg, 92%). An analytical sample was obtained by recrystallization from hexane–EtOAc as colorless needles: mp 126–128 °C; $[\alpha]_D^{20}$ +49 (*c* 0.10, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 277.2161, calcd for $C_{18}H_{29}O_2$ $[M+H]^+$: 277.2162. The IR and NMR data were in good accord with those of the reported.[10c]

4.1.9. (−)-(4a*R*,8*S*,8a*S*,10a*S*)-8-Hydroxy-1,1,4a,8a-tetramethyl-3,4,4a,6,7,8,8a,9,10,10a-decahydrophenanthren-2(1*H*)-one (−)-16. In the same manner as described above, (−)-**11** (2.86 g, 11.0 mmol) was converted into (−)-**16** (2.20 g, 72%): mp 123–125 °C; $[\alpha]_D^{26}$ −47 (*c* 0.14, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 277.2167, calcd for $C_{18}H_{29}O_2$ $[M+H]^+$: 277.2162.

4.1.10. (+)-(4a*R*,4b*R*,8*R*,8a*R*,10a*R*)-8-Hydroxy-1,1,4a,8a-tetramethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-2(1*H*)-one (+)-17. To a solution of (+)-**16** (827 mg, 2.99 mmol) in EtOH (15 ml) was added Pd–C (5%; 80 mg). This mixture was stirred for 24 h at room temperature under H_2. After filtration through Celite, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (+)-**17** (723 mg, 87%). An analytical sample was obtained by recrystallization from hexane–EtOAc as colorless needles: mp 124–125 °C; $[\alpha]_D^{20}$ +26 (*c* 0.11, $CHCl_3$); IR (nujol) 3350 (s, O–H), 1705 (s, C=O) cm^{-1}; ^1H NMR δ 0.90 (s, 3H, Me), 0.93 (s, 3H, Me), 1.03 (s, 3H, Me), 1.07 (s, 3H, Me), 1.05–1.98 (m, 15H), 2.34–2.57 (m, 2H, 3-H), 3.12 (dt, J=11.1, 4.5 Hz, 1H, 8-H); ^{13}C NMR δ 12.7, 16.1, 19.1, 20.3, 21.0, 24.4, 26.5, 30.1, 34.0, 36.6, 38.4, 39.1, 39.5, 47.3, 55.0, 56.1, 80.8, 217.8; HRTOFMS (ESI, positive) *m*/*z* found 279.2311, calcd for $C_{18}H_{31}O_2$ $[M+H]^+$: 279.2318.

4.1.11. (−)-(4a*S*,4b*S*,8*S*,8a*S*,10a*S*)-8-Hydroxy-1,1,4a,8a-tetramethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-2(1*H*)-one (−)-17. In the same manner as described above, (−)-**16** (554 mg, 2.00 mmol) was converted into (−)-**17** (478 mg, 86%): mp 122–124 °C; $[\alpha]_D^{24}$ −25 (*c* 0.12, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 279.2317, calcd for $C_{18}H_{31}O_2$ $[M+H]^+$: 279.2318. The IR and NMR data were identical to those of (+)-**17**.

4.1.12. (−)-(1*R*,4a*R*,4b*R*,8a*R*,10a*R*)-7,7-Ethylenedioxy-4b,8,8,10a-tetramethyl-1,2,3,4,4a,4b,5,6,7,8,8a,9,10,10a-tetradecahydrophenanthren-1-ol (−)-18. A mixture of (+)-**17** (637 mg, 2.29 mmol), ethylene glycol (1.1 ml, 20 mmol) and *p*-TsOH·H_2O (10 mg) in toluene (25 ml) was stirred for 2 h under reflux with a Dean–Stark water separator. After cooling, the reaction mixture was quenched with satd aq $NaHCO_3$ and extracted with Et_2O. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by recrystallization from hexane–EtOAc to give (−)-**18** (513 mg, 70%) as colorless needles: mp 190–192 °C; $[\alpha]_D^{26}$ −44 (*c* 0.10, $CHCl_3$); IR (nujol) 3450 (s, O–H) cm^{-1}; ^1H NMR δ 0.83 (s, 3H, Me), 0.87 (s, 6H, Me×2), 0.92 (s, 3H, Me), 1.02–1.63 (m, 15H), 1.70–1.92 (m, 3H), 3.10 (dt, J=11.1, 4.5 Hz, 1H, 1-H), 3.83–3.99 (m, 4H, O–CH_2CH_2–O); ^{13}C NMR δ 13.0, 16.2, 18.0, 19.8, 19.9, 22.9, 24.5, 26.8, 30.1, 36.8, 36.9, 39.1, 39.6, 42.1, 53.6, 56.6, 64.79, 64.83, 81.1, 113.2; HRTOFMS (ESI, positive) *m*/*z* found 323.2590, calcd for $C_{20}H_{35}O_3$ $[M+H]^+$: 323.2580.

4.1.13. (+)-(1*S*,4a*S*,4b*S*,8a*S*,10a*S*)-7,7-Ethylenedioxy-4b,8,8,10a-tetramethyl-1,2,3,4,4a,4b,5,6,7,8,8a,9,10,10a-tetradecahydrophenanthren-1-ol (+)-18. In the same manner as described above, (−)-**17** (697 mg, 2.50 mmol) was converted into (+)-**18** (509 mg, 63%): mp 190–193 °C; $[\alpha]_D^{26}$ +46 (*c* 0.11, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 323.2576, calcd for $C_{20}H_{35}O_3$ $[M+H]^+$: 323.2580. The IR and NMR data were identical to those of (−)-**18**.

4.1.14. (−)-(4a*R*,4b*R*,8a*R*,10a*R*)-7,7-Ethylenedioxy-4b,8,8,10a-tetramethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1(2*H*)-one (−)-19. To a solution of (−)-**18** (2.34 g, 7.26 mmol) in CH_2Cl_2 (60 ml) were added PDC (4.10 g, 10.9 mmol) and MS 4 Å (5 g; powdered). After stirring overnight, the reaction mixture was filtered through SiO_2, and the filtrate was concentrated under reduced pressure. The residue was purified by recrystallization from hexane–Et_2O to give (−)-**19** (88%) as colorless needles: mp 182–184 °C; $[\alpha]_D^{27}$ −26 (*c* 0.14, $CHCl_3$); IR (nujol) 1700 (s, C=O) cm^{-1}; ^1H NMR δ 0.83 (s, 3H, Me), 0.93 (s, 3H, Me), 0.97 (s, 3H, Me), 1.13 (s, 3H, 10a-Me), 1.14–1.28 (m, 3H), 1.32–1.75 (m, 9H), 1.81 (dt, J=3.9, 13.8 Hz, 1H, 6-H), 2.03 (m, 1H, 3-H), 2.13–2.22 (m, 1H, 2-H), 2.53 (dt, J=6.6, 13.8 Hz, 1H, 2-H), 3.83–3.99 (m, 4H, O–CH_2CH_2–O); ^{13}C NMR δ 16.5, 17.7, 19.76, 19.80, 20.0, 22.8, 26.1, 26.8, 34.4, 36.8, 37.6, 38.0, 42.1, 49.1, 52.9, 57.3, 64.8, 64.9, 112.9, 215.7; HRTOFMS (ESI, positive) *m*/*z* found 321.2417, calcd for $C_{20}H_{33}O_3$ $[M+H]^+$: 321.2424.

4.1.15. (+)-(4a*S*,4b*S*,8a*S*,10a*S*)-7,7-Ethylenedioxy-4b,8,8,10a-tetramethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1(2*H*)-one (+)-19. In the same

manner as described above, (+)-**18** (491 mg, 1.52 mmol) was converted into (+)-**19** (444 mg, 91%): mp 182–184 °C; $[\alpha]_D^{27}$ +27 (*c* 0.13, $CHCl_3$); HRTOFMS (ESI, positive) *m/z* found 321.2419, calcd for $C_{20}H_{33}O_3$ $[M+H]^+$: 321.2424. The IR and NMR data were identical to those of (−)-**19**.

4.1.16. (−)-(2*R*,4a*R*,4b*R*,8a*R*,10a*R*)-7,7-Ethylenedioxy-2,4b,8,8,10a-pentamethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1(2*H*)-one (−)-20. *n*-BuLi (1.58 M in hexane; 6.9 ml, 11 mmol) was added to a solution of $(i\text{-Pr})_2NH$ (1.8 ml, 13 mmol) in anhydrous THF (20 ml) at 0 °C under Ar. After stirring for 30 min at 0 °C, a solution of (−)-**19** (3.44 g, 10.7 mmol) in anhydrous THF (30 ml) was added at −78 °C. After stirring for 30 min, HMPA (5.6 ml, 32 mmol) and MeI (1.0 ml, 16 mmol) were added successively at −78 °C. After the mixture was stirred for 30 min with warming to 0 °C, the reaction mixture was quenched with satd aq NH_4Cl and extracted with Et_2O. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give a mixture of (−)-**20** and its (2*S*)-isomer (−)-**20′** (3.05 g, 95% based on the consumed SM) and the recovered (−)-**19** (0.38 g, 11%). The ratio of (−)-**20** and (−)-**20′** was estimated to be ca. 7:1 based on ^1H NMR analysis. An analytical sample of (−)-**20** was obtained by further chromatography and recrystallization from hexane as colorless flaky crystals: mp 188–190 °C; $[\alpha]_D^{24}$ −145 (*c* 0.207, $CHCl_3$); IR (nujol) 1705 (s, C=O) cm^{-1}; ^1H NMR δ 0.83 (s, 3H, Me), 0.93 (s, 3H, Me), 0.96 (s, 3H, Me), 1.03 (d, $J=6.6$ Hz, 3H, 2-Me) 1.07 (s, 3H, 10a-Me), 1.23–1.68 (m, 10H), 1.72–1.97 (m, 4H), 2.47 (septet-like, $J=5.7$ Hz, 1H, 2-H), 3.83–3.99 (m, 4H, O–CH_2CH_2–O); ^{13}C NMR δ 15.8, 16.0, 17.0, 18.0, 19.9, 20.3, 22.9, 26.7, 26.9, 36.1, 36.4, 37.4, 39.6, 42.1, 47.5, 51.0, 53.4, 64.8, 64.9, 113.0, 220.6; HRTOFMS (ESI, positive) *m/z* found 335.2570, calcd for $C_{21}H_{35}O_3$ $[M+H]^+$: 335.2580.

4.1.17. (+)-(2*S*,4a*S*,4b*S*,8a*S*,10a*S*)-7,7-Ethylenedioxy-2,4b,8,8,10a-pentamethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1(2*H*)-one (+)-20. In the same manner as described above, (+)-**19** (561 mg, 1.75 mmol) was converted into a mixture of (+)-**20** and its (2*R*)-isomer (+)-**20′** (541 mg, 92%). The ratio of (−)-**20** and (−)-**20′** was estimated to be ca. 8:1 based on ^1H NMR analysis. An analytical sample of (+)-**20** was obtained by further chromatography and recrystallization from hexane as colorless flaky crystals: mp 191–193 °C; $[\alpha]_D^{29}$ +149 (*c* 0.102, $CHCl_3$); HRTOFMS (ESI, positive) *m/z* found 335.2568, calcd for $C_{21}H_{35}O_3$ $[M+H]^+$: 335.2580. The IR and NMR data were identical to those of (−)-**20**.

4.1.18. (−)-(4a*R*,4b*R*,8a*R*,10a*R*)-7,7-Ethylenedioxy-2,4b,8,8,10a-pentamethyl-4a,4b,5,6,7,8,8a,9,10,10a-decahydrophenanthren-1(4*H*)-one (−)-21 and (−)-(2*S*,4a*R*,4b*R*,8a*R*,10a*R*)-7,7-ethylenedioxy-2,4b,8,8,10a-pentamethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1(2*H*)-one (−)-20′. *n*-BuLi (1.58 M in hexane; 6.8 ml, 11 mmol) was added to a solution of $(i\text{-Pr})_2NH$ (1.6 ml, 11 mmol) in anhydrous THF (20 ml) at 0 °C under Ar. After stirring for 30 min at 0 °C, a solution of (−)-**20**/(−)-**20′** (2.99 g, 8.94 mmol) in anhydrous THF (40 ml) was added at −78 °C. After stirring for 30 min with warming to −30 °C gradually, HMPA (4.7 ml, 27 mmol) and a solution of PhSeBr (2.43 g, 10.3 mmol) in THF (10 ml) were added successively at −40 °C. After the mixture was stirred for 30 min with warming to 0 °C, the reaction mixture was quenched with satd aq NH_4Cl and extracted with EtOAc. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give the crude product, which was employed for the next step without purification. To a solution of the yielded crude product in CH_2Cl_2 (30 ml), dil aq H_2O_2 (ca. 8%; 12.5 ml) was added dropwise at 0 °C. After stirring for 15 min at room temperature, the reaction mixture was poured into satd aq $NaHCO_3$ and extracted with Et_2O. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (−)-**21** (2.43 g, 89% based on the consumed SM) and the recovered (−)-**20′** (0.24 g, 8%). It was noted that the recovered SM was the pure (−)-**20′**, not a mixture of (−)-**20** and (−)-**20′**. An analytical sample of (−)-**21** was obtained by recrystallization from EtOAc as colorless plates: mp 210–213 °C; $[\alpha]_D^{25}$ −49 (*c* 0.13, $CHCl_3$); IR (nujol) 1670 (s, C=O) cm^{-1}; ^1H NMR δ 0.83 (s, 3H, Me), 0.93 (s, 3H, Me), 1.01 (s, 6H, Me×2), 1.19–1.65 (m, 8H) 1.72 (br s, 3H, 2-Me), 1.74–1.98 (m, 2H), 2.20–2.27 (m, 2H, 4-H_2), 3.83–3.99 (m, 4H, O–CH_2CH_2–O), 6.63 (br s, 1H, 3-H); ^{13}C NMR δ 16.3, 18.0, 18.4, 20.0, 22.8, 23.4, 26.6, 34.6, 36.1, 37.3, 42.1, 45.0, 52.8, 53.5, 64.81, 64.83, 112.8, 132.7, 143.2, 205.8; HRTOFMS (ESI, positive) *m/z* found 333.2419, calcd for $C_{21}H_{33}O_3$ $[M+H]^+$: 333.2424.

An analytical sample of (−)-**20′** was obtained by recrystallization from hexane as colorless flaky crystals: mp 176–178 °C; $[\alpha]_D^{24}$ −29 (*c* 0.24, $CHCl_3$); IR (nujol) 1705 (s, C=O) cm^{-1}; ^1H NMR δ 0.83 (s, 3H, Me), 0.93 (s, 3H, Me), 0.95 (d, $J=6.3$ Hz, 3H, 2-Me) 0.97 (s, 3H, Me), 1.12 (s, 3H, 10a-Me), 1.12–1.86 (m, 13H), 2.07 (m, 1H, 3-H), 2.62 (septet-like, $J=6.6$ Hz, 1H, 2-H), 3.83–3.99 (m, 4H, O–CH_2CH_2–O); ^{13}C NMR δ 14.9, 16.5, 17.8, 19.8, 20.0, 20.4, 22.8, 26.8, 34.5, 35.5, 36.8, 38.0, 40.0, 42.2, 48.8, 52.9, 58.1, 64.84, 64.87, 112.9, 216.4.

4.1.19. (+)-(4a*S*,4b*S*,8a*S*,10a*S*)-7,7-Ethylenedioxy-2,4b,8,8,10a-pentamethyl-4a,4b,5,6,7,8,8a,9,10,10a-decahydrophenanthren-1(4*H*)-one (+)-21 and (+)-(2*R*,4a*S*,4b*S*,8a*S*,10a*S*)-7,7-ethylenedioxy-2,4b,8,8,10a-pentamethyl-3,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1(2*H*)-one (+)-20′. In the same manner as described above, (+)-**20** (534 mg, 1.60 mmol) was converted into (+)-**21** (407 mg, 87% based on the consumed SM) and (+)-**20′** (64 mg, 12%). (+)-**21**: mp 211–213 °C; $[\alpha]_D^{31}$ +50 (*c* 0.15, $CHCl_3$); HRTOFMS (ESI, positive) *m/z* found 333.2436, calcd for $C_{21}H_{33}O_3$ $[M+H]^+$: 333.2424. The IR and NMR data were identical to those of (−)-**21**.

Compound (+)-**20′**: mp 176–178 °C; $[\alpha]_D^{29}$ +27 (*c* 0.10, $CHCl_3$). The IR and NMR data were identical to those of (−)-**20′**.

4.1.20. (−)-(1*R*,4a*R*,4b*R*,8a*R*,10a*R*)-7,7-Ethylenedioxy-1,2,4b,8,8,10a-hexamethyl-1,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1-ol (−)-22. To a solution of

(−)-**21** (2.17 g, 6.53 mmol) in anhydrous THF (50 ml), MeLi (0.98 M in Et_2O: 10 ml, 9.8 mmol) was added dropwise at 0 °C under Ar. After stirring for 20 min at 0 °C, the reaction mixture was quenched with satd aq NH_4Cl and extracted with EtOAc. The extract was washed with water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by recrystallization from hexane to give (−)-**22** (2.24 g, 98%) as colorless prisms: mp 143–145 °C; $[\alpha]_D^{24}$ −36 (*c* 0.20, $CHCl_3$); IR (nujol) 3550 (m, O–H) cm^{-1}; ^1H NMR δ 0.87 (s, 3H, Me), 0.95 (s, 6H, Me×2), 0.98 (s, 3H, Me), 1.13–1.91 (m, 13H), 1.26 (s, 3H, 1-Me), 1.69 (br s, 3H, 2-Me), 3.83–3.99 (m, 4H, O–CH_2CH_2–O), 5.28 (br s, 1H, 3-H); ^{13}C NMR δ 15.35, 15.39, 17.9, 18.2, 20.2, 22.93, 22.94, 23.7, 26.7, 33.0, 36.8, 37.0, 40.6, 42.0, 49.4, 53.5, 64.7, 64.8, 78.0, 113.1, 121.2, 138.0; HRTOFMS (ESI, positive) *m/z* found 331.2620, calcd for $C_{22}H_{35}O_2$ $[M-H]^+$: 331.2631.

4.1.21. (+)-(1*S*,4a*S*,4b*S*,8a*S*,10a*S*)-7,7-Ethylenedioxy-1,2,4b,8,8,10a-hexamethyl-1,4,4a,4b,5,6,7,8,8a,9,10,10a-dodecahydrophenanthren-1-ol (+)-22. In the same manner as described above, (+)-**21** (340 mg, 1.02 mmol) was converted into (+)-**22** (339 mg, 95%): mp 147–149 °C; $[\alpha]_D^{28}$ +37 (*c* 0.12, $CHCl_3$); HRTOFMS (ESI, positive) *m/z* found 331.2627, calcd for $C_{22}H_{35}O_2$ $[M-H]^+$: 331.2631. The IR and NMR data were identical to those of (−)-**22**.

4.1.22. (−)-(1*S*,2*S*,3*S*,4a*R*,4b*R*,8a*R*,10a*R*)-2,3-Epoxy-7,7-ethylenedioxy-1,2,4b,8,8,10a-hexamethyl-1,2,3,4,4a,4b,5,6,7,8,8a,9,10,10a-tetradecahydrophenanthren-1-ol (−)-23. To a mixture of (−)-**22** (2.04 g, 5.85 mmol) and $NaHCO_3$ (3.0 g, 36 mmol) in CH_2Cl_2 (40 ml), *m*-CPBA (77%: 2.0 g, 8.9 mmol) was added portionwise at 0 °C. After stirring for 4 h at room temperature, the reaction mixture was quenched with satd aq $Na_2S_2O_3$ and satd aq $NaHCO_3$ and extracted with Et_2O. The extract was washed with satd aq $NaHCO_3$, water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give (−)-**23** (1.71 g, 80%). An analytical sample was obtained by recrystallization from $(i\text{-Pr})_2O$ as colorless granules: mp 156–158 °C; $[\alpha]_D^{21}$ −16 (*c* 0.11, $CHCl_3$); IR (nujol) 3500 (m, O–H) cm^{-1}; ^1H NMR δ 0.84 (s, 3H, Me), 0.92 (s, 3H, Me), 0.94 (s, 3H, Me), 0.96 (s, 3H, Me), 1.09–1.95 (m, 13H), 1.24 (s, 3H, 1-Me), 1.29 (s, 3H, 2-Me), 3.08 (br t, *J*= 1.8 Hz, 1H, 3-H), 3.83–3.99 (m, 4H, O–CH_2CH_2–O); ^{13}C NMR δ 15.8, 16.0, 18.0, 19.9, 20.3, 21.9, 22.4, 23.0, 26.7, 33.2, 36.8, 40.2, 42.0, 44.7, 53.5, 61.5, 62.7, 64.7, 64.8, 76.0, 112.9; HRTOFMS (ESI, positive) *m/z* found 365.2697, calcd for $C_{22}H_{37}O_4$ $[M+H]^+$: 365.2686.

4.1.23. (+)-(1*R*,2*R*,3*R*,4a*S*,4b*S*,8a*S*,10a*S*)-2,3-Epoxy-7,7-ethylenedioxy-1,2,4b,8,8,10a-hexamethyl-1,2,3,4,4a,4b,5,6,7,8,8a,9,10,10a-tetradecahydrophenanthren-1-ol (+)-23. In the same manner as described above, (+)-**22** (311 mg, 892 μmol) was converted into (+)-**23** (259 mg, 80%): mp 157–158 °C; $[\alpha]_D^{28}$ +17 (*c* 0.10, $CHCl_3$); HRTOFMS (ESI, positive) *m/z* found 365.2698, calcd for $C_{22}H_{37}O_4$ $[M+H]^+$: 365.2686. The IR and NMR data were identical to those of (−)-**23**.

4.1.24. (−)-(2*R*,3*S*,4a*R*,4b*R*,8a*R*,10a*R*)-2,3-Epoxy-7,7-ethylenedioxy-2,4b,8,8,10a-pentamethyl-1-methylene-1,2,3,4,4a,4b,5,6,7,8,8a,9,10,10a-tetradecahydrophenanthrene (−)-24. To a solution of (−)-**23** (1.40 g, 3.84 mmol) in pyridine (70 ml), $SOCl_2$ (1.4 ml, 19 mmol) was slowly added dropwise at 0 °C. After stirring for 30 min at 0 °C, the reaction mixture was diluted with Et_2O and then poured into satd aq $NaHCO_3$. This mixture was extracted with Et_2O. The extract was washed with water, satd aq $CuSO_4$, water and brine, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography and recrystallization from hexane–EtOAc to give (−)-**24** (0.96 g, 72%) as colorless flaky crystals: mp 188–189 °C; $[\alpha]_D^{22}$ −19 (*c* 0.21, $CHCl_3$); IR (nujol) 1620 (w, C=C) cm^{-1}; ^1H NMR δ 0.84 (s, 3H, Me), 0.93 (s, 3H, Me), 0.95 (s, 3H, Me), 0.97 (s, 3H, Me), 1.17–1.62 (m, 8H) 1.46 (s, 3H, 2-Me), 1.70–1.88 (m, 3H), 2.02–2.11 (m, 1H, 4-H), 3.17 (br t, *J*=1.8 Hz, 1H, 3-H), 3.82–3.99 (m, 4H, O–CH_2CH_2–O), 5.11 (s, 1H, C=C*H*), 5.28 (s, 1H, C=C*H*); ^{13}C NMR δ 16.0, 18.5, 20.1, 21.8, 21.9, 22.7, 22.9, 26.6, 36.4, 36.8, 37.6, 38.6, 42.0, 45.6, 52.9, 56.1, 61.0, 64.76, 64.82, 110.8, 112.9, 156.9; HRTOFMS (ESI, positive) *m/z* found 347.2565, calcd for $C_{22}H_{35}O_3$ $[M+H]^+$: 347.2580.

4.1.25. (+)-(2*S*,3*R*,4a*S*,4b*S*,8a*S*,10a*S*)-2,3-Epoxy-7,7-ethylenedioxy-2,4b,8,8,10a-pentamethyl-1-methylene-1,2,3,4,4a,4b,5,6,7,8,8a,9,10,10a-tetradecahydrophenanthrene (+)-24. In the same manner as described above, (+)-**23** (311 mg, 892 μmol) was converted into (+)-**24** (259 mg, 80%): mp 192–194 °C; $[\alpha]_D^{28}$ +21 (*c* 0.11, $CHCl_3$); HRTOFMS (ESI, positive) *m/z* found 357.2573, calcd for $C_{22}H_{35}O_3$ $[M+H]^+$: 347.2580. The IR and NMR data were identical to those of (−)-**24**.

4.1.26. (+)-(1′*S*,4a'*R*,4b'*R*,8a'*R*,10a'*R*)-7′7′-Ethylenedioxy-4a′,4b′,5′,6′,7,8′,8a′,9′,10′,10a′-decahydro-6,7,2′,4b′,8′,8′,10a′-heptamethylspiro{4*H*-furo[3,2-*c*]pyran-2(3*H*),1′(4′*H*)-phenanthrene}-4-one (+)-25 and its 1′-epimer 25′. A mixture of (−)-**24** (180 mg, 519 μmol) and 7 (124 mg, 885 μmol) in distilled anisole (14 ml) was stirred for 5 h at 150 °C under Ar. After cooling, the reaction mixture was purified by silica gel chromatography to give a mixture of (+)-**25**/**25′** (124 mg, 51%) and also the major by-product **26** (28 mg, 16%). The ratio of **25**:**25′** was estimated to be ca. 7:1 based on ^1H NMR analysis. The yielded diastereomeric mixture was further purified by careful chromatography and recrystallization from hexane–Et_2O to separate the pure (+)-**25** (62 mg, 50% recovery) as colorless granules and an impure diastereomeric mixture (56 mg).

Compound (+)-**25**: mp 201–203 °C; $[\alpha]_D^{25}$ +41 (*c* 0.14, MeOH); IR (nujol) 1710 (s, C=0), 1650 (w, C=C) cm^{-1}; ^1H NMR δ 0.83 (s, 3H, Me), 0.90 (s, 3H, Me), 0.94 (s, 3H, Me), 0.99 (s, 3H, Me), 1.22–1.64 (m, 8H) 1.63 (br s, 3H, 2′-Me), 1.70–2.08 (m, 4H), 1.97 (s, 3H, 6-Me), 2.22 (s, 3H, 7-Me), 2.87 (d, *J*=15.9 Hz, 1H, 3-H), 3.04 (d, *J*=15.9 Hz, 1H, 3-H), 3.84–3.99 (m, 4H, O–CH_2CH_2–O), 5.63 (br s, 1H, 3′-H); ^{13}C NMR δ 9.9, 15.6, 16.3, 17.2, 17.9, 18.5, 20.2, 22.95, 23.03, 26.8, 28.8, 32.1, 36.4, 36.6, 40.9, 42.0, 47.2, 52.8, 64.7, 64.9, 99.5, 99.9, 103.1, 112.9, 128.0, 131.5, 160.1, 162.1, 171.3; HRTOFMS (ESI, positive) *m/z* found 469.2939, calcd for $C_{29}H_{41}O_5$ $[M+H]^+$: 469.2948.

Compound 1′-Epimer **25′**: ^{1}H NMR (only clearly observed peaks) δ 0.93 (s, Me), 1.06 (s, Me), 1.52 (br s, 2′-Me), 1.93 (s, Me), 2.76 (d, J=15.9 Hz, 3-H) 3.34 (d, J=15.9 Hz, 3-H), 5.34 (br s, 3′-H).

(4a*R*,4b*R*,8a*R*,10a*R*)-7,7-Ethylenedioxy-1,2,4b,8,8,10a-hexamethyl-4a,4b,5,6,7,8,8a,9,10,10a-decahydrophenanthren-3(4*H*)-one **26**: IR (nujol) 1650 (s, C=O), 1605 (m, C=C) cm^{-1}; ^{1}H NMR δ 0.84 (s, 3H, Me), 0.94 (s, 3H, Me), 0.96 (s, 3H, Me), 1.06 (s, 3H, Me), 1.24–2.02 (m, 12H) 1.72 (br s, 3H, Me), 1.82 (br s, 3H, Me), 2.34 (dd, J=17.4, 13.2 Hz, 1H, 4-H), 3.04 (dd, J=17.4, 4.5 Hz, 1H, 4-H), 3.83–3.99 (m, 4H, O–CH_2CH_2–O); ^{13}C NMR δ 11.4, 14.8, 15.8, 18.4, 18.5, 19.8, 22.7, 26.6, 34.3, 36.0, 36.7, 37.9, 40.4, 42.1, 52.8, 53.9, 64.8, 64.9, 112.9, 129.2, 164.8, 199.8.

4.1.27. (−)-(1′*R*,4a′*S*,4b′*S*,8a′*S*,10a′*S*)-7′,7′-Ethylenedioxy-4a′,4b′,5′,6′,7,8′,8a′,9′,10′,10a′-decahydro-6,7,2′,4b′,8′,8′,10a′-heptamethylspiro{4*H*-furo[3,2-*c*]pyran-2(3*H*),1′(4′*H*)-phenanthrene}-4-one (−)-25. In the same manner as described above, (+)-**24** (94 mg, 0.27 mmol) was converted into a mixture of (−)-**25**/**25′** (62 mg, 49%; ca. 7:1). The yielded diastereomeric mixture was further purified by careful chromatography and recrystallization from hexane–Et_2O to give the pure (−)-**25** (29 mg, 47% recovery) as colorless granules: mp 202–204 °C; $[\alpha]_D^{21}$ −39 (*c* 0.13, MeOH); HRTOFMS (ESI, positive) *m*/*z* found 469.2925, calcd for $C_{29}H_{41}O_5$ $[M+H]^+$: 469.2948. The IR and NMR data were identical to those of (+)-**25**.

4.1.28. (+)-(1′*S*,4a′*R*,4b′*R*,8a′*R*,10a′*R*)-4a′,5′,6′,8′,8a′,9′,10′,10a′-Octahydro-6,7,2′,4b′,8′,8′,10a′-heptamethylspiro{4*H*-furo[3,2-*c*]pyran-2(3*H*),1′(4′*H*)-phenanthrene}-4,7′(4b′*H*)-dione (brevione B) (+)-2. A solution of (+)-**25** (48 mg, 0.11 mmol) in AcOH (90%; 4 ml) was stirred for 36 h at 40 °C. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography and recrystallization from hexane–Et_2O to give (+)-**2** (37 mg, 85%) as colorless rods: mp 166–168 °C; $[\alpha]_D^{25}$ +120 (*c* 0.204, $CHCl_3$); IR ($CHCl_3$) 3000 (s, C–H), 1700 (s, C=O), 1650 (w, C=C), 1570 (m), 1440 (br m), 1390 (m), 1275 (m), 1120 (m), 930 (m) cm^{-1}; ^{1}H NMR δ 0.96 (s, 3H, 10a′-Me), 1.07 (s, 3H, 8′-Me), 1.09 (s, 6H, 4b′- and 8′-Me), 1.35 (m, 2H, 8a′- and 10′-H), 1.44–1.61 (m, 4H, 5′-and 10′-H, 9′-H_2), 1.65 (br d, J=1.5 Hz, 3H, 2′-Me), 1.75 (t-like, J=8.4 Hz, 1H, 4a′-H), 1.93 (s, 3H, 6-Me), 1.95 (m, 1H, 5′-H), 2.06 (m, 2H, 4′-H_2), 2.22 (s, 3H, 7-Me), 2.41 (ddd, J=15.9, 6.9, 3.9 Hz, 1H, 6′-H), 2.58 (ddd, J=15.9, 11.1, 7.2 Hz, 1H, 6′-H), 2.90 (d, J=15.9 Hz, 1H, 3-H), 3.05 (d, J=15.9 Hz, 1H, 3-H), 5.67 (1H, br s, 3′-H); ^{13}C NMR δ 9.7, 15.5, 16.0, 17.2, 18.4, 19.0, 21.5, 23.2, 26.4, 28.7, 31.8, 34.0, 36.5, 38.9, 40.9, 47.0, 47.4, 55.1, 99.5, 99.6, 102.8, 127.8, 131.8, 160.4, 162.0, 171.1, 217.2; HRTOFMS (ESI, positive) *m*/*z* found 425.2684, calcd for $C_{27}H_{37}O_4$ $[M+H]^+$: 425.2686.

4.1.29. (−)-(1′*R*,4a′*S*,4b′*S*,8a′*S*,10a′*S*)-4a′,5′,6′,8′,8a′,9′,10′,10a′-Octahydro-6,7,2′,4b′,8′,8′,10a′-heptamethylspiro{4*H*-furo[3,2-*c*]pyran-2(3*H*),1′(4′*H*)-phenanthrene}-4,7′(4b′*H*)-dione (brevione B) (−)-2. In the same manner as described above, (−)-**25** (14 mg, 30 μmol) was converted into (−)-**2** (11 mg, 87%): mp 167–170 °C; $[\alpha]_D^{23}$ −114 (*c* 0.254, $CHCl_3$); HRTOFMS (ESI, positive) *m*/*z* found 425.2689, calcd for $C_{27}H_{37}O_4$ $[M+H]^+$: 425.2686. The IR and NMR data were identical to those of (+)-**2**.

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11. Honda and his co-workers also reported that Robinson annulation of the OH-protected **10** was unsuccessful.[10c]
12. The other tested enzymes were as follows; Lipase AK (Amano), Lipase PS (Amano), Lipase AH (Amano), Lipase AY (Amano), PLE-A (Amano), Lipase PL (Meito), Lipase AL (Meito), Lipase (Shin-Nihon-Kagaku-Kogyo), Lipase(BSL) (Godo Syusei), etc. Acetylation proceeded much more slowly or did not proceed with those enzymes under the same conditions.
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14. The advantage of anisole as solvent was found in the course of our synthetic studies on decaturin D, a structurally similar spiro-fused diterpenoid. We would like to report the details concerning the solvent effect separately.
15. Although purification of **25**/**25**′ to the pure **25** was quite easy by recrystallization from Et_2O in our racemate synthesis, it did not work in this optically active form.
16. Prof. K. Shishido (Tokushima Univ.) and his co-workers have already announced the synthesis and absolute configuration of brevione C (**3**). Mitsuhashi, C.; Shindo, M.; Shishido, K. *Abstracts of Papers*, 48th TEAC, Yamaguchi, Japan, Oct 30–Nov 1, 2004; 1PII-4.

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Asymmetric addition of trimethylsilyl cyanide to ketones catalyzed by Al(salen)/triphenylphosphine oxide

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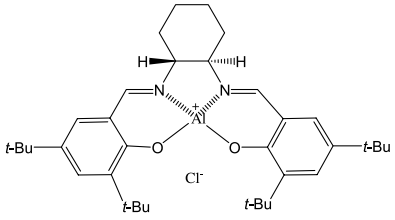
Abstract—Trimethylsilyl cyanide asymmetrically adds to various ketones by catalysis of **1**/triphenylphosphine oxide. This is a double activation where **1** acts as Lewis acid and Ph_3PO Lewis base. Various ketones were subjected to the enantioselective addition so as to give up to 92% ee and >90% yield.

1. Introduction

Asymmetric addition of trimethylsilyl cyanide (TMSCN) to carbonyl compounds and subsequent hydrolysis produces chiral cyanohydrins[1–3]. Such chiral compounds are useful intermediates for synthesis of pharmaceutics. The two functional groups (–OH and –CN) can be easily transformed into various homochiral ones including α-hydroxy acids[4,5], α-hydroxy aldehydes[6], α-hydroxy ketones[6], β-hydroxy amines[5,6] and α-amino acid derivatives[7].

A number of catalysts for the asymmetric addition of TMSCN to ketones are known. Belokon and North used a bimetallic titanium salen complex as a catalyst for the asymmetric addition of TMSCN to ketones[8]. Shibasaki has reported enantioselective catalytic additions of TMSCN to various ketones utilizing bifunctional ligand and $Ti(OiPr)_4$ or the lanthanide complexes[9]. The concept of dual activation has been pioneered by Shibasaki's group. Deng has first described method of cyanosilylation of ketones employing chiral Lewis bases which are free of metal ions[10]. Snapper and Hoveyda has described the addition of TMSCN to ketones catalyzed by peptide chiral ligand and $Al(OiPr)_3$[11]. Feng and Jiang has employed chiral *N*-oxide/titanium(IV) complex for the cyanosilylation of ketones[12]. Feng has utilized a catalytic double-activation method using chiral salen-Ti(IV) complex and various achiral *N*-oxides for the cyanosilylation of ketones[13]. Recently, Corey has shown that chiral oxazaborolidium salt is an excellent catalyst for the cyanosilylation of methyl ketones[14]. This method used TMSCN and diphenylmethyl phosphine oxide as co-reactants to generate $Ph_2MePOTMS(N{=}C{:})$ as a reactive intermediate. The cyanosilylations of aldehydes employing Al(salen)(**1**)/Ph_3PO and Mn(salen)/Ph_3PO, respectively were developed by us.[15] The former catalyst gives better % ee than the latter. We would like to herein report the cyanosilylation of ketones employing the same **1**/Ph_3PO as the catalysts.



1

2. Results and discussion

p-Chlorophenyl methyl ketone was chosen as a test substrate to find out the best conditions for the cyanosilylations. Amount of triphenylphosphine oxide was varied to find out the optimal conditions (entries 1–4). No reaction took place without triphenylphosphine oxide (entry 1). Entry 4 of 10 mol% Ph_3PO is shown to be the proper quantity for the cyanosilylation reaction. Various solvents were used for the cyanosilylations and CH_2Cl_2 proved to be the proper medium for the reactions (entries 4–7). The substrate concentration of 1 M appears adequate (entries 4, 8 and 9). The reactions were run at four different temperatures and rt offers the best outcome (entries 4, 10,

Keywords: Cyanohydrin; Ketone; Trimethylsilyl cyanide; Al(salen); Asymmetric addition.
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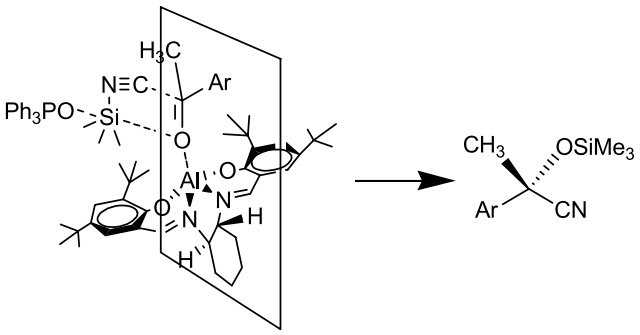


Figure 1. Transition state involved in the enantioselective cyanosilylation of ketones by double-activation catalysis.

11 and 12). The amount of **1** for the reactions should be 1 mol% that gives highest % yield and % ee(entries 4, 13, 14 and 15) (Table 1). The reactions appears to be catalyzed by double activation process occurring through the catalysis of both chiral Lewis acid and achiral Lewis base. Al(salen) complex functions as a Lewis acid to activate the ketonic oxygen and Ph_3PO acts as a Lewis base for activation of TMSCN (Fig. 1).

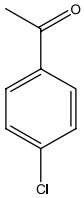
o-Chlorophenyl methyl ketone appears susceptible to steric effect compared to *p*-chloro- and *m*-chlorophenyl methyl ketone so as to give the much longer reaction time (23, 11 and 6 h) and lower % ee (62, 77 and 91%) although the % yield are nearly unaffected (entries 1, 2, 3 and 4). *p*-Chloro- and *m*-chloro group shorten the reaction time from 19 to 11 and 6 h (entries 1, 2 and 3). Electron-withdrawing substituents (entries 2, 4, 5, 6 and 7) exert favorable influence for the cyanosilylation in terms of reaction time (11, 6, 7, 5 and 2 h) to give excellent % yield and good % ee. On the other hand, electron-donating substituents indicate much longer reaction time (~20 h) and give a little sluggish effect on reactions (entries 8 and 9). The negative influence of longer reaction time is also shown in entry 10(25 h) and entry 11(40 h). However cyanosilylation of isobutylphenone (entry 11) produces excellent ee (92%). *p*-Methoxy phenyl acetone (entry 12), benzyl acetone (entry13) and 4-phenyl-3-buten-2-one (entry 14) take relatively short reaction time (3–4 h) (Table 2).

The % ee is quite good and % yield appears excellent that could be compared with other works.[9–14] The reaction temperature is milder (rt) than the previous cyanosilylations (below −20 °C).[9–11] Cyanosilylations by others[9–14] usually report quite longer reaction time. On the other hand *p*-nitrophenyl methyl ketone underwent cyanosilylation for only 2 h in our hand. The oxazaborolidium-catalyzed cyanosilylation of ketones[14] takes place at temperature of 25–40 °C that is higher than 0 °C at which similar reactions of the aldehydes[16] occur. Similar increase of reaction temperature from aldehydes (−40 to −50 °C)[15a] to ketones (rt)(present reactions) were also observed for our reactions. Compared to aldehydes, ketonic structure maintains steric hindrance so that TMSCN could add to the carbonyl at elevated temperature. However the steric effects render reaction time much longer for ketones[14] than aldehydes.[16] Such difference of reaction time between aldehydes[15a] and ketones (present work) appears not significant in our reactions.

3. Conclusion

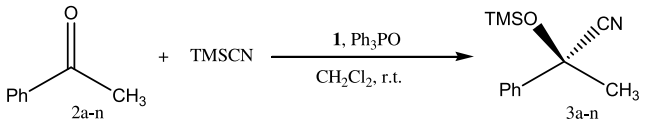
A highly efficient double activation catalysis by **1**/Ph_3PO has been developed for the enantioselective cyanosilylations of various ketones. The cyanosilylations take place under comparatively mild conditions in terms of temperature and reaction time. Several ketones with electron-withdrawing groups in phenyl ring (entries 2, 3 and 5–7) exhibit quite short reaction time that may indicate nucleophilic addition of CN^- group to carboxyl carbon as rate-determining process. Some other ketones without methyl group (entries 12, 13 and 14) undergo cyanosilylation also for quite short reaction time.

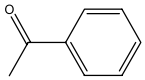
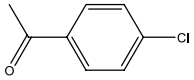
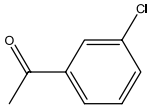
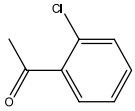
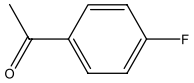
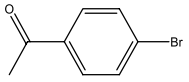
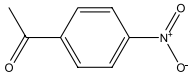
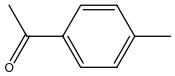
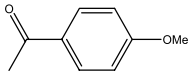
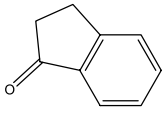
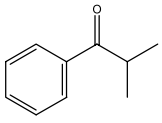
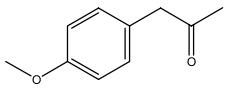
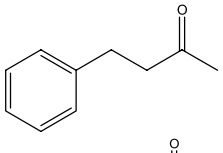
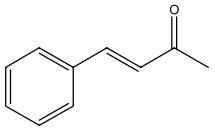
Table 1. Catalytic asymmetric cyanosilylation of methyl *p*-chlorophenyl ketone under various condition[a]

Entry	Substrate	Solvent	Temperature	1 (mol%)	Time (h)	Yield (%)	ee (%)	M (mol/l)
1[b]		CH_2Cl_2	rt	1	40	—	—	1
2[c]		CH_2Cl_2	rt	1	20	80	74	1
3[d]		CH_2Cl_2	rt	1	10	98	75	1
4		CH_2Cl_2	rt	1	11	98	77	1
5		$CHCl_3$	rt	1	12	92	68	1
6		THF	rt	1	16	97	72	1
7		Toluene	rt	1	20	67	69	1
8		CH_2Cl_2	rt	1	21	96	75	0.5
9		CH_2Cl_2	rt	1	7	96	73	2
10		CH_2Cl_2	0 °C	1	34	97	76	1
11		CH_2Cl_2	−10 °C	1	72	93	77	1
12		CH_2Cl_2	−40 °C	1	200	10	81	1
13		CH_2Cl_2	rt	3	9	97	68	1
14		CH_2Cl_2	rt	5	3	99	72	1
15		CH_2Cl_2	rt	10	3	98	57	1

[a] 10 mol% Ph_3PO has been used for all the cyanosilylation, except for otherwise stated.
[b] 0 mol% Ph_3PO has been used for the cyanosilylation.
[c] 5 mol% Ph_3PO has been used for the cyanosilylation.
[d] 20 mol% Ph_3PO has been used for the cyanosilylation.

Table 2. Catalytic Asymmetric Cyanosilylation of Ketones Catalyzed by **1**/$POPh_3$[a]



Entry	Substrate[b]	Time (h)	Yield (%)	ee (%)	Config.[c]
1		19	93	78	S[d]
2		11	97	77	S[d]
3		6	98	91	S[d]
4		23	98	62	—
5		7	95	73	—
6		5	96	73	—
7		2	95	72	—
8		21	90	66	S[d]
9		20	87	71	—
10		25	87	68	S[d]
11		40	80	92	—
12		3	85	65	—
13		3	90	75	S[d]
14		4	75	60	S[d]

[a] CH_2Cl_2 is the solvent for the reactions. The reactions were run at rt 1 mol% **1** was used for all the cyanosilylations. 30 mol% of Ph_3PO has been used except for entry 2. 10 mol% Ph_3PO was employed for the reaction of entry 2.
[b] Substrate concentration is 1 M.
[c] Determined by HPLC (see Refs. 9,11)
[d] The specific rotations carry minus values while the reported ones indicate positive values with R configuration.[9a,11,17]

4. Experimental

4.1. General methods

To a stirred CH_2Cl_2 solution of **1** (1 mol%), $POPh_3$ (30 mol%) was added an aldehyde (2 mmol) and stirred for 30 min at rt TMSCN (2.4 mmol) was added to the reaction mixture using syringe pump and the mixture was reacted at the same temperature for 2–25 h. The solvent was evaporated. The crude product was futher purified by flash chromatography (hexane: ethyl acetate=9:1) to give a cyanohydrin in more than 90% yield. The enantiomeric excesses of some products were determined after conversion to acetylester, ethylcarbonate, and *t*-butyl dimethylsilylether by the known methods. The sample was identified by ^1H, ^{13}C–MR, HRMS and ee % was determined by chiral HPLC column (DAICEL CHIRALCEL OJ-H, DAICEL CHIRALCEL OD-H and DAICEL CHIRALCEL OB-H). All ketones and Al(salen) were purchased from Sigma-Aldrich. ^1H and ^{13}C NMR were taken utilizing Varian Jemini 2000 (200 MHz) or Varian Unity Inova 400 (400 MHz) NMR spectrometer. Hewlett–Packard 5890A Gas Chromatograph/Jeol JMS-DX303 Mass Spectrometer was used for HRMS data. Analytical high performance liquid chromatography (HPLC) was performed on Gilson 305 series HPLC using the indicated chiral column. All data was in accordance with literature values. Absolute configurations were determined by optical rotation, see: (a) Hamashima, Y.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2000**, *122*, 7412; (b) Hamashima, Y.; Kanai, M.; Shibasaki, M. *Tetrahedron Lett*. **2001**, *42*, 691; (c) Yabu, K.; Masumoto, S.; Yamasaki, S.; Hamashima, Y.; kanai, M.; Du, W.; Curran, D.P.; Shibasaki, M. *J. Am. Chem. Soc.* **2001**, *123*, 9908;. and references cited therein.;(c) Deng, H. -B.; Isler, M. P.; Snapper, M. L.; Hoveyda, A. H. *Angew. Chem. Int. Ed*. **2002**, *41*, 1009.

4.1.1. 2-Trimethylsilyloxy-2-phenylpropanenitrile (3a). ^1H NMR ($CDCl_3$) δ 0.19 (s, 9H), 1.87 (s, 3H), 7.38–7.58 (m,5H, aromatic H) ^{13}C NMR ($CDCl_3$) δ 1.03, 33.5, 71.6, 121.6, 128.6, 142.0 $[\alpha]_D^{24}$ −16.0° (*c* 1.18, $CHCl_3$, 78% ee) [lit. $[\alpha]_D^{20}$ +21.9° (*c* 1.18, $CHCl_3$, for *R* enantiomer with 93% ee)][9a] HRMS(M+) calcd for $C_{12}H_{17}NOSi$: 219.1079; found: 219.1082 HPLC (DAICEL CHIRALCEL OB-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 14.9 and 15.4 min.

4.1.2. 2-Trimethylsilyloxy-2-(4′-chlorophenyl)propanenitrile (3b). ^1H NMR ($CDCl_3$) δ 0.21 (s, 9H), 1.83 (s, 3H), 7.38 (m, 2H), 7.49 (m,2H) ^{13}C NMR ($CDCl_3$) δ 1.05, 33.53, 71.06, 121.25, 126.07, 128.83, 134.60, 140.71 $[\alpha]_D^{24}$ −17.3° (*c* 1.78, $CHCl_3$, 77% ee) [lit. $[\alpha]_D^{20}$ +29.5° (*c* 1.04, $CHCl_3$, for *R* enantiomer with 92% ee)][9a] HRMS (M+) calcd for $C_{12}H_{16}ClNOSi$: 253.0690; found: 253.0687 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 19.5 and 20.5 min.

4.1.3. 2-Trimethylsilyloxy-2-(3′-chlorophenyl)propanenitrile (3c). ^1H NMR ($CDCl_3$) δ 0.22 (s, 9H), 1.86 (s, 3H), 7.34–7.55 (m, 4H aromatic H) ^{13}C NMR ($CDCl_3$) δ 1.0, 33.4, 70.9, 121.0, 122.7, 124.8, 128.8, 129.9, 134.6, 144.0 $[\alpha]_D^{24}$ −19.3° (*c* 1.28, $CHCl_3$, 91% ee) [lit. $[\alpha]_D^{26}$ +7.1° (*c* 0.34, $CHCl_3$, for *R* enantiomer with 33% ee)][17] HRMS(M+) calcd for $C_{12}H_{16}ClNOSi$: 253.0690; found: 253.0692 HPLC (DAICEL CHIRALCEL OB-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 14.6 and 17.4 min.

4.1.4. 2-Trimethylsilyloxy-2-(2′-chlorophenyl)propanenitrile (3d). ^1H NMR ($CDCl_3$) δ 0.23 (s, 9H), 1.88 (s, 3H), 7.37–7.62 (m, 4H aromatic H) ^{13}C NMR ($CDCl_3$) δ 1.0, 33.4, 70.9, 121.0, 122.7, 124.8, 128.8, 129.9, 134.6, 144.0 $[\alpha]_D^{24}$ −12.3° (*c* 1.58, $CHCl_3$, 62% ee) HRMS(M+) calcd for $C_{12}H_{16}ClNOSi$: 253.0691; found: 253.0687 HPLC (DAICEL CHIRALCEL OD-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 24.2 and 25.0 min.

4.1.5. 2-Trimethylsilyloxy-2-(4′-fluorophenyl)propanenitrile (3e). ^1H NMR ($CDCl_3$) δ 0.18 (s, 9H), 1.84 (s, 3H), 7.08 (m, 2H), 7.52 (m, 2H) ^{13}C NMR ($CDCl_3$) δ 1.0, 33.5, 71.0, 115.6, 121.4, 126.5, 138.0, 162.2 $[\alpha]_D^{22}$ −17.3° (*c* 1.4, $CHCl_3$, 73% ee) HRMS(M+) calcd for $C_{12}H_{16}FNOSi$: 237.0985; found: 237.0981 HPLC (DAICEL CHIRALCEL OB-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 16.4 and 17.8 min.

4.1.6. 2-Trimethylsilyloxy-2-(4′-bromophenyl)propanenitrile (3f). ^1H NMR ($CDCl_3$) δ 0.19 (s, 9H), 1.83 (s, 3H), 7.40–7.4 (m, 2H), 7.51–7.55 (m, 2H) ^{13}C NMR ($CDCl_3$) δ 1.0, 33.4, 71.0, 121.1, 122.7, 126.3, 131.7, 141.2 $[\alpha]_D^{22}$ −14.6° (*c* 1.69, $CHCl_3$, 73% ee) HRMS(M+) calcd for $C_{12}H_{16}BrNOSi$: 297.0185; found: 297.0181 HPLC (DAICEL CHIRALCEL OB-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 16.4 and 17.8 min.

4.1.7. 2-Trimethylsilyloxy-2-(4′-nitrophenyl)propanenitrile (3g). ^1H NMR ($CDCl_3$) δ 0.26 (s, 9H), 1.89 (s, 3H), 7.75 (d, 2H), 8.30 (d, 2H) ^{13}C NMR ($CDCl_3$) δ 1.0, 33.5, 71.0, 115.6, 121.4, 126.5, 138.0, 162.2 $[\alpha]_D^{22}$ −15.3° (*c* 1.65, $CHCl_3$, 72% ee) HRMS(M+) calcd for $C_{12}H_{16}N_2O_3Si$: 264.0930; found: 264.0933 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 51.38 and 54.61 min.

4.1.8. 2-Trimethylsilyloxy-2-(4′-methylphenyl)propanenitrile (3h). ^1H NMR ($CDCl_3$) δ 0.16 (s, 9H), 1.84 (s, 3H), 2.36 (s, 3H), 7.21 (m, 2H), 7.43 (m, 2H) ^{13}C NMR ($CDCl_3$) δ 1.1, 20.8, 33.5, 71.8, 121.9, 124.9, 128.3, 138.4, 139.8 $[\alpha]_D^{23}$ −16.1° (*c* 1.65, $CHCl_3$, 72% ee) [lit. $[\alpha]_D^{25}$ +21.3° (*c* 1.28, $CHCl_3$, for *R* enantiomer with 90% ee)]9a HRMS(M+) calcd for $C_{13}H_{19}NOSi$: 233.1236; found: 233.1240 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 51.38 and 54.61 min.

4.1.9. 2-Trimethylsilyloxy-2-(4′-methoxylphenyl)propanenitrile (3i). ^1H NMR ($CDCl_3$) δ 0.19(s, 9H), 1.87(s, 3H), 3.85(s, 3H), 6.93(m, 2H), 7.49(m, 2H) ^{13}C NMR ($CDCl_3$) δ 1.1, 33.41, 55.33, 71.28, 113.89, 121.81, 126.06, 134.04, 159.80 $[\alpha]_D^{23}$ −17.0° (*c* 1.44, $CHCl_3$, 71% ee) HRMS(M+) calcd for $C_{13}H_{19}NO_2Si$: 249.1185; found: 249.1182 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 23.61 and 25.54 min.

4.1.10. 2-(Trimethylsilyloxy)indane-1-carbonitrile (3j). ^1H NMR ($CDCl_3$) δ 0.20 (s, 9H), 2.43–2.47 (m, 1H),

2.70–2.74 (m, 1H), 2.97–3.02 (m, 1H), 3.10–3.15 (m, 1H), 7.28 (d, 1H), 7.31 (t, 1H), 7.36 (t, 1H), 7.55 (d, 1H) ^{13}C NMR ($CDCl_3$) δ 1.1, 29.4, 42.9, 76.8, 121.3, 124.5, 125.3, 127.8, 129.9, 142.7, 142.9 $[\alpha]_D^{23}$ −19.4° (*c* 1.4, $CHCl_3$, 68% ee) [lit. $[\alpha]_D^{20}$ +24.5° (*c* 2.18 CH_2Cl_2, for *R* enantiomer with 65% *ee*)][9a] HRMS(M+) calcd for $C_{13}H_{17}NOSi$: 231.1079; found: 231.1082 HPLC (DAICEL CHIRALCEL OD, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 3.27 and 3.64 min.

4.1.11. 2-Trimethylsilyloxy-2-phenyl-3-methyl-butanenitrile (3k). ^{1}H NMR ($CDCl_3$) δ 0.12 (s, 9H), δ 1.03 (q, *J*=7.4 Hz 6H), 1.97 (m, 1H), 7.38 (m, 3H), 7.50 (m, 2H) $[\alpha]_D^{20}$ −45.9° (*c* 4.1, $CHCl_3$, 92% ee) HRMS(M+) calcd for $C_{14}H_{21}NOSi$: 247.1392; found: 247.1395 HPLC (DAICEL CHIRALCEL OD, ^{i}PrOH/hexane=1/99, flow=0.5 mL/min) 6.36 and 6.84 min.

4.1.12. 2-Trimethylsilyloxy-3-(4-methoxyphenyl)-2-methyl-propanenitrile (3l). ^{1}H NMR ($CDCl_3$) δ 0.1 (s, 9H), δ 1.73 (s, 1H), 2.71–2.93 (m, 2H), 3.75 (s, 3H), 6.76–7.23 (m, 4H) $[\alpha]_D^{20}$ −1.2° (*c* 4.1, $CHCl_3$, 65% ee) HRMS(M+) calcd for $C_{14}H_{21}NO_2Si$: 263.1342; found: 263.1345 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0.25 mL/min) 24.72 and 27.38 min.

4.1.13. 2-Trimethylsilyloxy-2-methyl-4-phenylbutanenitrile (3m). ^{1}H NMR ($CDCl_3$) δ 0.27 (s, 9H), δ 1.61 (s, 3H), 2.02 (m, 2H), 2.78(d, 2H) 2.87 (d, 2H), 7.19–7.22 (m, 3H), 7.28–7.3 (m, 2H) ^{13}C NMR ($CDCl_3$) δ 1.3, 29.4, 30.7, 45.2, 69.4, 121.9, 126.1, 128.3, 128.5, 140.7 $[\alpha]_D^{20}$ −10.5° (*c* 4.8, $CHCl_3$, 75% ee) [lit. $[\alpha]_D^{24}$ +13.3° (*c* 1.15, $CHCl_3$, for *R* enantiomer with 81% ee)][9a] HRMS(M+) calcd for $C_{14}H_{21}NOSi$: 247.1392; found: 247.1390 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0 .5 mL/min) 13.90 and 17.34 min.

4.1.14. 2-Trimethylsilyloxy-2-methyl-4-phenyl-3-butenenitrile (3n). ^{1}H NMR ($CDCl_3$) δ 0.29 (s, 9H), δ 1.77 (s, 3H), 6.16 (d, 1H), 6.91(d, 1H) 7.31 (m, 1H), 7.39 (m, 2H), 7.44 (m, 2H) ^{13}C NMR ($CDCl_3$) δ 1.4, 30.8, 69.9, 120.6, 126.9, 128.6, 128.7, 129.5, 130.9, 135.1 $[\alpha]_D^{20}$ −17.5° (*c* 2.4, $CHCl_3$, 60% ee) [lit. $[\alpha]_D^{25}$ +21.3° (*c* 1.34, $CHCl_3$, for *R* enantiomer with 91% ee)][11] HRMS(M+) calcd for $C_{14}H_{19}NOSi$: 245.1236; found: 245.1233 HPLC (DAICEL CHIRALCEL OJ-H, ^{i}PrOH/hexane=1/99, flow=0.5 mL/min) 11.36 and 14.02 min.

Acknowledgements

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Asymmetric synthesis of phosphonic acid analogues for acylcarnitine

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Abstract—Phosphonic acid analogues of acylcarnitine were prepared in an optically active form expecting CPT I inhibitory activities. The synthetic methodology was based on catalytic asymmetric dihydroxylation of β,γ-unsaturated phosphonates and subsequent regioselective amination via the cyclic sulfates.

1. Introduction

Hyperglycemia of type II diabetes is considered to be primarily due to excess long-chain fatty acid oxidation, which is crucial to drive gluconeogenesis at higher rates.[1] Carnitine palmitoyltransferase I (CPT I) located on the outer mitochondrial membrane plays an important role in β-oxidation of long-chain free fatty acids, which catalyzes a formation of acylcarnitine from carnitine and long-chain fatty acids, then a translocase transports the fatty acids carnitine esters across the inner mitochondrial membrane.[2] CPT I inhibitors indirectly reduce gluconeogenesis by inhibiting the β-oxidation and are hence helpful in the treatment of type II diabetes as hypoglycemic agents.[3] Recent studies demonstrated modification of the functional groups of acylcarnitine was one access to the development of potent CPT I inhibitors. In these studies, aminoacylcarnitine derivatives, in which the β-oxygen atom of acylcarnitine was replaced with nitrogen, were discovered as good inhibitors.[4] The absolute configuration of these molecules was found to influence their inhibitory activities; the (*R*)-isomer inhibited CPT I more strongly than the corresponding enantiomer.

Since phosphonic acids are known to function as a bioisosteric group of carboxylic acids,[5] modification of the carboxylic moiety of acylcarnitine has been also examined. To date, three kinds of phosphonic acid analogues of acylcarnitine were prepared as racemate and showed modest activities.[4a] However, the structure–activity relationship of phosphonic acid analogues for acylcarnitine including effects of the chirality on activities has never been investigated probably due to lack of a general method for asymmetric synthesis of γ-amino-β-acyloxyphosphonic acid derivatives. Then, we investigated a new method for asymmetric synthesis of γ-amino-β-acyloxyphosphonates and their transformation to phosphonic acid analogues of acylcarnitine (Fig. 1). In this paper, we now describe full details of our study.[6]

2. Result and discussion

For obtaining the targeted molecules, a protected form of chiral γ-amino-β-hydroxyphosphonates would be required as synthetic intermediates. Inspection of the literature revealed that the key reactions used in this synthesis involved optical resolution,[7a] enzymatic resolution of γ-chloro-β-hydroxyphosphonates,[7b] hydrolytic kinetic resolution of epoxyphosphonates with an asymmetric catalyst,[7c] ring-opening of epichlorohydrin with silylphosphites,[7d] and diastereoselective reduction of γ-amino-β-ketophosphonates.[7e] Although

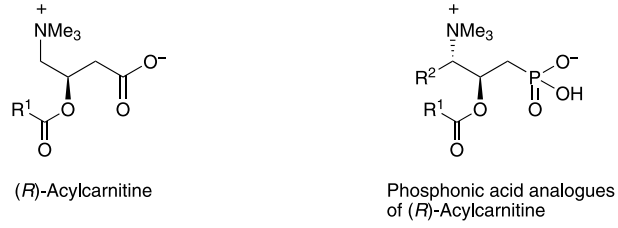

Figure 1.

Keywords: Acylcarnitine; Phosphonic acid; Asymmetric dihydroxylations; Regioselectivity.
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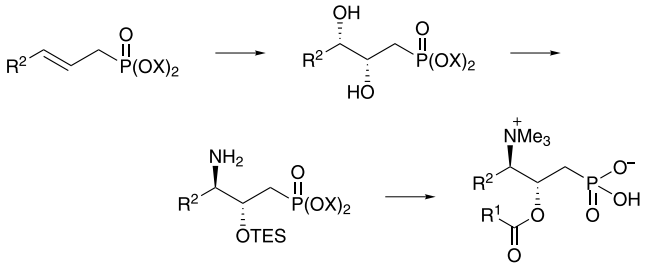
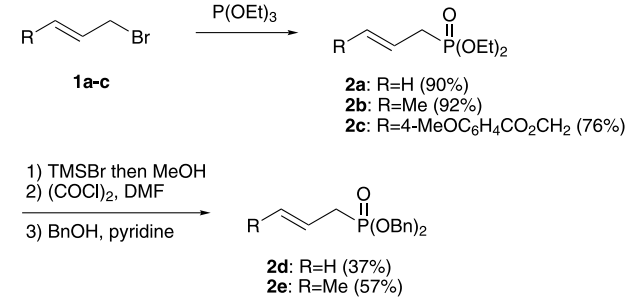
catalytic asymmetric aminohydroxylation of β,γ-unsaturated phosphonates developed by Sharpless et al. is an attractive method, the chemical yields were not satisfactory due to formation of the regioisomer of the desired amino alcohols and diols as byproducts.[7f] We examined asymmetric synthesis of γ-amino-β-acyloxyphosphonic acid derivatives by our own approach, which involved osmium-catalyzed asymmetric dihydroxylation (AD)[8] of β,γ-unsaturated phosphonates, followed by selective amination of the hydroxy group at the γ-position (Scheme 1).



Scheme 1.

2.1. AD reactions of β,γ-unsaturated phosphonates

The requisite starting materials **2a–c** were readily prepared by Arbuzov reactions of the corresponding allyl bromide derivatives **1a–c** with triethylphosphite (Scheme 2). The compounds having a dibenzylphosphonyl group, **2d** and **2e**, were prepared from **2a** and **2b**, respectively, through sequential deesterification, chlorination of the acid moiety, followed by treatment with benzyl alcohol and pyridine.



Scheme 2.

AD reaction of β,γ-unsaturated phosphonate having a phenyl group at the γ-position with AD-mix reagents was previously reported by us to provide diols in high ee (98% ee).[9] According to the reported protocol, AD reactions of **2a–e** were performed with AD-mix-α in the presence of $MeSO_2NH_2$ and additional $K_2OsO_4\cdot 2H_2O$ (0.8 mol%) in the *t*-BuOH–H_2O solvent system (1/1) at room temperature.[10] The results are summarized in Table 1.

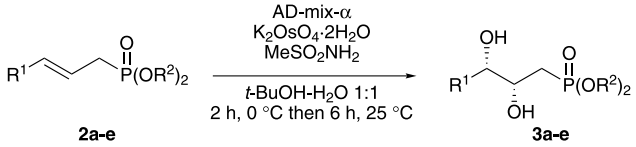
In the series of AD reactions of ethyl esters **2a–c** with AD-mix-α, very low enantioselectivity (10% ee) was observed when R^1 is a proton (entry 1). However, the enantioselectivity was slightly increased to 35% ee when R^1 is a methyl group (entry 2). Excellent selectivity (97% ee) was observed in the case of **2c** having a 4-methoxybenzoyloxymethylene group as R^1 substituent (entry 3). In the series of AD reactions of benzyl esters **2d**,**e**, the reaction with **2d** having a proton as R^1 substituent also proceeded in poor ee (10% ee) as in the case of the corresponding ethyl ester **2a** (entry 4).[11] However, **2e** having a methyl group as R^1 substituent reacted with AD-mix-α reagent to give **3e** in 61% ee (entry 5). It is worthy of note that the enantioselectivity for **2e** significantly improved in comparison to that for **2b** (35% ee) and crystalline **3e** could be obtained in an optically pure form (>99% ee) through one recrystallization from AcOEt (38% recovery).

Considering Corey's working model of the chemical architecture provided by the ligand of AD-mix-α, in which the ligand–osmium complex preferred the U-shaped conformation, the high ee of **3c** was accounted for by the 4-methoxybenzoyl group participating in π-stacking interactions with the methoxyquinoline ring of the catalyst.[12] An improved ee of **3e** may be ascribed to the enhanced hydrophobicity of substrate **2e** compared to **2b**, which facilitated substrates to be trapped into the lipophilic cavity of the ligand–osmium complex.[13]

Similar reaction of **2c** using AD-mix-β instead of AD-mix-α proceeded to give *ent*-**3c** in good selectivity (98% ee) (Scheme 3). The reaction of **2e** furnished product *ent*-**3e** in 68% ee, which could be increased to >99% ee by one recrystallization from AcOEt (59% recovery).

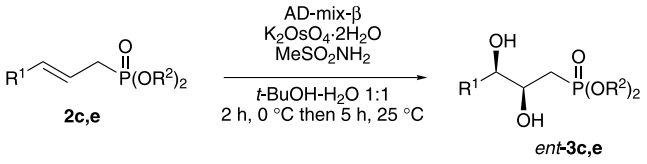
The absolute stereochemistry of **3a** was verified to be *R* by comparison of optical rotation with that reported.[14]

Table 1. AD reactions of β,γ-unsaturated phosphonates **2a–e** with AD-mix-α



Entry	**3**	R^1	R^2	Yield (%)	ee (%)[a]
1	**a**	H	Et	41	10
2	**b**	Me	Et	60	35
3	**c**	4-$MeOC_6H_4CO_2CH_2$	Et	58	97[b]
4	**d**	H	Bn	71	10
5	**e**	Me	Bn	69	61[c]

[a] Determined by ^{31}P NMR (121 MHz, $CDCl_3$) analysis of the corresponding bis-MTPA esters unless stated otherwise.
[b] Determined by HPLC analysis on a chiral phase (DAICEL CHIRALPAK AS column).
[c] Determined by HPLC analysis on a chiral phase (DAICEL CHIRALPAK OD column).



ent-**3**	R^1	R^2	yield (%)	ee (%)
c	4-$MeOC_6H_4CO_2CH_2$	Et	70	98
e	Me	Bn	64	68 (>99)[a]

[a] After one recrystallization from AcOEt

Scheme 3.

The absolute configuration of **3b** and **3c** was determined after conversion to 4-methoxybenzoate derivatives **5b** and **5c** (Scheme 4). The CD spectrum of **5b** showed a positive Cotton effect at 289 nm and a negative Cotton effect at 282 nm, which were analogous to that of methoxybenzoate **6** prepared from known β,γ-dihydroxyphosphonate **4**,[9] showing positive and negative Cotton effects at longer (286 nm) and shorter wavelengths (275 nm), respectively. The CD curve of **5c** (a positive Cotton effect at 284 nm and a negative Cotton effect at 276 nm) was also similar to that of **6**. Accordingly, **5b** and **5c** have the same absolute stereochemistry with **6**, indicating the stereochemistry of **3b** and **3c** was as shown in Scheme 4.

The absolute configuration of **3e** was verified after transformation to the corresponding phosphonic acid **7** through hydrogenolysis of the dibenzyl phosphonate moiety (Scheme 5). The sign of the optical rotation of **7** was identical with that of a sample derived from **3b**.

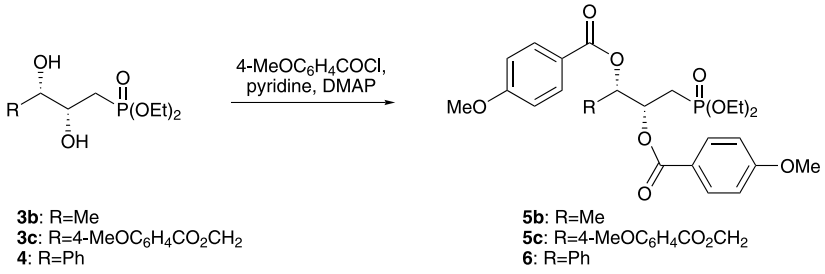
2.2. Synthesis of phosphonic acid analogues of acylcarnitine

With chiral β,γ-dihydroxyphosphonates in hand, we next directed our efforts toward the synthesis of phosphonic acid analogues of acylcarnitine. In these efforts, we first chose **3a** to tackle its transformation to phosphonic acid analogue **15** of acylcarnitine through the regioselective amination via a cyclic sulfate (Scheme 6).[15] Although the optical purity of **3a** is not sufficient, it will be valuable to obtain optically active **15** since the compound was previously prepared in racemic form.[4a]

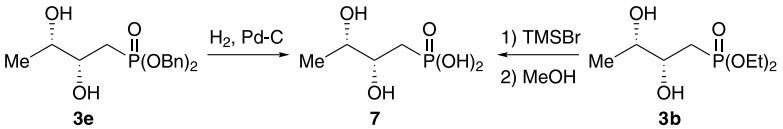
Treatment of **3a** with $SOCl_2$, followed by oxidation with $RuCl_3$–$NaIO_4$[16] afforded cyclic sulfate **8** in 80% yield. When **8** was treated with NaN_3 in acetone–H_2O at 50 °C, the starting material disappeared within 3 h on TLC. Subsequent treatment with 20% H_2SO_4 gave γ-azido-β-hydroxyphosphonate **9** with complete regioselectivity in 24% yield for two steps. Although an exact reason for the low chemical yield remained unclear, it might be associated with partial decomposition of the diethyl phosphonate moiety into the corresponding aqueous phosphonic acid or monoethyl ester in the reaction mixture. After transformation to TES ether **10**, catalytic hydrogenation was carried out to give γ-amino-β-silyloxyphosphonate **11**. Reductive dimethylation of **11** with formaldehyde and $NaBH_3CN$ provided **12** in 90% yield.[17] Deprotection of the silyl group, followed by treatment with myristoyl chloride in the presence of pyridine and DMAP afforded **13**, which underwent *N*-methylation with MeI to give **14**. Finally, **14** was deprotected with TMSBr and MeOH to give the desired **15** in 72% yield.

The methodology was next applied to the synthesis of γ-methyl-substituted phosphonic acid analogue **27** of acylcarnitine from diol **3e**. In this synthesis, γ-selective amination of the starting **3e** is a critical step because the corresponding cyclic sulfate **16** is prone to react with an azide anion at both β- and γ-positions. However, a molecular modeling of **16** reveals that the β-carbon is sterically more congested than the γ-carbon owing to the bulky dibenzyl phosphonate moiety in the most stable conformation and regioselective ring-opening reaction would be feasible due to the steric reason.[18] Then, we examined several representative conditions for ring-opening reaction of **16**, prepared from optically pure **3e** in an analogous manner to that for preparation of **8**, with metal azides (Table 2).

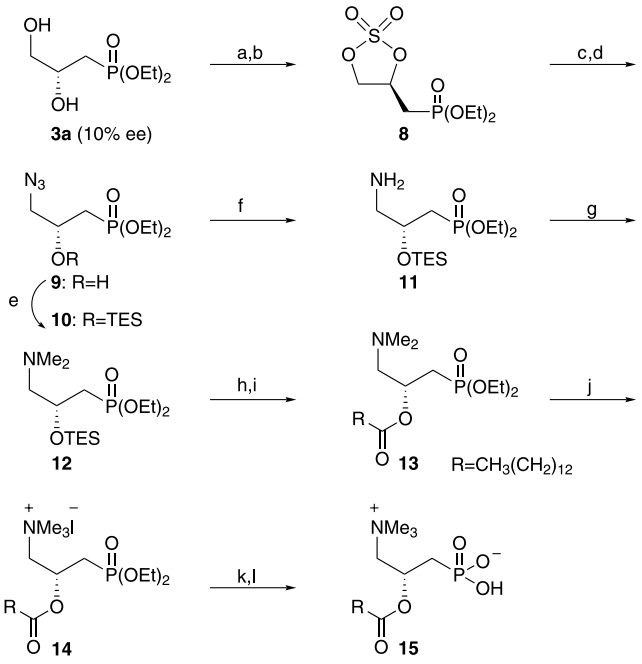
Upon treatment of **16** with LiN_3 in DMF at room temperature, the starting material disappeared within 12 h on TLC (entry 1). Quenching the reaction with 50% H_2SO_4



Scheme 4.



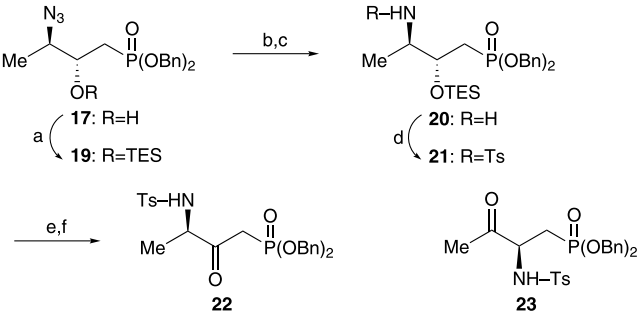
Scheme 5.



Scheme 6. Reagents and conditions: (a) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C; (b) $RuCl_3 \cdot nH_2O$, $NaIO_4$, CCl_4–CH_3CN–H_2O, 0 °C (80%, two steps); (c) NaN_3, acetone–H_2O, 50 °C; (d) 20% H_2SO_4, Et_2O, rt (24%, two steps); (e) TESCl, imidazole, DMF, rt (80%); (f) H_2, 10% Pd–C, MeOH, rt (97%); (g) 37% HCHO, $NaBH_3CN$, AcOH, CH_3CN, rt (90%); (h) TBAF, THF, 0 °C; (i) $CH_3(CH_2)_{12}COCl$, pyridine, DMAP, rt (52%, two steps); (j) MeI, acetone, rt (86%); (k) TMSBr, CH_2Cl_2, rt; (l) MeOH, rt (72%, two steps).

gave γ-azide **17** and β-azide **18** with a ratio of 5:1. Although the regioselectivity of the reaction was found to be the desired sense as expected, the chemical yield was poor (13%). Similar yield and ratio were observed when NaN_3 was used as an azide anion (entry 2). However, when the reaction was carried out in acetone–H_2O instead of DMF at 25 °C and subsequent treatment with 20% H_2SO_4, both yield (41%) and **17**/**18** ratio (10:1) were improved (entry 3). Increasing the reaction temperature up to 50 °C shortened the reaction time from 96 to 3 h (entry 4).

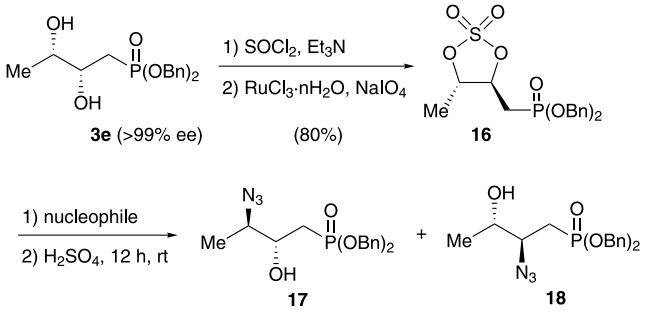
The structure of **17** was deduced after conversion into γ-amino-β-ketophosphonate **22** (Scheme 7). Staudinger reaction of TES ether **19** derived from **17** with PPh_3 and subsequent hydrolysis afforded γ-amino-β-siloxyphosphonate **20**. Tosylation of **20**, followed by desilylation, and oxidation with PDC gave **22**. In the ^1H NMR spectrum (400 MHz, $CDCl_3$) of **22**, a signal ascribed to the Me group at the γ-position was observed at 1.18 ppm as a doublet (J = 7.1 Hz) but not as a singlet corresponding to regioisomeric product **23**.



Scheme 7. Reagents and conditions: (a) TESCl, imidazole, DMF, rt (98%); (b) PPh_3, THF, rt; (c) H_2O, rt (97%, two steps); (d) TsCl, Et_3N, CH_2Cl_2, rt (13%); (e) TBAF, THF, 0 °C; (f) PDC, CH_2Cl_2, rt (47%, two steps).

Compound **20** was converted into **26** in the same manner to the case of **14** (Scheme 8). In this synthesis, deprotection of the benzyl ester moiety of **26** was attempted through hydrogenolysis in the presence of 10% Pd–C or 20% $Pd(OH)_2$–C. However, the hydrogenolysis did not work to give **27** in reproducible yield after several trials. Then, γ-methyl-substituted phosphonic acid analogue **27** of acylcarnitine was obtained through the TMSBr-mediated debenzylation of **26** in 48% yield (two steps).

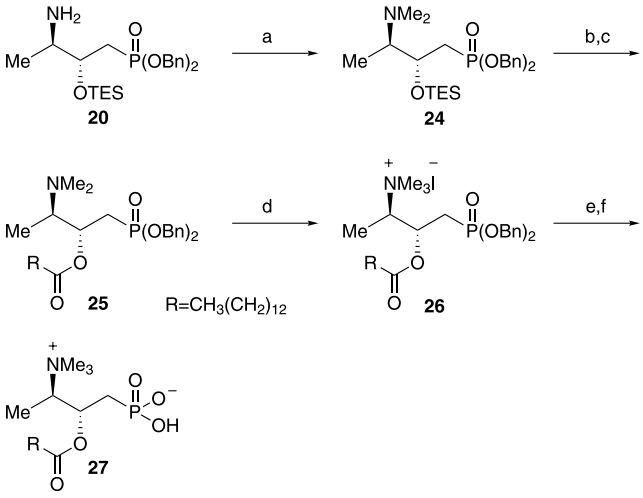
Table 2. Ring-opening reactions of **16** with metal azides



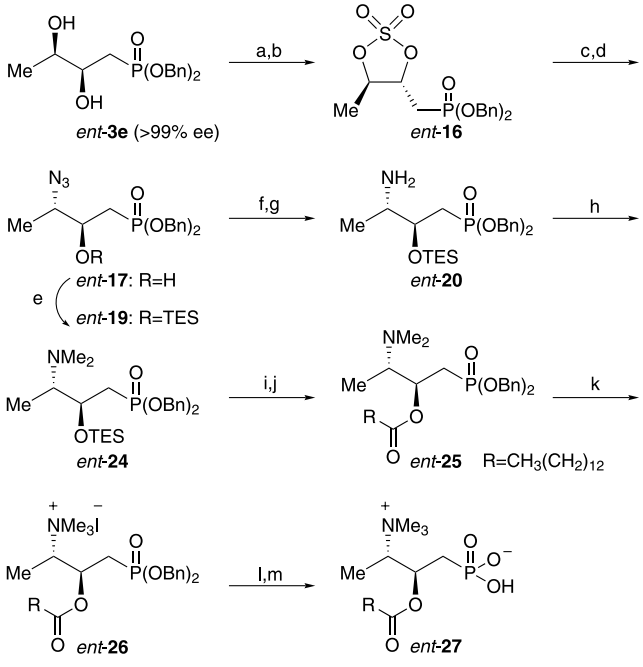
Entry	Nucleophile	Solvent	Temperature (°C)	Time (h)	H_2SO_4 (%)	Yield (%)[a]	**17**:**18**[b]
1	LiN_3	DMF	25	12	50	13	5:1
2	NaN_3	DMF	50	8	50	15	4:1
3	NaN_3	Acetone/H_2O 2:1	25	96	20	41	10:1
4	NaN_3	Acetone/H_2O 2:1	50	3	20	42	10:1

[a] Combined yield of **17** and **18**.
[b] Determined by ^{31}P NMR (121 MHz, $CDCl_3$) analysis of crude products.

The synthesis of enantiomer *ent*-**27** of **27** was also achieved starting from optically pure *ent*-**3e** through the same sequence (Scheme 9). Thus, we have obtained both enantiomers of γ-methyl substituted phosphonic acid analogues of acylcarnitine.



Scheme 8. Reagents and conditions: (a) 37% HCHO, $NaBH_3CN$, AcOH, CH_3CN, rt (85%); (b) TBAF, THF, 0 °C; (c) $CH_3(CH_2)_{12}COCl$, pyridine, DMAP, rt (23%, two steps); (d) MeI, acetone, rt (33%); (e) TMSBr, CH_2Cl_2, rt; (f) MeOH, rt (48%, two steps).



Scheme 9. Reagents and conditions: (a) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C; (b) $RuCl_3 \cdot nH_2O$, $NaIO_4$, CCl_4–CH_3CN–H_2O, 0 °C (76%, two steps); (c) NaN_3, acetone–H_2O, 50 °C; (d) 20% H_2SO_4 , Et_2O, rt (44%, two steps); (e) TESCl, imidazole, DMF, rt (97%); (f) PPh_3, THF, rt; (g) H_2O, rt (94%, two steps); (h) 37% HCHO, $NaBH_3CN$, AcOH, CH_3CN, rt (96%); (i) TBAF, THF, 0 °C; (j) $CH_3(CH_2)_{12}COCl$, pyridine, DMAP, rt (30%, two steps); (k) MeI, acetone, rt (30%); (l) TMSBr, CH_2Cl_2, rt; (m) MeOH, rt (34%, two steps).

3. Conclusion

In conclusion, we have developed a new method for preparing chiral γ-amino-β-acyloxyphosphonic acid derivatives through AD reactions of β,γ-unsaturated phosphonates and subsequent regioselective amination via cyclic sulfates. The method was applicable to the synthesis of optically active phosphonic acid analogues of acylcarnitine. A study on preparing a variety of derivatives and their CPT I inhibitory activities is underway.

4. Experimental

4.1. General

All melting points were taken on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 or P1030 digiital polarimeter. IR spectra were recorded on a JASCO FTIR-620. Mass spectra were measured on a Finnigan TSQ-700. Elemental analysis were recorded on an Elemental Vavio EL. NMR spectra were obtained on Bruker DPX400 NMR specrometer operating at 400 MHz for ^1H, 100 MHz for ^{13}C, and 162 MHz for ^{31}P. The chemical shift data for each signal on ^1H NMR are given in units of δ relative to $CHCl_3$ ($\delta=7.26$) for $CDCl_3$ solution. For ^{13}C NMR spectra, the chemical shifts in $CDCl_3$ are reported relative to the $CDCl_3$ resonance ($\delta=77.0$). The chemical shifts of ^{31}P are recorded relative to external 85% H_3PO_4 ($\delta=0$) with broad-band ^1H decoupling.

4.1.1. Diethyl allylphosphonate (2a). To a stirred suspension of **1a** (12.3 g, 100 mmol) and KI (16.6 g, 100 mmol) in acetone–CH_3CN 10:8 (500 mL) was added triethyl phosphite (17.2 mL, 100 mmol). The mixture was stirred for 12 h at room temperature and then stirred for 5 h at 60 °C. After filtration of the mixture, the filtrate was concentrated to give a residue, which was distilled (73–75 °C, 5 mmHg) to give **2a** (16.0 g, 90%). A colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.80–5.74 (1H, m), 5.23–5.16 (2H, m), 4.13–4.04 (4H, m), 2.59 (2H, dd, $J=21.9$, 7.4 Hz), 1.30 (6H, t, $J=7.1$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 127.4 (d, $J_{CP}=11.2$ Hz), 119.7 (d, $J_{CP}=14.4$ Hz), 61.7 (d, $J_{CP}=6.6$ Hz), 31.6 (d, $J_{CP}=139.3$ Hz), 16.2 (d, $J_{CP}=5.9$ Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.46; IR (neat) 2983, 1255, 1027 cm^{-1}; ESIMS *m/z* 179 (MH^+); HRMS (ESI) calcd for $C_7H_{16}O_3P$: 179.0873 (MH^+). Found: 179.0840.

4.1.2. Diethyl (2*E*)-but-2-enylphosphonate (2b). Compound **2b** was prepared from **1b** (13.8 g, 100 mmol) in an analogous manner to that for preparation of **2a**. Purification of the residue by distillation (85–88 °C, 7 mmHg) gave **2b** (16.4 g, 92%). A colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.64–5.58 (1H, m), 5.44–5.38 (1H, m), 4.14–4.05 (4H, m), 2.57–2.49 (2H, m), 1.71–1.64 (3H, m), 1.31 (6H, t, $J=$ 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.5 (d, $J_{CP}=$ 14.6 Hz), 119.5 (d, $J_{CP}=11.2$ Hz), 61.7 (d, $J_{CP}=6.6$ Hz), 30.3 (d, $J_{CP}=139.9$ Hz), 17.9 (d, $J_{CP}=2.2$ Hz), 16.3 (d, $J_{CP}=6.0$ Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.53; IR (neat) 2982, 1251, 1027 cm^{-1}; ESIMS *m/z* 193 (MH^+);

HRMS (ESI) calcd for $C_8H_{18}O_3P$: 193.0994 (MH^+). Found: 193.0993.

4.1.3. (2*E*)-4-(Diethoxyphosphoryl)but-2-enyl 4-methoxybenzoate (2c). A mixture of **1c** (9.36 g, 32.8 mmol) and triethyl phosphite (6.75 mL, 39.4 mmol) was stirred for 12 h at 160 °C. After being cooled, the mixture was chromatographed on silica gel (hexane/EtOAc=1:2) to give **2c** (10.2 g, 76%). A pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (2H, d, J=8.9 Hz), 6.90 (2H, d, J=8.9 Hz), 5.94–5.84 (2H, m), 4.78–4.75 (2H, m), 4.19 (4H, q, J=7.1 Hz), 3.85 (3H, s), 2.63 (2H, dd, J=21.8, 5.8 Hz), 1.28 (6H, t, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 163.4, 131.6, 129.5 (d, J_{CP}=14.5 Hz), 124.1 (d, J_{CP}=11.1 Hz), 122.4, 113.5, 64.4, 62.0 (d, J_{CP}=6.7 Hz), 55.3, 30.3 (d, J_{CP}=139.9 Hz), 16.3 (d, J_{CP}=5.9 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.72; IR (neat) 2983, 1715, 1257, 1167 cm^{-1}; ESIMS m/z 343 (MH^+); HRMS (ESI) calcd for $C_{16}H_{24}O_6P$: 343.1311 (MH^+). Found: 343.1289.

4.1.4. Dibenzyl allylphosphonate (2d). To a stirred solution of **2a** (1.60 g, 10 mmol) in CH_2Cl_2 (20 mL) was added TMSBr (5.3 mL, 40 mmol) at room temperature. The mixture was stirred for 9 h at the same temperature and then concentrated to give a residue. To an ice-cooled, stirred solution of this residue in CH_2Cl_2 (20 mL) was slowly added oxalyl chloride (3.0 mL, 35 mmol) and DMF (a few drops). After being stirred for 6 h at room temperature, the mixture was concentrated to give a residue. To a stirred solution of this residue in THF (30 mL) was added benzyl alcohol (2.3 mL, 22 mmol) and pyridine (1.8 mL, 22 mmol) at −21 °C. After the mixture was stirred for 30 min at the same temperature, stirring was continued for 6 h at room temperature. The mixture was poured into saturated aqueous $KHSO_4$ and extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (hexane/EtOAc=10:1–1:1) to give **2d** (1.10 g, 37%). A pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.29 (10H, m), 5.83–5.71 (1H, m), 5.19–5.13 (2H, m), 5.06 (2H, dd, J=11.9, 8.8 Hz), 4.99 (2H, dd, J=11.9, 8.2 Hz), 2.65 (1H, ddd, J=7.4, 1.1, 1.1 Hz), 2.60 (1H, ddd, J=7.4, 1.1, 1.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.2 (d, J_{CP}=5.7 Hz), 128.4, 128.2, 127.8, 126.9 (d, J_{CP}=11.5 Hz), 120.1 (d, J_{CP}=14.5 Hz), 67.3 (d, J_{CP}=6.6 Hz), 31.9 (d, J_{CP}=139.2 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.22; IR (neat) 1255 cm^{-1}; ESIMS m/z 325 (MNa^+); HRMS (ESI) calcd for $C_{17}H_{19}O_3NaP$: 325.0970 (MNa^+). Found: 325.0976.

4.1.5. Dibenzyl (2*E*)-but-2-enylphosphonate (2e). Compound **2e** (46.1 g, 57%) was prepared from **2b** (50.0 g, 260 mmol) in an analogous manner to that for preparation of **2a** after purification by column chromatography on silica gel (hexane/EtOAc=10:1–1:1). A pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.30 (10H, m), 5.58–5.51 (1H, m), 5.41–5.34 (1H, m), 5.06 (2H, dd, J=11.9, 8.7 Hz), 4.99 (2H, dd, J=11.9, 8.1 Hz), 2.59–2.52 (2H, m), 1.67–1.63 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.3 (d, J_{CP}=5.9 Hz), 131.0 (d, J_{CP}=15.0 Hz), 128.4, 128.2, 127.8, 119.0 (d, J_{CP}=11.5 Hz), 67.3 (d, J_{CP}=6.5 Hz), 30.6 (d, J_{CP}=139.7 Hz), 17.9 (d, J_{CP}=2.4 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.37; IR 2960, 1253 cm^{-1}; ESIMS m/z 317 (MH^+); HRMS (ESI) calcd for $C_8H_{22}O_3P$: 317.1307 (MH^+). Found: 317.1331.

4.2. General procedure for AD reactions of 2a–e

To a stirred suspension of AD-mix-α (5.60 g) in H_2O/*t*-BuOH 1:1 (32 mL) was added $K_2OsO_4\cdot 2H_2O$ (12 mg, 0.8 mol%) at room temperature. After the mixture was stirred until two clear phases were observed, $MeSO_2NH_2$ (380 mg, 4.0 mmol) was added at the same temperature. After the mixture was chilled with an ice-water bath, **2a–e** (4.0 mmol) was added slowly and this mixture was stirred for 2 h at the same temperature and then stirring was continued for 6 h at room temperature. Sodium sulfite (12.0 g) was added to quench the reaction and the mixture was stirred for 1 h at room temperature. The mixture was extracted with EtOAc and the combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (EtOAc to $CHCl_3$/MeOH=20:1) to give **3a–e**.

4.2.1. (2*R*)-Diethyl 2,3-dihydroxypropylphosphonate (3a). Compound **3a** (330 mg, 41%) was prepared from **2a** (712 mg, 4.0 mmol) with AD-mix-α. A colorless oil; $[\alpha]_D^{30}$ −0.67 (*c* 0.55, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17–4.06 (5H, m), 3.69 (1H, ddd, J=11.4, 3.6, 1.3 Hz), 3.53 (1H, dd, J=11.4, 5.6 Hz), 2.09–1.90 (2H, m), 1.34 (3H, t, J=7.1 Hz), 1.33 (3H, t, J=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 67.1 (d, J_{CP}=4.0 Hz), 66.7 (d, J_{CP}=16.1 Hz), 62.0 (d, J_{CP}=9.4 Hz), 61.9 (d, J_{CP}=9.4 Hz), 29.8 (d, J_{CP}=140.3 Hz), 16.3 (d, J_{CP}=5.2 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 30.44; IR (neat) 3346, 1221 cm^{-1}; ESIMS m/z 213 (MH^+); HRMS (ESI) calcd for $C_7H_8O_5P$: 213.0892 (MH^+). Found: 213.0883.

4.2.2. (2*R*,3*S*)-Diethyl 2,3-dihydroxybutylphosphonate (3b). Compound **3b** (540 mg, 60%) was prepared from **2b** (768 mg, 4.0 mmol) with AD-mix-α. A colorless oil; $[\alpha]_D^{26}$ −3.22 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21–4.07 (4H, m), 3.88–3.85 (1H, m), 3.66 (1H, dt, J=11.3, 5.4 Hz), 2.04–1.93 (2H, m), 1.34 (6H, t, J=7.1 Hz), 1.20 (3H, d, J=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.6 (d, J_{CP}=16.7 Hz), 70.4 (d, J_{CP}=5.5 Hz), 62.0 (d, J_{CP}=9.3 Hz), 30.0 (d, J_{CP}=140.1 Hz), 18.9, 16.3 (d, J_{CP}=5.9 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 30.87; IR (neat) 3356, 1220 cm^{-1}; ESIMS m/z 249 (MNa^+); HRMS (ESI) calcd for $C_8H_{19}O_5NaP$: 249.0868 (MNa^+). Found: 249.0864.

4.2.3. (2*S*,3*R*)-4-(Diethoxyphosphoryl)-2,3-dihydroxybutyl 4-methoxybenzoate (3c). Compound **3c** (840 mg, 58%) was prepared from **2c** (1.37 g, 4.0 mmol) with AD-mix-α. A colorless oil; $[\alpha]_D^{26}$ −2.14 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (2H, d, J=8.8 Hz), 6.87 (2H, d, J=8.8 Hz), 4.38 (2H, d, J=5.8 Hz), 4.09 (4H, q, J=6.9 Hz), 4.05–4.00 (1H, m), 3.87–3.85 (1H, m), 3.82 (3H, s), 2.22–1.96 (2H, m), 1.29 (6H, t, J=7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 163.5, 131.7, 122.2, 113.6, 72.4 (d, J_{CP}=14.9 Hz), 66.8 (d, J_{CP}=4.5 Hz), 65.5, 62.1 (d, J_{CP}=3.2 Hz), 55.4, 30.1 (d, J_{CP}=140.0 Hz), 16.3 (d, J_{CP}=5.9 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.36; IR (neat) 3356, 1713, 1258, 1168 cm^{-1}; ESIMS m/z 399 (MNa^+);

HRMS (ESI) calcd for $C_{16}H_{25}O_8NaP$: 399.1185 (MNa^+). Found: 399.1185.

4.2.4. (2*R*)-Dibenzyl 2,3-dihydroxypropylphosphonate (3d). Compound **3d** (850 mg, 71%) was prepared from **2d** (1.10 g, 3.6 mmol) with AD-mix-α. White needles; mp 55–58 °C; $[\alpha]_D^{25}$ −0.64 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.28 (10H, m), 5.06 (2H, ddd, *J*=11.8, 9.0, 4.4 Hz), 4.97 (2H, ddd, *J*=11.8, 8.1, 3.7 Hz), 4.10–4.03 (1H, m), 3.85 (1H, b), 3.63 (1H, dd, *J*=11.2, 1.7 Hz), 3.48 (1H, dd, *J*=11.2, 5.7 Hz), 2.95 (1H, b), 2.07 (1H, ddd, *J*=15.4, 16.8, 8.8 Hz), 1.97 (1H, ddd, *J*=15.4, 19.3, 4.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.9 (d, J_{CP}=5.7 Hz), 128.6–127.0 (aromatic), 67.6 (d, J_{CP}=6.9 Hz), 67.5 (d, J_{CP}=6.9 Hz), 67.0 (d, J_{CP}=4.0 Hz), 66.7 (d, J_{CP}=17.3 Hz), 30.2 (d, J_{CP}=139.9 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 31.43; IR (KBr) 3374, 1216 cm^{-1}; ESIMS *m*/*z* 359 (MNa^+); HRMS (ESI) calcd for $C_{17}H_{21}O_5NaP$: 359.1024 (MNa^+). Found: 359.1035.

4.2.5. (2*R*,3*S*)-Dibenzyl 2,3-dihydroxybutylphosphonate (3e). Compound **3e** (2.30 g, 69%) was prepared from **2e** (3.00 g, 9.6 mmol) with AD-mix-α. One recrystallization from EtOAc gave an optically pure **3e** (870 mg, 38%). White needles; mp 93–95 °C; $[\alpha]_D^{22}$ −7.92 (*c* 1.0, MeOH) for a sample of >99% ee; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.32 (10H, m), 5.12–4.96 (4H, m), 3.70 (1H, dq, *J*=14.9, 4.2 Hz), 3.59 (1H, dt, *J*=11.3, 5.6 Hz), 2.04–1.96 (2H, m), 1.13 (3H, d, *J*=6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.0 (d, J_{CP}=5.3 Hz), 135.9 (d, J_{CP}=5.3 Hz), 128.7, 128.6, 128.5, 128.1, 128.0, 70.7 (d, J_{CP}=17.4 Hz), 70.4 (d, J_{CP}=5.6 Hz), 67.6 (d, J_{CP}=6.4 Hz), 30.5 (d, J_{CP}=139.6 Hz), 18.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 31.91; IR (KBr) 3359, 2968, 1214 cm^{-1}; ESIMS *m*/*z* 351 (MH^+); HRMS (ESI) calcd for $C_{18}H_{24}O_5P$: 351.1361 (MH^+). Found: 351.1352.

4.2.6. (2*R*,3*S*)-4-(Diethoxyphosphoryl)-2,3-dihydroxybutyl 4-methoxybenzoate (*ent*-3c). Compound *ent*-**3c** (670 mg, 70%) was prepared from **2c** (1.37 g, 4.0 mmol) with AD-mix-β. A colorless oil; $[\alpha]_D^{25}$ +6.01 (*c* 1.0, MeOH). The ^{1}H NMR spectrum was identical with that of *ent*-**3c**.

4.2.7. (2*S*,3*R*)-Dibenzyl 2,3-dihydroxybutylphosphonate (*ent*-3e). Compound *ent*-**3e** (24.6 g, 64%) was prepared from **2e** (34.8 g, 110 mmol) with AD-mix-β. One recrystallization from EtOAc gave an optically pure *ent*-**3e** (14.6 g, 59%). White needles; $[\alpha]_D^{24}$ +7.76 (*c* 1.2, MeOH) for a sample of >99% ee. The ^{1}H NMR spectrum was identical with that of *ent*-**3e**.

4.2.8. (1*S*,2*R*)-3-(Diethoxyphosphoryl)-2-[(4-methoxybenzoyl)oxy]-1-methylpropyl 4-methoxybenoate (5b). To a stirred solution of 4-methoxybenzoyl chloride (1.40 g, 8.0 mmol) in CH_2Cl_2 (1 mL) was sequentially added a solution of **3b** (720 mg, 3.2 mmol) in CH_2Cl_2 (5 mL), pyridine (0.65 mL, 8.0 mmol) and DMAP (39 mg, 0.32 mmol). After stirring for 24 h at room temperature, the mixture was poured into saturated aqueous $KHSO_4$ and extracted with $CHCl_3$. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (hexane/EtOAc=1:1) to provide **5b** (600 mg, 38%). A colorless oil; $[\alpha]_D^{26}$ −5.01 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.99 (4H, m), 6.93–6.91 (4H, m), 5.65–5.61 (1H, m), 5.39 (1H, td, *J*=3.4, 3.1 Hz), 4.06–4.03 (4H, m), 3.86 (6H, s), 2.33–2.29 (2H, m), 1.38 (3H, t, *J*=6.4 Hz), 1.34 (6H, t, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 165.1, 163.5, 163.4, 131.8–113.6 (aromatic), 71.2 (d, J_{CP}=18.7 Hz), 71.0 (d, J_{CP}=10.4 Hz), 63.4 (d, J_{CP}=5.5 Hz), 55.4, 27.9 (d, J_{CP}=142.9 Hz), 16.2, 16.1 (d, J_{CP}=10.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.87; IR (neat) 1715, 1258 cm^{-1}; ESIMS *m*/*z* 495 (MH^+); HRMS (ESI) calcd for $C_{24}H_{32}O_9P$: 495.1784 (MH^+). Found: 495.1786.

4.2.9. (2*S*,3*R*)-4-(Diethoxyphosphoryl)-2,3-bis[(4-methoxybenzoyl)oxy]butyl 4-methoxybenzoate (5c). Compound **5c** (910 mg, 70%) was prepared from **3c** (730 mg, 2.0 mmol) in an analogous manner to that for preparation of **5b** after purification by column chromatography on silica gel (hexane/EtOAc=1:1). A colorless oil; $[\alpha]_D^{26}$ −7.25 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (4H, d, *J*=8.6 Hz), 7.91 (2H, d, *J*=8.8 Hz), 6.91–6.89 (4H, m), 6.85 (2H, d, *J*=8.8 Hz), 5.89–5.81 (1H, m), 5.76 (1H, td, *J*=4.7, 4.5 Hz), 4.56 (2H, d, *J*=2.2 Hz), 4.07–4.04 (4H, m), 3.86 (3H, s), 3.85 (3H, s), 3.83 (3H, m), 2.40–2.35 (2H, m), 1.23 (6H, t, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 165.1, 164.9, 163.7, 163.6, 163.4, 131.9–113.6 (aromatic), 71.8 (d, J_{CP}=10.0 Hz), 67.3, 62.5, 62.0, 55.4, 55.3, 28.0 (d, J_{CP}=143.3 Hz), 16.2; ^{31}P NMR (162 MHz, $CDCl_3$) δ 25.84; IR (neat) 1719. 1257 cm^{-1}; ESIMS *m*/*z* 645 (MH^+); HRMS (ESI) calcd for $C_{32}H_{38}O_{12}P$: 645.2101 (MH^+). Found: 645.2098.

4.2.10. (1*S*,2*R*)-3-(Diethoxyphosphoryl)-2-[(4-methoxybenzoyl)oxy]-1-phenylpropyl 4-methoxybenzoate (6). Compound **6** (553 mg, 50%) was prepared from **4** (576 mg, 2.0 mmol) in an analogous manner to that for preparation of **5b** after purification by column chromatography on silica gel (hexane/EtOAc=1:1). A colorless oil; $[\alpha]_D^{26}$ −9.24 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (2H, d, *J*=9.1 Hz), 7.28–7.20 (5H, m), 6.80 (4H, d, *J*=8.7 Hz), 6.13 (1H, d, *J*=6.7 Hz), 5.84 (1H, ddt, *J*=12.9, 6.5, 6.4 Hz), 3.98–3.90 (4H, m), 3.74 (6H, s), 2.15 (1H, d, *J*=6.4 Hz), 2.10 (1H, d, *J*=6.5 Hz), 1.13 (3H, t, *J*=7.1 Hz), 1.07 (3H, t, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 163.3, 163.2, 136.3–113.4 (aromatic), 76.6 (d, J_{CP}=14.1 Hz), 69.7 (d, J_{CP}=4.6 Hz), 61.6 (d, J_{CP}=6.3 Hz), 55.1, 27.4 (d, J_{CP}=143.3 Hz), 16.0 (d, J_{CP}=6.7 Hz), 15.9 (d, J_{CP}=6.7 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.18; IR (neat) 1719, 1258 cm^{-1}; ESIMS *m*/*z* 557 (MH^+); HRMS (ESI) calcd for $C_{29}H_{33}O_9P$: 557.1940 (MH^+). Found: 557.1927.

4.2.11. (2*R*,3*S*)-2,3-Dihydroxybutylphosphonic acid (7). To a solution of **3e** (350 mg, 1.0 mmol) in MeOH (20 mL) was added 10% Pd–C (40 mg) and the mixture was stirred for 12 h at room temperature under hydrogen atmosphere. The catalyst was removed by filtration through a pad of Celite, the filtrate was concentrated to give **7** (160 mg, 94%). Amorphous; $[\alpha]_D^{26}$ −0.23 (*c* 0.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90–3.81 (1H, m), 3.79–3.27 (1H, m), 2.21–1.86 (2H, m), 1.21 (3H, d, *J*=6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.4 (d, J_{CP}=4.4 Hz), 71.1

(d, J_{CP}=14.2 Hz), 31.5 (d, J_{CP}=137.9 Hz), 18.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.35; IR (neat) 3336, 997 cm^{-1}; ESIMS *m/z* 171 (MH^+); HRMS (ESI) calcd for $C_4H_{12}O_5P$: 171.0422 (MH^+). Found: 171.0410.

4.2.12. (2*R*,3*S*)-2,3-Dihydroxybutylphosphonic acid (7). To a stirred solution of **3b** (226 mg, 1.0 mmol) in CH_2Cl_2 (2 mL) was added TMSBr (0.53 mL, 4.0 mmol) and the mixture was stirred for 12 h at room temperature. Concentration of the mixture gave a residue, which was dissolved in MeOH (0.1 mL). After stirring for 2 h at room temperature, the solution was concentrated to provide **7** (158 mg, 93%). Amorphous; $[\alpha]_D^{26}$ −1.55 (*c* 0.9, MeOH). The ^{1}H NMR spectrum was identical to that of **7** prepared from **3e**.

4.2.13. (4*R*)-Diethyl (2,2-dioxido-1,3,2-dioxathiolan-4-yl)methylphosphonate (8). To a stirred solution of **3a** (990 mg, 5.0 mmol) in CH_2Cl_2 (30 mL) was added Et_3N (2.8 mL, 20 mmol) and a solution of $SOCl_2$ (0.50 mL, 15 mmol) in CH_2Cl_2 (3 mL) at 0 °C and the mixture was stirred for 1 h at the same temperature. The mixture was poured into H_2O and extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue. To a stirred solution of this residue in CCl_4–CH_3CN–H_2O 1:1:1.5 (50 mL) was added $NaIO_4$ (2.00 g, 10 mmol) and $RuCl_3\cdot nH_2O$ (52 mg) at 0 °C and the mixture was stirred for 2 h at the same temperature. The mixture was poured into H_2O and extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (EtOAc) to give **8** (1.09 g, 80%). A pale yellow oil; $[\alpha]_D^{25}$ −0.72 (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27–5.18 (1H, m), 4.85 (1H, dd, *J*=9.2, 6.0 Hz), 4.59 (1H, dd, *J*=9.2, 7.5 Hz), 4.15–4.07 (4H, m), 2.54 (1H, ddd, *J*=20.2, 15.0, 4.9 Hz), 2.33 (1H, ddd, *J*=18.4, 15.0, 9.9 Hz), 1.36 (6H, t, *J*=7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 77.6 (d, J_{CP}=3.5 Hz), 73.1 (d, J_{CP}=4.6 Hz), 63.6 (d, J_{CP}=5.8 Hz), 29.9 (d, J_{CP}=139.6 Hz), 16.3 (d, J_{CP}=6.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.55; IR (neat) 1386, 1212, 1163 cm^{-1}; ESIMS *m/z* 275 (MH^+); HRMS (ESI) calcd for $C_7H_{16}O_7PS$: 275.0354 (MH^+). Found: 275.0381.

4.2.14. (2*R*)-Diethyl 3-azido-2-hydroxypropylphosphonate (9). To a stirred solution of **8** (9.73 g, 35.5 mmol) in acetone (532 mL) was added NaN_3 (6.98 g, 106.5 mmol), followed by addition of H_2O (266 mL). The mixture was stirred for 3 h at 50 °C and concentrated to give a residue, which was dissolved in Et_2O (1.78 L). To the solution was added 20% aqueous H_2SO_4 (887 mL) and the mixture was stirred for 12 h at room temperature. The mixture was extracted with Et_2O and to the combined extracts was added K_2CO_3 (1.78 g). After stirring for 30 min at room temperature, this mixture was dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (hexane/EtOAc=3:1–1:1) to give **9** (2.02 g, 24%). A colorless oil; $[\alpha]_D^{25}$ −0.18 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22–4.07 (5H, m), 3.38 (1H, dd, *J*=12.4, 4.4 Hz), 3.34 (1H, dd, *J*=12.4, 5.7 Hz), 2.11–1.93 (2H, m), 1.35 (6H, t, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 65.9 (d, J_{CP}=4.1 Hz), 62.5 (d, J_{CP}=6.5 Hz), 62.3 (d, J_{CP}=6.5 Hz), 30.7 (d, J_{CP}=140.6 Hz), 16.3 (d, J_{CP}=5.6 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.28; IR (neat) 3361, 2104, 1220 cm^{-1}; ESIMS *m/z* 238 (MH^+); HRMS (ESI) calcd for $C_7H_{17}N_3O_4P$: 238.0957 (MH^+). Found: 238.0977.

4.2.15. (2*R*)-Diethyl 3-azido-2-[(triethylsilyl)oxy]propylphosphonate (10). To a stirred solution of **9** (2.09 g, 8.82 mmol) in DMF (30 mL) was added imidazole (1.44 g, 21.2 mmol) at 0 °C and the mixture was stirred for 15 min at the same temperature. To the solution was added TESCl (1.8 mL, 10.6 mmol) and the mixture was stirred for 5 h at room temperature. The mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (hexane/EtOAc=3:1) to provide **10** (2.48 g, 80%). A colorless oil; $[\alpha]_D^{26}$ +1.79 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23–4.17 (1H, m), 4.14–4.02 (4H, m), 3.49 (1H, dd, *J*=12.6, 3.1 Hz), 3.29 (1H, dd, *J*=12.6, 5.4 Hz), 2.19 (1H, ddd, *J*=16.8, 15.6, 9.3 Hz), 1.98 (1H, ddd, *J*=20.0, 15.6, 3.9 Hz), 1.33 (6H, t, *J*=7.1 Hz), 0.98 (9H, t, *J*=7.9 Hz), 0.65 (6H, q, *J*=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 67.5, 61.7, (d, J_{CP}=4.6 Hz), 56.6 (d, J_{CP}=3.1 Hz), 32.0 (d, J_{CP}=135.9 Hz), 16.3 (d, J_{CP}=6.1 Hz), 6.7, 4.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.87; IR (neat) 2157, 1228 cm^{-1}; ESIMS *m/z* 352 (MH^+); HRMS (ESI) calcd for $C_{13}H_{31}N_3O_4SiP$: 352.1821 (MH^+). Found: 352.1808.

4.2.16. (2*R*)-Diethyl 3-amino-2-[(triethylsilyl)oxy]propylphosphonate (11). A solution of **10** (100 mg, 0.28 mmol) in MeOH (5.7 mL) was stirred over 10% Pd–C (11 mg) for 12 h at room temperature under hydrogen atmosphere. The catalyst was removed by filtration through a pad of Celite, the filtrate was concentrated to give a residue, which was chromatographed on silica gel (EtOAc) to provide **11** (93.2 mg, 97%). A colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16–4.00 (5H, m), 2.92 (1H, dd, *J*=13.3, 3.6 Hz), 2.76 (1H, dd, *J*=13.3, 5.0 Hz), 2.13 (1H, ddd, *J*=17.3, 15.4, 9.1 Hz), 1.95 (1H, ddd, *J*=19.9, 15.4, 4.2 Hz), 1.32 (6H, t, *J*=7.1 Hz), 0.96 (9H, t, *J*=7.9 Hz), 0.62 (6H, q, *J*=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.8, 61.5 (d, J_{CP}=4.9 Hz), 47.8 (d, J_{CP}=3.4 Hz), 31.7 (d, J_{CP}=135.7 Hz), 16.3 (d, J_{CP}=6.0 Hz), 6.7, 4.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.10; IR (neat) 3416, 1237 cm^{-1}; ESIMS *m/z* 326 (MH^+); HRMS (ESI) calcd for $C_{13}H_{33}NO_4SiP$: 326.1917 (MH^+). Found: 326.1895.

4.2.17. (2*R*)-Diethyl 3-(dimethylamino)-2-[(triethylsilyl)oxy]propylphosphonate (12). To a stirred solution of **11** (91 mg, 0.28 mmol) in CH_3CN (1.4 mL) was added 37% aqueous formaldehyde (112 mg, 1.4 mmol) and $NaBH_3CN$ (31 mg, 0.45 mmol) and the mixture was stirred for 2 h at room temperature. To the solution was added acetic acid to be pH=3.0 and stirring was continued for 45 min. After pH was adjusted to be 9.0 with 1 M KOH, the mixture was extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (hexane/EtOAc=2:1 to $CHCl_3$/MeOH=20:1) to provide **12** (88 mg, 90%). A colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16–4.04 (5H, m), 2.38–2.28 (3H, m), 2.23 (6H, s), 1.89 (1H, ddd, *J*=18.3, 15.4, 6.1 Hz), 1.32 (6H, t,

J=7.1 Hz), 0.97 (9H, t, J=7.9 Hz), 0.63 (6H, q, J=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.6, 66.0 (d, J_{CP}=9.6 Hz), 61.3 (d, J_{CP}=3.2 Hz), 61.2 (d, J_{CP}=3.2 Hz), 46.4, 32.2 (d, J_{CP}=138.5 Hz), 16.4 (d, J_{CP}=6.1 Hz), 16.3 (d, J_{CP}=6.1 Hz), 6.8, 4.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.83; IR (neat) 1238 cm^{-1}; ESIMS m/z 354 (MH^+); HRMS (ESI) calcd for $C_{15}H_{37}NO_4SiP$: 354.2230 (MH^+). Found: 354.2238.

4.2.18. (1*R*)-2-(Diethoxyphosphoryl)-1-[(dimethylamino)methyl]ethyl myristate (13). To a stirred solution of **12** (402 mg, 1.14 mmol) in THF (6 mL) was added a 1 M THF solution of TBAF (2.3 mL, 2.3 mmol) at 0 °C and the mixture was stirred for 2 h at the same temperature. The mixture was concentrated to give a residue, which was dissolved in CH_2Cl_2 (2.5 mL). To this solution was added a solution of myristoyl chloride (0.63 mL, 2.3 mmol) in CH_2Cl_2 (2.5 mL), pyridine (0.16 mL, 2.1 mmol) and DMAP (14 mg, 0.11 mmol) at 0 °C and the mixture was stirred for 3.5 h at room temperature. The mixture was poured into H_2O and extracted with $CHCl_3$. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel ($CHCl_3$/MeOH=1:0–20:1) to provide **13** (207 mg, 52%). A colorless oil; $[\alpha]_D^{26}$ +5.19 (c 0.2, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (1H, dddd, J=12.5, 12.5, 6.2, 6.2 Hz), 4.16–4.06 (4H, m), 2.54 (1H, dd, J=12.8, 6.0 Hz), 2.46 (1H, dd, J=12.8, 5.5 Hz), 2.32–2.22 (9H, m), 2.07 (1H, ddd, J=18.2, 15.5, 7.0 Hz), 1.34–1.25 (28H, m), 0.88 (3H, t, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 66.9, 62.6 (d, J_{CP}=9.8 Hz), 61.7 (d, J_{CP}=7.2 Hz), 61.6 (d, J_{CP}=7.2 Hz), 46.0, 34.4, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 28.3, 24.8, 22.7, 16.4 (d, J_{CP}=7.1 Hz), 14.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.42; IR (neat) 1738, 1257 cm^{-1}; ESIMS m/z 450 (MH^+); HRMS (ESI) calcd for $C_{23}H_{49}NO_5P$: 450.3348 (MH^+). Found: 450.3344.

4.2.19. (2*R*)-3-(Diethoxyphosphoryl)-*N*,*N*,*N*-trimethyl-2-(tetradecanoyloxy)propan-1-aminium iodide (14). To a stirred solution of **13** (200 mg, 0.45 mmol) in acetone (1.9 mL) was added iodomethane (0.03 mL, 0.37 mmol) and the mixture was stirred for 10 h at room temperature. The solution was concentrated to give a residue, which was chromatographed on silica gel ($CHCl_3$/MeOH=1:0–10:1) to provide **14** (249 mg, 86%). A colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (1H, dddd, J=13.8, 13.8, 7.0, 7.0 Hz), 4.38–4.34 (1H, m), 4.18–4.09 (5H, m), 3.49 (9H, s), 2.43–2.24 (4H, m), 1.34–1.23 (28H, m), 0.86 (3H, t, J=6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 68.1 (d, J_{CP}=9.9 Hz), 62.6 (d, J_{CP}=6.4 Hz), 62.5 (d, J_{CP}=6.4 Hz), 54.5, 34.1, 31.7, 29.6, 29.4, 29.2, 29.1, 29.0, 28.9, 28.2, 24.2, 22.4, 16.2 (d, J_{CP}=5.8 Hz), 13.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.81; IR (neat) 1741, 1241 cm^{-1}; ESIMS m/z 464 (M^+-I); HRMS (ESI) calcd for $C_{24}H_{51}NO_5P$: 464.3505 (M^+-I). Found: 464.3471.

4.2.20. (2*R*)-*N*,*N*,*N*-Trimethyl-3-phosphono-2-(tetradecanoyloxy)propan-1-aminium (15). To a stirred solution of **14** (178 mg, 0.3 mmol) in CH_2Cl_2 (0.6 mL) was added TMSBr (0.06 mL, 0.48 mmol) and the mixture was stirred for 20 h at room temperature. Concentration of the mixture gave a residue, which was dissolved in MeOH (0.06 mL). After stirring for 12 h at room temperature, the solution was concentrated to provide **15** (88 mg, 72%). Amorphous; $[\alpha]_D^{26}$ +2.25 (c 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64–5.55 (1H, m), 3.95 (1H, dd, J=14.4, 8.7 Hz), 3.84 (1H, d, J=14.4 Hz), 3.23 (9H, s), 2.41 (2H, t, J=7.5 Hz), 2.24–2.17 (2H, m), 1.31–1.28 (22H, m), 0.89 (3H, t, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 71.4, 69.7, 65.7, 54.8, 35.1, 33.0, 31.2, 30.7, 30.6, 30.4, 30.2, 26.0, 25.5, 23.7, 14.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.01; IR (neat) 970 cm^{-1}; ESIMS m/z 408 (MH^+); HRMS (ESI) calcd for $C_{20}H_{43}NO_5P$: 408.2879 (MH^+). Found: 408.2913.

4.2.21. Dibenzyl [(4*R*,5*S*)-5-methyl-2,2-dioxido-1,3,2-dioxathiolan-4-yl]methylphosphonate (16). Compound **16** (850 mg, 80%) was prepared from **3e** (850 mg, 2.5 mmol) in an analogous manner to that for preparation of **8** after purification by column chromatography on silica gel (EtOAc). A pale yellow oil; $[\alpha]_D^{26}$ −12.95 (c 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.33 (10H, m), 5.10–4.95 (4H, m), 4.80 (1H, dt, J=12.9, 6.4 Hz), 4.68 (1H, qd, J=7.2, 7.1 Hz), 2.42–2.17 (2H, m), 1.47 (3H, d, J=6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.4 (d, J_{CP}=5.1 Hz), 135.3 (d, J_{CP}=5.1 Hz), 128.9, 128.8, 128.3, 128.2, 83.8 (d, J_{CP}=9.4 Hz), 82.6, 68.2 (d, J_{CP}=3.1 Hz), 68.1 (d, J_{CP}=3.1 Hz), 29.9 (d, J_{CP}=141.8 Hz), 17.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.12; IR (neat) 1383, 1260, 1211 cm^{-1}; ESIMS m/z 413 (MH^+); HRMS (ESI) calcd for $C_{18}H_{22}O_7PS$: 413.0840 (MH^+). Found: 413.0824.

4.2.22. (2*R*,3*R*)-Dibenzyl 3-azido-2-hydroxybutylphosphonate (17). Compound **17** (473 mg, 42%) was prepared from **16** (1.20 g, 3.0 mmol) in an analogous manner to that for preparation of **9** after purification by column chromatography on silica gel (hexane/EtOAc=3:1–1:1). A colorless oil; $[\alpha]_D^{30}$ −17.56 (c 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.32 (10H, m), 5.11–5.05 (2H, m), 5.02–4.96 (2H, m), 3.84–3.77 (1H, m), 3.66 (3H, s), 3.48 (1H, dt, J=12.6, 6.3 Hz), 2.07–1.88 (2H, m), 1.18 (3H, d, J=6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.9 (d, J_{CP}=5.7 Hz), 135.8 (d, J_{CP}=5.7 Hz), 128.7, 128.6, 128.0, 69.5 (d, J_{CP}=4.9 Hz), 67.7 (d, J_{CP}=6.3 Hz), 67.6 (d, J_{CP}=6.7 Hz), 61.4 (d, J_{CP}=17.3 Hz), 29.5 (d, J_{CP}=140.4 Hz), 14.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 31.56; IR (neat) 3347, 2098, 1220 cm^{-1}; ESIMS m/z 398 (MNa^+); HRMS (ESI) calcd for $C_{18}H_{22}N_3O_4NaP$: 398.1246 (MNa^+). Found: 398.1241.

4.2.23. (2*R*,3*R*)-Dibenzyl 3-azido-2-[(triethylsilyl)oxy]-butylphosphonate (19). Compound **19** (8.83 g, 98%) was prepared from **17** (6.90 g, 18.4 mmol) in an analogous manner to that for preparation of **10** after purification by column chromatography on silica gel (hexane/EtOAc=2:1). A colorless oil; $[\alpha]_D^{27}$ −1.20 (c 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.30 (10H, m), 5.04 (2H, dd, J=11.5, 9.7 Hz), 4.95 (1H, dd, J=8.5, 3.0 Hz), 4.92 (1H, dd, J=8.6, 3.1 Hz), 4.12–4.05 (1H, m), 3.63 (1H, qd, J=6.7, 2.7 Hz), 2.07–1.95 (2H, m), 1.13 (3H, d, J=6.7 Hz), 0.98–0.90 (9H, m), 0.60 (6H, t, J=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.2 (d, J_{CP}=5.5 Hz), 128.6–128.1 (aromatic), 70.2, 67.4 (d, J_{CP}=5.7 Hz), 67.3 (d, J_{CP}=5.6 Hz), 60.7 (d, J_{CP}=5.7 Hz), 31.5 (d, J_{CP}=137.3 Hz), 12.5, 6.8, 4.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.91; IR (neat) 2955, 2105, 1249 cm^{-1}; ESIMS m/z 490 (MH^+); HRMS (ESI) calcd for $C_{24}H_{37}N_3O_4SiP$: 490.2291 (MH^+). Found: 490.2283.

4.2.24. (2*R*,3*R*)-Dibenzyl 3-amino-2-[(triethylsilyl)oxy]-butylphosphonate (20). To a stirred solution of **19** (9.00 g, 18.4 mmol) in THF (180 mL) was added triphenylphosphine (5.30 g, 20 mmol) and the mixture was stirred for 2 h at room temperature. To the solution was added H_2O (50 mL) and the mixture was stirred for 12 h at room temperature. The mixture was extracted with $CHCl_3$ and then the combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (EtOAc to $CHCl_3$/MeOH=10:1) to provide **20** (7.84 g, 97%). A colorless oil; $[\alpha]_D^{24}$ −1.13 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.28 (10H, m), 5.03 (2H, dd, *J*=11.8, 9.1 Hz), 4.95 (2H, dd, *J*=11.8, 8.2 Hz), 4.02–3.87 (1H, m), 3.15–3.11 (1H, m), 2.06–1.97 (2H, m), 1.00 (3H, d, *J*=6.6 Hz), 0.91 (9H, t, *J*=7.9 Hz), 0.58 (6H, q, *J*=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.2 (d, J_{CP}=5.4 Hz), 128.5–127.7 (aromatic), 71.6, 67.2 (d, J_{CP}=6.6 Hz), 67.1 (d, J_{CP}=6.6 Hz), 51.3 (d, J_{CP}=7.6 Hz), 30.1 (d, J_{CP}=138.0 Hz), 17.0, 6.8, 4.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 30.81; IR (neat) 3457, 1608, 1240 cm^{-1}; ESIMS *m*/*z* 464 (MH^+); HRMS (ESI) calcd for $C_{24}H_{39}NO_4SiP$: 464.2386 (MH^+). Found: 464.2382.

4.2.25. (2*R*,3*R*)-Dibenzyl 3-{[(4-methylphenyl)sulfonyl]amino}-2-[(triethylsilyl)oxy]butylphosphonate (21). To a stirred solution of **20** (370 mg, 0.8 mmol) in CH_2Cl_2 (1 mL) was added Et_3N (0.14 mL, 0.97 mmol) and a solution of tosyl chloride (0.18 g, 0.97 mmol) in CH_2Cl_2 (1 mL) at 0 °C and the mixture was stirred for 12 h at room temperature. The mixture was poured into saturated aqueous $KHSO_4$ and extracted with Et_2O. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel (hexane/EtOAc=10:1) to provide **21** (60 mg, 13%). A colorless oil; $[\alpha]_D^{25}$ +29.28 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (2H, d, *J*=8.3 Hz), 7.38–7.31 (10H, m), 7.22 (2H, d, *J*=8.2 Hz), 5.04 (1H, dd, *J*=9.4, 5.6 Hz), 5.01 (1H, dd, *J*=9.4, 5.6 Hz), 4.95 (1H, dd, *J*=8.2, 3.5 Hz), 4.92 (1H, dd, *J*=8.2, 3.5 Hz), 4.79 (1H, d, *J*=8.3 Hz), 4.08–4.06 (1H, m), 3.64–3.62 (1H, m), 2.37 (3H, s), 1.97–1.90 (2H, m), 0.92 (3H, d, *J*=6.6 Hz), 0.87 (9H, t, *J*=7.9 Hz), 0.50 (6H, q, *J*=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.0, 138.3, 136.1 (d, J_{CP}=5.5 Hz), 129.6–127.0 (aromatic), 70.2 (d, J_{CP}=1.8 Hz), 67.5 (d, J_{CP}=6.5 Hz), 67.4 (d, J_{CP}=6.5 Hz), 53.2 (d, J_{CP}=2.7 Hz), 32.1 (d, J_{CP}=135.3 Hz), 21.4, 14.6, 6.8, 4.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 28.40; IR (neat) 3159, 1323, 1261, 1146 cm^{-1}; ESIMS *m*/*z* 618 (MH^+); HRMS (ESI) calcd for $C_{31}H_{45}NO_6SiPS$: 618.2475 (MH^+). Found: 618.2507.

4.2.26. (3*R*)-Dibenzyl 3-{[(4-methylphenyl)sulfonyl]-amino}-2-oxobutylphosphonate (22). To a stirred solution of **21** (60 mg, 0.1 mmol) in THF (0.5 mL) was added a 1 M THF solution of TBAF (0.22 mL, 0.22 mmol) at 0°C and the mixture was stirred for 2 h at the same temperature. The mixture was poured into H_2O and extracted with Et_2O. The combined extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent gave a residue, which was dissolved in CH_2Cl_2 (1 mL). To the solution was added PDC (41 mg, 0.11 mmol) and the mixture was stirred for 12 h at room temperature. After filtration of the mixture through a pad of Celite, the filtrate was concentrated to give a residue, which was chromatographed on silica gel (hexane/EtOAc=10:1–3:1) to give **22** (27 mg, 47%). A colorless oil; $[\alpha]_D^{25}$ +11.52 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (2H, d, *J*=8.2 Hz), 7.38–7.29 (10H, m), 7.24 (2H, d, *J*=8.2 Hz), 5.92 (1H, d, *J*=8.2 Hz), 5.08–4.91 (4H, m), 4.02 (1H, dt, *J*=15.2, 7.4 Hz), 3.45 (1H, dd, *J*=23.3, 13.7 Hz), 3.03 (1H, dd, *J*=22.5, 13.7 Hz), 2.36 (3H, s), 1.18 (3H, d, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.8 (d, J_{CP}=6.0 Hz), 143.6, 137.4, 135.5, 129.8–127.0 (aromatic), 68.4 (d, J_{CP}=6.0 Hz), 68.3 (d, J_{CP}=6.0 Hz), 58.2, 39.2 (d, J_{CP}=128.0 Hz), 21.5, 18.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.48; IR (neat) 3277, 1724, 1243 cm^{-1}; ESIMS *m*/*z* 502 (MH^+); HRMS (ESI) calcd for $C_{25}H_{29}NO_6PS$: 502.1453 (MH^+). Found: 502.1422.

4.2.27. (2*R*,3*R*)-Dibenyl 3-(dimethylamino)-2-[(triethylsilyl)oxy]butylphosphonate (24). Compound **24** (830 mg, 85%) was prepared from **20** (920 mg, 2.0 mmol) in an analogous manner to that for preparation of **12** after purification by column chromatography on silica gel (hexane/EtOAc=2:1–$CHCl_3$/MeOH=20:1). A colorless oil; $[\alpha]_D^{25}$ +5.26 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.28 (10H, m), 5.05–4.91 (4H, m), 4.08 (1H, dq, *J*=16.3, 5.5 Hz), 2.64 (1H, dt, *J*=12.2, 6.7 Hz), 2.31–2.21 (2H, m), 2.17 (6H, s), 0.93 (3H, d, *J*=2.5 Hz), 0.92 (9H, t, *J*=7.9 Hz), 0.59 (6H, q, *J*=7.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.5 (d, J_{CP}=5.9 Hz), 128.5–127.8 (aromatic), 69.7, 66.9 (d, J_{CP}=6.3 Hz), 63.3 (d, J_{CP}=6.2 Hz), 41.6, 32.6 (d, J_{CP}=137.2 Hz), 8.5, 6.9, 5.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 30.88; IR (neat) 2956, 1240, 1120 cm^{-1}; ESIMS *m*/*z* 492 (MH^+); HRMS (ESI) calcd for $C_{26}H_{43}NO_4SiP$: 492.2699 (MH^+). Found: 492.2701.

4.2.28. (1*R*,2*R*)-1-{[Bis(benzyloxy)phosphoryl]methyl}-2-(dimethylamino)propyl myristate (25). Compound **25** (487 mg, 23%) was prepared from **24** (1.76 g, 3.6 mmol) in an analogous manner to that for preparation of **13** after purification by column chromatography on silica gel ($CHCl_3$/MeOH=1:0–30:1). A colorless oil; $[\alpha]_D^{25}$ −0.97 (*c* 0.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.30 (10H, m), 5.31–5.23 (1H, m), 5.06–4.91 (4H, m), 4.12 (1H, q, *J*=7.0 Hz), 2.32 (2H, t, *J*=3.8 Hz), 2.22–2.08 (8H, m), 1.32–1.25 (25H, m), 0.88 (3H, t, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 172.9, 136.2, 128.7, 128.6, 128.4, 128.1, 128.0, 68.1, 67.5 (d, J_{CP}=6.5 Hz), 67.4 (d, J_{CP}=6.5 Hz), 61.6 (d, J_{CP}=10.7 Hz), 60.4, 40.5, 34.4, 34.3, 31.9, 29.6, 29.5, 29.3, 29.2, 28.3, 25.0, 24.6, 22.7, 21.0, 14.1, 8.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.33; IR (neat) 1736, 1249, 1115 cm^{-1}; ESIMS *m*/*z* 588 (MH^+); HRMS (ESI) calcd for $C_{34}H_{55}NO_5P$: 588.3818 (MH^+). Found: 588.3826.

4.2.29. (2*R*,3*R*)-4-[Bis(benzyloxy)phosphoryl]-*N*,*N*,*N*-trimethyl-3-(tetradecanoyloxy)butan-2-aminium iodide (26). Compound **26** (70 mg, 33%) was prepared from **25** (170 mg, 0.29 mmol) in an analogous manner to that for preparation of **14** after purification by column chromatography on silica gel ($CHCl_3$/MeOH=1:0–10:1). A colorless oil; $[\alpha]_D^{25}$ −5.65 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.31 (10H, m), 5.68 (1H, dt, *J*=7.5, 7.5 Hz), 5.08–4.91 (4H, m), 3.96 (1H, q, *J*=6.8 Hz), 3.08 (9H, s), 2.26 (2H, td, *J*=7.6, 2.1 Hz), 2.14 (2H, dd, *J*=19.4, 6.8 Hz), 1.45 (3H, d, *J*=6.8 Hz), 1.27–1.23 (22H, m), 0.88

(3H, t, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 135.7, 128.8, 128.7, 128.3, 128.2, 72.0 (d, J_{CP}=11.2 Hz), 68.1 (d, J_{CP}=6.5 Hz), 67.9 (d, J_{CP}=6.5 Hz), 64.5, 63.2, 51.6, 34.1, 31.9, 31.0, 30.9, 29.6, 29.4, 29.3, 29.2, 29.0, 24.4, 22.6, 15.2, 14.1, 8.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.34; IR (neat) 1739, 1224 cm^{-1}; ESIMS *m/z* 602 (M^+-I); HRMS (ESI) calcd for $C_{35}H_{57}NO_5P$: 602.3974 (M^+-I). Found: 602.4066.

4.2.30. (2*R*,3*R*)-*N*,*N*,*N*-Trimethyl-4-phosphono-3-(tetradecanoyloxy)butan-2-aminium (27). Compound **27** (38 mg, 48%) was prepared from **26** (139 mg, 0.19 mmol) in an analogous manner to that for preparation of **15**. Amorphous; $[\alpha]_D^{25}$ −4.03 (*c* 0.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (1H, dt, J=7.4, 7.4 Hz), 4.01–3.96 (1H, m), 3.67 (9H, s), 2.41 (2H, t, J=7.5 Hz), 2.22–2.14 (2H, m), 1.58 (3H, t, J=6.7 Hz), 1.30–1.22 (22H, m), 0.88 (3H, t, J=4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.8, 73.7 (d, J_{CP}=7.3 Hz), 53.6, 35.0, 32.9 (d, J_{CP}=6.5 Hz), 31.5, 30.7, 30.5, 30.4, 30.1, 25.5, 23.6, 14.4, 8.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.88; IR (neat) 1000 cm^{-1}; ESIMS *m/z* 422 (MH^+); HRMS (ESI) calcd for $C_{21}H_{45}NO_5P$: 422.3035 (MH^+). Found: 422.3040.

4.2.31. Dibenzyl [(4*S*,5*R*)-5-methyl-2,2-dioxido-1,3,2-dioxathiolan-4-yl]methylphosphonate (*ent*-16). Compound *ent*-**16** (13.0 g, 76%) was prepared from *ent*-**3e** (14.6 g, 41.6 mmol) in an analogous manner to that for preparation of **8** after purification by column chromatography on silica gel (hexane/EtOAc=10:1–1:1). A pale yellow oil; $[\alpha]_D^{20}$ +18.90 (*c* 1.1, MeOH). The ^{1}H NMR spectrum was identical with that of **16**.

4.2.32. (2*S*,3*S*)-Dibenzyl 3-azido-2-hydroxybutylphosphonate (*ent*-17). Compound *ent*-**17** (330 mg, 44%) was prepared from *ent*-**16** (824 mg, 2.0 mmol) in an analogous manner to that for preparation of **9** after purification by column chromatography on silica gel (hexane/EtOAc=3:1–1:1). A colorless oil; $[\alpha]_D^{24}$ +14.48 (*c* 1.1, MeOH). The ^{1}H NMR spectrum was identical with that of **17**.

4.2.33. (2*S*,3*S*)-Dibenzyl 3-azido-2-[(triethylsilyl)oxy]butylphosphonate (*ent*-19). Compound *ent*-**19** (6.27 g, 97%) was prepared from *ent*-**17** (4.96 g, 13.2 mmol) in an analogous manner to that for preparation of **10** after purification by column chromatography on silica gel (hexane/EtOAc=2:1). A colorless oil; $[\alpha]_D^{24}$ +3.06 (*c* 0.7, MeOH). The ^{1}H NMR spectrum was identical with that of **19**.

4.2.34. (2*S*,3*S*)-Dibenzyl 3-amino-2-[(triethylsilyl)oxy]-butylphosphonate (*ent*-20). Compound *ent*-**20** (5.80 g, 94%) was prepared from *ent*-**19** (6.46 g, 13.2 mmol) in an analogous manner to that for preparation of **11** after purification by column chromatography on silica gel (EtOAc to $CHCl_3$/MeOH=10:1). A colorless oil; $[\alpha]_D^{24}$ +2.38 (*c* 1.0, MeOH). The ^{1}H NMR spectrum was identical with that of **20**.

4.2.35. (2*S*,3*S*)-Dibenyl 3-(dimethylamino)-2-[(triethylsilyl)oxy]butylphosphonate (*ent*-24). Compound *ent*-**24** (830 mg, 96%) was prepared from *ent*-**20** (1.50 g, 3.4 mmol) in an analogous manner to that for preparation of **12** after purification by column chromatography on silica gel (hexane/EtOAc=2:1–$CHCl_3$/MeOH=20:1). A colorless oil; $[\alpha]_D^{24}$ −10.46 (*c* 1.1, MeOH). The ^{1}H NMR spectrum was identical with that of **24**.

4.2.36. (1*S*,2*S*)-1-{[Bis(benzyloxy)phosphoryl]methyl}-2-(dimethylamino)propyl myristate (*ent*-25). Compound *ent*-**25** (1.50 g, 30%) was prepared from *ent*-**24** (4.12 g, 8.38 mmol) in an analogous manner to that for preparation of **13** after purification by column chromatography on silica gel ($CHCl_3$/MeOH=1:0–30:1). A colorless oil; $[\alpha]_D^{25}$ +0.30 (*c* 0.6, MeOH). The ^{1}H NMR spectrum was identical with that of **25**.

4.2.37. (2*S*,3*S*)-4-[Bis(benzyloxy)phosphoryl]-*N*,*N*,*N*-trimethyl-3-(tetradecanoyloxy)butan-2-aminium iodide (*ent*-26). Compound *ent*-**26** (500 mg, 30%) was prepared from *ent*-**25** (1.50 g, 2.55 mmol) in an analogous manner to that for preparation of **14** after purification by column chromatography on silica gel ($CHCl_3$/MeOH=1:0–10:1). A colorless oil; $[\alpha]_D^{25}$ +7.58 (*c* 1.0, MeOH). The ^{1}H NMR spectrum was identical with that of **26**.

4.2.38. (2*S*,3*S*)-*N*,*N*,*N*-Trimethyl-4-phosphono-3-(tetradecanoyloxy)butan-2-aminium (*ent*-27). Compound *ent*-**27** (276 mg, 34%) was prepared from *ent*-**26** (487 mg, 1.15 mmol) in an analogous manner to that for preparation of **15**. Amorphous; $[\alpha]_D^{26}$ +3.34 (*c* 0.5, MeOH). The ^{1}H NMR spectrum was identical with that of **27**.

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Synthesis of methyl sulfomycinate, sulfomycinic amide and sulfomycinine, degradation products of the sulfomycin thiopeptide antibiotics

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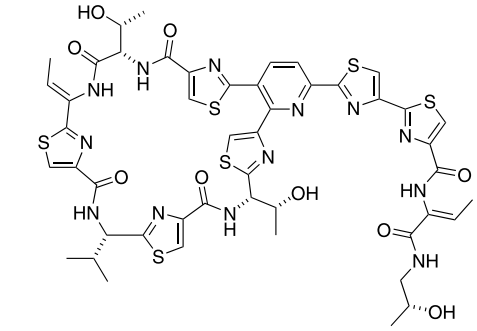
Abstract—The convergent synthesis of methyl sulfomycinate and sulfomycinic amide, two acidic methanolysis products of the sulfomycin thiopeptide antibiotics, is achieved starting from diethoxyacetonitrile. Further confirmation of structure is obtained by heating methyl sulfomycinate at 110 °C in hydrochloric acid to give (±)-sulfomycinine hydrochloride, the acid hydrolysate of sulfomycin I.

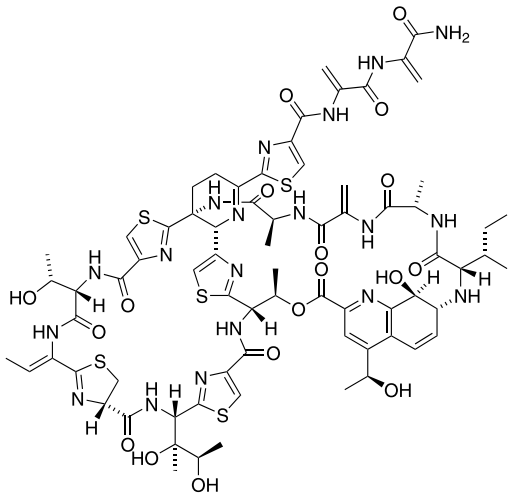
1. Introduction

The thiopeptide antibiotics are a class of biologically active natural products comprising 29 different families and at least 76 structurally distinct sulfur-containing cyclic peptide secondary metabolites isolated from the mycelial cake of actinomycetes. Their biological properties have attracted attention from a broad consortia of scientists and include features as diverse as the inhibition of bacterial protein synthesis, *tipA* promotion, renin inhibition and anti-malarial activity.[1] Since the first isolation of these natural products in 1948,[2] structure elucidation of metabolites such as micrococcin P_1 (**1**) by NMR[3] and the stunningly complex thiostrepton (**2**) by X-ray crystallography (Fig. 1)[4] has been supported by synthetic studies. The laboratory preparation of promothiocin A (**3**)[5,6] and amythiamicin D (**4**)[7] (Fig. 2) served to verify the constitution and stereochemistry of these thiopeptide families and recently the groundbreaking synthesis of thiostrepton (**2**) was reported,[8,9] demonstrating that the synthesis of any members of this antibiotic class is potentially within reach.

The sulfomycins (**5**) (Scheme 1) are one class of thiopeptide antibiotics isolated from *Streptomyces viridochromogenes* subsp. *sulfomycini* ATCC 29776 and MCRL-0368 with strong inhibitory activity against Gram-positive bacteria.[10] Chemical degradation studies,[11,12] in combination with ^1H and ^{13}C NMR spectroscopic and FAB mass spectrometric



micrococcin P_1 (**1**)



thiostrepton (**2**)

Figure 1. Micrococcin and thiostrepton thiopeptide antibiotics.

Keywords: Sulfomycin; Thiopeptide antibiotics; Chemical degradation; Thiazoles.
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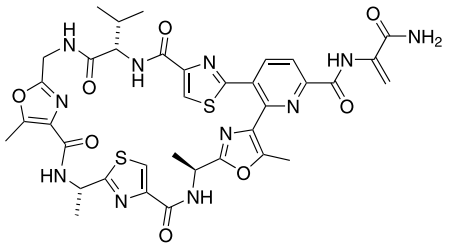


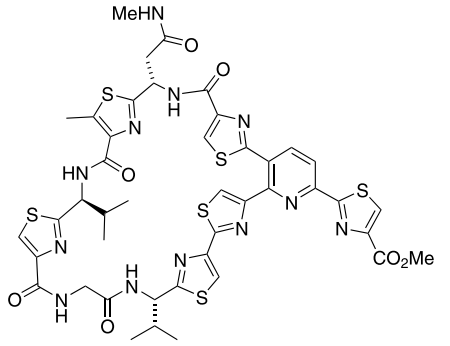
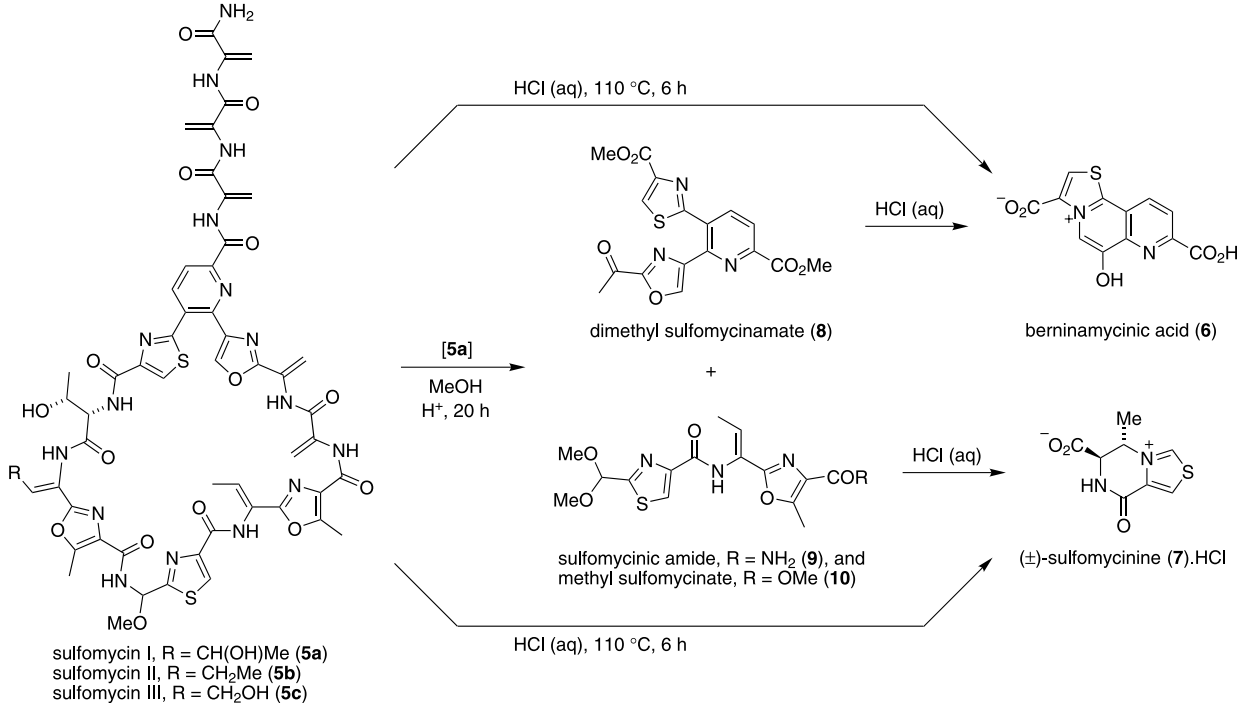


Figure 2. Promothiocin and amythiamicin thiopeptide antibiotics.

data,[13,14] elucidated their structure which contains an oxazole–thiazole–pyridine series *d* domain but differs between factors in the identity of one alkenyl substituent (*R*) located on the cyclic peptide backbone. In these degradation experiments, the acid hydrolysis of sulfomycin I in concentrated hydrochloric acid at 110 °C gave berninamycinic acid (**6**) and sulfomycinine (**7**) hydrochloride, both of which have been identified by X-ray crystallographic methods and chemical synthesis.[15,16] Acidic methanolysis of sulfomycin using Amberlyst 15 ion-exchange resin gave dimethyl sulfomycinamate (**8**), sulfomycinic amide (**9**), and methyl sulfomycinate (**10**), the latter of which was transformed to **7** in order to provide corroborating data on structure (Scheme 1). Our synthesis of dimethyl sulfomycinamate (**8**),[17,18] by Bohlmann-Rahtz reaction of an oxazolylenamine, further verified the identity of this acidic methanolysis product to complement the findings of Kelly,[19] but the structures of degradation products **9** and **10**, which were proposed on the basis of IR, UV, ^{1}H NMR spectroscopic and MS data for **10**,[11] are unconfirmed. In order to validate a route toward the sulfomycins, and as part of our interest in the total synthesis[20,21] and stereochemistry of thiopeptide antibiotics,[22] we set out to address this shortfall and establish a convergent synthesis of methyl sulfomycinate (**10**).

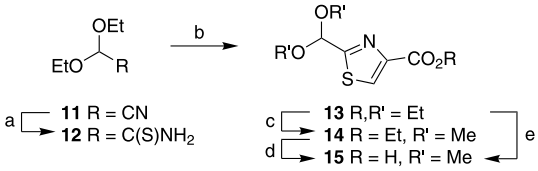
2. Results and discussion

Methyl sulfomycinate (**10**) is a modified tripeptide consisting of two fragments: an oxazole-containing dipeptide **20** related to a known (−)-muscoride A building block,[23] and a 2-formylthiazole-4-carboxylate residue **15** that is also a component of the antibiotic althiomycin,[24] present as its dimethyl acetal. Our convergent strategy assembled each heterocyclic component in turn and then coupled them together with subsequent dehydration to elaborate the aminopropenyl unit of **10**. Starting with diethoxyacetonitrile (**11**), treatment with ammonium sulfide in methanol at room temperature according to our recently reported procedure gave thioamide **12** in excellent yield.[25] Thiazole **13** was prepared by reaction with ethyl bromopyruvate in ethanol under Hantzsch conditions. Transacetalization under acidic conditions with *p*-toluenesulfonic acid (TsOH) in methanol gave dimethyl acetal **14**, which

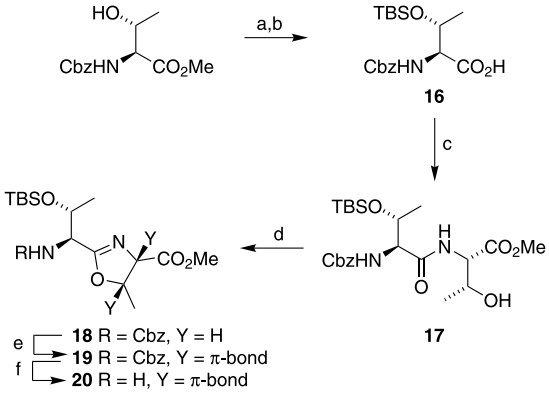


Scheme 1. Structure and chemical degradation of the sulfomycins.

was saponified using lithium hydroxide in methanol–water either directly or after isolation to give thiazole-4-carboxylic acid **15** in reasonable overall yield (Scheme 2).



Scheme 2. Synthesis of thiazole **15**. Reagents and conditions: (a) $(NH_4)_2S$, MeOH, RT, 18 h (100%); (b) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (c) TsOH, MeOH, reflux, 6 h (66%); (d) LiOH, MeOH–H_2O, RT, 18 h (64%); (e) TsOH, MeOH, reflux, 6 h; LiOH, MeOH–H_2O, 48 h (54%).



Scheme 3. Synthesis of oxazole **20**. Reagents and conditions: (a) TBDMSCl, imidazole, DMAP, DMF, RT, 36 h (100%); (b) LiOH, MeOH–H_2O, RT, 18 h (96%); (c) HCl·H-Thr-OMe, pyBOP, Et_3N, CH_2Cl_2, 0 °C–RT, 18 h (83%); (d) Deoxo-Fluor, CH_2Cl_2, −20 °C, 18 h (65%); (e) $CBrCl_3$, DBU, CH_2Cl_2, −20 °C, 18 h (52%); (d+e) without isolation of pure **18** (52%); (f) H_2, Pd–C, MeOH, RT, 3 h (93%).

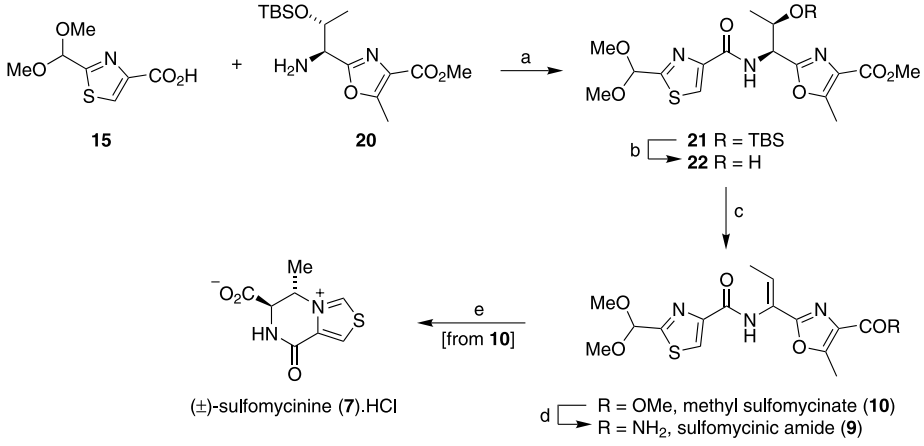
The other component, oxazole **20**, was prepared according to a modified method of Pattenden[23] from an *O*-silyl ether derivative of threonine **16** (Scheme 3).[26,27] Peptide coupling using benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) in dichloromethane gave dipeptide **17** which was cyclized to oxazoline **18** with [bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor)[28] and oxidized to the oxazole **19** with $CBrCl_3$–DBU.[29] Hydrogenolysis of the benzyloxycarbonyl (Cbz) protecting group over Pd–C in methanol gave amine **20** which was coupled with thiazole-acid **15** using pyBOP to give tripeptide **21** (Scheme 4). Finally protodesilylation by treatment with TBAF in THF gave alcohol **22**, which was dehydrated via the corresponding methanesulfonate derivative to give methyl sulfomycinate (**10**). The physical and spectroscopic properties of the synthetic material [mp 122–123 °C; UV (MeOH)/nm λ_{max} 246 (log ε 4.40)] were in very good agreement with literature data on the degradation product [lit.[11] mp 124–124.5 °C; lit.[11] UV (MeOH)/nm λ_{max} 247 (log ε 4.38)] confirming the outcome of Abe's methanolysis studies[11] and providing a viable route to this region of the sulfomycin cyclic peptides.

Further confirmation of structure was obtained by carrying out both the known ammonolysis and hydrolysis of **10**, in methanolic ammonia at room temperature for the former or in hydrochloric acid at 110 °C for the latter, to obtain sulfomycinic amide (**9**) and sulfomycinine (**7**) hydrochloride, respectively, with physical properties that corroborated known data.[16] This convergent synthesis thus confirms both the structure of methyl sulfomycinate (**10**) and the outcome of its chemical degradation, as well as providing a method for the preparation of this fragment applicable to the total synthesis of the sulfomycin thiopeptide antibiotics.

3. Experimental

3.1. General

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254} that were visualised under UV light (at 254 and/or 360 nm). Melting points (mp) were determined on a Kofler hot stage apparatus and are uncorrected. Infra-red (IR) spectra were



Scheme 4. Synthesis of methyl sulfomycinate (**10**). Reagents and conditions (a) pyBOP, CH_2Cl_2, 0 °C–RT, 18 h (88%); (b) TBAF, THF, 0 °C–RT, 5 h (77%); (c) $MeSO_2Cl$, CH_2Cl_2, RT, 1 h; Et_3N, CH_2Cl_2, RT, 18 h (60%); (d) NH_3, MeOH, RT, 2 d (24%); (e) HCl (aq), 110 °C, 2 h (27%).

recorded in the range 4000–600 cm^{-1} on a Perkin-Elmer 1600 series FTIR spectrometer using KBr disks for solid samples and thin films between NaCl plates for liquid samples and are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ at 25 °C unless stated otherwise, using either a Bruker DPX 400 or 500 Avance instrument and were reported in ppm; *J* values were recorded in Hz and multiplicities were expressed by the usual conventions (s=singlet, d=doublet, t=triplet, app=apparent, b=broad, m=multiplet). Low-resolution mass spectra (MS) were determined using a Fisons VG Platform II Quadrupole instrument using atmospheric pressure chemical ionization (APcI) unless stated otherwise. ES refers to electrospray ionization, CI refers to chemical ionization (ammonia) and EI refers to electron ionization. High-resolution mass spectra denote the mass of the ion (mass of electron is 0.00055 Da) unless stated otherwise. Specific rotations were measured at the indicated temperature using an AA-1000 (Optical Activity Ltd) polarimeter at the sodium D line and are given in deg $cm^3\ g^{-1}\ dm^{-1}$ with concentration *c* in $10^{-2}\ gcm^{-3}$. Microanalyses were recorded using a Perkin-Elmer 240C Elemental Analyzer. In vacuo refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by the removal of trace volatiles using a vacuum (oil) pump.

3.1.1. 2,2-Diethoxythioacetamide (12). A mixture of diethoxyacetonitrile (1.12 ml, 8.06 mmol) and ammonium sulfide (50 wt% in H_2O; 1.5 ml, 11.0 mmol) in MeOH (100 ml) was stirred overnight. The mixture was evaporated in vacuo to give the title compound as a pale yellow solid (1.5 g, 100%), mp 92–93 °C (lit.[30] mp 81–82 °C) (Found: MH^+, 164.0743. $C_6H_{13}NO_2S$ requires MH^+, 164.0740) (Found: C, 44.1; H, 8.0; N, 8.4; S, 19.5. Calcd for $C_6H_{13}NO_2S$: C, 44.2; H, 8.0; N, 8.6; S, 19.6%); IR (KBr)/cm^{-1} ν_{max} 3371, 3256, 2971, 2916, 1604, 1424, 1368, 1246, 1121, 1057, 960, 918, 824, 724; ^1H NMR (400 MHz; $CDCl_3$) δ 7.87 (1H, bs, NH), 7.62 (1H, bs, NH), 5.05 (1H, s, CH), 3.76 (2H, dq, *J*=9.5, 7 Hz, 2OC*H*H), 3.67 (2H, dq, *J*=9.5, 7 Hz, 2OCH*H*), 1.18 (6H, t, *J*=7 Hz, 2Me); ^{13}C NMR (125 MHz; $CDCl_3$) δ 202.2 (C), 103.0 (CH), 62.9 (CH_2), 15.1 (Me); *m/z* (EI) 163 (M^+, 2%), 118 (15), 103 (87).

3.1.2. Ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (13). Ethyl bromopyruvate (1.71 ml, 13.6 mmol) was added to a stirred solution of 2,2-diethoxythioacetamide (**12**) (1.22 g, 7.47 mmol) in EtOH (16 ml) over 4 Å molecular sieves (14 g) and the mixture was heated at reflux for 1 h. The solution was allowed to cool, filtered through Celite® and evaporated in vacuo. Purification twice by column chromatography on SiO_2, eluting with Et_2O–light petroleum (4:1) and Et_2O–light petroleum (1:1) (R_f 0.28), gave the title compound as a pale yellow oil (1.94 g, 100%) (Found: MH^+, 260.0956. $C_{11}H_{17}NO_4S$ requires MH^+, 260.0951); IR (film)/cm^{-1} ν_{max} 3441, 3113, 2983, 2359, 1694, 1556, 1454, 1391, 1368, 1332, 1216, 1101, 1020, 953, 882, 860, 767, 704; ^1H NMR (400 MHz; $CDCl_3$) 8.10 (1H, s, 5-H), 5.63 (1H, s, CH), 4.32 (2H, q, *J*=7.1 Hz, CH_2), 3.69 (2H, dq, *J*=9.5, 7 Hz, 2OC*H*H), 3.60 (2H, dq, *J*=9.5, 7 Hz, 2OCH*H*), 1.35 (3H, t, *J*=7.1 Hz, Me), 1.18 (6H, app t, *J*=7.1 Hz, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.2 (C), 161.4 (C), 147.1 (C), 128.5 (CH), 98.7 (CH), 62.8 (CH_2), 61.5 (CH_2), 15.1 (Me), 14.4 (Me); *m/z* (APcI) 260 (MH^+, 37%).

3.1.3. Ethyl 2-(dimethoxymethyl)thiazole-4-carboxylate (14). *p*-TsOH monohydrate (30 mg, 20 mol%) was added to a stirred solution of ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (**13**) (200 mg, 0.77 mmol) in dry MeOH (5 ml) and the mixture heated at reflux for 6 h. After evaporating in vacuo, the mixture was partition between saturated aqueous $NaHCO_3$ solution (15 ml) and CH_2Cl_2 (30 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×30 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with EtOAc–light petroleum (1:1) (R_f 0.41), gave the title compound as a brown oil (118 mg, 66%) (Found: MH^+, 232.0638. $C_9H_{13}NO_4S$ requires MH^+, 232.0637); IR (film)/cm^{-1} ν_{max} 3439, 3106, 2981, 1730, 1454, 1369, 1332, 1248, 1216, 1095, 1021, 950.1, 860, 766; ^1H NMR (400 MHz; $CDCl_3$) δ 8.14 (1H, s, 5-H), 5.52 (1H, s, CH), 4.35 (2H, q, *J*=7.1 Hz, CH_2), 3.39 (6H, s, MeO), 1.34 (3H, t, *J*=7.1 Hz, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.9 (C), 161.4 (C), 147.3 (C), 128.6 (CH), 100.3 (CH), 61.6 (CH_2), 54.0 (Me), 14.4 (Me); *m/z* (APcI) 232 (MH^+, 73%).

3.1.4. 2-(Dimethoxymethyl)thiazole-4-carboxylic acid (15). LiOH monohydrate (108 mg, 2.6 mmol) was added to a stirred solution of ethyl ester **14** (100 mg, 0.43 mmol) in MeOH–$_2$O (5:1) (4 ml) and the solution was stirred overnight. After evaporating in vacuo, the mixture was partitioned between citric acid (1 M; 8 ml) and CH_2Cl_2 (35 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×20 ml) and the organic extracts were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated in vacuo to give the title compound as a brown solid (56 mg, 64%), mp 112–113 °C (EtOAc) (Found: MH^+ 204.0325. $C_7H_9NO_4S$ requires MH^+ 204.0325); IR (KBr)/cm^{-1} ν_{max} 3391, 2885, 2698, 2586, 2511, 1726, 1503, 1484, 1459, 1399, 1320, 1205, 1092, 1062, 984, 953, 905, 872, 820, 770, 732, 670; ^1H NMR (400 MHz; $CDCl_3$) δ 9.40 (1H, bs, OH), 8.25 (1H, s, 5-H), 5.52 (1H, s, CH), 3.36 (6H, s, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.5 (C), 161.3 (C), 147.4 (C), 129.1 (CH), 100.2 (CH), 53.1 (Me); *m/z* (APcI) 204 (MH^+, 100%).

3.1.5. 2-(Dimethoxymethyl)thiazole-4-carboxylic acid (15) from 13. *p*-TsOH monohydrate (300 mg, 156 mmol) was added to a stirred solution of ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (**13**) (2 g, 7.7 mmol) in MeOH (60 ml) and the mixture heated at reflux for 6.5 h. After cooling to room temperature, LiOH monohydrate (1.9 g, 46 mmol) and water (12 ml) were added and the solution was stirred for 48 h at room temperature. After evaporating in vacuo, the mixture was partitioned between citric acid (1 M; 40 ml) and CH_2Cl_2 (80 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×60 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo to give the title compound as an off-white solid (0.85 g, 54%), mp 115–116 °C, with identical spectroscopic properties.

3.1.6. Z-L-Thr(TBS)-OMe. Imidazole (1.5 g, 22 mmol), DMAP (0.92 g, 7.5 mmol) and TBDMSCl (2.3 g, 15 mmol) were added successively to a solution of Z-L-Thr-OMe (3.0 g, 11 mmol) in DMF (11 ml). The mixture was stirred at room temperature for 36 h, acidified with hydrochloric acid (1 M; 15 ml) and extracted with ether (3×40 ml). The combined organic extracts were washed with H_2O (3×60 ml), dried (Na_2SO_4) and evaporated in vacuo to give the title compound as a pale yellow oil (4.3 g, 100%) (Found: MH^+, 382.2048. $C_{19}H_{31}NO_5Si$ requires MH^+, 382.2044); $[\alpha]_D^{30}$ −6.6 (*c*2.05, $CHCl_3$) {lit.[31] $[\alpha]_D^{24}$ −7.31 (*c*3.55, $CHCl_3$)}; IR (film)/cm^{-1} ν_{max} 3449, 2954, 2856, 1730, 1507, 1472, 1436, 1378, 1344, 1315, 1255, 1209, 1174, 1129, 1101, 1071, 1030, 1004, 963, 838, 810, 777; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.35 (5H, PhH), 5.42 (1H, bd, J=9.8 Hz, NH), 5.13 (2H, s, CH_2O), 4.42 (1H, dq, J=6.3, 1.7 Hz, β-CH), 4.25 (1H, dd, J=9.8, 1.7 Hz, α-CH), 3.67 (3H, s, MeO), 1.17 (3H, d, J=6.3 Hz, Me) 0.8 (9H, s, CMe_3), −0.01 (3H, s, *Me*SiMe), −0.05 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.4 (C), 156.8 (C), 136.3 (C), 128.6 (CH), 128.5 (CH), 122.3 (CH), 69.8 (CH), 67.2 (CH_2), 59.9 (CH), 52.3 (Me), 25.6 (Me), 20.8 (Me), 17.8 (C), −4.39 (Me), −5.34 (Me); *m*/*z* (APcI) 382 (MH^+, 100%).

3.1.7. Z-L-Thr(TBS)-OH 16. LiOH monohydrate (1.05 g, 25.0 mmol) was added to a stirred solution of Z-L-Thr(TBS)-OMe (1.5 g, 3.93 mmol) in MeOH–H_2O (5:1) (40 ml). After stirring for 18 h, the mixture was partitioned between hydrochloric acid (1 M; 40 ml) and CH_2Cl_2 (60 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×60 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo to give the title compound as a colourless solid (1.38 g, 96%), mp 149–150 °C (triturated with light petroleum–EtOAc) (lit.[27] mp 154–157 °C) (Found: MH^+, 368.1883. $C_{18}H_{29}NO_5Si$ requires MH^+, 368.1888); $[\alpha]_D^{31}$ +10.3 (*c*2.82, $CHCl_3$) {lit.[27] $[\alpha]_D^{22}$ +10.5 (*c*1.69, $CHCl_3$)}; IR (KBr)/cm^{-1} ν_{max} 3440, 3035, 2956, 2856, 1702, 1600, 1514, 1455, 1413, 1344, 1308, 1257, 1102, 1042, 1005, 975, 836, 813, 778, 733, 696; ^{1}H NMR (500 MHz; $CDCl_3$) δ 9.6 (1H, bs, CO_2H), 7.3 (5H, PhH), 5.43 (1H, bd, J=8.6 Hz, NH), 5.04 (2H, s, CH_2), 4.40 (1H, qd, J=6.3, 2.4 Hz, β-H), 4.24 (1H, dd, J=8.6, 2.4 Hz, α-H), 1.11 (3H, d, J=6.3 Hz, Me), 0.75 (9H, s, CMe_3), −0.02 (3H, s, *Me*SiMe), −0.03 (3H, s, MeSi*Me*); ^{13}C NMR (125 MHz; $CDCl_3$) δ 175.7 (C), 156.7 (C), 136.1 (C), 128.6 (CH), 128.3 (CH), 128.2 (CH), 68.5 (CH), 67.3 (CH_2), 59.4 (CH), 25.7 (Me), 20.3 (Me), 17.9 (C), −4.6 (Me) and −5.1 (Me); *m*/*z* (CI) 368 (MH^+, 28%), 277 (100).

3.1.8. Z-L-Thr(TBS)-L-Thr-OMe 17. Et_3N (0.95 ml, 6.8 mmol) was added dropwise over 30 min to a stirred solution of acid **16** (1.0 g, 2.7 mmol), HCl.H-L-Thr-OMe (0.509 g, 3.0 mmol) and pyBOP (1.56 g, 3.0 mmol) in dry CH_2Cl_2 (13 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating in vacuo, purification by column chromatography on silica, eluting with light petroleum–EtOAc (1:1) (R_f 0.49), gave the title compound as a colourless solid (1.1 g, 83%), mp 133–134 °C (light petroleum–EtOAc) (Found: MH^+, 483.2521. $C_{23}H_{38}N_2O_7Si$ requires MH^+, 483.2521); $[\alpha]_D^{32}$ +13.1 (*c*1.05, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3446, 3357, 2955, 1752, 1723, 1670, 1507, 1253, 1207, 1132, 1102, 1078, 967, 839, 780; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.43 (1H, bd, J=8.8 Hz, NH), 7.20 (5H, PhH), 5.74 (1H, bd, J=5.7 Hz, NH), 5.01 (1H, d, J=12 Hz, C*H*H), 4.92 (1H, d, J=12 Hz, CH*H*), 4.42 (1H, dd, J=8.8, 1.7 Hz, C*H*CO_2Me), 4.21 (2H, 2β-H), 4.14 (1H, m, α-H), 3.60 (3H, s, Me), 2.05 (1H, bs, OH), 1.05 (3H, d, J=6.4 Hz, Me), 1.01 (3H, d, J=6.2 Hz, Me), 0.78 (9H, s, CMe_3), 0.04 (3H, s, *Me*SiMe), 0.00 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.1 (C), 170.1 (C), 156.2 (C), 136.1 (C), 128.6 (CH), 128.2 (CH), 128.1 (CH), 68.4 (CH), 67.6 (CH), 67.0 (CH_2), 59.1 (CH), 57.3 (CH), 52.5 (Me), 25.7 (C), 19.9 (Me), 17.9 (Me), 17.4 (Me), −4.8 (Me), −5.0 (Me); *m*/*z* (APcI) 483 (MH^+, 100%).

3.1.9. (4*S*, 5*S*, 1′*S*, 2′*R*)-Methyl *N*-(benzyloxy)carbonyl-2-[1-amino-2-(*tert*-butyldimethylsilyloxy)prop-1-yl]-5-methyloxazoline-4-carboxylate (18). [Bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor) (0.37 ml, 2.0 mmol) was added dropwise to a stirred solution of Z-L-Thr(TBS)-L-Thr-OMe **17** (0.95 g, 1.97 mmol) in dry CH_2Cl_2 (30 ml) at −20 °C. The solution was stirred for 18 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (30 ml). After warming to room temperature, the mixture was extracted with CH_2Cl_2 (3×40 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (R_f 0.39), gave the title compound as a colourless oil (595 mg, 65%); $[\alpha]_D^{32}$ +7.4 (*c*1.05, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3330, 3034, 2954, 2856, 2358, 1731, 1674, 1504, 1383, 1258, 1212, 1101, 836, 778, 698; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.33 (5H, PhH), 5.45 (1H, bd, J=9.5 Hz, NH), 5.11 (1H, d, J=13.7 Hz, C*H*H), 5.08 (1H, d, J=13.7 Hz, CH*H*), 4.90 (1H, dq, J=10.4, 6.3 Hz, 5-H), 4.78 (1H, d, J=10.4 Hz, 4-H), 4.42 (1H, d, J=9.5 Hz, NHC*H*), 4.35 (1H, q, J=6.3 Hz, CH), 3.75 (3H, s, OMe), 1.25 (3H, d, J=6.3 Hz, 5-Me), 1.17 (3H, d, J=6.3 Hz, Me), 0.80 (9H, s, CMe_3), 0.00 (3H, s, *Me*SiMe), −0.06 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.0 (C), 168.8 (C), 156.4 (C), 136.4 (C), 128.6 (CH), 128.5 (CH), 128.1 (CH), 78.1 (CH), 71.3 (CH), 69.3 (CH), 67.0 (CH_2), 55.5 (CH), 52.1 (Me), 25.7 (Me), 20.6 (Me), 17.9 (C), 16.3 (Me), −4.4 (Me), −5.0 (Me); *m*/*z* (APcI) 483 (100%), 465 (MH^+, 50).

3.1.10. (1′*S*, 2′*R*)-Methyl *N*-(benzyloxy)carbonyl-2-[1-amino-2-(*tert*-butyldimethylsilyloxy)prop-1-yl]-5-methyloxazole-4-carboxylate (19). $BrCCl_3$ (0.88 ml, 8.9 mmol) and DBU (0.88 ml, 5.9 mmol) were added successively to a stirred solution of the oxazoline **18** (0.60 g, 1.28 mmol) in dry CH_2Cl_2 (24 ml) at −20 °C. After stirring for 18 h, the mixture was poured into saturated aqueous $NaHCO_3$ solution (40 ml) and extracted with ethyl acetate (3×60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (R_f 0.63), gave the title compound as a colourless oil (0.31 g, 52%) (Found: MH^+ 463.2258. $C_{23}H_{34}N_2O_6Si$ requires MH^+, 463.2259); $[\alpha]_D^{33}$ −5.7 (*c*2.75, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3436, 3352, 2954, 2925, 2857, 2361, 1732, 1622, 1587, 1504, 1442, 1352, 1253, 1211, 1100, 965, 915, 838, 810, 778, 739, 698; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.35 (5H, PhH), 5.72 (1H, bd, J=9.4 Hz, NH), 5.15 (2H, s, CH_2), 4.93 (1H, dd, J=9.4, 2.1 Hz, 1′-H),

4.42 (1H, qd, J=6.2, 2.1 Hz, 2′-H), 3.90 (3H, s, OMe), 2.60 (3H, s, 5-Me), 1.26 (3H, d, J=6.2 Hz, 2′-Me) 0.78 (9H, s, CMe_3), 0.03 (3H, s, *Me*SiMe), −0.17 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 162.6 (C), 160.9 (C), 156.4 (C), 156.4 (C), 136.2 (C), 128.6 (CH), 128.2, (CH), 127.8 (CH), 127.5 (C), 70.0 (CH), 67.3 (CH_2), 55.5 (CH), 52.0 (Me), 25.5 (C), 20.4 (Me), 17.8 (C), 11.9 (Me), −4.7 (Me), −5.5 (Me); *m/z* (APcI) 463 (MH^+, 100%).

3.1.11. Oxazole 19 from Z-L-Thr(TBS)-L-Thr-OMe 17. Deoxo-Fluor (0.72 ml, 3.9 mmol) was added dropwise to a stirred solution of Z-L-Thr(TBS)-L-Thr-OMe **17** (1.87 g, 3.87 mmol) in dry CH_2Cl_2 (60 ml) at −20 °C. The solution was stirred for 18 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (30 ml). After warming to room temperature, the mixture was extracted with CH_2Cl_2 (3×100 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo to give the crude oxazoline **18** as a brown oil (2.1 g). The residue was dissolved in dry CH_2Cl_2 (70 ml) and cooled to −20 °C. $BrCCl_3$ (1.7 ml, 17.4 mmol) and DBU (2.6 ml, 17.4 mmol) were added and the solution was stirred at −20 °C for 18 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution (70 ml) and extracted with ethyl acetate (3× 60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (R_f 0.15), gave the title compound as a colourless oil (0.94 g, 52%) with identical physical and spectroscopic properties.

3.1.12. (1′*S*, 2′*R*)-Methyl 2-[1-amino-2-(*tert*-butyldimethylsilyloxy)prop-1-yl]-5-methyloxazole-4-carboxylate (20). A solution of oxazole **19** (0.94 g, 2.0 mmol) in MeOH (34 ml) was stirred over Pd–C (10 wt%; 817 mg) under an atmosphere of H_2 for 3 h. The mixture was filtered through Celite® and evaporated in vacuo to give the title compound as a colourless solid, mp 75–76 °C (623 mg, 93%) (Found: MH^+329.1890, $C_{15}H_{28}N_2O_4Si$ requires MH^+329.1891); $[\alpha]_D^{31}$ −29.3 (*c*2.78, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3387, 2955, 2857, 1721, 1621, 1441, 1351, 1256, 1205, 1098, 970, 837.0, 776, 666; ^{1}H NMR (500 MHz; $CDCl_3$) δ 9–6 (2H, bs, NH), 4.45 (2H, 1′,2′-H), 3.85 (3H, s, OMe), 2.53 (3H, s, 5-Me) 1.35 (3H, bs, Me) 0.75 (9H, s, CMe_3), 0.05 (3H, s, *Me*SiMe), −0.1 (3H, s, MeSi*Me*); ^{13}C (100 MHz; $CDCl_3$) δ 164.0 (C), 162.8 (C), 156.3 (C), 127.2 (C), 70.7 (CH), 56.5 (CH), 52.0 Me), 25.7 (Me), 20.5 (Me), 17.8 (C), 11.9 (Me), −4.5 (Me), −5.3 (Me); *m/z* (APcI) 329 (MH^+, 100%).

3.1.13. Amide 21. Et_3N (0.50 ml, 3.6 mmol) was added dropwise over 30 min to a stirred solution of acid **15** (325 mg, 1.6 mmol), amine **20** (420 mg, 1.3 mmol) and pyBOP (830 mg, 1.6 mmol) in dry CH_2Cl_2 (13 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating in vacuo, purification by column chromatography on silica, eluting with light petroleum–EtOAc (1:1) (R_f 0.31), gave the title compound as a colourless oil (580 mg, 88%) (Found: MH^+514.2044, $C_{22}H_{35}N_3O_7SSi$ requires MH^+, 514.2038); $[\alpha]_D^{25}$ +16 (*c*2.3, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3409, 3116, 2956, 2857, 2400, 1731, 1682, 1621, 1538, 1499, 1471, 1352, 1258, 1186, 1098, 973, 910, 838, 756, 666; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.3 (1H, s, CH), 8.2 (1H, d, J=8.9 Hz, NH) 5.72 (1H, s, CH), 5.41 (1H, dd, J=8.9, 2.8 Hz, CH), 4.65 (1H, qd, J=6.1, 2.8 Hz, C*H*Me), 4.07 (3H, s, OMe), 3.68 (3H, s, OMe) 3.4 (3H, s, OMe), 2.75 (3H, s, Me), 1.41 (3H, d, J=6.1 Hz, CH*Me*), 1.05 (9H, s, CMe_3), 0.22 (3H, s, *Me*SiMe), 0.03 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 167.9 (C), 162.7 (C), 161.1 (C), 160.7 (C), 156.4 (C), 149.5 (C), 127.5 (C), 125.0 (CH), 100.3 (CH), 70.0 (CH), 54.1 (Me), 53.8 (Me), 53.4 (CH), 52.0 (Me), 25.5 (Me), 20.7 (Me), 17.8 (C), 12.0 (Me), −4.6 (Me), −5.4 (Me); *m/z* (CI^+) 514 (MH^+, 95%), 159 (100).

3.1.14. Alcohol 22. A solution of TBAF (1.0 M; 1.8 mmol) in THF (1.8 ml) was added to a stirred solution of silyl ether **21** (580 mg, 1.1 mmol) in dry THF (21 ml) at 0 °C. After warming to room temperature, the mixture was stirred for 4.5 h and then partitioned between H_2O and EtOAc. The aqueous layer was further extracted with EtOAc (twice) and the combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.31), gave the title compound as a colourless solid (344 mg, 77%), mp 159–160 °C (25% EtOAc–light petroleum) (Found: MH^+400.1171. $C_{16}H_{21}N_3O_7S$ requires MH^+, 400.1173); $[\alpha]_D^{27}$ −14.7 (*c*2.3, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3458, 3277, 3123, 2956, 2825, 2362, 1715, 1668, 1540, 1497, 1453, 1391, 1376, 1334, 1247, 1217, 1192, 1148, 1098, 1063, 982, 828, 797; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.11 (1H, s, CH), 8.03 (1H, d, J=9.3 Hz, NH) 5.45 (1H, s, CH), 5.24 (1H, dd, J=9.3, 2.9 Hz, CH), 4.51 (1H, qd, J=6.3, 2.9 Hz, C*H*Me), 3.82 (3H, s, OMe), 3.61 (1H, s, OH), 3.37 (6H, s, OMe), 2.53 (3H, s, Me), 1.22 (3H, d, J=6.3 Hz, CH*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 162.4 (C), 161.3 (C), 160.6 (C), 156.9 (C), 149.3 (C), 127.3 (C), 125.5 (CH), 99.9 (CH), 67.7 (CH), 53.7 (Me), 52.0 (Me), 51.9 (CH), 19.2 (Me), 12.1 (Me); *m/z* (CI^+) 400 (MH^+, 100%).

3.1.15. Methyl sulfomycinate (10). A solution of alcohol **22** (344 mg, 0.86 mmol), Et_3N (1.2 ml, 8.6 mmol) and MsCl (0.53 ml, 6.9 mmol) in dry CH_2Cl_2 (20 ml) was stirred for 1 h at room temperature. The solution was partitioned between H_2O (45 ml) and CH_2Cl_2 (30 ml) and the aqueous layer was further extracted with $CHCl_3$ (2×40 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was dissolved in dry CH_2Cl_2 (20 ml), Et_3N (2.0 ml, 14.4 mmol) was added and the mixture was stirred for 18 h. After evaporating in vacuo, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.44), gave the title compound as a colourless solid (198 mg, 60%), mp 122–123 °C (EtOAc) (lit.[11] mp 124–124.5 °C) (Found: MH^+382.1066. $C_{16}H_{19}N_3O_6S$ requires MH^+, 382.1067); IR (KBr)/cm^{-1} ν_{max} 3372, 3116, 2924, 2845, 2363, 2344, 1719, 1684, 1618, 1527, 1469, 1439, 1351, 1233, 1189, 1101, 1062; UV (MeOH)/nm λ_{max} 246 (log ε 4.40) [lit.[11] 247 (log ε 4.38)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.65 (1H, bs, NH), 8.14 (1H, s, CH) 6.68 (1H, q, J=7.2 Hz, CH), 5.55 (1H, s, CH), 3.82 (3H, s, OMe), 3.41 (6H, s, OMe), 2.57 (3H, s, Me), 1.84 (3H, d, J=7.2 Hz, CH*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.1 (C), 162.7 (C), 159.0 (C), 157.5 (C), 156.5 (C), 149.7 (C), 128.9 (CH), 128.1 (C), 125.7 (CH), 122.1 (C), 99.9 (CH), 53.6 (Me), 52.0 (Me), 14.5 (Me), 12.2 (Me); *m/z* (CI^+) 382 (MH^+, 100%).

3.1.16. Sulfomycinic amide (9). A saturated solution of methanolic NH_3 (20 ml) was added to methyl sulfomycinate (**10**) (70 mg, 0.18 mmol) at room temperature. The mixture was stirred at this temperature for 2 days and then evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with EtOAc, gave the title compound as a colourless solid (16 mg, 24%), mp 188–189 °C (EtOAc) (lit.[11] mp 194.5–195 °C); (Found: MH^+ 367.1070. $C_{15}H_{18}N_4O_5S$ requires MH^+, 367.1071); IR (KBr)/cm^{-1} ν_{max} 3474, 3360, 3284, 3153, 3090, 2940, 2842, 1687, 1636, 1604, 1555, 1531, 1497, 1482, 1449, 1419, 1369, 1333, 1305, 1229, 1211, 1195, 1168, 1104, 1082, 1042, 1016, 990, 968, 956, 836, 789, 762, 721, 707, 684; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.71 (1H, bs, NH), 8.13 (1H, s, CH) 6.84 (1H, bs, N*H*H), 6.61 (1H, q, J=7.2 Hz, CH), 5.58 (1H, bs, NH*H*), 5.53 (1H, s, CH), 3.37 (6H, s, OMe), 2.55 (3H, s, Me), 1.84 (3H, d, J=7.2 Hz, CH*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 164.0 (C), 159.0 (C), 156.7 (C), 153.9 (C), 149.7 (C), 129.4 (C), 128.7 (CH), 125.7 (CH), 122.2 (C), 99.8 (CH), 53.6 (Me), 14.5 (Me), 11.9 (Me); *m/z* (APcI) 367 (MH^+, 18%).

3.1.17. (±)-Sulfomycinine (7)·HCl (6-carboxy-5-methyl-8-oxo-5,6,7,8-tetrahydrothiazolo[3,4-*a*]pyrazinium chloride). Methyl sulfomycinate (**10**) (67 mg, 0.76 mmol) was stirred in hydrochloric acid (6 M; 9 ml) in a Carius tube at 110 °C for 2 h. After cooling, the mixture was evaporated in vacuo, by forming an azeotrope with MeOH. The residue was triturated with MeOH to give the title compound as a colourless solid (12 mg, 27%), mp 203–204 °C (dec.) (EtOAc) (lit.[12] mp 205–207 °C) (Found: $[M-Cl]^+$ 213.0325. $C_8H_8N_2O_3S$.HCl requires $[M-Cl]^+$, 213.0328); IR (KBr)/cm^{-1} ν_{max} 3455, 3197, 3096, 3055, 2370, 2288, 1740, 1686, 1575, 1436, 1192, 887, 761; UV (MeOH)/nm λ_{max} 230 (log ε 3.76) [lit.[12] 230 (log ε 3.85)]; ^{1}H NMR (500 MHz; D_2O) δ 10.15 (1H, d, J=2.4 Hz, exch D_2O, 3-H), 8.77 (1H, d, J=2.4 Hz, 1-H), 5.51 (1H, dq, J=1.5, 6.9 Hz, 5-H), 4.46 (1H, d, J=1.5 Hz, 6-H), 1.61 (3H, d, J=6.9 Hz, Me); ^{13}C NMR (125 MHz; D_2O) δ 172.3 (C), 159.7 (CH), 156.7 (C), 136.1 (C), 131.5 (CH), 59.8 (CH), 57.5 (CH), 19.6 (Me); *m/z* (ES) 215 ($[M-Cl]^+$, 100%).

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A concise enantioselective synthesis of the fungal metabolite (+)-decarestrictine L

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Abstract—A stereoselective 10-step synthesis of the fungal metabolite (+)-decarestrictine L from commercially available ethyl (*R*)-3-hydroxybutyrate is described in which tandem oxonium ylide formation and rearrangement is used to construct the tetrahydropyranyl core of the natural product.

1. Introduction

The decarestrictines are secondary metabolites that were isolated from various *Penicillium* strains and identified as bioactive compounds by chemical screening.[1] Several members of the decarestrictine family of natural products have been shown to inhibit the biosynthesis of cholesterol in both a HEP-G2 liver cell assay and in vivo.[1c,d] Of the 15 decarestrictines isolated to date, 13 possess a 10-membered lactone core,[1a,b] a structural feature that is common to other fungal metabolites (Fig. 1). Decarestrictines L and M differ substantially in structure from the other decarestrictines—decarestrictine L contains a tetrahydropyranyl system whilst decarestrictine M is an ether-bridged bicyclic lactone (Fig. 1).[1c]

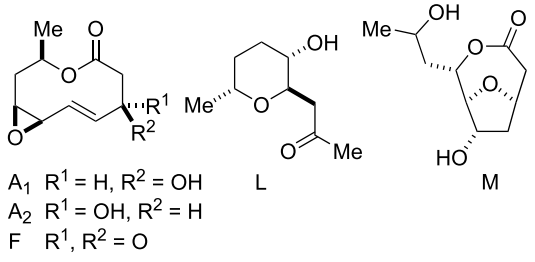
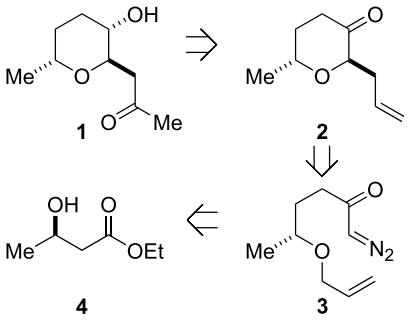


Figure 1. Representative members of the decarestrictine family of natural products.

Decarestrictine L was isolated as a minor component from a culture broth of *Penicillium simplicissimum* (strain FH-A 6090) by Thiericke and co-workers in 1992,[1c] and the relative configurations of the stereogenic centres were established using NMR spectroscopy. Although it is a relatively simple natural product, decarestrictine L has aroused considerable synthetic interest. The first synthesis of (+)-decarestrictine L was reported by Kobayashi and co-workers in 1993 and this established the absolute configuration of the natural product as (2*R*,3*S*,6*R*).[2] Subsequently, four other syntheses of the natural (+) enantiomer have been reported,[3–6] and in addition we have synthesised the compound as its racemate.[7] The syntheses of (+)-decarestrictine L published to date all suffer from one or more shortcomings with regard to following issues: the number of steps, overall chemical yield, stereoselectivity or the availability and expense of the starting materials.[2–6] We now wish to report a short (10-step) stereoselective synthesis of (+)-decarestrictine L that commences from the relatively inexpensive and readily available chiral pool material ethyl (*R*)-3-hydroxybutyrate.

2. Results and discussion

The synthesis of (+)-decarestrictine L was conceived following the retrosynthetic analysis outlined in Scheme 1.



Scheme 1. Retrosynthetic analysis of (+)-decarestrictine L (**1**).

Keywords: Decarestrictine; Stereoselective synthesis; Metal carbenoid; Oxonium ylide; Rearragement.
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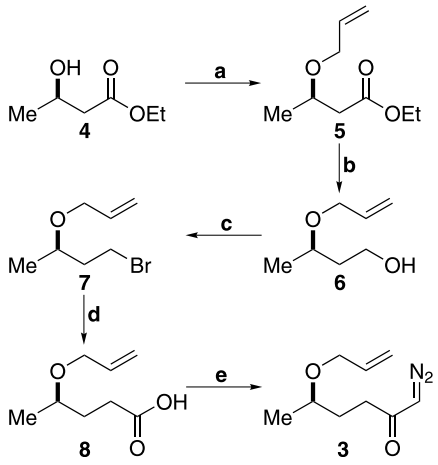
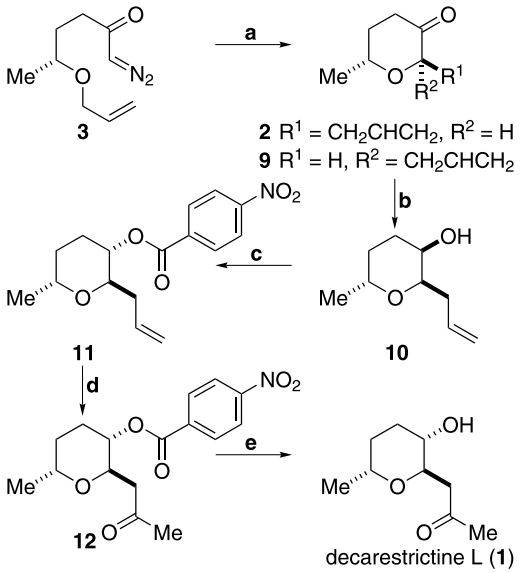
Functional group interconversion in the propyl side chain and on the ring led to the dihydro-2*H*-pyran-3(4*H*)-one **2** as the key intermediate. Recognition of the ketone **2** as the rearrangement product of allylic oxonium ylide then led to the diazo ketone **3** as an acyclic precursor,[7] and further disconnections revealed ethyl (*R*)-3-hydroxybutyrate (**4**) as an ideal chiral pool starting material.

Our synthesis commenced with the allylation of ethyl (*R*)-3-hydroxybutyrate (**4**) using the procedure of Bundle and co-workers that we have employed previously for the allylation of related alcohols (Scheme 2).[7,8] Treatment of the alcohol **4** with allyl 2,2,2-trichloroacetimidate and a sub-stoichiometric amount of triflic acid afforded the allyl ether **5** in good yield. The ester group of allyl ether **5** was then reduced using lithium aluminium hydride to give the alcohol **6** and this was converted into the bromide **7** by treatment with carbon tetrabromide and triphenylphosphine. The bromide was converted into the corresponding Grignard reagent by reaction with magnesium turnings, and subsequent treatment with solid carbon dioxide delivered the carboxylic acid **8** in excellent yield. The diazo ketone **3**, the key cyclisation precursor, was then obtained by conversion of the carboxylic acid **8** into the corresponding acid chloride and subsequent reaction with an ethereal solution of diazomethane.



Scheme 2. Reagents and conditions: (a) $CH_2CHCH_2OC(NH)CCl_3$, CF_3SO_3H, CH_2Cl_2–C_6H_{14}, rt (82%); (b) $LiAlH_4$, THF, 0 °C (89%); (c) CBr_4, PPh_3, CH_2Cl_2, 0 °C→rt (94%); (d) (i) Mg, THF, reflux, (ii) CO_2(s), −78 °C→rt (92%); (e) (i) $(COCl)_2$, DMF (cat.), CH_2Cl_2, (ii) CH_2N_2, Et_2O, 0 °C (72%).

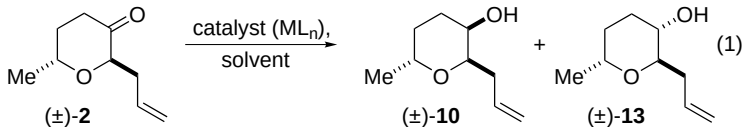
The key ring-forming reaction was effected by treatment of the diazo ketone **3** with copper(II) trifluoroacetylacetonate (2 mol%) in dichloromethane at reflux (Scheme 3).[7,9] Tandem catalytic carbenoid generation, ylide formation and rearrangement delivered a mixture of the isomeric pyranones **2** and **9** (91:9) in 60% yield with the required diastereoisomer (**2**) predominating. The use of copper(II) trifluoroacetylacetonate as catalyst provided the best combination of yield and diastereoselection from the cyclisation reaction, a result that is consistent with our previous work.[9]



Scheme 3. Reagents: (a) $Cu(tfacac)_2$, CH_2Cl_2, reflux (60% **2** and **9**, 91:9); (b) L-Selectride®, THF, −78 °C (95%); (c) *p*-$O_2NC_6H_4CO_2H$, DEAD, PPh_3, THF, rt (46%); (d) $PdCl_2$, CuCl, O_2, DMF aq, rt (89%); (e) K_2CO_3, MeOH aq, rt (96%).

Reduction of the ketone **2** to give the required diastereoisomer (**13**) proved to be problematic (Eq. 1, Table 1). The reduction reaction was effected using a variety of reducing agents, but in most cases the undesired diastereoisomer **10** was obtained as the major product (entries 1–3, Table 1).[10] In two cases (entries 4 and 5, Table 1) attempted reduction, using procedures developed for the stereoselective reduction of related cyclic ketones,[11,12] resulted in decomposition of the ketone **2** and neither the required alcohol **13** nor the diastereoisomeric compound **10** was obtained. At this stage we decided to perform the reaction at low temperature using L-Selectride® as the reducing agent

Table 1.



Entry	Reducing agent	Solvent	Temperature (°C)	Ratio (**10**:**13**)[a]	Yield (**10**+**13**)[b]
1	$NaBH_4$	EtOH	25	80:20	67%
2	MAD, *t*-BuMgCl	PhMe	−78	95:5	58%
3	L-Selectride	THF	−78	>99:1	88%
4	Na–NH_3	NH_3	−40	—	—
5	Et_3SiH, $(Ph_3P)_3RhCl$	C_6H_6	80	—	—

[a] Isomer ratio determined by ^1H NMR analysis.
[b] Yield of purified product.

in order to obtain a single diastereoisomeric product (**10**). Subsequent protection of the alcohol as the 4-nitrobenzoyl ester using Mitsunobu conditions delivered the ester **11** as the sole isolable product with clean inversion of configuration at the hydroxyl-bearing stereogenic centre, albeit in modest yield.[13] The side chain carbonyl group was then installed by palladium-catalysed Wacker oxidation of the terminal alkene.[14] Finally, treatment of the ketone **12** with potassium carbonate in methanol removed the 4-nitrobenzoyl ester group and furnished (+)-decarestrictine L in good yield. The spectroscopic and optical rotation data {$[\alpha]_D^{26}$ +22 (c=0.40, MeOH)} of synthetic (+)-decarestrictine L closely matched that reported for the natural product.[1c]

3. Conclusions

In summary, a stereoselective synthesis of the fungal metabolite (+)-decarestrictine L from commercially available ethyl (*R*)-3-hydroxybutyrate has been completed in 10-steps and in an overall yield of 9%. The important features of the synthesis are the use of tandem oxonium ylide formation and rearrangement from a catalytically generated copper carbenoid for diastereoselctive construction of the tetrahydropyranyl core, and conversion of the allyl side chain into the required methyl ketone using a Wacker oxidation reaction.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform at ambient temperature using Bruker AM 400, AV 400 or DRX 500 instruments. Chemical shift values are quoted in parts per million (ppm) and J values are given in Hertz. ^{1}H NMR signals are described as singlets (s), doublets (d), triplets (t), quartets (q), multiplets (m), or broad (br) or a combination of these. Signals in ^{13}C NMR spectra are quoted in parts per million (ppm), with residual chloroform (δ=77.1 ppm) as the internal standard. Signals are given as (C), (CH), (CH_2), (CH_3) indicating the number of protons attached to each carbon atom as determined using DEPT sequences. IR spectra were recorded using a Perkin–Elmer 1600 series FT-IR spectrometer with internal calibration using solution cells unless otherwise stated. Mass spectra and accurate mass measurements were recorded using a Fisons VG Autospec, VG Micromass 70E or Micromass LCT instrument. Optical rotations were determined using a Jasco DIP-370 digital polarimeter. Reactions were monitored by TLC performed on Merck Kieselgel 60 F_{254} plates, and TLC plates were visualized by a combination of UV light and ethanolic anisaldehyde with heat. Flash column chromatography was performed using Fluka silica gel 60. Solvents and reagents were distilled using standard methods prior to use and air sensitive compounds were handled under either nitrogen or argon. All moisture-sensitive reactions were performed in flame-dried glassware under nitrogen or argon.

4.1.1. Ethyl (*R*)-3-(allyloxy)butanoate (5). Trifluoromethanesulfonic acid (300 μL, 3.39 mmol) was added dropwise to a solution of ethyl (*R*)-3-hydroxybutyrate (6.58 g, 50.0 mmol) and allyl 2,2,2-trichloroacetimidate (20.3 g, 100 mmol) in dry dichloromethane (50 mL) and hexane (100 mL). The mixture was stirred at room temperature for 16 h and then quenched by addition of excess triethylamine. The mixture was filtered through Celite and the filtrate was evaporated. The resulting liquid was purified by distillation to give the title compound as a colourless liquid (7.05 g, 82%): bp 31–33 °C at 0.23 mmHg; $[\alpha]_D^{27}$ −9.1 (c=0.86, $CHCl_3$); ν_{max} ($CHCl_3$) 2972, 2933, 2904, 2872, 1732, 1373, 1646, 994, 943, 913, 871 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (ddt, 1H, J=17.2, 10.4, 5.6 Hz, C*H*=CH_2), 5.26 (ddt, 1H, J=17.2, 1.7, 1.6 Hz, CH=C*H*$_2$ *trans*), 5.14 (ddt, 1H, J=10.4, 1.7, 1.3 Hz, CH=C*H*$_2$ *cis*), 4.13 (q, 2H, J=7.1 Hz, O*CH*$_2$CH$_3$), 4.03 (dddd, 1H, J=12.6, 5.6, 1.6, 1.3 Hz, O*CH*$_2$CH=CH_2), 3.95 (dddd, 1H, J=12.6, 5.6, 1.6, 1.3 Hz, O*CH*$_2$CH=CH_2), 3.96–3.87 (m, 1H, O*CH*CH$_3$), 2.58 (dd, 1H, J=15.0, 7.2 Hz, C*H*$_2$CO$_2$), 2.37 (dd, 1H, J=15.0, 5.9 Hz, C*H*$_2$CO$_2$), 1.25 (t, 3H, J=7.1 Hz, OCH$_2$C*H*$_3$), 1.21 (d, 3H, J=6.2 Hz, OCHC*H*$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.5 (C), 135.1 (CH), 116.7 (CH_2), 71.8 (CH), 69.8 (CH_2), 60.4 (CH_2), 42.1 (CH_2), 19.9 (CH_3), 14.2 (CH_3); LRMS (CI) *m/z* 173 (M^++H, 100); HRMS (CI) for $C_9H_{17}O_3$ [M^++H] calcd 173.1178, found 173.1175.

4.1.2. (*R*)-3-(Allyloxy)butan-1-ol (6). A solution of $LiAlH_4$ (27 mL of 1 M solution in THF, 27 mmol) was added to a solution of ester **5** (4.40 g, 25.6 mmol) in THF (50 mL) at 0 °C. The mixture was stirred at room temperature for 16 h and excess $LiAlH_4$ was quenched by careful addition of 2 M NaOH at 0 °C. The biphasic mixture was stirred vigorously for 2 h and the aqueous layer extracted with diethyl ether. The combined organic extracts were dried ($MgSO_4$), filtered and the solvent was evaporated (caution, volatile product). The residue was purified by flash column chromatography on silica gel (*n*-pentane/diethyl ether, 1:1) to afford the alcohol **6** as a colourless liquid (2.96 g, 89%): $[\alpha]_D^{26}$ −59 (c=0.75, $CHCl_3$); ν_{max} ($CHCl_3$) 3497, 2972, 2932, 2868, 1646, 994, 965, 908 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92, (dddd, 1H, J=17.2, 10.4, 5.8, 5.4 Hz, C*H*=CH_2), 5.28 (dddd, 1H, J=17.2, 1.6, 1.5, 1.4 Hz, CH=C*H*$_2$ *trans*), 5.18 (dddd, 1H, J=10.4, 1.6, 1.5, 1.4 Hz, CH=C*H*$_2$ *cis*), 4.11 (ddt, 1H, J=12.6, 5.4, 1.5 Hz, C*H*$_2$CH=CH_2), 3.93 (ddt, 1H, J=12.6, 5.8, 1.4 Hz, C*H*$_2$CH=CH_2), 3.84–3.70 (m, 3H, CH$_3$C*H*, C*H*$_2$OH), 2.56 (br, 1H, O*H*), 1.81–1.70 (m, 2H, C*H*$_2$CH$_2$OH), 1.21 (d, 3H, J=6.2 Hz, C*H*$_3$CH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 134.9 (CH), 116.8 (CH_2), 74.8 (CH), 69.4 (CH_2), 61.0 (CH_2), 38.7 (CH_2), 19.4 (CH_3); LRMS (ES) *m/z* 153 (M^++Na, 100); HRMS (ES) for $C_7H_{14}O_2Na$ [M^++Na] calcd 153.0891, found 153.0881.

4.1.3. (*R*)-3-(Allyloxy)-1-bromobutane (7). Carbon tetrabromide (8.95 g, 27.0 mmol) and triphenylphosphine (7.08 g, 27.0 mmol) were added sequentially to a solution of alcohol **6** (2.34 g, 18.0 mmol) in dichloromethane (60 mL) at 0 °C. The mixture was then stirred at 0 °C for 1 h and at room temperature for a further 2 h. Silica gel (~10 g) was added and the solvent was evaporated at 0 °C (caution, volatile product) and the solid residue was dry

loaded on a silica flash column. Elution (*n*-pentane/diethyl ether, 10:1) afforded the desired compound as a colourless liquid (3.27 g, 94%). The product was used in the next reaction without further purification: $[\alpha]_D^{29}$ −49.6 (c=2.65, $CHCl_3$); ν_{max} ($CHCl_3$) 2973, 2932, 2865, 1646, 994, 941, 915, 875 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.94 (ddt, 1H, J=17.2, 10.4, 5.6 Hz, C*H*=CH_2), 5.30 (dddd, 1H, J=17.2, 1.6, 1.5, 1.4 Hz, CH=CH_2 *trans*), 5.19 (dddd, 1H, J=10.4, 1.6, 1.5, 1.4 Hz, CH=CH_2 *cis*), 4.13–4.08 (m, 1H, CH_2CH=CH_2), 3.98–3.93 (m, 1H, CH_2CH=CH_2), 3.71–3.66 (m, 1H, CH_3C*H*), 3.60–3.47 (m, 2H, CH_2Br), 2.14–2.05 (m, 1H, CH_2CH_2Br), 1.99–1.90 (m, 1H, CH_2CH_2Br), 1.20 (d, 3H, J=6.1 Hz, CH_3CH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 135.2 (CH), 116.7 (CH_2), 72.6 (CH), 69.7 (CH_2), 40.0 (CH_2), 30.4 (CH_2), 19.4 (CH_3).

4.1.4. (*R*)-4-(Allyloxy)pentanoic acid (8). A crystal of iodine was added to a suspension of magnesium turnings (392 mg, 16.3 mmol) in dry THF under gentle heating (water bath). The bromide **7** (3.00 g, 15.5 mmol) was then added dropwise to the suspension causing an exotherm. After addition was complete, the mixture was heated at 50 °C for 30 min, before being cooled to −78 °C. Solid carbon dioxide (2 g, 3 equiv) was then added in one portion to the solution and the mixture was allowed to warm to room temperature. The resulting white suspension was stirred for 1 h and 1 M aqueous HCl was added until the pH of the aqueous layer was acidic. The aqueous layer was extracted with ethyl acetate and combined organic extracts were dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography on silica gel (petrol 40–60/ethyl acetate, 1:1) to afford the carboxylic acid **8** as a colourless liquid (2.25 g, 92%): $[\alpha]_D^{25}$ −31 (c=0.45, $CHCl_3$); ν_{max} ($CHCl_3$) 3174, 2972, 2931, 2864, 1745, 1710, 995, 943, 914 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.92 (dddd, 1H, J=17.2, 10.4, 5.7, 5.5 Hz, C*H*=CH_2), 5.27 (ddt, 1H, J=17.2, 1.6, 1.4 Hz, CH=CH_2 *trans*), 5.16 (ddt, 1H, J=10.4, 1.6, 1.4 Hz, CH=CH_2 *cis*), 4.06 (ddt, 1H, J=12.6, 5.5, 1.4 Hz, CH_2CH=CH_2), 3.92 (ddt, 1H, J=12.6, 5.7, 1.4 Hz, CH_2CH=CH_2), 3.58–3.48 (m, 1H, CH_3C*H*), 2.51–2.45 (m, 2H, CH_2C=O), 1.85–1.79 (m, 2H, CH_2CH_2C=O), 1.18 (d, 3H, J=6.2 Hz, CH_3CH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 179.7 (C), 135.1 (CH), 116.7 (CH_2), 73.8 (CH), 69.5 (CH_2), 31.3 (CH_2), 30.2 (CH_2), 19.4 (CH_3); LRMS (ES) m/z 181 (M^++Na, 100); HRMS (ES) for $C_8H_{14}O_3Na$ [M^++Na] calcd 181.0841, found 181.0842.

4.1.5. (*R*)-5-(Allyloxy)-1-diazohexan-2-one (3). Oxalyl chloride (0.29 mL, 3.3 mmol) was added to a solution of the carboxylic acid **8** (0.40 g, 2.5 mmol) in dichloromethane (5 mL), followed by two drops of dry DMF. The mixture was stirred at room temperature for 6 h and transferred to an ethereal solution of diazomethane at 0 °C. The mixture was stirred at room temperature for 3 h and excess diazomethane was quenched by careful addition of acetic acid (2 mL). The ether solution was washed with saturated aqueous $NaHCO_3$ (20 mL) and the aqueous layer was back-extracted with diethyl ether (20 mL). The combined organic layers and extracts were dried ($MgSO_4$), filtered and evaporated to give a yellow oil. Purification by flash column chromatography (hexane/diethyl ether, 2:1) afforded the diazo ketone **3** as a bright yellow liquid (0.33 g, 72%): $[\alpha]_D^{25}$ −28 (c=0.43, $CHCl_3$); ν_{max} ($CHCl_3$) 3117, 2973, 2930, 2863, 2109, 1639, 995, 914 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.92 (ddt, 1H, J=17.2, 10.4, 5.6 Hz, C*H*=CH_2), 5.29–5.23 (br, 1H, C*H*N_2), 5.28 (ddt, 1H, J=17.2, 1.6, 1.5 Hz, CH=CH_2 *trans*), 5.17 (ddt, 1H, J=10.4, 1.6, 1.4 Hz, CH=CH_2 *cis*), 4.06 (dddd, 1H, J=12.7, 5.6, 1.5, 1.4 Hz, CH_2CH=CH_2), 3.90 (dddd, 1H, J=12.7, 5.6, 1.5, 1.4 Hz, CH_2CH=CH_2), 3.56–3.47 (m, 1H, CH_3C*H*), 2.51–2.38 (m, 2H, CH_2C=O), 1.92–1.73 (m, 2H, CH_2CH_2C=O), 1.18 (d, 3H, J=6.1 Hz, CH_3CH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.0 (C), 135.2 (CH), 116.5 (CH_2), 73.8 (CH), 69.4 (CH_2), 54.2 (CH), 36.7 (CH_2), 31.6 (CH_2), 19.5 (CH_3); LRMS (ES) m/z 205 (M^++Na, 100), 183 [(M^++H, 10)]; HRMS (ES) for $C_9H_{14}N_2O_2Na$ [M^++Na] calcd 205.0953, found 205.0946.

4.1.6. (2*R*,6*R*)-2-Allyl-6-methyldihydro-2*H*-pyran-3(4*H*)-one (2) and (2*S*,6*R*)-2-allyl-6-methyldihydro-2*H*-pyran-3(4*H*)-one (9). A solution of diazo ketone **3** (0.30 g, 1.6 mmol) in dichloromethane (5 mL) was added dropwise to a solution of $Cu(tfacac)_2$ (25 mg, 5 mol%) in dichloromethane (10 mL) at reflux. The mixture was stirred under reflux for 20 min and the solvent was evaporated. Flash column chromatography (petrol 40–60/diethyl ether, 10:1) of the residue afforded a mixture of the ketones **2** and **9** (91:9) as a colourless liquid (0.15 g, 60%). *Ketone* **2**. $[\alpha]_D^{23}$ +157 (c=0.65, $CHCl_3$); ν_{max} ($CHCl_3$) 2976, 2936, 2873, 1722, 1642, 997, 915 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.83 (ddt, 1H, J=17.1, 10.2, 6.9 Hz, C*H*=CH_2), 5.15 (dq, 1H, J=17.1, 1.5 Hz, CH=CH_2) 5.19–5.10 (m, 1H, CH=CH_2), 4.21–4.12 (m, 1H, CH_3C*H*), 4.10 (dd, 1H, J=8.3, 5.6 Hz, OC*H*C=O), 2.59–2.46 (m, 4H, CH_2C=O, CH_2CH=CH_2), 2.18–2.09 (m, 1H, CH_2CH_2C=O), 1.88–1.76 (m, 1H, CH_2CH_2C=O), 1.29 (d, 3H, J=6.2 Hz, CH_3CH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 210.6 (C), 133.6 (CH), 117.6 (CH_2), 78.9 (CH), 66.6 (CH), 35.6 (CH_2), 34.4 (CH_2), 31.1 (CH_2), 20.5 (CH_3); HRMS (EI) for $C_9H_{14}O_2$ [M^+] calcd 154.0994, found 154.0978. *Ketone* **9**. $[\alpha]_D^{23}$ −67 (c=0.60, $CHCl_3$); ν_{max} ($CHCl_3$) 2977, 2927, 2855, 1722, 1642, 996, 908 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.83 (dddd, 1H, J=17.1, 10.2, 6.9, 6.8 Hz, C*H*=CH_2), 5.13 (dddd, 1H, J=10.2, 1.6, 1.4, 1.3 Hz, CH=CH_2) 5.07 (dddd, 1H, J=17.1, 1.6, 1.4, 1.3 Hz, CH=CH_2), 3.91–3.86 (m, 2H, CH_3C*H*, OC*H*C=O), 2.59 (dddt, 1H, J=14.6, 6.9, 4.5, 1.3 Hz, CH_2CH=CH_2), 2.55 (ddd, 1H, J=16.0, 6.3, 3.5 Hz, CH_2C=O), 2.45 (dddd, 1H, J=16.0, 11.3, 7.0, 0.8 Hz, CH_2C=O), 2.34 (dddt, 1H, J=14.6, 8.0, 6.8, 1.4 Hz, CH_2CH=CH_2), 2.08 (dddd, 1H, J=13.6, 7.0, 3.5, 3.0 Hz, CH_2CH_2C=O), 1.88 (dddd, 1H, J=13.6, 11.3, 10.5, 6.3, Hz, CH_2CH_2C=O), 1.30 (d, 3H, J=6.2 Hz, CH_3CH); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 208.7 (C), 134.4 (CH), 117.2 (CH_2), 82.4 (CH), 72.6 (CH), 37.7 (CH_2), 34.1 (CH_2), 33.5 (CH_2), 21.4 (CH_3); HRMS (EI) for $C_9H_{14}O_2$ [M^+] calcd 154.0994, found 154.1000.

4.1.7. (2*R*,3*R*,6*R*)-2-Allyl-6-methyltetrahydro-2*H*-pyran-3-ol (10). A solution of L-Selectride® (4.7 mL of a 1 M solution in THF, 4.7 mmol) was added dropwise to a solution of ketone **2** (0.24 g, 1.6 mmol) in THF (20 mL) at −78 °C. The mixture was stirred at −78 °C for 3 h and H_2O_2 (4 mL of a 30% aqueous solution) was added, followed by 2 M NaOH until pH ~10. The biphasic mixture was stirred vigorously at room temperature for 1 h and the layers separated. The aqueous layer was extracted

with diethyl ether (3×20 mL) and the combined extracts were dried, filtered and evaporated. The residue was purified by flash column chromatography (petrol 40–60/diethyl ether, 1:1) to afford the alcohol **10** as a colourless oil (0.23 g, 95%): $[\alpha]_D^{23}$ +51 (c=0.65, $CHCl_3$); ν_{max} ($CHCl_3$) 3611, 2975, 2938, 1642, 997, 970, 910, 864 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.85 (ddt, 1H, J=17.1, 10.2, 6.9 Hz, C*H*=CH_2), 5.15 (dddd, 1H, J=17.1, 1.7, 1.6, 1.5 Hz, CH=C*H*$_2$ *trans*), 5.09 (ddd, 1H, J=10.2, 1.6, 1.5, 1.3 Hz, CH=C*H*$_2$ *cis*), 4.04–3.94 (m, 1H, CH_3C*H*), 3.83–3.73 (m, 2H, OC*H*C*H*OH), 2.45–2.37 (m, 1H, C*H*$_2$CH=CH_2), 2.35–2.26 (m, 1H, C*H*$_2$CH=CH_2), 2.00–1.91 (m, 1H, CH_3CHC*H*$_2$), 1.90–1.82 (m, 2H, C*H*HCHO*H*), 1.79–1.70 (m, 1H, C*H*$_2$CHOH), 1.34–1.25 (m, 1H, CH_3CHC*H*$_2$), 1.21 (d, 3H, J=6.6 Hz, C*H*$_3$CH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 135.0 (CH), 116.8 (CH_2), 72.7 (CH), 67.1 (CH), 67.0 (CH), 33.3 (CH_2), 27.0 (CH_2), 26.5 (CH_2), 18.3 (CH_3); LRMS (EI) m/z 156 (M^+, 1), 141 (1), 115 (100), 97 (15), 79 (10), 71 (35), 57 (36); HRMS (EI) for $C_9H_{16}O_2$ [M^+] calcd 156.1150, found 156.1149.

4.1.8. (2*R*,3*S*,6*R*)-2-Allyl-6-methyltetrahydro-2*H*-pyran-3-yl 4-nitrobenzoate (11). To a solution of the alcohol **10** (60 mg, 0.38 mmol) in dry THF (10 mL) was added triphenylphosphine (0.20 g, 0.77 mmol), 4-nitrobenzoic acid (0.13 g, 0.77 mmol), followed by DEAD (120 μl, 0.77 mmol), and the reaction was stirred at room temperature for 16 h. The solvent was then evaporated, and the residue was purified by flash column chromatography on silica gel (hexane/diethyl ether, 10:1) to give the ester **11** (54 mg, 46%) as a colourless oil: $[\alpha]_D^{26}$ +27.4 (c=1.75, $CHCl_3$); ν_{max} ($CHCl_3$) 3112, 2947, 2872, 1721, 1642, 1609, 970, 908, 873, 859 cm^{-1}; ^1H NMR (400 MHz) δ 8.30 (d, 2H, J=9.0 Hz, Ar-*H*), 8.24 (d, 2H, J=9.0 Hz, Ar-*H*), 5.84 (ddt, 1H, J=17.1, 10.2, 6.9 Hz, CH_2C*H*C=CH_2), 5.16–5.09 (m, 2H, CH_2CH=C*H*$_2$), 4.97 (dt, 1H, J=5.0, 3.5 Hz, C*H*O_2CAr), 4.05–3.99 (m, 1H, C*H*OCH_2CH=CH_2), 3.95–3.89 (m, 1H, CH_3C*H*O), 2.58–2.48 (m, 1H, CHOC*H*$_2$CH=CH_2), 2.44–2.36 (m, 1H, CHOC*H*$_2$CH=CH_2), 2.12–2.02 (m, 1H, C*H*$_2$CHO_2CAr), 2.01–1.92 (m, 1H, C*H*$_2$CHO_2CAr), 1.71–1.63 (m, 2H, CH_3CHOC*H*$_2$), 1.24 (d, 3H, J=6.3 Hz, C*H*$_3$CHO); ^{13}C NMR (100.6 MHz) δ 164.1 (C), 150.6 (C), 135.9 (C), 133.9 (CH), 130.8 (CH), 123.5 (CH), 117.4 (CH_2), 73.9 (CH), 71.4 (CH), 66.0 (CH), 34.8 (CH_2), 28.1 (CH_2), 23.8 (CH_2), 20.6 (CH_3); HRMS (EI) for $C_{13}H_{14}NO_5$ [$M^+-C_3H_5$] calcd 264.0872, found 264.0885.

4.1.9. (2*R*,3*S*,6*R*)-6-Methyl-2-(2-oxopropyl)tetrahydro-2*H*-pyran-3-yl 4-nitrobenzoate (12). $PdCl_2$ (5.8 mg, 20 mol%) and CuCl (16 mg, 0.16 mmol) were added to a solution of the ester **11** (50 mg, 0.16 mmol) in DMF (2.5 mL) and water (0.25 mL) under an atmosphere of oxygen and the reaction was stirred vigorously at room temperature until consumption of the starting material was complete (~1.5 h). A saturated aqueous solution of sodium chloride was added and the mixture was then extracted with dichloromethane (4×20 mL). The combined organic extracts were washed with brine (25 mL) and the organic layer was dried (Na_2SO_4). The solvent was evaporated and the residue was purified by flash chromatography on silica gel (hexane/diethyl ether, 1:1) to give the ketone **12** (47 mg, 89%) as a colourless oil: $[\alpha]_D^{26}$ +12 (c=0.60, $CHCl_3$); ν_{max} ($CHCl_3$) 2953, 2872, 1721, 1609, 971, 908, 873 cm^{-1}; ^1H NMR (400 MHz) δ 8 31 (d, 2H, J=9.0 Hz, Ar-*H*), 8.23 (d, 2H, J=9.0 Hz, Ar-*H*), 4.92–4.87 (m, 1H, C*H*O_2CAr), 4.46 (ddd, 1H, J=8.8, 5.0, 4.9 Hz, C*H*OCH_2C=O); 4.01–3.92 (m, 1H, CH_3C*H*O), 2.86 (dd, 1H, J=15.5, 8.8 Hz, C*H*$_2$C=O), 2.64 (dd, 1H, J=15.5, 4.9 Hz, C*H*$_2$C=O), 2.21 (s, 3H, C*H*$_3$C=O), 2.10–1.93 (m, 2H, C*H*$_2$CHO_2CAr), 1.82–1.74 (m, 1H, CH_3CHOC*H*$_2$$CH_2$), 1.70–1.61 (m, 1H, CH_3CHOC*H*$_2$$CH_2$), 1.28 (d, 3H, J=6.4 Hz, C*H*$_3$CHO); ^{13}C NMR (100.6 MHz) δ 205.8 (C), 164.0 (C), 150.7 (C), 135.5 (C), 130.8 (CH), 123.6 (CH), 72.0 (CH), 70.0 (CH), 67.0 (CH), 45.2 (CH_2), 30.5 (CH_3), 28.1 (CH_2), 24.0 (CH_2), 19.5 (CH_3); HRMS (EI) for $C_{13}H_{14}NO_5$ [$M^+-C_3H_5O$] calcd 264.0872, found 264.0848.

4.1.10. 1-[(2*R*,3*S*,6*R*)-3-Hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]-acetone [(+)-decarestrictine L] (1). To a solution of the ketone **12** (39 mg, 12 mmol) in a mixture of methanol (5 mL) and water (0.1 mL) was added K_2CO_3 (17 mg, 12 mmol), and the reaction was stirred at room temperature for 45 min. The solvent was evaporated, and the residual material was purified by flash column chromatography on silica gel (diethyl ether) to give decarestrictine L (**1**) (20 mg, 96%) as a colourless oil: $[\alpha]_D^{26}$ +22 (c=0.40, MeOH) {lit. $[\alpha]_D^{20}$ +21.8 (c=0.5, MeOH)}; ν_{max} ($CHCl_3$) 3627, 3477, 2943, 2838, 1712, l602 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.04–3.98 (m, 1H, C*H*OCHOH), 3.97–3.92 (m, 1H, CH_3C*H*O), 3.43–3.37 (m, 1H, C*H*OH), 2.75 (dd, 1H, J=15.6, 5.6 Hz, C*H*$_2$C=O), 2.70 (dd, 1H, J=15.6, 7.4 Hz, C*H*$_2$C=O), 2.20 (s, 3H, C*H*$_3$C=O), 2.17 (br, 1H, O*H*), 1.92–1.82 (1H, m, C*H*$_2$CHOH), 1.77–1.65 (m, 2H, C*H*$_2$C*H*$_2$), 1.61–1.52 (m, 1H, CH_3CHOC*H*$_2$), 1.22 (d, 3H, J=6.6 Hz, C*H*$_3$CHO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.8 (C), 72.1 (CH), 69.4 (CH), 67.5 (CH), 46.3 (CH_2), 30.5 (CH_3), 28.3 (CH_2), 27.1 (CH_2), 18.4 (CH_3); HRMS (EI) for $C_9H_{14}O_2$ [M^+-H_2O] calcd 154.0994, found 154.0995.

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Use of Stille-type cross-coupling as a route to oligopyridylimines

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Abstract—The new tin reagents, 2-(*n*-Bu_3Sn)-6-{C(R)OCH_2CH_2O}-C_5H_3N, (R = H **a**, Me **b**), have been employed in Stille-type cross-coupling reactions with a range of oligopyridylbromides generating, following a facile deprotection step, a series of formyl- and acetyl-functionalised oligopyridines. Condensation reactions with 2,6-diisopropylaniline has allowed access to families of novel sterically bulky multidentate *N*,*N*,*N*,*N* (tetradentate), *N*,*N*,*N*,*N*,*N* (pentadentate), *N*,*N*,*N*,*N*,*N*,*N* (sexidentate) and *N*,*N*,*N*,*N*,*N*,*N*,*N* (heptadentate) nitrogen donor ligands. This work represents a straightforward and rapid synthetic route for the preparation of oligopyridylimines, which are expected to act as useful components for the self-assembly of polymetallic complexes.

1. Introduction

The chemistry of 2,6-oligopyridines, $C_5H_4N(C_5H_3N)_nC_5H_4N$ (n = 1–7), has been widely developed in recent years due, in the main, to its connection with supramolecular chemistry, in which the ligand in combination with a suitable metal ion can self-assemble to form helical or grid-like superstructures.[1–4] In the same way, it would be anticipated that the structurally related 2,6-oligopyridylimines, $C_5H_4N(C_5H_3N)_nCR{=}NR'$ or $R'N{=}RC(C_5H_3N)_mCR{=}NR'$ (m or $n \geq 1$; R = H or hydrocarbyl; R′ = hydrocarbyl), in which the pyridyl chain is terminated by either one or two imino units, could be employed to develop a similar chemistry. Moreover, the presence of a functionalisable imino-substituent (R or R′) would offer a convenient method of modifying both the electronic and steric properties of the ligand manifold. However, an absence of suitable synthetic protocols has meant the chemistry of the higher members (m or $n \geq 2$) of the oligopyridylimine family is not nearly as well developed.[5–10] Nevertheless, multistep procedures[5,8] have been carried out that have allowed access to bis(imino)bipyridine (m = 2) and bis(imino)terpyridine (m = 3) compounds and indeed their capacity to form double helicate structures has been recognised.[8,9] In stark contrast, the lower members of the family, that is, bis(imino)pyridine (m = 1), have been widely used in coordination chemistry and more recently as supports for olefin polymerisation catalysts.[11–13] Notably, in the latter case an appreciation of the steric properties of the arylimino *N*-substituent has been paramount in the development of the area.

Herein, we introduce a simple general strategy for preparing a wide variety of new multitopic nitrogen donor oligopyridylimines ligands containing either one or two sterically demanding aryl-aldimino [–C(H)=NAr] or aryl-ketimino [–C(Me)=NAr] end-groups. The ligand synthesis proceeds via the synthesis of formyl- and acetyl-functionalised oligopyridines (using Stille-type coupling methodologies[14]) which can be readily converted to their aryl-imine counterparts.

2. Results and discussion

2.1. Synthesis of 2-(*n*-Bu_3Sn)-6-{C(R)OCH_2CH_2O}-C_5H_3N (R = H a, Me b)

The tin reagents, 2-(*n*-Bu_3Sn)-6-{C(R)OCH_2CH_2O}-C_5H_3N (R = H **a**, Me **b**), can be prepared in good overall yield in two steps from 2-bromo-6-formylpyridine and 2-bromo-6-acetylpyridine, respectively (Scheme 1). Firstly, the protected ketal derivatives 2-Br-6-{C(R)OCH_2CH_2O}-C_5H_3N (R = H, Me) can be prepared by reaction of 2-bromo-6-formylpyridine and 2-bromo-6-acetylpyridine with ethane-1,2-diol under azeotropic reflux in benzene.[15] Secondly, treatment of 2-Br-6-{C(R)OCH_2CH_2O}-C_5H_3N (R = H, Me) with *n*-BuLi at −100 °C in diethylether followed by reaction of the lithiated intermediates with

Keywords: Stille-type; Cross-coupling; Oligopyridylimines; Nitrogen donor.
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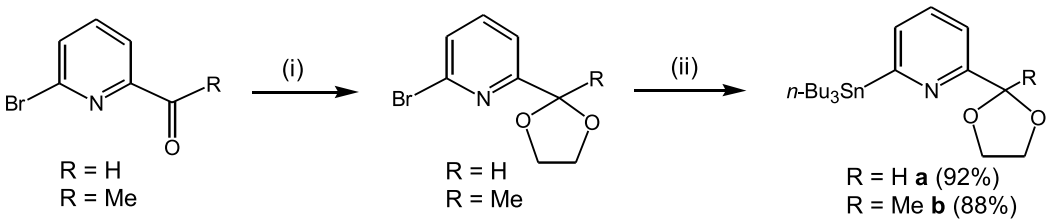


Scheme 1. Reagents and conditions: (i) $(CH_2OH)_2$, cat. *p*-TsOH, benzene, reflux;[15] (ii) *n*-BuLi, $ClSn(n\text{-}Bu)_3$, Et_2O, $-100\,^{\circ}C$.

tributyltin chloride affords air stable **a** and **b** in good yield. Notably, the reactions can be readily scaled-up allowing access to up to 30 g of the tin reagents.

2.2. Stille-type reaction of a and b with oligopyridylbromides

Stille-type cross-coupling reactions of **a** or **b** were carried out with a range of oligopyridylbromides in toluene at elevated temperature in the presence of catalytic quantities of $Pd(PPh_3)_4$ (loadings between 6–8 mol%) to yield, following acid-mediated deprotection, the formyl- and acetyl-oligopyridines **1–5**, respectively (Table 1). All the new compounds have been characterised by ^1H, ^{13}C NMR, IR spectroscopy and ESI mass spectrometry (see Section 4).

The IR spectra for **1–5** showed characteristic absorption bands for their carbonyl functionalities (ca. 1705 cm^{-1}) while molecular ion peaks were revealed in their ESI mass spectra. In the $^{13}C\{^1H\}$ NMR spectra, the carbonyl carbon atoms were seen in the range δ 193.8 and 200.4 with the C*H*=O and C*Me*=O protons being seen at ca. δ 10.1 (**1a**, **2a**, **3a**, **4a**) and δ 2.8 (**1b**, **2b**, **3b**, **4b**) in their ^1H NMR spectra, respectively.

The overall yields obtained for the combination of both the coupling reaction and the acid deprotection are generally good (>60%) but start to decrease as the pyridyl chain length is increased (Table 1). This observation can be, in part, attributed to the reduction in solubility of the higher members of the carbonyl-containing oligopyridines in the organic solvents employed, which is particularly significant in the case of the diformyl-substituted quartepyridine **4a** where the yield drops to 45%. Moreover, we have not been able to isolate the diformylquinquepyridine (**5a**) despite being able to characterise the protected form. It is noteworthy that alternative procedures for compounds **1a**,[16] **1b**[17] and **3a**[5] have been previously reported although no spectroscopic data was included for **1b**; the yields described here are higher or comparable with the previous reports.

2.3. Condensation reaction of carbonyl compounds with 2,6-diisopropylaniline

Treatment of **1–5** with 2,6-diisopropylaniline in the presence of a catalytic amount of acid (glacial acetic or formic acid) gave the imine compounds **6–10** (see Table 2) as pale yellow or white solids in moderate to good yield. The aldimine compounds (**6a**, **7a**, **8a**, **9a**) were most conveniently prepared by carrying out the reaction in ethanol at 50 °C overnight (method A) while the ketimine compounds (**6b**, **7b**, **8b**, **9b**, **10b**) could be prepared by using 2,6-diisopropylaniline as both reactant and solvent (method B) at elevated temperatures over short time periods. All the new compounds have been characterised by ^1H NMR, ^{13}C NMR, IR spectroscopy and ESI mass spectrometry (see Section 4).

The molecular structures of **6b**, **8a** and **9a** are depicted in Figures 1–3; selected bond distances and angles are listed in Table 3. Each structure consists of a chain of 2,6-linked pyridine rings (three for **6a** and **6b**; four for **9a**) containing either one (**6a**) or two (**8a** and **9a**) 2,6-diisopropylphenyl-substituted imino groups as the chain-ends. In each compound a mutually transoid conformation of the nitrogen atoms is observed throughout the imine–pyridine backbone as has been observed for the structurally related 2,6-oligopyridines.[18] The C–N bond lengths of the imine groups range from 1.256(2) to 1.275(4) Å and are consistent with double bond character. While the pyridine and imine groups in **6a** are essentially coplanar some twisting is evident within the backbone of **8a** [tors.: N(3)–C(19)–C(18)–N(2) 14.0°], which is even more noticeable in **9a** [tors.: N(2)–C(18)–C(19)–N(3) 28.9°]. For all three structures the 2,6-diisopropylphenyl rings are inclined essentially orthogonally to the plane of the adjacent pyridyl-imine units.

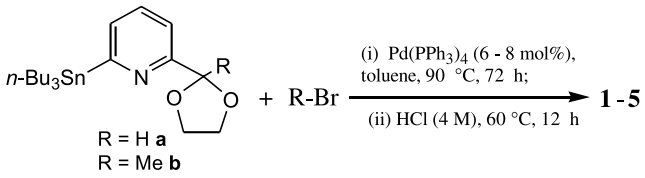
Compounds **6–10** gave molecular ion peaks in their mass spectra and IR spectroscopy showed characteristic absorption bands for their imino functionalities (ca. 1635 cm^{-1}). In the ^1H NMR spectra, the aldimine compounds (**6a**, **7a**, **8a**, **9a**) gave singlets at ca. δ 8.1 consistent with the presence of C*H*=N protons while the C*Me*=N protons could be seen at ca. δ 2.2 for the ketimine compounds (**6b**, **7b**, **8b**, **9b**, **10b**).

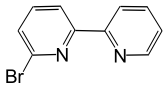
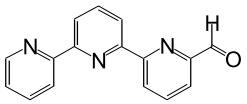
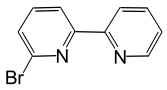
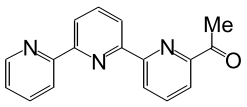
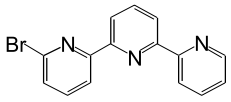
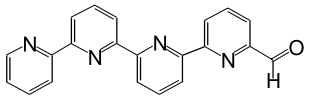
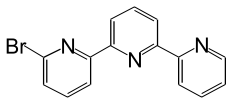
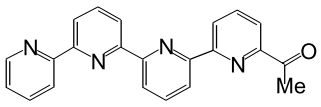
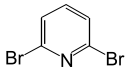
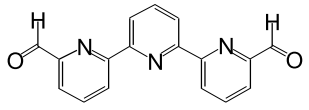
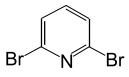
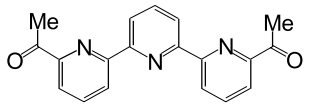
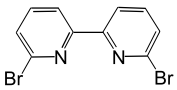
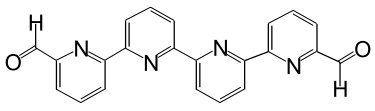
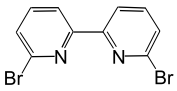
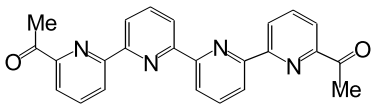
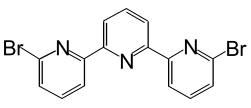
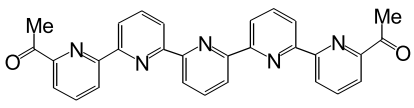
It is noteworthy that the introduction of the 2,6-diisopropylphenyl group to the oligopyridyl chain not only changes the steric and electronic attributes of the compounds but significantly also enhances the solubility of the longer chain members of the series. For example, **9a** is highly soluble in chlorinated solvents while the corresponding formyl precursor **4a** is scarcely soluble.

3. Conclusions

In summary, we have described a straightforward and efficient synthesis for a broad range of formyl- and acetyl-oligopyridines via a palladium-mediated Stille-type cross-coupling approach. Their subsequent condensation reactions with 2,6-diisopropylaniline have allowed access to several novel families of sterically encumbered

Table 1. Synthesis of formyl- and acetyl-functionalised oligopyridines via Stille-type cross-coupling of **a** or **b** with oligopyridylbromides



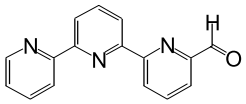
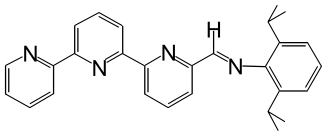
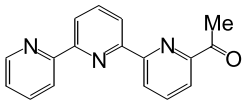
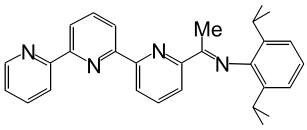
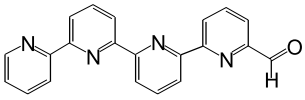
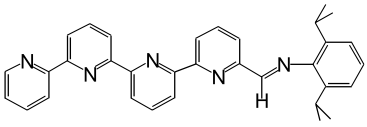
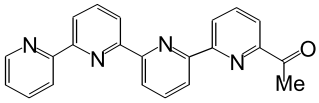
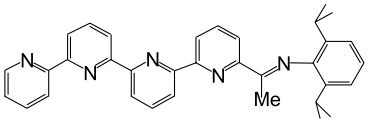
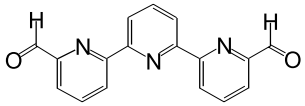
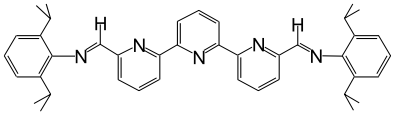
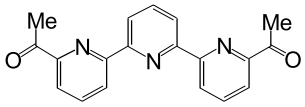
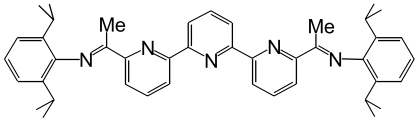
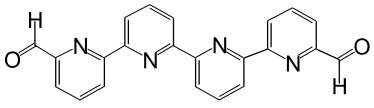
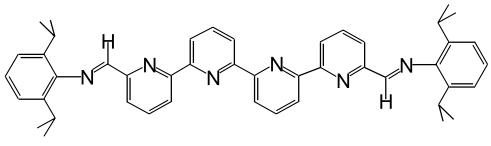
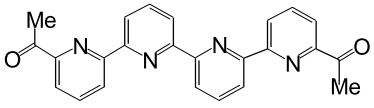
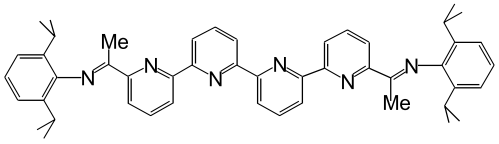
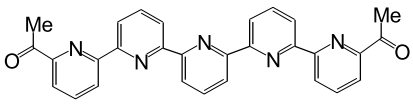
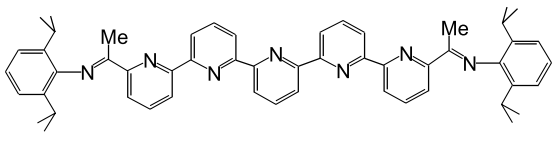
Entry	Tin reagent	R-Br	Deprotected product	Yield (%)[a]
1	**a**		 **1a**	60[b]
2	**b**		 **1b**	62
3	**a**		 **2a**	70
4	**b**		 **2b**	75
5	**a**		 **3a**	80[c]
6	**b**		 **3b**	75
7	**a**		 **4a**	45
8	**b**		 **4b**	72
9	**b**		 **5b**[d]	50

[a] Isolated yields from both steps.
[b] See Ref. 16 for an alternative procedure.
[c] See Ref. 5 for an alternative procedure.
[d] Note **5a** proved too insoluble.

Table 2. Condensation reactions of **1–5** with 2,6-diisopropylaniline

Method A R-C(H)=O $\xrightarrow[\text{EtOH, 50 °C, 18 h}]{\text{2,6-}i\text{-Pr}_2\text{C}_6\text{H}_3\text{NH}_2\text{ (1.1 - 2.2 equiv.), cat. H}^+}$ **6a, 7a, 8a, 9a**

Method B R-C(Me)=O $\xrightarrow[\text{160 °C, 20 min}]{\text{2,6-}i\text{-Pr}_2\text{C}_6\text{H}_3\text{NH}_2\text{ (solvent), cat. H}^+}$ **6b, 7b, 8b, 9b, 10**

Entry	Method	R-C(R)=O	Oligopyridylimine product	Yield (%)[a]
10	A	 **1a**	 **6a**	45
11	B	 **1b**	 **6b**	50
12	A	 **2a**	 **7a**	50
13	B	 **2b**	 **7b**	60
14	A	 **3a**	 **8a**	65
15	B	 **3b**	 **8b**	60
16	A	 **4a**	 **9a**	50
17	B	 **4b**	 **9b**	58
18	B	 **5b**	 **10b**	45

[a] Isolated yields.

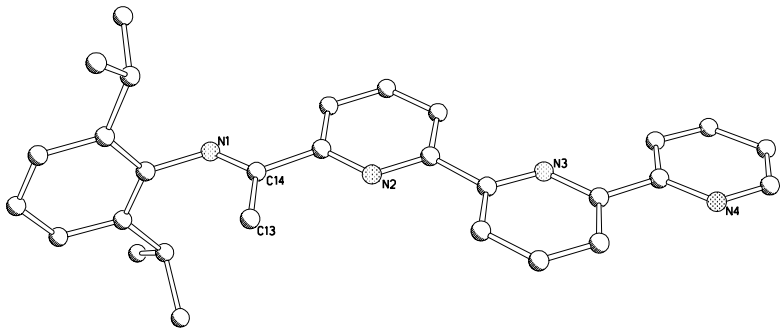


Figure 1. Molecular structures of **6b** including a partial atom numbering scheme. All hydrogen atoms have been omitted for clarity.

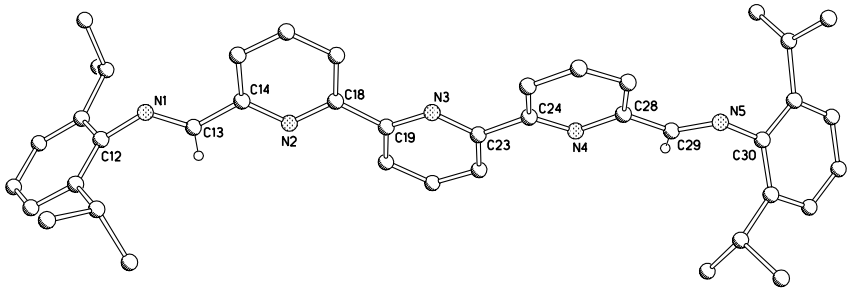


Figure 2. Molecular structures of **8a** including a partial atom numbering scheme. All hydrogen atoms apart from H13 and H29 have been omitted for clarity.

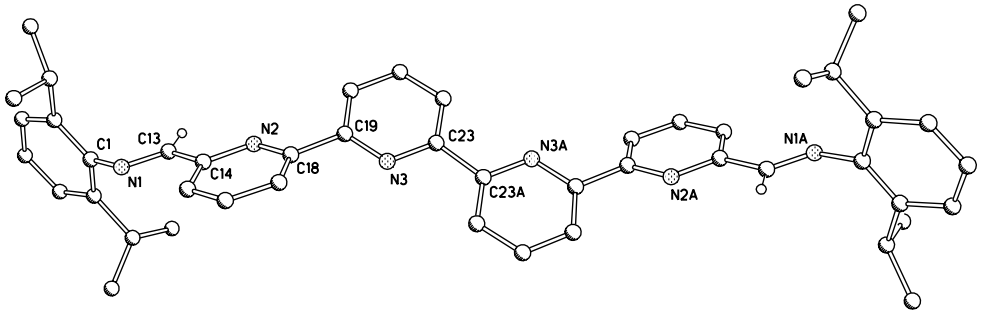


Figure 3. Molecular structures of **9a** including a partial atom numbering scheme. All hydrogen atoms apart from H13 have been omitted for clarity.

Table 3. Selected bond distances (Å) and angles (°) for **6b**, **8a** and **9a**

6b		**8a**		**9a**	
N(1)–C(14)	1.275(4)	N(1)–C(13)	1.256(2)	N(1)–C(13)	1.265(6)
N(1)–C(12)	1.434(4)	N(5)–C(29)	1.260(2)	C(1)–N(1)	1.437(6)
C(13)–C(14)	1.493(4)	C(28)–C(29)	1.470(2)	C(18)–C(19)	1.489(7)
C(14)–C(15)	1.495(4)	C(18)–C(19)	1.488(2)	C(23)–C(23A)	1.514(12)
C(19)–C(20)	1.484(4)	C(23)–C(24)	1.487(2)	C(13)–C(14)	1.478(7)
C(24)–C(25)	1.484(4)	C(13)–C(14)	1.472(2)		
C(14)–N(1)–C(12)	121.1(3)	C(13)–N(1)–C(12)	119.64(14)	C(1)–N(1)–C(13)	120.7(5)
N(1)–C(14)–C(13)	125.0(3)	C(29)–N(5)–C(30)	117.84(14)	N(1)–C(13)–C(14)	120.7(6)

multidentate oligopyridylimines. The coordination chemistry of these new ligand types will be explored elsewhere.

4. Experimental

4.1. General

All reactions, unless otherwise stated, were carried out under an atmosphere of dry, oxygen-free nitrogen, using standard Schlenk techniques. Solvents were distilled under nitrogen from appropriate drying agents and degassed prior to use.[19] The infrared spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer on solid samples. The electrospray ionisation (ESI) and the fast atom bombardment (FAB) mass spectra were recorded using a micromass Quattra LC mass spectrometer and a Kratos Concept spectrometer with chloroform or NBA as the matrix, respectively. High resolution FAB mass spectra were recorded on Kratos Concept spectrometer (xenon gas,

7 kV) with NBA as matrix. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX spectrometer (250 or 300 MHz); chemical shifts (ppm) are referred to the residual protic solvent peaks; coupling constants are in Hertz (Hz). Melting points (mp) were measured on a Gallenkamp melting point apparatus (model MFB-595) in open capillary tubes and were uncorrected. Elemental analyses were performed at the Department of Chemistry, University of North London by Dr. Boyer.

The reagents, 2,6-diisopropylaniline, tributyltinchloride, 2,6-dibromopyridine and *n*-BuLi were purchased from Aldrich Chemical Co. and used without further purification. The compounds, 6-bromo-2-(1,3-dioxolan-2-yl)pyridine,[15] 6-bromo-2-(2-methyl-1,3-dioxolan-2-yl)pyridine,[15] tetrakis(triphenylphosphine)palladium(0),[20] 6-bromo-2,2′-bipyridine,[21] 6-bromo-2,2′;6′,2″-terpyridine,[22] 6,6′-dibromo-2,2′-bipyridine[23] and 6,6″-dibromo-2,2′;6′,2″-terpyridine[24] were prepared according to previously reported procedures. All other chemicals were obtained commercially and used without further purification.

4.2. Synthesis of tin reagents

4.2.1. 6-Tributylstannyl-2-(1,3-dioxolan-2-yl)pyridine (a). Under an atmosphere of nitrogen, 6-bromo-2-(1,3-dioxolan-2-yl)pyridine (16.02 g, 69.65 mmol) was dissolved in anhydrous diethyl ether (160 ml) and cooled to −100 °C. *n*-BuLi (43.5 ml, 69.65 mmol, 1 equiv, 1.6 M in hexanes) was added dropwise over 15 min. After stirring the mixture for 10 min, the solution was warmed to −78 °C and stirred for a further 30 min. The solution was re-cooled to −100 °C before addition of freshly distilled tributyltinchloride (20.4 ml, 76.62 mmol, 1.1 equiv). The solution was stirred for 1 h at −100 °C, allowed to warm to room temperature and left to stir overnight. The resulting mixture was filtered through Celite and washed with dichloromethane. The reaction mixture was concentrated under reduced pressure to give a brown/black oil. The resulting oil was distilled (Kugelrohr, 210 °C/0.1 mmHg) to afford 6-tributylstannyl-2-(1,3-dioxolan-2-yl)pyridine (28.0 g, 92%) as a clear brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, $^{3}J_{H-H}=7.3$ Hz, 9H, CH_3), 1.17 (dd, $^{3}J_{H-H}=8.2$ Hz, $^{3}J_{H-H}=8.5$ Hz, 6H, CH_2), 1.38 (app. sex, $^{3}J_{H-H}=7$ Hz, 6H, CH_2), 1.63 (m, 6H, CH_2), 4.1–4.3 (m, 4H, CH_2), 5.91 (s, 1H, CH), 7.3–7.5 (m, 2H, Py-H), 7.57 (app. t, $^{3}J_{H-H}=7.6$ Hz, 1H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 10.1 (CH_2), 13.7 (CH_3), 27.3 (CH_2), 28.0 (CH_2), 65.6 (CH_2), 104.8 (CH), 118.9 (Py), 132.4 (Py), 133.1 (Py), 157.3 (Py), 173.5 (Py). IR (cm^{-1}): 2955, 2922, 1575, 1557, 1463, 1411, 1340, 1123, 1093, 960, 944, 794, 665. FABMS: *m/z* 438 [M+H (^{116}Sn)]$^{+}$. HRMS (FAB): calcd for $C_{20}H_{36}NO_2{}^{116}Sn$ [M+H]$^{+}$438.17670, found 438.17663.

4.2.2. Synthesis of 6-tributylstannyl-2-(2-methyl-1,3-dioxolan-2-yl)pyridine (b). A similar procedure to that described for **a** was followed, using 6-bromo-2-(2-methyl-1,3-dioxolan-2-yl)pyridine (16.99 g, 69.65 mmol) as the protected ketal derivative. The resulting oil was distilled (Kugelrohr, 230 °C/0.1 mmHg) to afford 6-tributylstannyl-2-(2-methyl-1,3-dioxolan-2-yl)pyridine (27.64 g, 88%) as a clear brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, $^{3}J_{H-H}=7.3$ Hz, 9H, CH_3), 1.02 (dd, $^{3}J_{H-H}=8.2$ Hz, $^{3}J_{H-H}=8.5$ Hz, 6H, CH_2), 1.25 (app. sex, $^{3}J_{H-H}=7$ Hz, 6H, CH_2), 1.49 (m, 6H, CH_2), 1.70 (s, 3H, CH_3), 3.6–4.0 (m, 4H, CH_2), 7.2–7.3 (m, 2H, Py-H), 7.41 (app. t, $^{3}J_{H-H}=7.6$ Hz, 1H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 10.0 (CH_3), 13.6 (CH_2), 24.4 (CH_2), 27.9 (CH_2), 28.0 (CH_3), 65.0 (CH_2), 109.2, 118.9 (Py), 131.4 (Py), 133.6 (Py), 160.4 (Py), 173.5 (Py). IR (cm^{-1}): 2955, 2923, 1569, 1556, 1463, 1411, 1376, 1192, 1099, 1042, 947, 872, 800, 752, 690, 661. FABMS: *m/z* 452 [M+H (^{116}Sn)]$^{+}$. HRMS (FAB): calcd for $C_{21}H_{38}NO_2Sn$ [M+H (^{116}Sn)]$^{+}$452.19235, found 452.19242.

4.3. Stille-type cross-coupling reactions

4.3.1. Formyl-2,2′:6′,2″-terpyridine (1a). 6-Bromo-2,2′-bipyridine (0.971 g, 4.13 mmol), **a** (1.99 g, 4.54 mmol, 1.1 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.286 g, 0.25 mmol, 0.06 equiv) were loaded in a Schlenk tube under N_2 and the contents stirred in dry toluene (30 ml) for 72 h at 90 °C. After removal of the solvent under reduced pressure, the reaction mixture was stirred overnight at 60 °C in 4 M HCl (20 ml), cooled to room temperature and carefully neutralised by addition of 2 M $NaHCO_3$. The suspension was extracted with $CHCl_3$ (3×30 ml) and the organic phase separated, washed with water (3×30 ml), brine (1×40 ml) and dried over magnesium sulphate. Following filtration, the solvent was removed under reduced pressure and the crude product crystallised from ethanol at −30 °C. The resulting precipitate was collected by filtration to afford **1a** (0.647 g, 60%) as a pale yellow solid. The melting point and ^{1}H NMR spectroscopic data of **1a** were consistent with the data previously reported.[16a] ^{13}C {^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 121.6 (Py), 121.9 (Py), 122.0 (Py), 124.1 (Py), 124.3 (Py), 125.6 (Py), 137.3 (Py), 138.5 (Py), 138.8 (Py), 147.1 (Py), 149.6 (Py), 152.6 (Py), 154.1 (Py), 156.0 (Py), 156.4 (Py), 194.2 (*C*=O). IR (cm^{-1}) 1725 (C=O). ESIMS: *m/z* 262 [M+H]$^{+}$.

4.3.2. 6-Acetyl-2,2′:6′,2″-terpyridine (1b). A similar procedure to that described for **1a** was followed, using 6-bromo-2,2′-bipyridine (0.971 g, 4.13 mmol), **b** (2.06 g, 4.54 mmol, 1.1 equiv) and tetrakis(triphenylphosphine)-palladium(0) (0.286 g, 0.25 mmol, 0.06 equiv). The product was crystallised from ethanol at −30 °C and collected by filtration to afford **1b** (0.704 g, 62%) as a pale yellow solid. Mp: 185–187 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.79 (s, 3H, CH_3C=O), 7.27 (m, 1H, Py-H), 7.78 (m, 1H, Py-H), 7.87 (m, 1H, Py-H), 7.98 (m, 1H, Py-H), 8.38 (dd, $^{3}J_{H-H}=8.0$ Hz, $^{4}J_{H-H}=2.8$ Hz, 1H, Py-H) 8.41 (m, 1H, Py-H), 8.51 (dd, $^{3}J_{H-H}=7.5$ Hz, $^{4}J_{H-H}=2.6$ Hz, 1H, Py-H), 8.56 (d, $^{3}J_{H-H}=7.4$ Hz, Py-H), 8.64 (d, $^{3}J_{H-H}$ H = 7.5 Hz, 1H, Py-H), δ 8.76 (dd, $^{3}J_{H-H}=7.7$ Hz, $^{4}J_{H-H}=2.5$ Hz, 1H, Py-H) ^{13}C {^{1}H} NMR (62.5 MHz, $CDCl_3$): δ 24.8 (CH_3C=O), 120.0 (Py), 120.1 (Py), 120.4 (Py), 120.5 (Py), 122.8 (Py), 122.9 (Py), 135.9 (Py), 136.7 (Py), 137.0 (Py), 148.2 (Py), 151.9 (Py), 153.5 (Py), 154.4 (Py), 154.5 (Py), 155.9 (Py), 199.4 (*C*=O). IR (cm^{-1}) 1700 (C=O), 1564, 1426, 1078, 790, 748, 668. ESIMS: *m/z* 276 [M+H]$^{+}$. HRMS (FAB): calcd for $C_{17}H_{14}N_3O$ [M+H]$^{+}$276.11369, found 276.11364.

4.3.3. 6-Formyl-2,2′:6′,2″:6″,2‴-quaterpyridine (2a). A similar procedure to that described for **1a** was followed,

using 6-bromo-2,2′:6′,2″-terpyridine (1.28 g, 4.13 mmol), **a** (2.00 g, 4.54 mmol, 1.1 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.286 g, 0.25 mmol, 0.06 equiv). The product was crystallised from ethanol at −30 °C and collected by filtration to afford **2a** (0.977 g, 70%) as a white solid. Mp: 210–213 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.2–7.3 (m, 1H, Py-H), 7.82 (ddd, $^3J_{H-H}$=7.9, 7.9 Hz, $^4J_{H-H}$=1.7 Hz, 1H, Py-H), 7.9–8.0 (m, 4H, Py-H), 8.43 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.2 Hz, 1H, Py-H), 8.5–8.7 (m, 5H, Py-H), 8.85 (dd, $^3J_{H-H}$=7.6 Hz, $^4J_{H-H}$=1.7 Hz, 1H, Py-H), 10.18 (s, 1H, *H*C=O). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 121.0 (Py), 121.2 (Py), 121.5 (Py), 121.7 (Py), 123.8 (Py), 125.3 (Py), 136.9 (Py), 137.9 (Py), 138.0 (Py), 149.2 (Py), 193.8 (H*C*=O). IR (cm^{-1}) 1715 (C=O), 1564, 1427, 1260, 1076, 799, 774, 683. ESIMS: *m/z* 339 $[M+H]^+$. HRMS (FAB): calcd for $C_{21}H_{15}N_4O$ $[M+H]^+$339.12471, found 339.12549.

4.3.4. 6-Acetyl-2,2′:6′,2″:6″,2‴-quaterpyridine (2b). A similar procedure to that described for **1a** was followed, using 6-bromo-2,2′:6′,2″-terpyridine (1.50 g, 4.82 mmol), **b** (2.410 g, 5.30 mmol, 1.1 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.334 g, 0.29 mmol, 0.06 equiv). The product was crystallised from ethanol at −30 °C and collected by filtration to afford **2b** (1.270 g, 75%) as a white solid. Mp: 215–217 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (s, 3H, C*H*$_3$C=O), 7.2–7.3 (m, 1H, Py-H), 7.82 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.7 Hz, 1H, Py-H), 7.9–8.0 (m, 4H, Py-H), 8.43 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=0.9 Hz, 1H, Py-H), 8.54 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.7 Hz, 1H, Py-H), 8.5–8.7 (m, 4H, Py-H), 8.80 (dd, $^3J_{H-H}$=7.6 Hz, $^4J_{H-H}$=1.2, 1H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 25.9 (*C*H$_3$C=O), 121.1 (Py), 121.5 (Py), 123.9 (Py), 124.4 (Py), 136.9 (Py), 137.8 (Py), 137.9 (Py), 149.2 (Py), 154.5 (Py), 155.5 (Py), 200.4 (CH$_3$*C*=O). IR (cm^{-1}) 1697 (C=O), 1562, 1426, 1352, 1267, 1109, 1075, 992, 804, 775, 740. ESIMS: *m/z* 353 $[M+H]^+$. HRMS (FAB): calcd for $C_{22}H_{17}N_4O$ $[M+H]^+$353.14024, found 353.14016.

4.3.5. 6,6″-Diformyl-2,2′:6′,2″-terpyridine (3a). A similar procedure to that described for **1a** was followed, using 2,6-dibromopyridine (1.10 g, 4.65 mmol), **a** (4.50 g, 10.23 mmol, 2.2 equiv) and tetrakis(triphenylphosphine)-palladium(0) (0.429 g, 0.37 mmol, 0.08 equiv). The product was crystallised from ethanol at −30 °C and collected by filtration to afford **3a** (1.08 g, 80%) as a white solid. The ^{1}H and ^{13}C NMR spectroscopic data of **3a** were consistent with those previously reported.[5]

4.3.6. 6,6″-Diacetyl-2,2′:6′,2″-terpyridine (3b). A similar procedure to that described for **1a** was followed, using 2,6-dibromopyridine (1.21 g, 5.12 mmol), **b** (5.11 g, 11.26 mmol, 2.2 equiv) and tetrakis(triphenylphosphine)-palladium(0) (0.473 g, 0.41 mmol, 0.08 equiv). The product was crystallised from ethanol at −30 °C and collected by filtration to afford **3b** (1.22 g, 75%) as a white solid. Mp: 192–194 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (s, 6H, CH_3), 7.8–8.1 (m, 5H, Py-H), 8.51 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.75 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.7 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 24.7 (CH_3), 120.4 (Py), 120.6 (Py), 123.3 (Py), 136.7 (Py), 137.0 (Py), 151.9 (Py), 153.6 (Py), 154.2 (Py), 199.2 (C=O). IR (cm^{-1}): 1697 (C=O), 1577, 1429, 1349, 1268, 1111, 1071, 992, 951, 793, 740. ESIMS: *m/z* 318 $[M+H]^+$. Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.92; H, 4.73; N, 13.25. Found C, 72.00; H, 4.82; N, 13.15.

4.3.7. 6,6‴-Diformyl-2,2′:6′,2″:6″,2‴-quaterpyridine (4a). 2,6′-Dibromo-2,2″-bipyridine (1.42 g, 4.52 mmol), **a** (4.38 g, 9.95 mmol, 2.2 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.417 g, 0.36 mmol, 0.08 equiv) were loaded in a Schlenk tube under N_2 and the contents stirred in dry toluene (35 ml) for 72 h at 100 °C. After removal of the solvent under reduced pressure, the reaction mixture was stirred overnight at 60 °C in 4 M HCl (20 ml), cooled to room temperature and carefully neutralised by addition of 2 M $NaHCO_3$. The suspension was extracted with $CHCl_3$ (3×30 ml) and the organic phase separated, washed with water (3×30 ml), brine (1×40 ml) and dried over magnesium sulphate. Following filtration, the solvent was removed under reduced pressure and the crude product recrystallised from ethanol at −30 °C. The resulting precipitate was collected by filtration to afford **4a** (0.746 g, 45%) as a white solid. Mp: 202–204 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.9–8.1 (m, 6H, Py-H), 8.60 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.7 Hz, 2H, Py-H), 8.67 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.7 Hz, 2H, Py-H), 8.85 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.7 Hz, 2H, Py-H), 10.13 (s, 1H, HC=O). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): poor quality spectrum (compound too insoluble). IR (cm^{-1}): 1721 (C=O), 1565, 1429, 1348, 1259, 1213, 992, 878, 786, 666. EIMS: *m/z* 367 $[M+H]^+$.

4.3.8. 6,6‴-Diacetyl-2,2′:6′,2″:6″,2‴-quaterpyridine (4b). A similar procedure to that described for **4a** was followed, using 2,6′-dibromo-2,2″-bipyridine (1.32 g, 4.20 mmol), **a** (4.19 g, 9.24 mmol, 2.2 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.388 g, 0.34 mmol, 0.08 equiv). The product was crystallised from ethanol at −30 °C and collected by filtration to afford **4b** (1.19 g, 72%) as a white solid. Mp: 242–244 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (s, 6H, CH_3), 7.95 (dd, $^3J_{H-H}$=7.6, 7.6 Hz, 2H, Py-H), 7.99 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.04 (dd, $^3J_{H-H}$=7.6 Hz, $^4J_{H-H}$=1.2 Hz, 2H, Py-H), 8.55 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.2 Hz, 2H, Py-H), 8.65 (dd, $^3J_{H-H}$=7.6 Hz, $^4J_{H-H}$=1.2 Hz, 2H, Py-H), 8.80 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.2 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 25.8 (CH_3), 121.2 (Py), 121.5 (Py), 121.6 (Py), 124.4 (Py), 137.8 (Py), 137.9 (Py), 153.0 (Py), 154.6 (Py), 155.4 (Py), 155.5 (Py), 200.3 (C=O). IR (cm^{-1}): 1697 (C=O), 1565, 1429, 1355, 1293, 1269, 1112, 1066, 992, 952, 792, 740. ESIMS: *m/z* 395 $[M+H]^+$. HRMS (FAB): calcd for $C_{24}H_{19}N_4O_2$ $[M+H]^+$395.15094, found 395.15080.

4.3.9. Attempted synthesis of 6,6⁗-diformyl-2,2′:6′,2″:6″,2‴:6‴:2⁗-quinquepyridine (5a). A similar procedure to that described for **4a** was followed, using 2,6″-dibromo-2,2′:6′,2″-terpyridine (1.64 g, 4.20 mmol), **a** (4.07 g, 9.24 mmol, 2.2 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.388 g, 0.34 mmol, 0.08 equiv). Following the deprotection step and subsequent neutralisation, the resultant suspension was insoluble in the chlorinated solvents employed for the extraction and could not be isolated and characterised.

4.3.10. 6,6′′′′-Diacetyl-2,2′:6′,2″:6″,2‴:6‴:2′′′′-quinquepyridine (5b). A similar procedure to that described for **4a**, using 2,6″-dibromo-2,2′:6′,2″-terpyridine (1.82 g, 4.65 mmol), **a** (4.64 g, 10.23 mmol, 2.2 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.430 g, 0.37 mmol, 0.08 equiv). The product was crystallised from ethanol at −30 °C. The resulting precipitate was collected by filtration to afford **5b** (1.030 g, 50%) as a white solid. Mp: >260 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.81 (s, 6H, CH_3C=O), 7.9–8.1 (m, 7H, Py-H), 8.55 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.2 Hz, 2H, Py-H), 8.64 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.69 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=0.9 Hz, 2H, Py-H), 8.81 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=1.2 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): poor quality spectrum (compound too insoluble). IR (cm^{-1}): 1705 (C=O), 1562, 1465, 1426, 1360, 1269, 1108, 1076, 992, 805, 788, 742. ESIMS: *m/z* 472 [M+H]$^+$.

4.4. Synthesis of the imino-substituted oligopyridines

Method A. The monoformyl- or diformyl-substituted oligopyridine was suspended in absolute ethanol and 2,6-diisopropylaniline (1.1 or 2.2 equiv) introduced along with a catalytic amount of glacial acetic acid. The mixture was stirred and heated vigourously at 50 °C for 18 h. The solution was cooled and the resulting precipitate collected by filtration and washed with ethanol.

Method B. The monoacetyl- or diacetyl-substituted oligopyridine was suspended in an excess of 2,6-diisopropylaniline (10 equiv) and stirred for 15 min at 160 °C on a heating mantle. A catalytic amount of formic acid was added, and the reaction mixture was stirred for an additional 20 min at this temperature. Following removal of the excess 2,6-diisopropylaniline under reduced pressure (130 °C, 0.5 mmHg), the resulting brown residue was stirred in ethanol at room temperature and the resultant precipitate filtered and washed with ethanol.

4.4.1. 6-Iminoformyl-2,2′:6′,2″-terpyridine (2,6-diisopropylanil) (6a). The procedure outlined in method A was followed, using **1a** (0.630 g, 2.41 mmol) and 2,6-diisopropylaniline (0.483 g, 2.73 mmol, 1.1 equiv) in ethanol (15 ml). The residue was crystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate filtered, washed with hexane and dried under reduced pressure to afford **6a** (0.453 g, 45%) as a pale yellow solid. Mp: 223–225 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.14 (d, $^3J_{H-H}$=7 Hz, 12H, CH(*Me*)$_2$), 2.94 (sept, 2H, C*H*(Me)$_2$), 7.0–7.2 (m, 3H, Ar-H), 7.2–7.3 (m, 1H, Py-H), 7.7–7.8 (m, 1H, Py-H), 7.90 (dd, $^3J_{H-H}$=7.9 Hz, $^3J_{H-H}$=7.6 Hz, 1H, Py-H), 7.93 (dd, $^3J_{H-H}$=7.9, 7.6 Hz, 1H, Py-H), 8.26 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.37 (s, 1H, HC=N), 8.41 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.49 (d, $^3J_{H-H}$=7.6 Hz, 1H, Py-H), 8.56 (d, $^3J_{H-H}$=8.2 Hz, 1H, Py-H), 8.63 (m, 1H, Py-H), 8.67 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 22.4 (CH_3), 26.8 (CH), 120.0 (Py), 120.1 (Py), 120.2 (Py), 121.6 (Ar), 122.0 (Ar), 122.8 (Py), 123.4 (Py), 135.9 (Py), 136.2 (Py), 136.5 (Py), 136.9 (Py), 147.5 (Py), 148.1 (Py), 152.8 (Py), 153.8 (Py), 154.4 (Py), 155.1 (Py), 162.4 (C=N). IR (cm^{-1}): 2952, 1629 (C=N), 1577, 1562, 1422, 1263, 1180, 1104, 1076, 990, 856, 775, 758. FABMS: *m/z* 421 [M+H]$^+$. HRMS (FAB): calcd for $C_{28}H_{29}N_4$ [M+H]$^+$421.23922, found 421.23936.

4.4.2. 6-Iminoacetyl-2,2′:6′,2″-terpyridine (2,6-diisopropylanil) (6b). The procedure outlined in method B was followed, using **1b** (0.300 g, 1.09 mmol) in 2,6-diisopropylaniline (3.00 g, 10.90 mmol, 10 equiv). The product was recrystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate was filtered, washed with hexane and dried under reduced pressure to afford **6b** (0.237 g, 50%) as a pale yellow solid. Crystals suitable for the X-ray determination were grown by slow cooling of a hot *n*-BuOH solution containing **6b**. Mp: 242–245 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (d, $^3J_{H-H}$=6.7 Hz, 12H, CH(*Me*)$_2$), 2.28 (s, 3H, CH_3C=N), 2.81 (sept, 2H, C*H*(Me)$_2$), 7.0–7.2 (m, 3H, Ar-H), 7.2–7.3 (m, 1H, Py-H), 7.7–7.9 (m, 1H, Py-H), 7.91 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.35 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.42 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.53 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.59 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.67 (m, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 16.3 (*C*H_3C=N), 21.9 (CH_3), 22.2 (CH_3), 27.3 (CH), 120.1 (Py), 120.2 (Py), 121.0 (Py), 122.5 (Ar), 122.8 (Ar), 134.8 (Py), 136.3 (Py), 136.8 (Py), 142.0 (Ar), 148.1 (Py), 154.4 (Py), 154.5 (Py), 155.1 (Py), 155.9 (Py), 166.1 (C=N). IR (cm^{-1}) 2952, 1630 (C=N), 1562, 1422, 1360, 1263, 1075, 990, 777, 744. ESIMS: *m/z* 435 [M+H]$^+$. HRMS (FAB): calcd for $C_{29}H_{31}N_4$ [M+H]$^+$435.25490, found 435.25487.

4.4.3. 6-Iminoformyl-2,2′:6′,2″:6″,2‴-quaterpyridine (2,6-diisopropylanil) (7a). The procedure outlined in method A was followed, using **2a** (0.836 g, 2.48 mmol) and 2,6-diisopropylaniline (0.483 g, 2.73 mmol, 1.1 equiv) in ethanol (15 ml). The residue was recrystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate was filtered, washed with hexane and dried under reduced pressure to afford **7a** (0.616 g, 50%) as a pale yellow solid. Mp: >260 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.17 (d, $^3J_{H-H}$=7 Hz, 12H, CH(*Me*)$_2$), 2.95 (sept, 2H, C*H*(Me)$_2$), 7.0–7.2 (m, 3H, Ar-H), 7.2–7.3 (m, 1H, Py-H), 7.81 (dd, $^3J_{H-H}$=7.9, 7.6 Hz, 1H, Py-H), 7.9–8.0 (m, 3H, Py-H), 8.27 (d, $^3J_{H-H}$=7.6 Hz, 1H, Py-H), 8.37 (s, 1H, HC=N), 8.43 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.52 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.5–8.7 (m, 4H, Py-H), 8.72 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 23.5 (CH_3), 28.0 (CH), 121.1 (Py), 121.2 (Py), 122.7 (Py), 123.1 (Py), 123.8 (Ar), 124.4 (Ar), 136.9 (Py), 137.3 (Ar), 137.6 (Py), 137.9 (Py), 149.2 (Py), 153.9 (Py), 154.8 (Py), 155.4 (Py), 156.2 (Py), 155.1 (Py), 163.5 (C=N). IR (cm^{-1}): 2959, 1636 (C=N), 1565, 1425, 1263, 1181, 1077, 991, 772. ESIMS: *m/z* 498 [M+H]$^+$. HRMS (FAB): calcd for $C_{33}H_{32}N_5$ [M+H]$^+$498.26577, found 498.26570.

4.4.4. 6-Iminoacetyl-2,2′:6′,2″:6″,2‴-quaterpyridine (2,6-diisopropylanil) (7b). The procedure outlined in method B was followed, using **2b** (1.50 g, 4.44 mmol) and 2,6-diisopropylaniline (7.86 g, 44.40 mmol, 10 equiv). The residue was crystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate filtered, washed with hexane and dried under reduced pressure to afford **7b** (0.629 g, 60%) as a pale yellow solid. Mp: >260 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ

1.10 (d, $^3J_{H-H}$=6.7 Hz, 12H, CH(*Me*)$_2$), 2.28 (s, 3H, CH_3C=N), 2.71 (sept, 2H, C*H*(Me)$_2$), 7.0–7.2 (m, 3H, Ar-H), 7.2–7.3 (m, 1H, Py-H), 7.8–7.9 (m, 1H, Py-H), 7.91 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.35 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.42 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.53 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.59 (d, $^3J_{H-H}$=7.9 Hz, 1H, Py-H), 8.67 (m, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 16.3 (s, *C*H_3C=N), 21.9 (CH_3), 22.2 (CH_3), 27.3 (CH), 120.1 (Py), 120.2 (Py), 121.0 (Py), 122.0 (Py), 122.5 (Ar), 122.8 (Ar), 134.8 (Py), 135.9 (Py), 136.3 (Ar), 136.7 (Py), 136.9 (Py), 148.1 (Py), 154.2 (Py), 154.4 (Py), 154.6 (Py), 166.1 (C=N). IR (cm^{-1}): 2950, 1634 (C=N), 1562, 1425, 1362, 1292, 1269, 1188, 1111, 1080, 991, 807, 759, 684. ESIMS: *m*/*z* 512 [M+H]$^+$. HRMS (FAB): calcd for $C_{34}H_{34}N_5$ [M+H]$^+$512.28142, found 512.28155.

4.4.5. 6,6″-Bis-(iminoformyl)-2,2′:6′,2″-terpyridine-bis-(2,6-diisopropylanil) (8a). The procedure outlined in method A was followed, using **3a** (1.031 g, 3.57 mmol) and 2,6-diisopropylaniline (1.390 g, 7.85 mmol, 2.2 equiv) in ethanol (25 ml). The residue was recrystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate filtered, washed with hexane and dried under reduced pressure to afford **8a** (1.411 g, 65%) as a pale yellow solid. Crystals suitable for the X-ray determination were grown by slow evaporation of a dichloromethane solution containing **8a**. Mp: 220–222 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14 (d, $^3J_{H-H}$=7 Hz, 24H, CH(*Me*)$_2$), 2.92 (sept, 4H, C*H*(Me)$_2$), 7.0–7.2 (m, 6H, Ar-H), 7.91 (app. t, $^3J_{H-H}$=7.6, 7.6 Hz, 1H, Py-H), 7.96 (app. t, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.29 (d, $^3J_{H-H}$=7.6 Hz, 2H, Py-H), 8.36 (s, 2H, HC=N), 8.52 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H) and 8.71 (d, $^3J_{H-H}$=7.6 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 23.5 (CH_3), 28.0 (CH), 121.1 (Py), 121.4 (Py), 122.7 (Py), 122.8 (Py), 123.1 (Ar), 124.5 (Ar), 137.3 (Py), 137.6 (Py), 148.6 (Ar), 154.0 (Ar), 155.0 (Py), 156.1 (Py), 163.5 (C=N). IR (cm^{-1}): 2952, 1648 (C=N), 1563, 1434, 1361, 1190, 1115, 795, 786, 760. ESIMS: *m*/*z* 608 [M+H]$^+$. HRMS (FAB): calcd for $C_{41}H_{45}N_5H^+$ [M+H]$^+$608.37532, found 608.37532. Anal. Calcd for ($C_{41}H_{45}N_5\cdot 0.5H_2O$): C, 79.87; H, 7.46; N, 11.36. Found C, 79.62; H, 7.63; N, 10.81.

4.4.6. 6,6″-Bis-(iminoacetyl)-2,2′:6′,2″-terpyridine-bis-(2,6-diisopropylanil) (8b). The procedure outlined in method B was followed, using **3b** (1.050 g, 3.31 mmol) and 2,6-diisopropylaniline (5.86 g, 33.10 mmol, 10 equiv). The residue was recrystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate filtered, washed with hexane and dried under vacuum to afford **8b** (1.263 g, 60%) as a pale yellow solid. Mp: 252–254 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (d, $^3J_{H-H}$=6.7 Hz, 24H, CH(*Me*)$_2$), 2.29 (s, 6H, CH_3C=N), 2.72 (sept, 4H, C*H*(Me)$_2$), 7.00–7.14 (m, 6H, Ar-H), 7.91 (app. t, $^3J_{H-H}$=7.9, 7.9 Hz, 1H, Py-H), 7.92 (app. t, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.37 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=0.9 Hz, 2H, Py-H), 8.54 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.71 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=0.9 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 16.3 (*C*H_3C=N), 21.9 (CH_3), 22.2 (CH_3), 27.3 (CH), 120.0 (Py), 120.3 (Py), 121.0 (Py), 122.0 (Ar), 122.6 (Ar), 134.8 (Ar), 136.3 (Py), 136.8 (Py), 145.5 (Py), 136.3 (Py), 136.8 (Py), 145.4 (Ar), 154.0 (Py), 154.2 (Py), 154.6 (Py), 166.1 (C=N). IR (cm^{-1}): 2955, 1642 (C=N), 1563, 1430, 1362, 1256, 1184, 1108, 800, 784, 760. ESIMS: *m*/*z* 636 [M+H]$^+$. Anal. Calcd for ($C_{43}H_{49}N_5$): C, 81.26; H, 7.72; N, 11.02. Found C, 81.16; H, 7.86; N, 10.92.

4.4.7. 6,6‴-Bis-(iminoformyl)-2,2′:6′,2″:6″,2‴-quaterpyridine-bis-(2,6-diisopropylanil) (9a). The procedure outlined in method A was followed, using **4a** (0.875 g, 2.05 mmol) and 2,6-diisopropylaniline (0.798 g, 4.51 mmol, 2.2 equiv) in ethanol (12 ml). The residue was recrystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate filtered, washed with hexane and dried under reduced pressure to afford **9a** (0.701 g, 50%) as a white solid. Crystals suitable for the X-ray determination were grown by slow evaporation of a dichloromethane solution containing **9a**. Mp: >260 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14 (d, $^3J_{H-H}$=6.7 Hz, 24H, CH(C*H*$_3$)$_2$), 2.90 (sept, 4H, C*H*(CH$_3$)$_2$), 7.0–7.2 (m, 6H, Ar-H), 7.95 (app. t, $^3J_{H-H}$=7.9, 7.9 Hz, 4H, Py-H), 8.27 (d, $^3J_{H-H}$=7.3 Hz, 2H, Py-H), 8.63 (s, 2H, *H*C=N), 8.52 (d, $^3J_{H-H}$=7.3 Hz, 2H, Py-H), 8.64 (dd, $^3J_{H-H}$=7.9 Hz, $^4J_{H-H}$=0.9 Hz, 2H, Py-H), 8.72 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 23.5 (*C*H$_3$), 28.0 (CH), 121.1 (Py), 121.2 (Py), 121.4 (Py), 122.7 (Ar), 123.1 (Ar), 134.5 (Ar), 137.3 (Py), 137.6 (Py), 137.9 (Py), 148.6 (Ar), 154.0 (Py), 154.9 (Py), 155.5 (Py), 156.2 (Py), 163.5 (C=N). IR (cm^{-1}): 2960, 1644 (C=N), 1566, 1456, 1427, 1321, 1262, 1108, 1098, 1076, 990, 885, 794, 768, 743, 692. FABMS: *m*/*z* 685 [M+H]$^+$. HRMS (FAB): calcd for $C_{46}H_{49}N_6$ [M+H]$^+$685.40190, found 685.40187.

4.4.8. 6,6″-Bis-(iminoacetyl)-2,2′:6′,2″:6″,2‴-quaterpyridine-bis-(2,6-diisopropylanil) (9b). The procedure outlined in method B was followed, using **4b** (0.854 g, 2.17 mmol) and 2,6-diisopropylaniline (3.84 g, 21.7 mmol, 10 equiv). The residue was recrystallised from a dichloromethane–hexane (1/9) mixture at room temperature and the resulting precipitate was filtered, washed with hexane and dried under vacuum to afford **9b** (0.896 g, 58%) as a white solid. Mp: >260 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (d, $^3J_{H-H}$=6.7 Hz, 24H, CH(*Me*)$_2$), 2.30 (s, 6H, CH_3C=N), 2.78 (sept, 4H, C*H*(Me)$_2$), 7.0–7.1 (m, 6H, Ar-H), 7.93 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 7.97 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.36 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.56 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.66 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.72 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 17.3 (*C*H_3C=N), 22.9 (CH_3), 23.3 (CH_3), 28.3 (CH), 121.1 (Py), 121.2 (Py), 122.0 (Py), 123.0 (Ar), 123.6 (Ar), 135.9 (Ar), 137.4 (Py), 137.8 (Py), 146.6 (Ar), 155.0 (Py), 155.3 (Py), 155.5 (Py), 155.7 (Py), 167.1 (C=N). IR (cm^{-1}): 2959, 1646, 1565, 1458, 1429, 1363, 1261, 1192, 1111, 1080, 1042, 991, 860, 764, 740, 686. ESIMS: *m*/*z* 713 [M+H]$^+$. Anal. Calcd for ($C_{48}H_{52}N_6$): C, 80.90; H, 7.30; N, 11.80. Found C, 80.63; H, 7.40; N, 11.66.

4.4.9. 6,6⁗-Bis-(iminoformyl)-2,2′:6′,2″:6″,2‴:6‴,2⁗-quinquepyridine-bis-(2,6-diisopropylanil) (10b). The procedure outlined in method B was followed, using **5b** (0.645 g, 1.42 mmol) and 2,6-diisopropylaniline (2.51 g, 14.20 mmol, 10 equiv). The residue was recrystallised from a dichloromethane–hexane (1/9) mixture at room

temperature and the resulting precipitate filtered, washed with hexane and dried under vacuum to afford **10b** (0.504 g, 45%) as a white solid. Mp: >260 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (d, $^3J_{H-H}$=7.1 Hz, 12H, CH(*Me*)$_2$), 2.31 (s, 6H, CH_3), 2.74 (sept, 4H, C*H*(Me)$_2$), 7.0–7.2 (m, 6H, Ar-H), 7.94 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 7.97 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 2H, Py-H), 8.02 (dd, $^3J_{H-H}$=7.9, 7.9 Hz, 1H, Py-H), 8.38 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.56 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.67 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.68 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H), 8.73 (d, $^3J_{H-H}$=7.9 Hz, 2H, Py-H). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$): δ 16.3 (CH), 21.9 (CH_3), 22.2 (CH_3), 27.3 (*C*H$_3$C=O), 120.0 (Py), 120.2 (Py), 121.0 (Py), 122.0 (Ar), 122.5 (Ar), 134.8 (Py), 136.3 (Py), 136.8 (Py), 145.5 (Ar), 154.0 (Py), 154.3 (s, Py), 154.5 (s, Py), 154.6 (Py), 167.1 (C=N). IR (cm^{-1}): 2960, 1643 (C=N), 1565, 1425, 1363, 1262, 1241, 1110, 1066, 935, 700, 743, 687. FABMS: *m/z* 790 $[M+H]^+$. HRMS (FAB): calcd for $C_{53}H_{56}N_7$ $[M+H]^+$790.45979, found 790.45972.

4.5. Crystallography

Data for **6b**, **8a** and **9a** were collected on a Bruker APEX 2000 CCD diffractometer. Details of data collection, refinement and crystal data are listed in Table 4. The data were corrected for Lorentz and polarisation effects and empirical absorption corrections applied. Structure solution by Patterson methods and structure refinement on F^2 employed SHELXTL version 6.10.[25] Hydrogen atoms were included in calculated positions (C–H=0.96 Å) riding on the bonded atom with isotropic displacement parameters set to 1.5 U_{eq}(C) for methyl H atoms and 1.2 U_{eq}(C) for all other H atoms. All non H atoms were refined with anisotropic displacement parameters. In the case of **9a**, the small crystals were weak diffractors; the data was refined with full matrix and anisotropic parameters resulting in a poor ratio of observed data:parameter (4.5:1).

CCDC reference numbers 279915–279917 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ; fax: (internet) +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk].

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Table 4. Crystallographic and data processing parameters for **6b**, **8a** and **9a**

Complex	**6b**	**8a**	**9a**
Formula	$C_{29}H_{30}N_4$	$C_{41}H_{45}N_5\cdot CDCl_3$	$C_{46}H_{48}N_6$
M	434.57	728.19	684.90
Crystal size (mm^3)	0.59×0.38×0.18	0.37×0.32×0.25	0.22×0.15×0.03
Temperature (K)	200(2)	160(2)	150(2)
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	*P* bar 1	*P*2(1)/*n*	*P*2(1)/*c*
a (Å)	8.851(4)	14.2131(9)	11.445(15)
b (Å)	11.016(5)	20.9441(13)	11.289(14)
c (Å)	12.976(5)	14.3123(9)	15.38(2)
α (°)	97.48(3)	—	—
β (°)	102.49(4)	112.2860(10)	98.12(3)
γ (°)	93.17(4)	—	—
U (Å^3)	1220.2(9)	3942.2(4)	1967(4)
Z	2	4	2
D_c (Mg m^{-3})	1.183	1.225	1.156
F(000)	464	1536	732
μ(Mo K$_\alpha$)(mm^{-1})	0.071	0.268	0.069
Reflections collected	4373	30,472	13,854
Independent reflections	4233	7739	3454
R_{int}	0.0791	0.0240	0.4098
Restraints/parameters	0/298	0/477	0/239
Final *R* indices ($I>2\sigma(I)$)	R_1=0.0674, wR_2=0.1579	R_1=0.0444, wR_2=0.1257	R_1=0.0969, wR_2=0.1450
All data	R_1=0.1446, wR_2=0.1983	R_1=0.0574, wR_2=0.1305	R_1=0.2716, wR_2=0.2079
Goodness of fit on F^2 (all data)	1.023	1.086	0.860

Data in common: graphite-monochromated Mo K$_\alpha$ radiation, λ=0.71073 Å; $R_1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$, $wR_2=[\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$, $w^{-1}=[\sigma^2(F_o)^2+(aP)^2]$, $P=[\max(F_o^2,0)+2(F_c^2)]/3$, where a is a constant adjusted by the program; goodness of fit=$[\Sigma(F_o^2-F_c^2)2/(n-p)]^{1/2}$ where *n* is the number of reflections and *p* the number of parameters.

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Synthesis, structural determination and photo-antiproliferative activity of new 3-pyrazolyl or -isoxazolyl substituted 4-hydroxy-2(1*H*)-quinolinones

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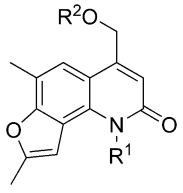
Abstract—A convenient route to new 3-quinolinonyl-pyrazoles and isoxazoles is described through cyclization of 3-[(*E*)-3-(dimethylamino)-2-propenoyl]-4-hydroxy-1-methyl-2(1*H*)-quinolinone. The phototoxicity as well as the cytotoxic activities of the title compounds are evaluated against leukemia- and adenocarcinoma-derived cell lines in comparison to the normal human keratinocytes.

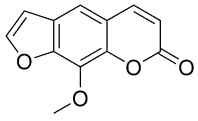
1. Introduction

4-Hydroxy-2(1*H*)-quinolinones and their derivatives represent a class of heterocyclic compounds that have been associated with several pharmacological,[1] medicinal and industrial applications;[2] platinum-containing derivatives were found useful for the treatment of a malignant tumor,[3] some *N*-benzyl derivatives were useful against HSV-1 and HSV-2 viruses[4] and 1-benzyl-3-formyl-4-hydroxy-2(1*H*)-quinolinone inhibited, in vitro, herpes simplex 2 virus.[5]

Recently, 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]-quinolin-2(1*H*)-one (HOFQ) was prepared in a new profitable way, which allowed also 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (MOFQ), and 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOHFQ) to be synthesized.[6] These compounds show strong photoantiproliferative activity, higher than that one of 8-methoxypsoralen (8-MOP) the most widely employed drug for photochemotherapy. Moreover, their activity is devoid of mutagenicity and skin phototoxicity.[7] For these features, furoquinolinones and other analogues appeared to be very promising photochemotherapeutic agents.



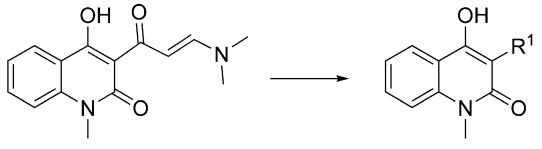
HOFQ R^1=Me, R^2=H
MOFQ R^1=Me, R^2=Me
HOHFQ R^1=H, R^2=Me

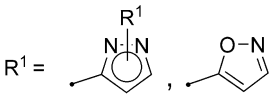


8-MOP

The great importance of this category of heterocycles oriented our attention to the synthesis of a series of new heterocyclic derivatives combining quinolinones and pyrazole or isoxazole moieties in one molecular frame as new possible antitumoral or photochemically reactive compounds.

We wish to report here a new and convenient method for the preparation of quinolinones bearing a pyrazole or isoxazole ring at position 3, starting from the corresponding enaminone, together with the results on their photobiological activity.





Keywords: 4-Hydroxy-2(1*H*)-quinolinones; Pyrazoles; Isoxazoles; Structural determination; ^{15}N NMR; Antitumoral compounds.
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2. Results and discussion

2.1. Chemistry

It is well known that enaminone moieties can be used as starting materials for the preparation of 5-[8] or 3-substituted isoxazoles[9] and *N*-1 or/and *N*-2 substituted pyrazoles[8–10] but it must be noted that in many cases, the authors were not worried about verifying the assigned regiochemistry to the reaction products.

In fact, the reaction between enaminones and 1,2-bis-nucleophiles may led to two possible regioisomers because the cyclization may proceed via two possible mechanisms that differ in their sequential nucleophilic attack/amine exchange reaction. Thus, the new enaminone **2**, obtained by condensation of 3-acetylquinolinone **1**[11] with dimethylformamide dimethylacetal (DMFDMA) in refluxing xylene, may afford *N*-1 or/and *N*-2 substituted pyrazoles or 3- or 5-isoxazole by reaction with *N*-substituted-hydrazines or hydroxylamine, respectively (see Scheme 1).

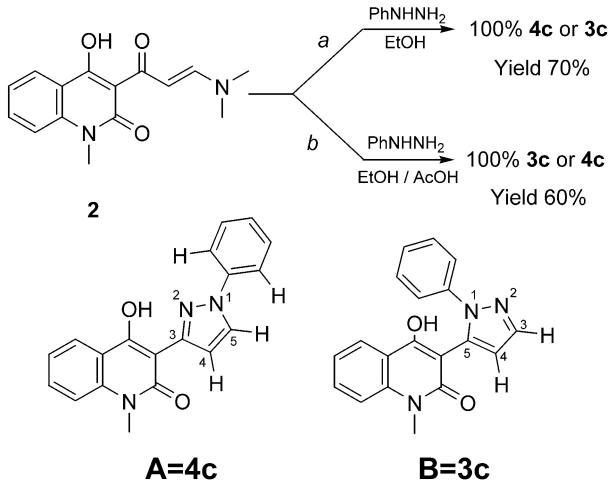
In particular, the regiochemistry of the reaction with methyl- and phenylhydrazine depends on the reaction conditions while only one regioisomer is obtained employing hydroxylamine as shown in Table 1.

Table 1. Cyclization reaction of enaminone **2** with 1,2-bisnucleophiles

Compounds	R^1	Yield (%)		Ratio **3**:**4** or **5a**:**5b**	
		EtOH	EtOH/H^+	EtOH	EtOH/H^+
3a, **4a**	H	70	75	—	—
3b, **4b**	Me	86	90	45:55[a]	55:45[a]
3c, **4c**	Ph	70	60	0:100	100:0
5	—	84	80	100:0	100:0

[a] In AcOH/AcONa **3b**:**4b**=40:60.

To understand the nature of the reaction product(s), we started investigating the reaction of the enaminone **2** with 1,2-nitrogen bisnucleophiles (hydrazine, phenylhydrazine and methylhydrazine) in different reaction conditions (EtOH reflux or EtOH/AcOH reflux). Thus, reaction of enaminone **2** with phenylhydrazine (Table 1) gives rise, regioselectively, to different products (see Scheme 2).

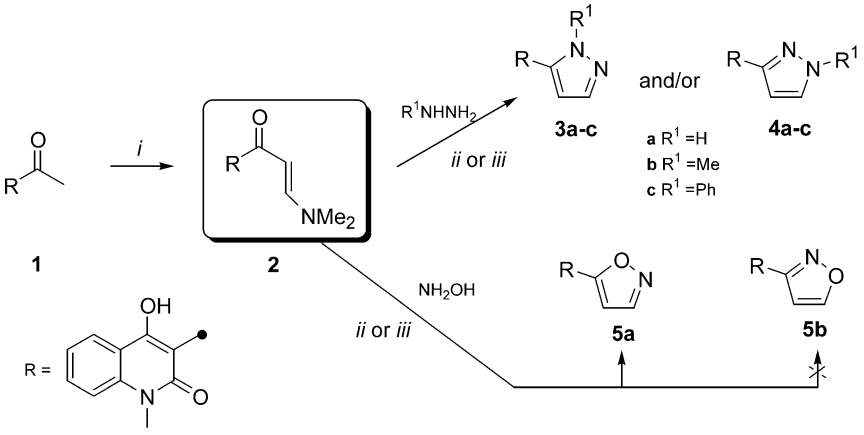


Scheme 2.

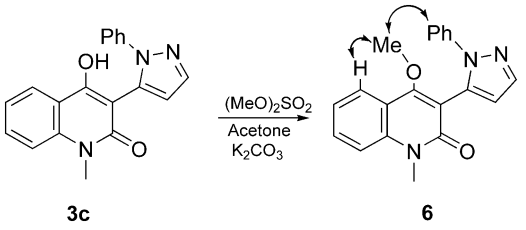
The regiochemistry of the reaction was easily determined by NOE experiments on the isolated product. To this end, we started assigning the pyrazole proton signals in both compounds to H-4 and H-5 (or H-3) on the basis of their different chemical shifts. Then, irradiation of the proton resonating at the highest frequency (δ 8.71 ppm) in compound obtained from route *a* leads to a significant enhancement of the *ortho* protons of the phenyl group, allowing us to assign the structure **A** to this product (**4c**). Similarly, irradiation of the proton resonating at the highest frequency (δ 7.76 ppm) in compound obtained from route *b*, gives rise to a significant enhancement only of the other pyrazole proton, thus confirming the pyrazol-5-yl nature of compound **B** (**3c**) (Scheme 2).

Structure of compound **3c** was confirmed also by NOE experiments carried out on the *O*-methyl derivative **6** obtained by reaction of **3c** with dimethyl sulfate (DMS) (see Scheme 3).

Thus, irradiation of the *O*-methyl singlet, that appears at lower frequencies with respect to the *N*-Me one (δ 3.53 vs 3.60 ppm, *g*-HSQC), gives a positive effect on the *ortho*- and *meta* protons of the phenyl group, in addition to *H*-5 of the quinolinone ring.

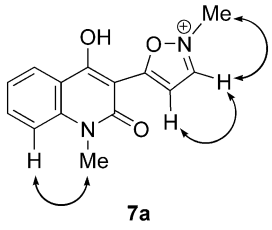


Scheme 1. Reagents and conditions: (i) DMFDMA, xylene, reflux; (ii) EtOH, reflux; (iii) EtOH, AcOH, reflux.



3c 6

Scheme 3.



7a

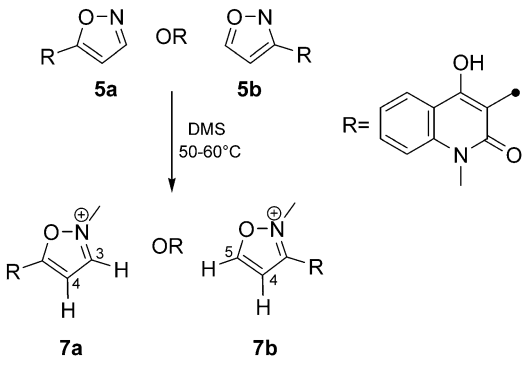
Figure 1.

So, we demonstrated that route *a* (Scheme 2) gives rise to 1,3-disubstituted pyrazole **4c** whereas route *b* affords the 1,5-disubstituted one **3c**.

Continuing our study, we examined the same reaction between compound **2** and methylhydrazine in both the reaction conditions (see Scheme 1 and Table 1). The reaction always gave a mixture of both regioisomers. The compounds were separated by flash chromatography and the regiochemistry determined again via analysis by NMR spectroscopy. Due to the close proximity of the 1-methyl substituent to pyrazole *H*-5 in compound **4b**, an interaction is observed between these two groups in a NOESY 1D experiment.

As for the reaction with *N*,*N*′-1,2 bisnucleophiles we examined also the reaction of the enaminone **2** with hydroxylamine again in both the reaction conditions (EtOH, EtOH/AcOH). To establish the correct structure, the isolated product was treated with DMS to give the corresponding isoxazolium salt **7**.[12]

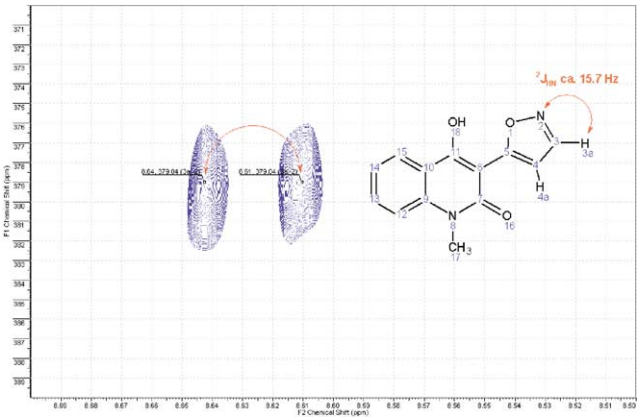
Then, we attributed the isoxazole proton resonances to *H*-4 and *H*-3 or *H*-5 on the basis of their diagnostic chemical shifts. Homonuclear NOE experiments (NOESY 1D) carried out by irradiation of the highest proton resonance of isoxazolium salt is then proposed as a simple method to distinguish between the two regioisomers. According to our knowledge, this approach has never been used for structural determination of isoxazoles obtained from enaminones (Scheme 4).



Scheme 4.

Thus, upon irradiation of the highest hydrogen resonance at δ 9.20 ppm, enhancements of signals at δ 7.55 (H-4) and δ 4.24 ppm (N^+-Me) were observed. On this basis, structure **7a** is easily assigned to the reaction product (see Fig. 1).

In a more elegant way, the previous result can be confirmed working directly on the reaction product through ^{15}N NMR spectroscopy; from the proton-detected ^{1}H–^{15}N *g*-HMBC experiment it appears that the nitrogen atom presents only one ^{3}J N–H of ca. 3 Hz and a diagnostic coupling of 15.7 Hz that must be attributed to a two bonds *J* N–H.[13] These results agree only with structure **5a**.



2.2. Biological results

The phototoxicity of title compounds, was investigated first on a cell line of human tumor HL-60 (human promyelocytic leukemia). Table 2 shows the extent of cell survival expressed as GI_{50}, which is the concentration, expressed in μM, which induces 50% of inhibition of cell growth, after irradiation at different UVA doses.

Table 2. Photocytotoxicity of test compounds against HL-60 cell line

Compounds	GI_{50} (μM)[a]	
	1.25[b] J cm^{-2}	2.5 J cm^{-2}
3a, **4a**	>10	>10
3b	>10	>10
3c	>10	7.8±2.2
4b	>10	7.3±1.9
4c	6.2±0.7	0.9±0.1
5	>10	>10

[a] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[b] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole-Parmer radiometer.

Control experiments with UVA light or compounds alone were carried out without significant cytotoxic effects (data not shown). The results, shown in Table 2, indicate that compound **3a** is not active, instead for 3-pyrazolyl

derivative **4c** we observe the highest activity. Interestingly, the isomeric compound **3c** is only slightly active and substitution for a methyl group lead to an inactive derivative **3b**. From this preliminary screening the most active compounds were also evaluated on a human intestinal adenocarcinoma cell line (LoVo) and one line of immortalized, not tumorigenic, human keratinocytes (NCTC 2544). From Table 3 it appears that the phototoxicity of the most active compounds, in particular **4c**, is higher in the tumor cell lines in comparison to the normal ones (NCTC 2544). In preliminary experiments devoted to the search for a possible molecular target, compound **4c** was evaluated for its potential capability to induce single strand breaks in a plasmid DNA, as a model.

Table 3. Photocytotoxicity of test compounds against NCTC 2544 and LoVo cell lines[a]

Compounds	Cell lines GI_{50} (μM)[b]			
	NCTC 2544		LoVo	
	1.25[c] J cm^{-2}	2.5 J cm^{-2}	1.25 J cm^{-2}	2.5 J cm^{-2}
4c	7.0±1.3	2.5±0.7	3.7±0.8	1.8±0.2
3c	>20	>20	>20	12.1±1.9
4b	>20	>20	18.2±2.1	10.1±1.3

[a] Human cell lines: NCTC 2544 Human keratinocytes; LoVo intestinal adenocarcinoma.
[b] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[c] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole-Parmer radiometer.

The obtained results (data not shown) indicate that **4c**, after irradiation in the presence of DNA, is not able to induce any significant damage to DNA thus suggesting that another target at cellular level may be involved in its phototoxic effect. In parallel to the cytotoxic evaluation, flow cytometry was employed to study cell cycle variations upon irradiation. The effects of the most active compound **4c** were evaluated after 24, 48 and 72 h from irradiation in the leukemic cell line. The percentage of the cells in the different phases of the cell cycle is shown in Table 4.

Table 4. Percentage of HL-60 in the different phases of the cell cycle[a]

Treatment	G1	G2	S	Apoptotic cells[b]
Non irradiated cells	39.0	9.0	51.7	0.8
UVA irradiated cells without drug (2.5 J cm^{-2})				
24 h	36.7	13.0	50.3	8.6
48 h	48.2	10.9	40.9	11.7
72 h	35.0	10.5	54.4	9.4
4c 2.5 μM+UVA (2.5 J cm^{-2})				
24 h	30.8	10.8	58.4	13.2
48 h	44.7	10.3	45.0	27.0
72 h	44.2	12.0	43.8	40.5
4c 5.0 μM+UVA (2.5 J cm^{-2})				
24 h	34.6	11.1	54.3	21.0
48 h	49.6	11.7	38.6	26.0
72 h	56.0	6.9	37.1	46.0

[a] The percentage of each phase of the cell cycle (G1, S and G2/M) were calculated on living cells.
[b] The percentage of apoptotic cells is referred to cells population characterized by the appearance of a sub G1 peak.

It can be observed that treatment with **4c** in combination with UVA induces a reduction of the S phase at 48 and 72 h after irradiation especially for the highest dose utilized. This is accompanied by a concomitant block in G1 phase. This event is followed at 48 and 72 h after the irradiation by massive induction of apoptosis, as observed by the appearance of a sub G1 peak (apoptotic cells) that refers to cells with DNA content lower than G1.[14,15] In fact, apoptosis induces the activation of endogenous nucleases, which are responsible for nucleic acid degradation.

3. Conclusions

In summary, we have unambiguously determined the structures of the reaction products of 3-quinolinonylenaminone **2** with 1,2-bisnucleophiles such as phenylhydrazine, methylhydrazine and hydroxylamine under different reaction conditions. Whereas from the reaction with phenylhydrazine both the regioisomers (**3c** and **4c**) can be selectively obtained according to the reaction conditions, reaction with methylhydrazine always gives rise to a mixture of the regioisomers **3b** and **4b**; moreover, we establish that reaction of the enaminone **2** with hydroxylamine affords only the 5-substituted isoxazole **5**. On the basis of the biological evaluation, compound **4c** seems to be very attractive as a potential drug for photochemotherapy. Hence, experiments aimed at defining the target(s) at cellular level and the phototoxicity mechanism are in progress.

4. Experimental

4.1. General

Melting points were taken on a Büchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercuryplus 400 spectrometer (operating at 100.58 MHz for ^{13}C) in the Fourier transform mode at 21±0.5 °C in $CDCl_3$ or DMSO-d_6. Chemical shifts (δ) are reported in ppm relative to TMS as secondary reference standard; coupling constants in Hz. Mass spectra were registered with a Carlo Erba QMD 1000 instrument at 70 eV. IR spectra were obtained with a Perkin-Elmer 881 spectrophotometer for dispersion in KBr. Elemental analyses were obtained by Elemental Analyzer Perkin-Elmer 240C apparatus. Silica gel plates (Merck F_{254}) and silica gel 60 (Merck 230–400 mesh) were used for analytical TLC and for flash chromatography, respectively. Solvents were removed under reduced pressure.

4.1.1. 3-[(*E*)-3-(Dimethylamino)-2-propenoyl]-4-hydroxy-1-methyl-2(1*H*)-quinolinone (2). Dimethylformamide dimethylacetal (DMFDMA) (1.19 g, 10 mmol) was added to a solution of 3-acetylquinolinone (2.17 g, 10 mmol) in xylene (25 ml), and the reaction mixture was refluxed for 4 h. Removal of the solvent under reduced pressure yielded the crude product (2.31 g, 85%). An analytical sample (yellowish crystals) was obtained by recrystallization from benzene; mp 178–179 °C (from benzene); IR (KBr) 3150, 2921, 1655, 1620, 1580 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (1H, d, ^{3}J=12.0 Hz, H-3′), 8.07 (1H, d,

^3J=7.6 Hz, H-5), 7.67 (1H, dd, ^3J=8.2, 7.6 Hz, H-7), 7.43 (1H, d, ^3J=8.2 Hz, H-8), 7.22 (1H, pt, ^3J=7.6 Hz, H-6), 7.05 (1H, d, ^3J=12.0 Hz, H-2′), 3.52 (3H, s, N–CH_3), 3.28 (3H, s, N–CH_3), 3.01 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, DMSO-d_6) δ 186.40 (s, C-1′), 177.48 (s, C-4), 161.65 (s, C-2), 157.52 (d, C-3′), 140.98 (s, C-8a), 134.09 (d, C-7), 128.80 (d, C-5), 125.62 (d, C-6), 121.77 (s, C-4a), 118.06 (d, C-8), 115.11 (s, C-3), 92.84 (d, C-2′), 45.90 (q, N–CH_3), 38.14 (q, N–CH_3), 29.12 (q, N–CH_3); EI-MS *m/z* (%) 272 (M^+, 34), 254 (14), 228 (100), 202 (12), 98 (28). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.12; H, 5.85; N, 10.19.

4.2. General procedure for the preparation of compounds 3, 4 and 5

The 1,2-bisnucleophile (1 mmol) was added in one portion to a stirred solution of compound **2** (0.272 g, 1 mmol) in EtOH (6 ml) or acidified EtOH (6 ml containing 0.1 ml of AcOH) and the reaction mixture refluxed for 3 h. After cooling and removal of the solvent produced a solid that was crystallized (compound **3a**, **3c**, **4c** and **5**) or subjected to flash cromatography with ethanol/ethyl acetate=1:20, as eluant (compounds **3b** and **4b**).

4.2.1. 4-Hydroxy-1-methyl-3-(1*H*-pyrazol-5-yl)-2(1*H*)-quinolinone (3a). Yellowish crystals; mp >300 °C (from methanol); IR (KBr) 3350–3210, 1645, 1610, 1590 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (1H, dd, ^3J=8.0 Hz, ^4J=1.2 Hz, H-5), 7.96 (1H, d, ^3J=2.3 Hz, H-3′), 7.67 (1H, ddd, ^3J=8.6, 7.0 Hz, ^4J=1.6 Hz, H-7), 7.55 (1H, dd, ^3J=8.6 Hz, ^4J=1.0 Hz, H-8), 7.34 (1H, d, ^3J=2.3 Hz, H-4′), 7.29 (1H, ddd, ^3J=8.0, 7.0 Hz, ^4J=1.0 Hz, H-6), 3.66 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, DMSO-d_6) δ 160.47 (s, C-2), 159.12 (s, C-4), 148.23 (s, C-5′), 138.47 (s, C-8a), 131.44 (d, C-7), 129.03 (d, C-3′), 123.49 (d, C-5), 121.61 (d, C-6), 115.32 (s, C-4a), 114.55 (d, C-8), 105.28 (d, C-4′), 100.39 (s, C-3), 28.97 (q, N–CH_3); EI-MS *m/z* (%) 241 (M^+, 100), 212 (15), 184 (8), 149 (2), 134 (20). Anal. Calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.40; H, 4.58; N, 17.33.

4.2.2. 4-Hydroxy-1-methyl-3-(1-methyl-1*H*-pyrazol-5-yl)-2(1*H*)-quinolinone (3b). Yellowish needles; mp 232–233 °C (from methanol); IR (KBr) 2946, 1632, 1580, 1326 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (1H, dd, ^3J=8.0 Hz, ^4J=1.6 Hz, H-5), 7.71 (1H, ddd, ^3J=8.6. 7.2 Hz, ^4J=1.6 Hz, H-7), 7.56 (1H, d, ^3J=2.0 Hz, H-3′), 7.46 (1H, dd, ^3J=8.6 Hz, ^4J=1.0 Hz, H-8), 7.35 (1H, ddd, ^3J=8.0, 7.2 Hz, ^4J=1.0 Hz, H-6), 6.39 (1H, d, ^3J=2.0 Hz, H-4′), 3.80 (3H, s, N–CH_3), 3.77 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, $CDCl_3$) δ 161.20 (s, C-2), 159.05 (s, C-4), 140.08 (s, C-8a), 139.13 (d, C-3′), 133.62 (s, C-5′), 132.24 (d, C-7), 124.66 (d, C-5), 122.06 (d, C-6), 115.18 (s, C-4a), 114.10 (d, C-8), 107.17 (d, C-4′), 101.07 (s, C-3), 37.47 (q, N–CH_3), 29.58 (q, N–CH_3); EI-MS *m/z* (%) 255 (M^+, 100), 228 (32), 200 (58), 134 (38), 77 (59). Anal. Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 66.08; H, 4.94; N, 16.72.

4.2.3. 4-Hydroxy-1-methyl-3-(1-phenyl-1*H*-pyrazol-5-yl)-2(1*H*)-quinolinone (3c). Yellowish needles; mp >260 °C (from ethanol); IR (KBr) 2890, 1600, 1557, 1381 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (1H, s, OH), 7.98 (1H, dd, ^3J=8.0 Hz, ^4J=1.6 Hz, H-5), 7.76 (1H, d, ^3J=1.8 Hz, H-3′), 7.66 (1H, ddd, ^3J=8.6, 7.0 Hz, ^4J=1.6 Hz, H-7), 7.50 (1H, d, ^3J=8.6 Hz, H-8), 7.43–7.21 (6H, m, H-6, H-2″, H-3″, H-4″), 6.42 (1H, d, ^3J=1.8 Hz, H-4′), 3.51 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, DMSO-d_6) δ 161.04 (s, C-2), 159.09 (s, C-4), 140.46 (s, C-1″), 139.74 (d, C-3′), 139.55 (s, C-8a), 134.67 (s, C-5′), 131.86 (d, C-7), 128.66 (d, C-3), 126.88 (d, C-4″), 123.82 (d, C-5), 123.11 (d, C-2″), 121.56 (d, C-6), 115.48 (s, C-4a), 114.66 (d, C-8), 110.02 (d, C-4′), 102.17 (s, C-3), 29.14 (q, N–CH3); EI-MS *m/z* (%) 317 (M^+, 96), 300 (100), 107 (50), 77 (92). Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 72.20; H, 4.72; N, 13.44.

4.2.4. 4-Hydroxy-1-methyl-3-(1-methyl-1*H*-pyrazol-3-yl)-2(1*H*)-quinolinone (4b). Yellowish needles; mp 139–140 °C (from methanol); IR (KBr) 2934, 1634, 1592, 1516 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (1H, dd, ^3J=8.0 Hz, ^4J=1.6 Hz, H-5), 7.59 (1H, ddd, ^3J=8.6, 7.2 Hz, ^4J=1.6 Hz, H-7), 7.45 (2H, AB system, H-4′ and H-5′), 7.35 (1H, dd, ^3J=8.6 Hz, ^4J=1.0 Hz, H-8), 7.27 (1H, ddd, ^3J=8.0, 7.2 Hz, ^4J=1.0 Hz, H-6), 3.98 (3H, s, N–CH_3), 3.76 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, $CDCl_3$) δ 162.22 (s, C-2), 160.36 (s, C-4), 149.50 (d, C-3′), 139.10 (s, C-8a), 131.36 (d, C-7), 130.60 (s, C-5′), 124.68 (d, C-5), 121.90 (d, C-6), 116.78 (s, C-4a), 114.07 (d, C-8), 107.33 (d, C-4′), 101.29 (s, C-3), 39.20 (q, N–CH_3), 29.53 (q, N–CH_3); EI-MS *m/z* (%) 255 (M^+, 100), 226 (17), 212 (19), 134 (29), 77 (19). Anal. Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.73; H, 5.38; N, 16.24.

4.2.5. 4-Hydroxy-1-methyl-3-(1-phenyl-1*H*-pyrazol-3-yl)-2(1*H*)-quinolinone (4c). Yellowish needles; mp 211–212 °C (from ethanol); IR (KBr) 3418–3220, 1643, 1620 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (1H, d, ^3J=2.7 Hz, H-5′), 8.12 (1H, dd, ^3J=8.0 Hz, ^4J=1.6 Hz, H-5), 7.90 (2H, m, H-2″), 7.71 (1H, ddd, ^3J=8.6, 7.0 Hz, ^4J=1.6 Hz, H-7), 7.62–7.55 (4H, H-4′, H-3″, H-8), 7.39 (1H, m, H-4″), 7.35 (1H, ddd, ^3J=8.0, 7.0 Hz, ^4J=1.0 Hz, H-6), 3.69 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, DMSO-d_6) δ 160.93 (s, C-2), 158.94 (s, C-4), 150.59 (s, C-3′), 139.24 (s, C-8a), 138.93 (s, C-1″), 132.47 (d, C-7), 130.37 (d, C-3″), 129.10 (d, C-5′), 127.39 (d, C-4″), 124.13 (d, C-5), 122.37 (d, C-6), 118.91 (d, C-2″), 155.54 (s, C-4a), 115.25 (d, C-8), 109.33 (d, C-4′), 100.38 (s, C-3), 29.59 (q, N–CH_3); EI-MS *m/z* (%) 317 (M^+, 100), 288 (20), 274 (14), 212 (14), 115 (12), 77 (94). Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.88; H, 4.69; N, 13.18.

4.2.6. 4-Hydroxy-3-(5-isoxazolyl)-1-methyl-2(1*H*)-quinolinone (5). Yellowish needles; mp 280–282 °C (from ethanol); IR (KBr) 3298, 1642, 1618 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (1H, d, ^3J=2.0 Hz, H-3′), 8.15 (1H, dd, ^3J=8.2 Hz, ^4J=1.6 Hz, H-5), 7.74 (1H, ddd, ^3J=8.0, 7.0 Hz, ^4J=1.6 Hz, H-7), 7.58 (1H, d, ^3J=8.0 Hz, H-8), 7.35 (1H, dd, ^3J=8.2, 7.0 Hz, H-6), 6.86 (1H, d, ^3J=2.0 Hz, H-4′), 3.62 (3H, s, N–CH_3); ^{13}C NMR (100.58 MHz, DMSO-d_6) δ 163.74 (s, C-5′), 159.85 (s, C-2), 159.55 (s, C-4), 150.48 (d, C-3′), 139.50 (s, C-8a), 132.56 (d, C-7), 124.20 (d, C-5), 121.82 (d, C-6), 115.54 (s, C-4a), 114.85 (d, C-8), 104.97 (d, C-4′), 99.82 (s, C-3), 28.97 (q, N–CH_3); EI-MS *m/z* (%) 242 (M^+, 37), 215 (40), 158 (95), 101 (26),

76 (84), 68 (100). Anal. Calcd for $C_{13}H_{10}N_2O_3$: C, 64.46; H, 4.16; N, 11.56. Found: C, 64.32; H, 4.18; N, 11.47.

4.2.7. 4-Methoxy-1-methyl-3-(1-phenyl-1*H*-pyrazol-5-yl)-2(1*H*)-quinolinone (6). Yellowish needles; mp 175–176 °C (from ethanol); IR (KBr) 2947, 1640, 1595, 1500, 1355 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.86 (1H, dd, $^3J=8.0$ Hz, $^4J=1.6$ Hz, H-5), 7.81 (1H, d, $^3J=1.8$ Hz, H-3′), 7.69 (1H, ddd, $^3J=8.4$, 7.0 Hz, $^4J=1.6$ Hz, H-7), 7.57 (1H, d, $^3J=8.4$ Hz, H-8), 7.47–7.22 (6H, m, H-6 and ArH_5), 6.57 (1H, d, $^3J=1.8$ Hz, H-4′), 3.60 (3H, s, N–CH_3), 3.53 (3H, s, OCH_3); ^{13}C NMR (100.58 MHz, $CDCl_3$) δ 161.93 (s, C-2), 161.71 (s, C-4), 140.71 (s, C-1″), 140.55 (d, C-3′), 139.93 (s, C-8a), 135.13 (s, C-5′), 132.67 (d, C-7), 129.55 (d, C-3″), 127.68 (d, C-4″), 124.48 (d, C-5), 123.34 (d, C-2″), 122.64 (d, C-6), 116.77 (s, C-4a), 115.41 (d, C-8), 111.52 (d, C-4′), 107.77 (s, C-3), 60.00 (q, OCH_3), 30.13 (q, N–CH_3); EI-MS *m/z* (%) 331 (M^+, 100), 300 (88), 226 (17), 77 (67). Anal. Calcd for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.21; H, 5.56; N, 12.54.

4.3. Irradiation procedure

Two HPW 125 Philips lamps, mainly emitting at 365 nm, were used for irradiation experiments. The spectral irradiance of the source was 4.0 mW cm^{-2} as measured, at the sample level, by a Cole-Parmer Instrument Company radiometer (Niles, IL), equipped with a 365-CX sensor.

4.4. Cellular phototoxicity

Human promyelocytic leukemia cells (HL-60) were grown in RPMI-1640 medium (Sigma Co Mo USA), human keratinocytes (NCTC 2544) were grown in DMEM medium (Sigma Co Mo USA), intestinal adenocarcinoma cells (LoVo) were grown in Ham's F12 medium (Sigma Co Mo USA) all supplemented with 115 units/ml of penicillin G (Invitrogen, Milano- Italy), 115 μg/ml streptomycin (Invitrogen, Milano Italy) and 10% fetal bovine serum (Invitrogen, Milano- Italy). Individual wells of a 96-well tissue culture microtiter plate (Falcon BD) were inoculated with 100 μl of complete medium containing 8×10^3 HL-60 cells or 5×10^3 NCTC 2544 and LoVo cells. The plates were incubated at 37 °C in a humidified 5% incubator for 18 h prior the experiments. After medium removal, 100 μl of the drug solution, dissolved in DMSO and diluted with Hank's Balanced Salt Solution (HBSS pH=7.2), was added to each well and incubated at 37 °C for 30 min and then irradiated. After irradiation, the solution was replaced with the medium, and the plates were incubated for 72 h. Cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] test, as described previously.[16,17]

4.5. Cell cycle analysis

For flow cytometric analysis of DNA content, 5×10^5 HL-60 cells in exponential growth were treated at different concentrations of the test compounds for 24, 48 and 72 h. After the incubation period, the cells were centrifuged and fixed with ice-cold ethanol (70%), treated with lysis buffer containing RNAseA, and then stained with propidium iodide. Samples were analyzed on a Becton Coulter Epics XL-MCL flow cytometer. For cell cycle analysis, DNA histograms were analyzed using MultiCycle for Windows (Phoenix Flow Systems, San Diego, CA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.tet.2005.09.135.

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Stereocontrolled synthesis of thiohydantoin spironucleosides from sugar spiroacetals

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

Abstract—5-Epithiohydantocidin, *N*-alkyl and *N*-glycosylthiohydantoin spironucleosides are prepared from glycosylaminoesters and from furanoid and pyranoid methyl isothiocyanatoulosonates. The aminoesters and the isothiocyanates are obtained, in a stereocontrolled manner, from sugar spiroacetals through a high-yielding sequence involving ring opening with trimethyl azide, formation of an ester group, reduction of the azide, and, in the case of isothiocyanates, reaction with thiophosgene.

1. Introduction

The chemistry of spironucleosides, a type of nucleoside in which the anomeric carbon belongs simultaneously to the sugar ring and to the nitrogenated heterocyclic moiety,[1] has received considerable development in the last decade. This interest is due to the isolation from culture broths of *Streptomices hygroscopicus* of (+)-hydantocidin (**1**), the first natural spironucleoside.[2] The (+)-hydantocidin shows low toxicity for mammals and has herbicidal and plant growth-regulatory activities, which have been related to its inhibitory activity of adenylsuccinate synthase.[3] Other spiro-anulated compounds[4] also have biological interest due to their activity as inhibitors of glycogen phosphorilase and α-amylase.[5] Syntheses of (+)-hydantocidin have been reported,[6] and starting from 1993 many syntheses of hydantocidin analogues and related carbocyclic derivatives have been described.[1b,7]

Recently, we reported our preliminary results on the preparation of pyranoid and furanoid isothiocyanatoulosonates, a new type of sugar isothiocyanate which is used for the stereocontrolled preparation of thiohydantoin spironucleosides.[8] Some data on related ulosononitriles[9] and furanoid ulosonoisothiocyanates[10] have been reported later.

In this paper, we report the full data on a synthetic procedure to prepare furanoid and pyranoid spirothiohydantoins using methyl isothiocyanatoulosonates (**11**, **18**) and methyl aminoulosonates (**9**, **10**, and **17**) as key intermediates. We have previously shown[7f] that ketofuranosyl isothiocyanates are transient intermediates in the preparation of spironucleosides of 1,3-*O*,*N* five member heterocycles.

2. Results and discussion

The starting materials to prepare the pyranoid and furanoid 2-isothiocyanatoulosonic esters **11** and **18** (Scheme 1) were the β-azido-1-trimethyl ethers **3** and **13**, respectively, which we have previously reported,[7f] and were obtained by reaction of trimethylsilyl azide with the corresponding spiroacetal[11] **2** or **12** in freshly distilled acetonitrile, under stringently anhydrous conditions. Desilylation of **3** with a catalytic amount of TBAF (tetrabutyl ammonium fluoride). $3H_2O$ produced **4** in high yield. Swern[12] oxidation of **4** afforded the azido aldehyde **5**, whose NMR data showed the signals for **5** and additional signals corresponding to a hydrate as is described for related aldehydes.[13] Further oxidation ($NaClO_2$) of **5** followed by treatment with diazomethane gave the 3-*O*-benzylazido ester **7**. Treatment of **4** with ruthenium chloride-sodium metaperiodate produced simultaneous oxidation of the formyl and benzyl groups with formation of the 3-*O*-benzoyl ulosonic acid derivative **6**, which was not isolated, and in situ converted (reaction with CH_2N_2) into the methyl 3-*O*-benzoyl azido

Keywords: Spironucleosides; Thiohydantoins; Thioureas; Isothiocyanates.
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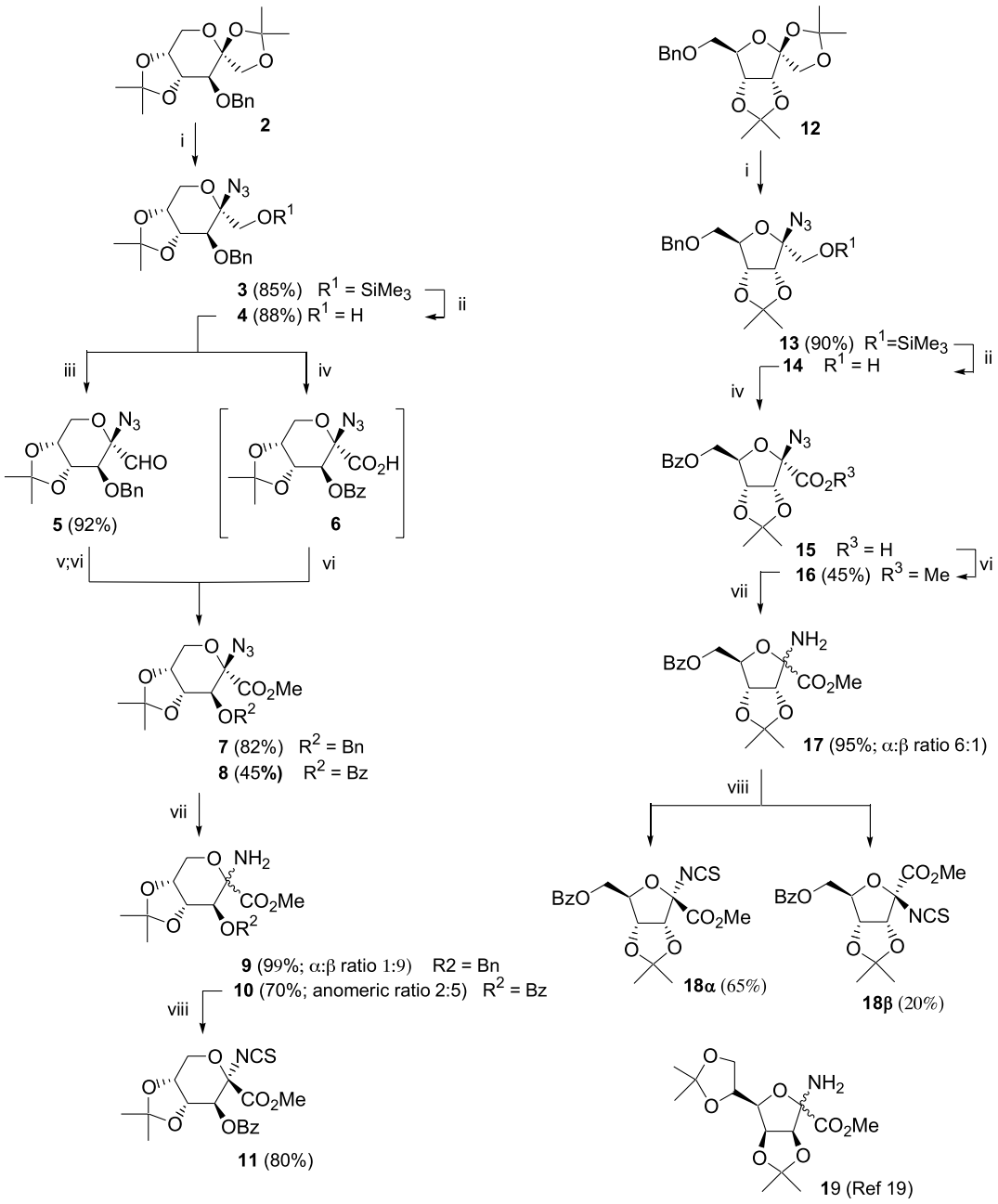
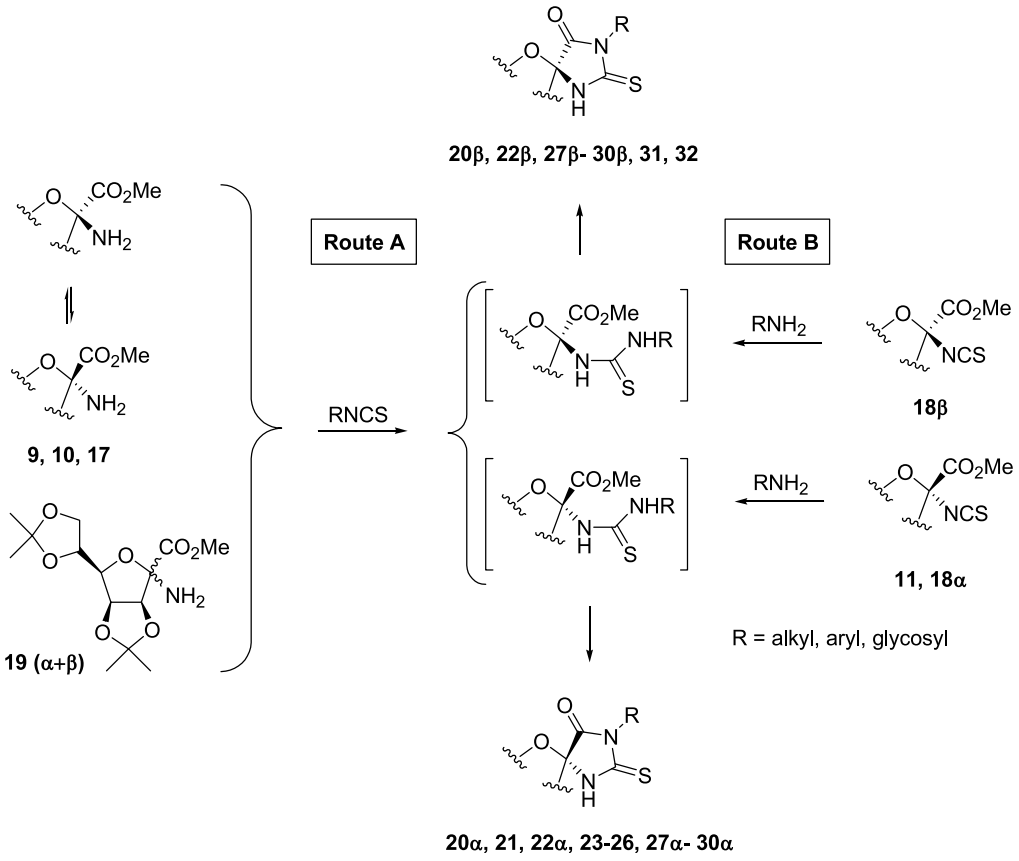


Scheme 1. Preparation of new glycosyl aminoulosonates and glycosyl isothiocyanatoulosonates from sugar spiroacetals. Reagents and conditions: (i) $TMSN_3$, TMSOTf, CH_3CN, 0 °C; (ii) TBFA.$3H_2O$, THF, rt; (iii) DMSO, CH_2Cl_2, −70 °C; (iv) $RuCl_3 \cdot H_2O$, $NaIO_4$, CH_3CN, H_2O, CCl_4, rt; (v) $NaClO_2$, $(CH_3)_2C{=}CH{-}CH_3$, $NaH_2PO_4 \cdot 2H_2O$, ^{t}BuOH, H_2O, 0 °C; (vi) CH_2N_2, Et_2O, MeOH, 0 °C; (vii) H_2/C–Pd, MeOH, rt; (viii) $CSCl_2$, $CHCl_3$, H_2O, $CaCO_3$, rt.

ester **8**. Catalytic hydrogenation of **7** and **8** gave the glycosylaminoester anomeric mixtures **9** and **10**, respectively, in good yields. The anomeric ratio for **9** was 1:9 (α:β, $CDCl_3$, rt, equilibrium), whereas in the case of **10** the ratio of the two anomers was 2:5 (same conditions), although the anomeric (C-2) configuration of each anomer for **10** was not determined. The anomeric mixture **9** was used only in route A (Scheme 2) for the synthesis of spirothiohydantoins. Compound **10** was also transformed, by reaction with thiophosgene in basic medium, into an anomeric mixture (α:β 16:1) of glycosyl isothiocyanates, from which only the α anomer **11** was isolated. This compound was used in route B of spirothiohydantoins (Scheme 2).

In a similar way, the reaction of **13** with TBAF· $3H_2O$ gave the known[6b] 1-*O*-unprotected furanosyl azide **14**, which by oxidation with ruthenium chloride–sodium metaperiodate (→**15**), followed by estherification with CH_2N_2, produced the methyl ulosonate **16**. Catalytic hydrogenation of **16** yielded the anomeric mixture (α:β ratio 6:1) of aminoesters **17** in virtually quantitative yield. Reaction of **17** with thiophosgene in the presence of $CaCO_3$ gave, after column chromatography, the α (65%) and β (20%) isothiocyanato ulosonates **18α** and **18β** as isolated products.

Table 1 shows selected spectroscopic data for the structural assignments of compounds **4**–**5**, **7**–**11**, and **16**–**18**. Thus the



Scheme 2. Preparation of 5-*epi*thiohydantocidin, *N*-alkyl and *N*-glycosylthiohydantoin spironucleosides from glycosylaminoesters (route A) and from methyl isothiocyanatoulosonates (route B).

IR spectra of the azido derivatives **4**–**5**, **7**, **8**, and **16** had absorption for the N_3 group at 2116–2128 cm^{-1}. The NMR spectra of **4** showed no signals for a $SiMe_3$ group, and the OH was evident from the IR absorption at 3497 cm^{-1} and from the interchangeable (D_2O) double doublet at 2.12 ppm in the ^1H NMR spectrum. The hydrated form of **5** appeared in the ^1H NMR spectrum in a 1:4 ratio with respect to the free aldehyde, the chemical shift for the resonance of H-1 of the hydrate being 5.22 ppm. The carbonyl groups of the β-azido ulosonic esters **7**, **8** and **16**, resonated at 165.4–166.7 ppm (Table 1) as is reported[14] for related azido esters. The signal for the resonance of the anomeric carbon in **4**, **5**, **7**, and **8** was close to 91 ppm, as is described for glycopyranosyl azides;[15] the same carbon for the furanoid derivative **16** resonated at 100.5 ppm. The β configuration for the major compound in the anomeric mixture **9** is proposed according to the order of formation, and the anomeric ratio is given in the equilibrium (NMR measurements). In the 6:1 anomeric mixture **17** the major compound was the α anomer. The anomeric configuration being supported on the order of formation, and on the differences of chemical shifts of H-3 and C-3 for the two anomers (H-3 is relatively deshielded in the α anomer whereas C-3 is relatively shielded in the same anomer), which is in agreement with reported data for related compounds.[16] The isothiocyanato group of **11**, **18α** and **18β** was evident from the corresponding IR absorptions

Table 1. Selected spectroscopic data (ν cm^{-1}, δ ppm, J Hz) for compound **3**–**5**, **7**–**11**, and **16**–**18**[a]

Compound	ν_{N3}[b]/ν_{NCS}[b]	δ C-1	δ C-2	δ C-3	δ NCS
4	2118	64.9	91.7	76.2	—
5	2122	192.5	90.9	74.6	—
7	2126	166.7	91.1	76.7	—
8	2128	165.8	90.3	70.8	—
9[c]	—	171.4	86.1	77.2	—
10[c]	—	170.9	85.4	72.2	—
11	2002	165.0	87.7	72.9	145.1
16	2116	165.4	100.5	86.9	—
17[c]	—	169.9	93.6	81.7	—
18α	2018	166.0	95.1	84.4	144.9
18β	2016	164.9	97.7	88.8	145.1

[a] NMR data are obtained in $CDCl_3$.
[b] KBr discs.
[c] Data for the major anomer.

Table 2. Synthesis of thiohydantoin spironucleosides

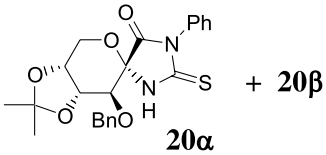
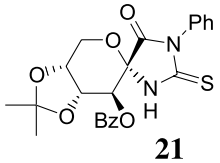
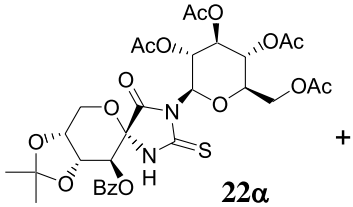
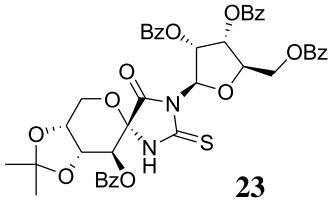
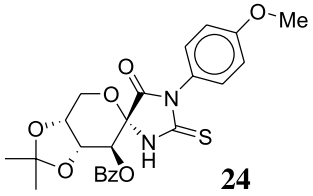
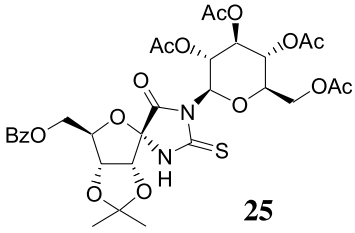
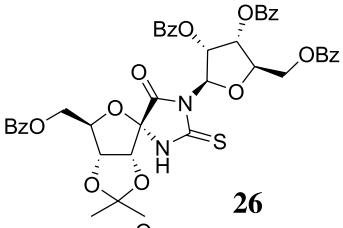
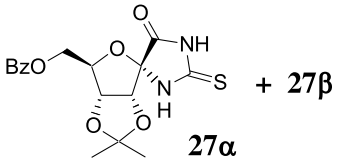
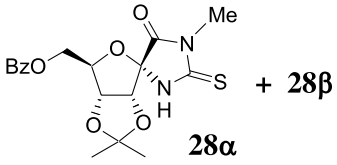
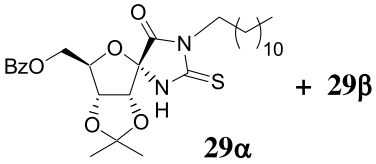
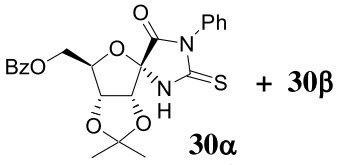
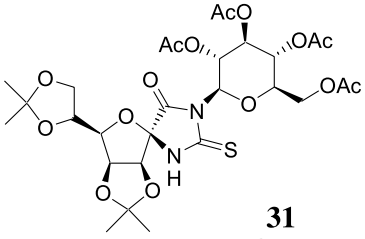
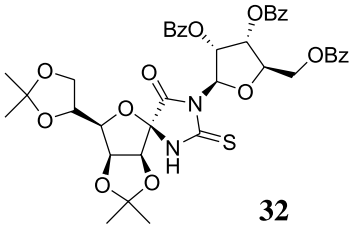
Entry	Starting aminoester or isothiocyanatoulosonate (anomeric ratio)	Route	Product (s)	Yield (%)
1	**9** (α:β 1:9)	A	 **20α** + **20β**	65 (**20α**) 31 (**20β**)
2	**10** (5:2)	A	 **21**	70
3	**10** (5:2)	A	 **22α** + **22β**	76 (**22α**) 9 (**22β**)
4	**10** (5:2)	A	 **23**	81
5	**11** (only α anomer)	B	 **24**	72
6	**17** (α:β 6:1)	A	 **25**	79
7	**17** (α:β 6:1)	A	 **26**	83
8	**18** (α:β 13:4)	B	 **27α** + **27β**	74 (**27α**) 20 (**27β**)
9	**18** (α:β 13:4)	B	 **28α** + **28β**	64 (**28α**) 21 (**28β**)

Table 2 (*continued*)

Entry	Starting aminoester or isothiocyanatoulosonate (anomeric ratio)	Route	Product (s)	Yield (%)
10	**18** (α:β 13:4)	B	 **29α** + **29β**	60 (**29α**) 17 (**29β**)
11	**18** (α:β 13:4)	B	 **30α** + **30β**	73 (**30α**) 21 (**30β**)
12	**19** (1:5)	A	 **31**	94
13	**19** (1:5)	A	 **32**	94

at 2000–2018 cm^{-1}, and from the ^{13}C resonances at roughly 145 ppm, characteristic of glycosyl isothiocyanates.[17] The vicinal coupling constants between the protons of the pyranoid ring of **11** were indicative of $^5C_2$ conformation, slightly distorted by the dioxolane ring (the value of $J_{5,6'}$, for example, was 6.9 Hz). The anomeric configuration (the NCS group is in α position) of this compound was deduced from its transformation into the spiranic compound **24** and from the NMR data of **24** (see below). The $^3J_{HH}$ values for the protons of the furanoid ring of **18β** were very close to that for the β-D-psicofuranosyl azide.[6b] The anomeric carbon in **18α** resonated at higher field (95.1 ppm) than the same carbon in **18β** (97.7 ppm), as in related pairs of anomers.[14b,16a,18] This assignment of the anomeric configurations were confirmed by the NMR data of **27–30** (see below).

The glycosylamines **9**, **10** and **17**, together with described[19] methyl 2-aminohept-2-ulofuranosonate **19** were used in the route A to prepare spirothiohydantoins (Scheme 2). Both anomers of **19** had been reported as isolated products. In our first attempt to prepare spirothiohydantoins from **19**, pure α and β anomers were used; but, due to the anomeric equilibrium under the reaction conditions, in both cases the same spirothiohydantoin was obtained, consequently, the anomeric mixture **19 α+β** was used in subsequent reactions. A related cyclization using phenylisocyanate as sole heterocumulene has been reported.[16a]

Treatment of the anomeric mixture of aminoesters **9** with phenyl isothiocyanate in DMF at 85 °C for 23 h resulted in 96% yield the resoluble mixture of spirothiohydantoins **20** (Scheme 2, route A and Table 2, entry 1). This reaction was low-yielding under milder conditions, probably due to the stabilization by hydrogen bondings between the amino group and the oxygen atoms on C-1 and C-3 in the starting aminoester. The reaction involves the formation of an intermediate thiourea which spontaneously cyclates to the thiohydantoin.[20]

The IR spectra of **20α** and **20β** had the signal for the carbonyl group ($\nu_{C=O}$) at 1765 cm^{-1}, and the C═S and C═O groups resonated (Table 3) at roughly 184 and 169.5 ppm, respectively, in accord with that described for related spirohydantoins.[21] The $^3J_{HH}$ values between protons of the pyranose ring were indicative of the $_4C^1$ conformation. The anomeric configuration is based on the value of the $^3J_{CH}$ between C-4 and H-10 (Fig. 1), which was in the range[18,21] for antiperiplanar nuclei in **20α** (6.8 Hz) and in the range for *gauche* nuclei in **20β** (2.6 Hz). Additionally, the resonance for H-1 (NH) of **20β** was at lower field than that for **20α**, as in spirohydantoins of other pyranoid sugars.[21]

The α configuration of **21** was deduced from the value (5.9 Hz) of the coupling constant between H-10 and C-4, indicative of *anti* relationship between the corresponding nuclei.

Similarly, the reaction of **10** with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate[22] and with 2,3,5-tri-*O*-

benzoyl-β-D-ribofuranosyl isothiocyanate[23] gave the *N*-glycosyl spirothiohydantoins **22α**, **22β** (entry 3) and **23** (entry 4).

The treatment of the ulofuranosonic aminoester **17** with the same glycosyl isothiocyanates gave, in high yield, the furanoid spirothiohydantoins **25** and **26** only as α anomers (Table 2, entries 6 and 7). Selected structural data, including representative diaxial $^{3}J_{H,H}$ values of pyranoids derivatives, of **22**, **23**, **25** and **26** are shown in Table 3. The anomeric configurations were supported on HMBC[24] and carbon-proton coupled experiments in a similar way to that above commented for **20**.

The last spirothiohydantoins prepared through route A were **31** and **32**, which were obtained, in almost quantitative yield, by reaction of the sugar aminoester anomeric mixture[19] **19** (see above) with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate[22] and with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl isothiocyanate[23] respectively, under mild conditions (Table 2, entries 12 and 13). As the configuration of C-4 and C-3 in **31** and **32** are the contrary to those for **25**–**28**, and the spectroscopic data for the hydration

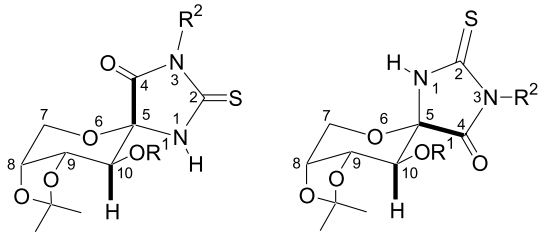


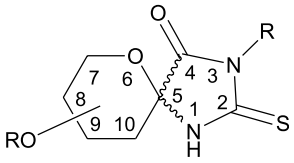
Figure 1. Relationship between H-10 and C-4 in C-5 epimers of pyranoid spirothiohydantoins.

moiety and C-4 of **31**–**32** practically coincide with those for the α anomers **25**, **26**, **27α**, **28α**, **29α**, and **30α**, we propose that in both cases the same spatial relationship exists, that is, **31** and **32** have β anomeric configuration.

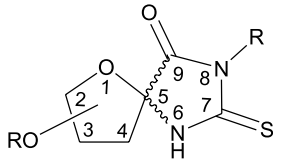
Compounds **22**–**26**, **31** and **32** had a glycosyl radical on N-8, consequently their structures are simultaneously those of *N*- and spiro-nucleoside.

The route B (Scheme 2) to prepare thiohydantoin spironucleosides is the reaction of methyl isothiocyanatoulosonates with ammonia, alkyl, and aryl amines.

Table 3. Selected NMR data (δ ppm, *J* Hz) for spironucleosides **20**–**35** at 500 MHz in $CDCl_3$



Compound[a]	δ H-1 (NH)	δ H-10	$J_{9,10}$	δ C-2 (C=S)	δ C-4 (C=O)	δ C-5	δ C-10
20α	7.06	3.70	7.1	183.6	169.4	86.6	77.7
20β	9.09	4.10	7.9	184.9	169.5	87.1	77.0
21(α)	8.04–7.18	5.67	7.4	182.8	168.7	85.6	71.2
22α	7.65	5.52	7.8	182.1	167.1	84.4	71.1
22β	8.24	5.79	6.9	183.5	166.7	84.5	68.4
23(α)	8.13–7.26	5.52	7.8	181.2	168.2	84.8	71.2
24(α)	7.52	5.65	7.4	183.2	168.9	85.5	71.1
33(α)	8.72	5.75	9.7	182.1	166.7	84.5	71.5
34(α)[b]	—	3.95	10.1	185.6	172.0	88.0	70.3



Compound[a]	δ H-6	δ H-4	$J_{3,4}$	δ C-7	δ C-9	δ C-5	δ C-4
25(α)	7.38	4.88	6.0	181.6	170.5	91.5	80.2
26(α)	8.13–7.34	4.63	6.0	181.2	169.9	92.1	80.6
27α	8.09–7.43	4.88	6.1	181.2	171.2	94.7	80.7
27β	7.49	4.93	6.4	180.8	168.1	95.8	86.2
28α	7.41	4.83	6.0	183.5	170.9	92.8	80.7
28β	7.43	4.92–4.87	—	183.3	168.0	93.8	86.1
29α	7.27	4.82	6.0	183.2	171.0	92.4	80.6
29β	7.32	4.92–4.85	—	183.1	168.2	93.6	86.1
30α	8.09–7.31	4.99	6.1	182.8	170.4	93.0	80.9
30β	7.76	5.00	6.1	182.7	167.5	94.1	86.3
31β	7.34	4.84	5.9	181.6	170.5	89.7	80.0
32β	7.31	4.89	5.9	181.4	170.6	90.2	80.1
35α	—	4.32	4.8	185.0	167.8	95.5	74.6

In MeOH-d_4.
[a] In the second part of the table (furanoid derivatives) the numbering of formulas changes, but homologous nuclei are in the same column as in the first part.
[b] In D_2O.

Thus, the treatment of the pyranoid isothiocyanate **11** with 4-methoxyaniline, in DMF at 85 °C, produced (Table 2, entry 5) the spirothiohydantoin **24**, whose spectroscopic data (Table 3) supported the indicated structure. The coupling constant (5.8 Hz) between C-4 and H-10 confirmed[18] the α configuration not only for **24**, but also for the starting isothiocyanate **11**.

Reactions of a mixture of the anomers **18α** and **18β** with ammonia, methylamine, dodecylamine, and aniline, gave the corresponding thioureido derivative, which spontaneously cyclates, in the reaction medium, and under mild conditions, to afford the corresponding spironucleosides (**27**–**30**) in high yields (Table 2, entries 8–11). In all cases, mixtures of spiroanomers were obtained, which could be resolved. Table 3 shows selected spectroscopic data for these compounds. The most significant differences between the NMR data for the α and the β anomers are the chemical shifts of H-2, C-4, and C-5, which resonated at lower field in the minor β anomers, than in the major α anomers. The difference in the chemical shift of H-2 has been previously reported,[16b] and has been used to define the anomeric configuration of thiohydantocidin.[16c] The δ values for C-2 and C-3 are virtually identical in all the α anomers, and differ by 2 ppm in the β anomers. The coupling constants between H-2 and CH_2OBz are higher in the α anomers than in the β anomers. These data further confirmed the anomeric configurations of **18α** and **18β**.

With the goal of having *O*-unprotected spirothiohydantoins, the deprotections of **22α** and **27α** have been carried out (Scheme 3).

The isopropylidene group of **22α** was removed with DDQ[25] in acetonitrile:water 9:1 at 45 °C, obtaining **33**, which was *O*-debenzoylated by treatment with sodium methoxide in methanol. No measurable anomerization[14a] was observed, and the *O*-unprotected *N*- and spironucleoside **34** was obtained in 85% yield after preparative HPLC.

In the case of **27α** the acetal group was removed (→**35**) in 91% yield, by treatment with TFA:H_2O 2:3 as reported for its C-7 oxo analogue.[16b] The *O*-debenzoylation with sodium methoxide of **35** produced the 5-*epi*thiohydantocidin **36α**, together with its β anomer **36β** (α:β anomeric ratio 6:1, global yield 85%). Related spiroepimerizations have been reported.[7f,26] The spectroscopic data of **36α** and **36β** coincide with those previously reported[16c] for the same compounds prepared through an iminophosphorane intermediate.

3. Conclusion

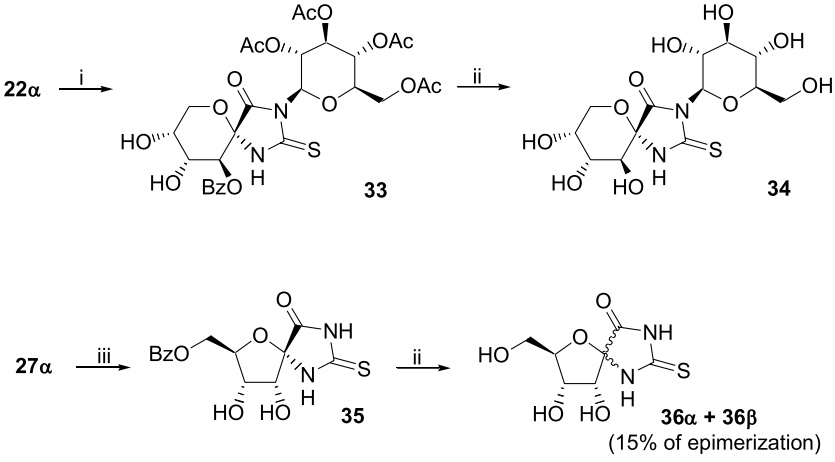
Glycosylaminoesters and 2-deoxy-2-isothiocyanato-hex-2-ulofura(pyra)nosonates —a new class of glycosyl isothiocyanate- are easily and stereoselectively prepared from sugar spiroacetals. Both types of compound can be transformed under mild conditions and in high yields, into glycosylspirothiohydantoins, including 5-*epi*thiohydantocidin. The target compounds are spironucleosides, and in the case of *N*-glycosyl derivatives are simultaneously *N*-nucleosides.

4. Experimental

4.1. General methods

Unless otherwise noted, starting materials were obtained for commercial suppliers and used without purification. All manipulations of air-sensitive compounds were carried out in an inert atmosphere under recirculation of nitrogen or argon. The following reaction solvents were distilled under nitrogen immediately before use: THF and Et_2O from Na/benzophenone; CH_2Cl_2 from CaH_2; toluene from Na; and MeOH from Mg. Et_2O and petroleum ether for column chromatography were also distilled under nitrogen from Na/benzophenone before use. TLC were performed on silica gel HF_{254}, with visualization by UV light or charring with 10% H_2SO_4 (EtOH) or 1% $Ce(SO_4)_2$. $4H_2O$-5% ammonium molybdate-6% H_2SO_4. Silica gel 60 (Merck, 70–230 or 230–400 mesh) was used for preparative chromatography.

A Perkin-Elmer model 141 MC polarimeter, tubes of 1 cm, and solutions in CH_2Cl_2, unless other stated, at 589 nm, were used for measurements of specific rotations. IR were



Scheme 3. Total deprotection of spirohydantoins **22α** and **27α**. Reagents and conditions: (i) DDQ, CH_3CN:H_2O 9:1, 45 °C, 36 h, 83%; (ii) NaOMe 1M, MeOH, 91% (**36α**+**36β**), 85% (**34**); (iii) TFA:H_2O 2:3, rt., 1 h, 91%.

recorded for KBr discs or films on a Bomen Michelson MB 120 FTIR spectrophotometer.

Mass spectra (EI, CI and FAB) were recorded with a Kratos MS-80RFA or a Micromass AutoSpecQ instrument with a resolution of 1000 or 60000 (10% valley resolution). For the FAB spectra ions were produced by a beam of xenon atoms (6–7 keV), using 3-nitrobenzyl alcohol or thioglycerol as matrix and NaI as salt.

A Waters 2690 instrument, with a PDA 996 detector, and a μBondpack C18 column (7,8×300 mm) was used for HPLC.

NMR experiments were recorded on a Bruker AMX 500 (500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C) or on a Bruker AMX300 (300.5 MHz for ^{1}H and 75.50 MHz for ^{13}C). Sample concentrations were typically in the range 10–15 mg per 0.5 mL of $CDCl_3$. Chemical shifts are given in parts per million, and tetramethylsilane was the internal standard. 2D COSY, HMQC, TOCSY, HMBC and 1D NOESY experiments were carried out to assist in NMR signal assignments.

Compounds **2**[11], **12**[11] and **19**[19] were prepared according to the described literature procedures. Compounds **3** and **13** were prepared as we described in a previous work.[7f]

4.2. 2-Azido-3-*O*-benzyl-2-deoxy-4,5-*O*-isopropylidene-β-D-fructopyranose (4) and 2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-β-D-psicofuranose (14)

To a solution of **3** (for **4**) or **13** (for **14**) (100 mg, 0.25 mmol) a catalytic amount of TBAF·$3H_2O$ in THF (3 mL) was added. The mixture was stirred at room temperature for 2 h, evaporated, and purified by column chromatography (Et_2O/petroleum ether 1:5).

Data for **4**. Yield: 0.072 g, 88% (syrup). $[\alpha]_D^{27}$ −158 (*c* 1.0). IR: ν_{max} 3497, 3032, 2986, 2934, 2880, 2118 (N_3), 1458, 1375, 1248, 1221, 1115, 1078 and 1022 cm^{-1}. ^{1}H RMN (500 MHz, CDCl3, δ ppm, *J* Hz): δ 7.38–7.26 (m, 5H, Ar), 4.92 (d, 1H, $^{2}J_{H,H}$=11.7 Hz, CHHPh), 4.68 (d, 1H, CHHPh), 4.38 (dd, 1H, $J_{4,5}$=5.89 Hz, $J_{3,4}$=7.2 Hz, H-4), 4.28 (dt, 1H, $J_{5,6a}$=$J_{5,6b}$=1.9 Hz, H-5), 4.14 (m, 2H, H-6a, H-6b), 3.88 (dd, 1H, $J_{1a,1b}$=11.7 Hz, $J_{1a,OH}$=6.3 Hz, H-1a), 3.81 (dd, 1H, $J_{1b,OH}$=7.9 Hz, H-1b), 3.65 (d, 1H, H-3), 2.12 (dd, OH), 1.50, 1.39 (each s, each 3H, 2CH3). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 128.6–127.4 (Ar), 109.1 (CCH_3), 91.7 (C-2), 76.5 (C-4), 76.2 (C-3), 73.2 (C-5), 73.0 (CH_2Bn), 64.9 (C-1), 61.6 (C-6), 27.8, 26.0 ($2CH_3$). HREIMS *m/z* calcd for C16H21O5N3 ([M]+): 335.1481, found: 335.1483.

Data for **14**. Yield: 0.088 g (92%). The spectroscopic data for **14** were coincident with those reported[6b] for the same compound prepared in a different way.

4.3. 2-Azido-3-*O*-benzyl-1-dehydro-2-deoxy-4,5-*O*-isopropylidene-β-D-fructopyranose (5)

To a cold solution (−70 °C) of oxalyl chloride (0.1 mL, 1.2 mmol) in CH_2Cl_2 (1 mL), under argon, a solution of DMSO (0.17 mL, 2.4 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was stirred for 5 min, and a solution of **4** (100 mg, 0.3 mmol) in CH_2Cl_2 (1.5 mL) was then added dropwise. After 30 min, Et_3N (0.42 mL, 3 mmol) was added. The mixture was stirred at −70 °C for another 5 min, and then raised to room temperature slowly. A solution of saturated $NaHCO_3$ (2 mL) was added and the mixture was extracted with AcOEt (3×4 mL), dried ($MgSO_4$), evaporated, and purified by column chromatography (Et_2O/petroleum ether 1:2). Yield: 0.091 g, 92% (amorphous solid). IR: ν_{max} 2984, 2122 (N3), 1576, 1417, 1117 and 1101 cm^{-1}. ^{1}H NMR (500 MHz, CDCl3, δ ppm, *J* Hz): δ 9.28 (s, 1H, H-1), 7.36–7.30 (m, 5H, Ar), 4.77 (s, 2H, CH2Ph), 4.40 (dd, 1H, $J_{4,5}$=6.5 Hz, H-4), 4.31 (ddd, 1H, H-5), 4.24 (dd, 1H, $J_{5,6a}$=2.5 Hz, $J_{6a,6b}$=13.1 Hz, H-6a), 4.12 (dd, 1H, $J_{5,6}$=1.1 Hz, H-6b), 3.91 (d, 1H, $J_{3,4}$=5.7 Hz, H-3), 1.48, 1.35 (each s, each 3H, 2CH3). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 192.5 (C-1), 137.5–127.7 (Ar), 109.9 (CCH_3), 90.9 (C-2), 74.6 (C-3), 73.9 (C-4, CH_2Ph), 72.4 (C-5), 62.4 (C-6), 27.0, 25.3 ($2CH_3$). HRFABMS *m/z* calcd. for $C_{16}H_{21}N_3O_6Na$ ([M+H_2O+Na]$^+$): 374.1328, found: 374.1331.

^{1}H NMR data for the hydrate of **5**. (500 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 7.35–7.30 (m, 5H, Ar), 5.22 (s, 1H, H-1), 4.93 (d, 1H, $^{2}J_{H,H}$=11.5 Hz, C*H*HPh), 4.67 (d, 1H, CH*H*Ph), 4.40 (dd, 1H, $J_{4,5}$=5.8 Hz, H-4), 4.30 (m, 1H, H-5), 4.20 (dd, 1H, $J_{5,6a}$=1.5 Hz, $J_{6a,6b}$=13.4 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$=3.0 Hz, H-6b), 3.92 (d, 1H, $J_{3,4}$=7.3 Hz, H-3), 1.52, 1.38 ($2CH_3$).

4.4. Methyl (3-*O*-benzyl-2-deoxy-4,5-*O*-isopropylidene-β-D-*arabino*-hex-2-ulopyranosyl)onate azide (7)

To a solution of **5** (100 mg, 0.3 mmol) and 2-methylbut-2-ene (0.32 mL, 3 mmol) in 2-methylpropan-2-ol (2 mL) at 0 °C, another solution of $NaClO_2$ (81 mg, 0.9 mmol) and $NaH_2PO_4.2H_2O$ (140 mg, 0.9 mmol) in H_2O (1 mL) was added. The mixture was stirred at room temperature for 2 h, evaporated to half, extracted with Et_2O (3×8 mL), washed with HCl (2%, 20 mL) and then with brine, dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in Et_2O:MeOH (5 mL) and stirred at 0 °C with a solution of CH_2N_2 in Et_2O (5 mL) for 20 min, then evaporated and purified by column chromatography (Et_2O/petroleum ether 2:1). Yield: 0.089 g, 82% (amorphous solid). $[\alpha]_D^{24}$ −106 (*c* 2.0). IR: ν_{max}. 2988, 2953, 2126(N_3), 1755, 1381, 1244, 1121 and 1074 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 7.33–7.27 (m, 5H, Ar), 4.84 (d, 1H, $^{2}J_{H,H}$=11.8 Hz, C*H*HPh), 4.69 (d, 1H, CH*H*Ph), 4.37 (dd, 1H, H-4), 4.30 (ddd, 1H, $J_{4,5}$=6.0 Hz, $J_{5,6a}$=2.5 Hz, $J_{5,6b}$=1.8 Hz, H-5), 4.16 (m, 2H, H-6a, H-6b), 4.01 (d, 1H, $J_{3,4}$=6.8 Hz, H-3), 3.75 (s, 3H, OCH_3), 1.52, 1.37 (each s, each 3H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 166.7 (C-1), 137.3–127.7 (Ar), 109.4 (CCH_3), 91.1 (C-2), 76.7 (C-3), 75.8 (C-4), 73.5 (CH_2Ph), 72.6 (C-5), 63.0 (C-6), 53.2 (OCH_3), 27.5, 25.8 ($2CH_3$). HRFABMS *m/z* calcd for $C_{17}H_{21}O_6N_3Na$ ([M+Na]$^+$): 386.1, found 386.1331. Anal. Calcd for $C_{17}H_{21}O_6N_3$: C, 56.19; H, 5.83; N, 11.56. Found: C, 56.29; H, 5.88; N, 11.65.

4.5. General procedure for the oxidation of 4 and 14 with RuO_4. Preparation of compounds 8 and 16

To a stirred solution of **4** (for **8**) or **14** (for **16**) (335 mg, 1.0 mmol), CH_3CN (5.4 mL), CCl_4 (5.4 mL), H_2O (8 mL) and $NaIO_4$ (1.12 g, 5.2 mmol) $RuCl_3$. H_2O (0.12 g, 0.52 mmol) was added. The mixture was stirred vigorously for 15 min at room temperature, diluted with buffer AcOH/AcO^- (1 M; pH=4), filtered over Celite, extracted with CH_2Cl_2 (20 mL), dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in Et_2O:MeOH 1:1 (15 mL) and stirred at 0 °C with a solution of CH_2N_2 in Et_2O for 20 min, evaporated, and purified by column chromatography (Et_2O/petroleum ether 1:6).

4.5.1. Methyl (3-*O*-benzoyl-2-deoxy-4,5-*O*-isopropylidene-β-D-*arabino*-hex-2-ulopyranosyl)onate azide (8). Yield: 0.170 g, 45% (syrup). $[\alpha]_D^{25}$ −95 (*c* 0.9). IR: ν_{max} 2988, 2948, 2128(N_3), 1755, 1732, 1385, 1223, 1076 and 1101 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.06–7.42 (m, 5H, Ar), 5.69 (d, 1H, $J_{3,4}$=7.3 Hz, H-3), 4.44 (dd, 1H, $J_{4,5}$=5.6 Hz, H-4), 4.35 (m, 2H, H-5, H-6a), 4.24 (dd, 1H, $J_{5,6b}$=2.8 Hz, $J_{6a,6b}$=13.5 Hz, H-6b), 3.79 (s, 3H, OCH_3), 1.61, 1.39 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 165.8, 164.9 (C-1, C=O), 133.5–127.8 (Ar), 110.2 (*C*CH_3), 90.3 (C-2), 73.5 (C-4), 72.7 (C-5), 70.8 (C-3), 62.9 (C-6), 53.6 (OCH_3), 27.3, 26.0 ($2CH_3$). HRCIMS *m/z* calcd for $C_{17}H_{20}O_7N_3$ ($[M+H]^+$): 378.1301, found: 378.1304.

4.5.2. Methyl (6-*O*-benzoyl-2-deoxy-3,4-*O*-isopropylidene-β-D-*ribo*-hex-2-ulofuranosyl)onate azide (16). Yield: 0.170 g, 45% (syrup). $[\alpha]_D^{25}$ −104 (*c* 0.85). IR: ν_{max} 2990, 2116(N_3), 1763, 1724, 1453, 1375, 1273, 1107 and 1070 cm^{-1}. ^{1}H NMR: (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.09–7.43 (m, 5H, Ar), 4.89 (dd, 1H, $J_{4,5}$=1.5 Hz, H-4), 4.82 (dt, 1H, H-5), 4.70 (d, 1H, $J_{3,4}$=5.8 Hz, H-3), 4.55 (dd, 1H, $J_{5,6a}$=6.0 Hz, $J_{6a,6b}$=11.8 Hz, H-6a), 4.48 (dd, 1H, $J_{5,6b}$=6.1 Hz, H-6b), 3.89 (s, 3H, OCH_3), 1.48, 1.32 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 166.0, 165.4 (C-1, C=O), 133.2–128.1 (Ar), 114.3 (*C*CH_3), 100.5 (C-2), 86.9 (C-3), 85.7 (C-5), 81.9 (C-4), 63.9 (C-6), 53.1 (OCH_3), 26.0, 25.1 ($2CH_3$). HRFABMS *m/z* calcd for $C_{17}H_{19}O_7N_3Na$ ($[M+Na]^+$): 400.1121, found: 400.1124. Anal. Calcd for $C_{17}H_{19}O_7N_3$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.32; H, 5.09; N, 11.14.

4.6. General procedure for the reduction of azides 7, 8 and 16. Preparation of amines 9, 10 and 17

A solution of the corresponding azide **7** (for **9**), **8** (for **10**) or **16** (for **17**) (*x* mg, 0.3 mmol) in MeOH (10 mL) was stirred at room temperature in the presence of Pd-C 10% (20 mg) and hydrogen (balloon pressure) for 1 h. The mixture was filtered over Celite, evaporated, and purified by column chromatography.

4.6.1. Methyl (3-*O*-benzyl-2-deoxy-4,5-*O*-isopropylidene-α,β-D-*arabino*-hex-2-ulopyranosyl)onate amine (9). *x*=0.109 g. Relationship of diastereoisomers in C-2 ratio (α:β) 1:9. Column chromatography: Et_2O/petroleum ether 1:6. Yield: 0.10 g, 99% (syrup). IR: ν_{max}. 3395, 3335, 2984, 1743, 1433, 1381, 1249, 1167 and 1068 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm *J* Hz): δ 7.33–7.27 (m, 5H, Ar), 4.85 (d, 1H, $^2J_{H,H}$=11.7 Hz, C*H*HPh), 4.65 (d, 1H, CH*H*Ph), 4.39 (dd, 1H, $J_{5,6a}$=2.9 Hz, $J_{6a,6b}$=13.1 Hz, H-6a), 4.30 (t, 1H, H-4), 4.23 (dd, 1H, $J_{4,5}$=6.4 Hz, H-5), 3.98 (d, 1H, $J_{3,4}$=7.1 Hz, H-3), 3.97 (d, 1H, H-6b), 3.70 (s, 3H, OCH_3), 2.17 (bs, 2H, NH_2), 1.54, 1.38 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 171.4 (C-1), 137.8–127.7 (Ar), 108.9 (*C*CH_3), 86.1 (C-2), 77.2 (C-3), 77.0 (C-4), 73.4 (C-5), 72.9 (CH_2Ph), 59.8 (C-6), 52.8 (OCH_3), 27.9, 26.2 ($2CH_3$). HRFABMS *m/z* calcd for $C_{17}H_{23}O_6NNa$ ($[M+Na]^+$): 360.1423, found: 360.1423.

4.6.2. Methyl (3-*O*-benzoyl-2-deoxy-4,5-*O*-isopropylidene-α,β-D-*arabino*-hex-2-ulopyranosyl)onate amine (10). *x*=0.113 g. Diastereoisomers in C-2 ratio 5:2 (configuration not determinated). Column chromatography: Et_2O/petroleum ether 2:3. Yield: 0.074 g, 70% (syrup). HRCIMS: *m/z* calcd for ($[M+H]^+$): 352.1396, found: 352.1399. IR: $\nu_{max.}$ 3412, 3309, 2986, 1743, 1734, 1437, 1249, 1119 and 1099 cm^{-1}.

NMR data for the major anomer. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.03–7.41 (m, 5H, Ar), 5.66 (d, 1H, $J_{3,4}$=7.7 Hz, H-3), 4.42 (dd, 1H, $J_{5,6a}$=2.9 Hz, H-6a), 4.39 (dd, 1H, $J_{4,5}$=5.5 Hz, H-4), 4.30 (dd, 1H, H-5), 4.13 (d, 1H, $J_{6a,6b}$=13.2 Hz, H-6b), 3.74 (s, 3H, OCH_3), 2.11 (bs, 2H, NH_2), 1.65, 1.37 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (170.9 (C-1), 164.7 (COPh), 133.3–128.3 (Ar), 109.6 (*C*CH_3), 85.4 (C-2), 74.3 (C-4), 73.3 (C-5), 72.2 (C-3), 59.9 (C-6), 53.26 (OCH_3), 27.6, 26.2 ($2CH_3$).

NMR data for the minor anomer. ^{1}H NMR (500 MHz, $CDCl_3$, (ppm, *J* Hz): (8.03–7.40 (m, 5H, Ar), 5.37 (d, 1H, $J_{3,4}$=7.1 Hz, H-3), 4.84 (t, 1H, $J_{4,5}$=6.1 Hz, H-4), 4.33 (dd, 1H, H-5), 4.20 (dd, 1H, $J_{5,6a}$=2.0 Hz, $J_{6a,6b}$=13.7 Hz, H-6a), 3.88 (dd, 1H, $J_{5,6b}$=3.1 Hz, H-6b), 3.78 (s, 3H, OCH_3), 2.38 Method, 2H, NH_2), 1.62, 1.57 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (167.6 (C-1), 165.2 (COPh), 133.3–128.3 (Ar), 109.3 (*C*CH_3), 87.6 (C-2), 74.7 (C-4), 73.5 (C-3), 72.8 (C-5), 63.1 (C-6), 52.4 (OCH_3), 27.7, 26.0 ($2CH_3$).

4.6.3. Methyl (6-*O*-benzoyl-2-deoxy-3,4-*O*-isopropylidene-α,β-D-*ribo*-hex-2-ulofuranosyl)onate amine (17). *x*=0.113 g. Diastereoisomers in C-2 ratio (α:β) 6:1. Column chromatography: Et_2O/petroleum ether 1:2. Yield: 0.10 g, 95% (syrup). IR: ν_{max} 3422, 3343, 2988, 1734, 1720, 1655, 1383, 1273 and 1097 cm^{-1}. HRCIMS *m/z* calcd for $C_{17}H_{22}O_7N_1$($[M+H]^+$): 352.1396, found: 352.1398. Anal. Calcd for $C_{17}H_{21}O_7N$: C, 58.11; H, 6.02; N, 3.99, found C, 57.94; H, 6.06; N, 3.82.

NMR data for the α anomer. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.05–7.46 (m, 5H, Ar), 5.02 (d, 1H, $J_{3,4}$= 6.1 Hz, H-3), 4.85 (dd, 1H, $J_{4,5}$=3.4 Hz, H-4), 4.45 (td, 1H, $J_{5,6a}$=$J_{5,6b}$=5.7 Hz, H-5), 4.34 (m, 2H, H-6a, H-6b), 3.73 (s, 3H, OCH_3), 2.56 (bs, 2H, NH_2), 1.59, 1.38 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 169.9 (C-1), 166.1 (COPh), 133.1–128.3 (Ar), 113.8 (*C*CH_3), 93.6 (C-2), 82.4 (C-4), 81.8 (C-5), 81.7 (C-3), 64.2 (C-6), 52.7 (OCH_3), 26.8, 25.2 ($2CH_3$).

NMR data for the β anomer. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.05–7.42 (m, 5H, Ar), 4.89 (dd, 1H, $J_{4,5}$= 1.9 Hz, H-4), 4.71 (td, 1H, $J_{5,6a}=J_{5,6b}=6.4$ Hz, H-5), 4.61 (d, 1H, $J_{3,4}=6.1$ Hz, H-3), 4.56 (ddd, 2H, $J_{6a,6b}=11.4$ Hz, H-6a, H-6b), 3.83 (s, 3H, OCH_3), 1.48, 1.32 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 170.9 (C-1), 166.1 (COPh), 133.0–128.0 (Ar), 113.9 (CCH_3), 96.2 (C-2), 88.4 (C-3), 84.7 (C-5), 82.7 (C-4), 65.3 (C-6), 52.6 (OCH_3), 26.2, 25.1 ($2CH_3$).

4.7. General procedure for the preparation of the isothiocyanatoulosonates 11, 18α, and 18β

To a mixture of a solution of the glycosylaminoester **10** (for **11**) or **17** (for **18α** and **18β**) (150 mg, 0.43 mmol) in CH_2Cl_2 (3 mL), and $CaCO_3$ (300 mg, 3.0 mmol) in H_2O (0.75 mL), was added $CSCl_2$ (120 μl, 1.5 mmol). The mixture was stirred vigorously at room temperature for 3 days, diluted with CH_2Cl_2 (30 mL), washed with water and then brine, dried ($MgSO_4$), and evaporated to dryness. The residue was purified by column chromatography.

4.7.1. Methyl (2-deoxy-3-*O*-benzoyl-4,5-*O*-isopropylidene-α-D-*arabino*-hex-2-ulopyranosyl)onate isothiocyanate (11). The residue was a 16:1 mixture of anomers. Column chromatography: Et_2O/petroleum ether 1:6.Yield: 0.135 g, 80% (syrup). $[\alpha]_D^{26}$ +79 (*c* 1.0). IR: $\nu_{max.}$ 2986, 2002 (NCS), 1740, 1736, 1314, 1261, 1103 and 1070 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 7.98–7.43 (m, 5H, Ar), 5.70 (d, 1H, $J_{3,4}=4.4$ Hz, H-3), 4.50 (dd, 1H, $J_{4,5}=6.1$ Hz, H-4), 4.43 (dd, 1H, H-5), 4.15 (d, 1H, $J_{5,6a}$= 5.4 Hz, H-6a), 4.04 (dd, 1H, $J_{6a,6b}=12.6$ Hz, $J_{5,6b}=6.9$ Hz, H-6b), 377 (s, 3H, OCH_3), 1.61, 1.37 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (165.0 (C-1), 164 (C=O), 145.1 (NCS), 133.8–127.6 (Ar), 110.7 (CCH_3), 87.7 (C-2), 72.9 (C-4), 71.3 (C-3), 69.3 (C-5), 63.6 (C-6), 53.6 (OCH_3), 27.3, 25.6 ($2CH_3$). HRFABM *m/z* calcd for ($[M+Na]^+$): 416.0780, found: 416.0772. Anal. Calcd for $C_{18}H_{19}O_7NS$: C, 54.95; H, 4.87; N, 3.56. Found: C, 54.91; H, 4.89; N, 3.59.

4.7.2. Methyl (2-deoxy-6-*O*-benzoyl-3,4-*O*-isopropylidene-α-D-*ribo*-hex-2-ulofuranosyl)onate isothiocyanate (18α). Column chromatography: Et_2O/petroleum ether 1:9. Yield: 0.110 g, 65% (syrup). $[\alpha]_D^{28}$ −62 (*c* 0.86). IR: ν_{max}. 2986, 2018 (NCS), 1757, 1724, 1601, 1271 and 1099 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.05–7.45 (m, 5H, Ar), 5.07 (d, 1H, $J_{3,4}=6.8$ Hz, H-3), 4.84 (dd, 1H, $J_{4,5}=3.1$ Hz, H-4), 4.68 (dd, 1H, H-5), 4.58 (dd, 1H, $J_{5,6a}=3.8$ Hz, $J_{6a,6b}=12.2$ Hz, H-6a), 4.49 (dd, 1H, $J_{5,6b}=4.2$ Hz, H-6b), 3.80 (s, 3H, OCH_3), 1.71, 1.40 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 166.0 (C-1), 165.9 (C=O), 144.9 (NCS), 133.5–128.1 (Ar), 117.3 (CCH_3), 95.1 (C-2), 84.4 (C-3), 82.6 (C-5), 80.9 (C-4), 63.5 (C-6), 53.8 (OCH_3), 26.1, 25.3 ($2CH_3$). HRCIMS *m/z* calcd for $C_{18}H_{20}O_7NS$ ($[M+H]^+$): 394.0961, found: 394.0959. Anal. Calcd for $C_{18}H_{19}O_7NS$: C, 54.95; H, 4.87; N, 3.56. Found: C, 55.10; H, 4.76; N, 3.60.

4.7.3. Methyl (2-deoxy-6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-*ribo*-hex-2-ulofuranosyl)onate isothiocyanate (18β). Column chromatography: Et_2O/Hex 1:9. Yield: 0.034 g, 20%. $[\alpha]_D^{25}$ −115 (*c* 0.5). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.09–7.45 (m, 5H, Ar), 4.98 (d, 1H, $J_{3,4}=5.8$ Hz, H-3), 4.94 (dd, 1H, $J_{4,5}=1.3$ Hz, H-4), 4.81 (td, 1H, H-5), 4.55 (dd, 1H, $J_{5,6a}=6.1$ Hz, H-6a), 4.50 (dd, 1H, $J_{5,6b}=5.6$ Hz, $J_{6a,6b}=12.0$ Hz, H-6b), 3.92 (s, 3H, OCH_3), 1.47, 1.33 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 165.9 (C=O), 164.9 (C-1), 145.1 (NCS), 133.2–128.4 (Ar), 114.6 (CCH_3), 97.7 (C-2), 88.8 (C-3), 85.8 (C-5), 81.8 (C-4), 63.5 (C-6), 53.4 (OCH_3), 25.8, 25.0 ($2CH_3$). HRCIMS *m/z* calcd for $C_{18}H_{20}O_7NS$ ($[M+H]^+$): 394.0961, found: 394.0971.

4.8. General procedures for the reactions of the glycosylaminoesters 9, 10, 17, and 19 (α+β) with alkyl or aryl isothiocyanates (Route A). Preparation of the hydantocidin-related spironucleosides 20α, 20β, 21, 22α, 22β, 23, 25, 26, 31, and 32[†]

Method I (used for the preparation of compounds **20α** and **20β**). A solution of **9** (100 mg, 0.3 mmol) in DMF was stirred with phenylisothiocyanate (53 μL, 0.45 mmol) at 85 °C for 23 h. The solvent was evaporated and the residue was purified by column chromathography.

Method II (used for the preparation of compounds **21**, **22α**, **22β**, **23**, **25**, **26**, **31**, and **32**). A solution of the aminoester **10** (for **21**, **22α**, **22β** and **23**), **17** (for **25** and **26**), or **19**(α+β) (for **31** and **32**) (0.3 mmol) in THF (2 mL) was stirred at 40 °C with phenylisothiocyanate (for **21**), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylisothiocyanate (for **22α**, **22β**, **25**, and **31**) or 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosylisothiocyanate (for **23**, **26**, and **32**) (0.33 mmol). After *t* days, the solvent was evaporated and the residue was purified by column chromatography.

4.8.1. (5*R*,8*R*,9*R*,10*S*)-10-Benzyloxy-8,9-dimethylmethylenedioxy-4-oxo-3-phenyl-2-thioxo-6-oxa-1,3-diazaspiro-[4.5]decane (20α). *Method I*. Column chromatography: Et_2O/petroleum ether 1:4. Yield: 0.086 g, 65% (amorphous solid). $[\alpha]_D^{22}$ −84 (*c* 0.5). IR: ν_{max} 2986, 2916, 1765, 1726, 1402, 1159 and 1030 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, α ppm, *J* Hz): (7.47–7.22 (m, 10H, Ar), 7.06 (bs, 1H, NH), 4.91 (d, 1H, $^2J_{H,H}=11.8$ Hz, C*H*HPh), 4.73 (d, 1H, CH*H*Ph), 4.72 (t, 1H, H-9), 4.59 (dd, 1H, $J_{7a,8}=3.2$ Hz, $J_{7a,7b}=13.5$ Hz, H-7a), 4.39 (ddd, 1H, $J_{8,9}=5.6$ Hz, H-8), 4.16 (dd, 1H, $J_{7b,8}=2.2$ Hz, H-7b), 3.70 (d, 1H, $J_{9,10}$= 7.1 Hz, H-10), 1.54, 1.41 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (183.6 (C-2), 169.4 (C-4), 136.9–128.2 (Ar), 109.7 (CCH_3), 86.6 (C-5), 77.7 (C-10), 76.8 (C-9), 73.3 (CH_2Ph), 72.9 (C-8), 63.0 (C-7), 27.9, 25.9 ($2CH_3$). HRFABMS *m/z* calcd for $C_{23}H_{24}O_5N_2SNa$ ($[M+Na]^+$): 463.1304, found: 463.1293.

4.8.2. (5*S*,8*R*,9*R*,10*S*)-10-Benzyloxy-8,9-dimethylmethylenedioxy-4-oxo-3-phenyl-2-thioxo-6-oxa-1,3-diazaspiro-[4.5]decane (20β). *Method I*. Column chromatography: Et_2O/petroleum ether 1:4. Yield: 0.041 g, 31% (amorphous

[†] For nomenclature of the spironucleosides we have followed the IUPAC rules for spiro compounds (see rule B-10.1 in IUPAC Nomenclature of organic Chemistry, Edition of 1979, Pergamon Press). Alternatively, the second part of the rule 2-Carb-35.3 of Nomenclature of Carbohydrates, recomendations 1996 (*Carbohydr. Res. 1997*, **297**,1) would be used.

solid). $[\alpha]_D^{26}$ −110 (*c* 1.1). IR: $\nu_{max.}$ 2986, 1768, 1591, 1406, 1107 and 1022 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 9.09 (bs, 1H, NH), 7.47–7.15 (m, 10H, Ar), 4.93 (d, 1H, C*H*HPh), 4.60 (d, 1H, $^2J_{H,H}$=10.0 Hz, CH*H*Ph), 4.43 (dd, 1H, $J_{9,10}$=7.9 Hz, $J_{8,9}$=5.5 Hz, H-9), 4.30 (dd, 1H, $J_{7a,8}$=1.6 Hz, H-7a), 4.28 (m, 1H, H-8), 4.13 (dd, 1H, $J_{7a,7b}$=10.9 Hz, $J_{7b,8}$=3.2 Hz, H-7b), 4.10 (d, 1H, $J_{9,10}$=7.9 Hz, H-10), 1.54, 1.38 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (184.9 (C-2), 169.5 (C-4), 137.1–127.6 (Ar), 109.9 (CCH_3), 87.1 (C-5), 77.0 (C-10), 76.0 (C-9), 73.5 (CH_2Ph), 72.5 (C-8), 63.2 (C-7), 27.8, 25.9 ($2CH_3$). HRFABMS *m/z* calcd for $C_{23}H_{24}O_5N_2SNa$ ([M+Na]$^+$): 463.1304, found 463.1292.

4.8.3. (5*R*,8*R*,9*R*,10*S*)-10-Benzoyloxy-8,9-dimethylmethylenedioxy-4-oxo-3-phenyl-2-thioxo-6-oxa-1,3-diazaspiro[4.5]decane (21). *Method II*. *t*=3 days. Column chromatography: Et_2O/Hex 1:5. Yield: 0.095 g, 70% (amorphous solid). $[\alpha]_D^{20}$ −77 (*c* 1.1). IR: $\nu_{max.}$ 3291, 2986, 1755, 1732, 1593, 1491, 1252 and 1103 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.04–7.18 (m, 11H, Ar, NH), 5.67 (d, 1H, $J_{9,10}$=7.4 Hz, H-10), 4.88 (dd, 1H, $J_{8,9}$=5.9 Hz, H-9), 4.65 (dd, 1H, $J_{7a,8}$=2.9 Hz, H-7a), 4.50 (m, 1H, H-8), 4.34 (d, 1H, $J_{7a,7b}$=13.6 Hz, H-7b), 1.67, 142 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, CDCl3, δ ppm): δ 182.8 (C-2), 168.7 (C-4), 165.5 (C=O), 133.8–128.1 (Ar), 110.3 (CCH_3), 85.6 (C-5), 73.7 (C-9), 72.9 (C-8), 71.2 (C-10), 63.4 (C-7), 27.6, 25.9 ($2CH_3$). HRCIMS *m/z* calcd for $C_{23}H_{22}O_6N_2S$ ([M+H]$^+$): 455.1277, found: 455.1264.

4.8.4. (5*R*,8*R*,9*R*,10*S*)-10-Benzoyloxy-8,9-dimethylmethylenedioxy-4-oxo-3-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thioxo-6-oxa-1,3-diazaspiro[4.5]decane (22α). *Method II*. *t*=4 days. Column chromatography: Et_2O/petroleum ether 1:1. Yield: 0.161 g, 76% (amorphous solid). $[\alpha]_D^{24}$ −46 (*c* 0.9). IR: ν_{max} 2986, 2942, 1957, 1953, 1499, 1375, 1223 and 1099 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 7.97–7.37 (m, 5H, Ar), 7.65 (bs, 1H, NH), 5.97 (t, 1H, $J_{2',3'}$=9.4 Hz, H-2′), 5.77 (d, 1H, $J_{1',2'}$=9.6 Hz, H-1′), 5.52 (d, 1H, $J_{9,10}$=7.8 Hz, H-10), 5.33 (t, 1H, $J_{3',4'}$=9.3 Hz, H-3′), 5.24 (t, 1H, $J_{4',5'}$=9.6 Hz, H-4′), 4.88 (dd, 1H, $J_{8,9}$=5.5 Hz, H-9), 4.56 (dd, 1H, $J_{7a,8}$=2.9 Hz, H-7a), 4.42 (m, 1H, H-8), 4.31 (d, 1H, $J_{7a,7b}$=13.7 Hz, H-7b), 4.20 (m, 2H, H-6′a, H-6′b), 3.76 (dt, 1H, $J_{5',6'a}$=$J_{5',6'b}$=3.3 Hz, H-5′), 2.04, 2.03, 2.02, 1.97, 1.61, 1.38 (each s, each 3H, $6CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 182.1 (C-2), 170.5, 169.9, 169.4, 169.3, 165.6 (5C=O), 167.1 (C-4), 133.7–128.4 (Ar), 110.1 (*C*CH_3), 84.4 (C-5), 81.0 (C-1′), 74.4 (C-5′), 74.1 (C-9), 73.2 (C8, C3′), 71.1 (C-10), 68.0 (C-4′), 67.1 (C-2′), 63.0 (C-7), 61.7 (C-6′), 27.8, 26.0, 20.5, 20.4, 20.3 ($6CH_3$). HRCIMS *m/z* calcd for $C_{31}H_{37}O_{15}N_2S$ ([M+H]$^+$): 709.1915, found: 709.1916. Anal. Calcd for $C_{31}H_{36}O_{15}N_2S$: C, 52.54; H, 5.12; N, 3.95. Found: C, 52.62; H, 5.23; N, 4.00.

4.8.5. (5*S*,8*R*,9*R*,10*S*)-10-Benzoyloxy-8,9-dimethylmethylenedioxy-4-oxo-3-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thioxo-6-oxa-1,3-diazaspiro[4.5]decane (22β). *Method II*. *t*=4 days. Column chromatography: Et_2O/petroleum ether 1:1. Yield: 0.019 g, 9% (amorphous solid). $[\alpha]_D^{22}$ −83 (*c* 0.6). IR: ν_{max} 2986, 2942, 1957, 1953, 1499, 1375, 1223 and 1099 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.24 (bs, 1H, NH), 8.05–7.40 (m, 5H, Ar), 5.94 (t, 1H, H-2′), 5.80 (d, 1H, $J_{2',3'}$=9.3 Hz, H-1′), 5.79 (d, 1H, $J_{9,10}$=6.9 Hz, H-10), 5.23 (t, 1H, $J_{2',3'}$=9.3 Hz, H-3′), 5.16 (t, 1H, $J_{3',4'}$=9.8 Hz, H-4′), 4.39 (m, 2H, H-8, H-9), 4.33 (dd, 1H, $J_{7a,8}$=2.1 Hz, $J_{7a,7b}$=13.8 Hz, H-7a), 4.21 (d, 1H, $J_{6'a,6'b}$=12.3 Hz, H-6′a), 4.16 (dd, 1H, $J_{5',6'b}$=3.0 Hz, H-6′b), 4.07 (dd, 1H, $J_{7b,8}$=3.2 Hz, H-7b), 3.77 (m, 1H, H-5′), 2.08, 2.01, 1.95, 1.67, 1.38 and 1.27 (each s, each 3H, $6CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 183.5 (C-2), 170.8, 170.1, 169.2, 168.9, 166.7, 164.0 (6C=O), 133.4–128.4 (Ar), 111.1 (CCH_3), 84.5 (C-5), 81.2 (C-1′), 74.4*, 74.2* (C-9, C5′), 73.4 (C-3′), 71.9 (C-8), 68.4 (C-10), 67.7 (C-4′), 67.4 (C-2′), 62.9 (C-7), 61.5 (C-6′), 27.2, 25.9, 20.7, 20.5 (2C), 19.2 ($6CH_3$). HRCIMS *m/z* calcd for $C_{31}H_{37}O_{15}N_2S$ ([M+H]$^+$): 709.1915, found: 709.1910.

4.8.6. (5*R*,8*R*,9*R*,10*S*)-10-Benzoyloxy-8,9-dimethylmethylenedioxy-4-oxo-2-thioxo-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-oxa-1,3-diazaspiro[4.5]decane (23). *Method II*. *t*=5 days. Column chromatography: Et_2O/petroleum ether 1:3. Yield: 0.20 g, 81% (amorphous solid). $[\alpha]_D^{27}$ −31 (*c* 0.9). IR: ν_{max} 2991, 1730, 1599, 1489, 1379, 1265, 1105 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.13–7.26 (m, 21H, Ar, NH), 6.41 (d, 1H, $J_{1',2'}$=2.4 Hz, H-1′), 6.26 (dd, 1H, $J_{2',3'}$=6.3 Hz, H-2′), 6.22 (t, 1H, $J_{3',4'}$=6.6 Hz, H-3′), 5.52 (d, 1H, $J_{9,10}$=7.8 Hz, H-10), 4.88 (dd, 1H, $J_{4',5'a}$=3.0 Hz, $J_{5'a,5'b}$=11.8 Hz, H-5′a), 4.64 (dd, 1H, H-4′), 4.63 (dd, 1H, $J_{7b,8}$=2.7 Hz, H-7b), 4.61 (dd, 1H, $J_{4',5'b}$=3.8 Hz, H-5′b), 4.60 (dd, 1H, $J_{8,9}$=5.8 Hz, H-9), 4.38 (m, 1H, H-8), 4.31 (d, 1H, $J_{7a,7b}$=13.6 Hz, H-7a), 1.62, 1.40 (each s, each 3H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 181.2 (C-2), 168.2, 166.1, 165.6, 165.1, 165.0 (5C=O), 133.6–128.0 (Ar), 110.1 (*C*CH_3), 86.2 (C-1′), 84.8 (C-5), 78.9 (C-4′), 73.7 (C-9), 72.9 (C-8), 72.5 (C-2′), 71.2 (C-10), 70.3 (C-3′), 63.2 (C-7), 62.6 (C-5′), 27.6, 25.9 ($2CH_3$). Anal. Calcd for $C_{43}H_{38}N_2O_{13}S$: C, 62.77; H, 4.65; N, 3.40. Found: C, 62.74; H, 4.82; N, 3.21.

4.8.7. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-9-oxo-8-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (25). *Method II*. *t*=5 days. Column chromatography: Et_2O/petroleum ether 2:3. Yield: 0.168 g, 79% (amorphous solid). $[\alpha]_D^{25}$ −36 (*c* 1.2). IR: ν_{max} 2988, 2936, 1755, 1491, 1273, 1215 and 1097 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.09–7.42 (m, 5H, Ar), 7,38 (bs, 1H, NH), 5.92 (t, 1H, $J_{2',3'}$=9.5 Hz, H-2′), 5.82 (d, 1H, $J_{1',2'}$=9.5 Hz, H-1′), 5.30 (t, 1H, H-3′), 5.19 (t, 1H, $J_{3',4'}$=9.8 Hz, H-4′), 4.94 (dd, 1H, $J_{2,3}$=1.6 Hz, H-3), 4.88 (d, 1H, $J_{3,4}$=6.0 Hz, H-4), 4.56 (m, 2H, H-2, C*H*HOBz), 4.48 (dd, 1H, $^2J_{H,H}$=13.3 Hz, $J_{2,H}$=8.6 Hz, CH*H*OBz), 4.19 (m, 2H, H-6′a, H-6′b), 3.83 (ddd, 1H, $J_{5',6'a}$=2.8 Hz, $J_{5',6'b}$=4.4 Hz, H-5′), 2.09, 2.04, 2.01, 1.90, 1.60, 1.40 (each s, each 3H, $6CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 181.6 (C-7), 170.5, 170.0, 169.2, 169.1, 168.7, 165.9 (6C=O), 133.1–128.3 (Ar), 114.5 (*C*CH_3), 91.5 (C-5), 82.8 (C-2), 82.6 (C-3), 80.9 (C-1′), 80.2 (C-4), 74.6 (C-5′), 73.2 (C-3′), 67.7 (C-4′), 66.9 (C-2′), 64.1 (CH_2OBz), 61.5 (C-6′), 26.5, 24.7, 20.6, 20.4 (for 2C), 20.1

($6CH_3$). Anal. Calcd for $C_{31}H_{36}O_{15}N_2S$ C, 52.54; H, 5.12; N, 3.95. Found: C, 52.38; H, 5.44; N, 3.99.

4.8.8. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-9-oxo-7-thioxo-8-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1-oxa-6,8-diazaspiro[4.4]nonane (26). *Method II*. $t=5$ days. Column chromatography: Et_2O/petroleum ether 1:3. Yield: 0.205 g, 83% (amorphous solid). $[\alpha]_D^{27}$ −47 (*c* 1.0). IR: ν_{max} 2996, 1773, 1724, 1601, 1269 and 1099 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, *(* ppm, *J* Hz): 8.13–7.34 (m, 21H, Ar, NH), 6.48 (d, 1H, $J_{1',2'}=3.0$ Hz, H-1′), 6.28 (dd, 1H, $J_{2',3'}=6.2$ Hz, H-2′), 6.20 (t, 1H, $J_{3',4'}=6.5$ Hz, H-3′), 4.89 (dd, 1H, $J_{4',5'a}=3.6$ Hz, $J_{5'a,5'b}=12.2$ Hz, H-5′a), 4.75 (dd, 1H, $J_{2,3}=2.4$ Hz, H-3), 4.65 (ddd, 1H, $J_{4',5'b}=4.7$ Hz, H-4′), 4.63 (d, 1H, $J_{3,4}=6.0$ Hz, H-4), 4.57 (td, 1H, $J_{2,CH2}=6.0$ Hz, H-2), 4.53 (dd, 1H, H-5′b), 4.53 (m, 2H, CH_2OBz), 1.58, 1.33 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (181.2 (C-7), 169.9 (C-9), 166.1, 166.0, 165.0 (4C=O), 133.5–128.3 (Ar), 114.6 (CCH_3), 92.1 (C-5), 86.4 (C-1′), 83.1 (C-2), 82.2 (C-3), 80.6 (C-4), 79.6 (C-4′), 72.6 (C-2′), 70.6 (C-3′), 64.1 (CH_2OBz), 62.9 (C-5′), 26.6, 24.8 ($2CH_3$). HRCIMS *m/z* calcd for $C_{43}H_{39}O_{13}N_2S$ ($[M+H]^+$): 823.217, found: 823.2173. Anal. Calcd for $C_{43}H_{38}O_{13}N_2S$: C, 62.77; H, 4.65; N, 3.40. Found: C, 62.52; H, 4.64; N, 3.94.

4.8.9. (2*R*,3*S*,4*S*,5*S*,4″*R*)-2-(2″,2″-Dimethyl-1″,3″-dioxolan-4″-yl)-3,4-dimethylmethylenedioxy-9-oxo-8-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-7-thioxo-1-oxa-6,8-dizaspiro[4.4]nonane (31). *Method II*. $t=5$ days. Column chromatography: Et_2O/petroleum ether 1:6 $[\alpha]_D^{25}$ +31 (*c* 1.0). Yield: 0.190 g, 94% (amorphous solid). IR: $\nu_{max.}$ 2985, 2942,1757, 1753, 1491, 1377,1227, 1223, 1098 and 1068 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, *(* ppm, *J* Hz): 7.34 (bs, 1H, NH), 5.82 (d, 1H, $J_{1',2'}=9.2$ Hz, H-1′), 5.71 (t, 1H, $J_{2',3'}=9.2$ Hz, H-2′), 5.34 (t, 1H, $J_{3',4'}=9.4$ Hz, H-3′), 5.16 (t, 1H, $J_{4',5'}=9.8$ Hz, H-4′), 4.99 (m, 1H, H-3), 4.84 (d, 1H, $J_{3,4}=5.9$ Hz, H-4), 4.33 (m, 2H, H-2, H-4″), 4.22 (m, 2H, H-6′a, H-6′b), 4.05 (m, 2H, H-5″a, H-5″b), 3.82 (dt, 1H, $J_{5',6'a}=J_{5',6'b}=3.6$ Hz, H-5′), 2.10, 2.04, 2.02, 1.97, 1.54, 1.44, 1.39, 1.36 (each s, each 3H, $8CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, (ppm): (181.6 (C-7),170.5, 170.1, 169.8, 169.5, 169.3 (5C=O), 113.9, 109.5 ($2CCH_3$), 89.7 (C-5), 81.2 (C-1′), 80.0 (C-3+C-4), 79.0 (C-2), 74.6 (C-5′), 72.7*, 72.6* (C-3′, C-4″), 68.1 (C-2′), 67.8 (C-4′), 66.6 (C-5″), 61.6 (C-6′), 26.8, 25.8, 25.0, 24.3, 20.6, 20.5, 20.4 ($8CH_3$). Anal. Calcd for $C_{28}H_{38}O_{15}N_2S_1$: C, 49.85; H, 5.68; N, 4.15, found: C, 49.28; H, 5.63; N, 4.18.

4.8.10. (2*R*,3*S*,4*S*,5*S*,4″*R*)-2-(2″,2″-Dimethyl-1″,3″-dioxolan-4″-yl)-3,4-dimethylmethylenedioxy-9-oxo-7-thioxo-8-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1-oxa-6,8-diazaspiro[4.4]nonane (32). *Method II*. $t=4$ days. Column chromatography: Et_2O/petroleum ether 1:3. Yield: 0.177 g, 75% (amorphous solid). $[\alpha]_D^{26}$ +18 (*c* 1.1). IR: ν_{max} 2988, 2930, 1730, 1719, 1489, 1269, 1099 and 1068 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, *(* ppm, *J* Hz): *(* 8.08–7.31 (m, 15H, Ar), 7.31 (s, 1H, NH), 6.43 (d, 1H, $J_{1',2'}=4.0$ Hz, H-1′), 6.32 (dd, 1H, $J_{2',3'}=6.3$ Hz, H-2′), 6.02 (t, 1H, $J_{3',4'}=6.3$ Hz, H-3′), 5.02 (dd, 1H, $J_{2,3}=3.3$ Hz, $J_{3,4}=5.9$ Hz, H-3), 4.89 (d, 1H, H-4), 4.76 (dd, 1H, $J_{4',5'a}=3.8$, $J_{5'a,5'b}=11.3$ Hz, H-5′a), 4.67 (ddd, 1H, H-4′), 4.64 (dd, 1H, $J_{4',5'b}=5.4$ Hz, H-5′b), 4.34 (m, 2H, H-2, H-4″), 4.08 (m, 1H, H-5″a), 3.96 (m, 1H, H-5″b), 1.56, 1.46, 1.40, 1.38 (each s, each 3H, $4CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): 181.4 (C-7), 170.6 (C-9), 166.1, 165.2, 165.1 (3C=O), 133.5–128.3 (Ar), 114.1, 109.5 ($2CCH_3$), 90.2 (C-5), 86.2 (C-1′), 80.1 (C-3+C-4), 79.4 (C-2), 79.2 (C-4′), 72.7 (C-4″), 71.8 (C-2′), 71.2 (C-3′), 66.9 (C-5″), 63.4 (C-5′), 26.8, 25.8, 25.1, 24.2 ($4CH_3$). HRCIMS *m/z* calcd for $C_{40}H_{41}O_{13}N_2S$ ($[M+H]^+$): 789.2329, found: 789.2329. Anal. Calcd for $C_{40}H_{40}O_{13}N_2S$: C, 60.90; H, 5.11; N, 3.55. Found: C, 60.84; H, 4.98; N, 3.47.

4.9. General procedures for the reactions of the isothiocyanatoulosonates 11, 18α, and 18β with ammonia, alkyl, aryl, and glycosyl amines (Route B). Preparation of the hydantocidin-related spironucleosides 24, 27α, 27β, 29α, 28α, 28β, 29α, 29β, 30α, and 30β

Method III (starting from free amines; used for the preparation of compounds **24**, **27α**, **27β**, **29α**, **29β**, **30α**, and **30β**). A solution of the isothiocyanatoulosonate **11** (for **24**) or a mixture 6.5:2 of **18α** and **18β** (for **27α**, **27β**, **29α**, **29β**, **30α**, and **30β**) (90 mg, 0.23 mmol) in THF (3 mL) was stirred with NH_3 (for **27α** and **27β**), dodecylamine (for **29α** and **29β**) or aniline (for **30α** and **30β**) (*x* mmol) at *T* °C for *t* min. The solvent was evaporated and the residue was purified by column chromatography.

Method IV (starting from amines as ammonium salts; used for the preparation of compounds **28α and 28β**). To a solution of a mixture 6.5:2 of the isothiocyanatoulosonates **18α** and **18β** (90 mg, 0.23 mmol), a solution of CH_3NH_2. HCl (17 mg, 0.25 mmol) and $NaHCO_3$ (21 mg, 0.25 mmol) in H_2O (0.2 mL) was added. The mixtue was stirred for 10 min at room temperature, concentrated to half, diluted with CH_2Cl_2 (15 mL), washed with brine, dried ($MgSO_4$), and evaporated to dryness. The residue was purified by column chromatography.

4.9.1. (5*R*,8*R*,9*R*,10*S*)-10-Benzoyloxy-8,9-dimethylmethylenedioxy-3-*p*-methoxyphenyl-4-oxo-2-thioxo-6-oxa-1,3-diazaspiro[4.5]decane (24). *Method III*. $x=$ 31 mg, 0.25 mmol. $T=40$ °C. $t=2$ h. Column chromatography: Et_2O/petroleum ether 1:4. Yield: 0.08 g, 72%. $[\alpha]_D^{22}$ −88 (*c* 1.3). IR: ν_{max} 3291, 2984, 1755, 1732, 1599, 1489, 1379, 1252 and 1105 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.04–6.93 (m, 9H, Ar), 7.52 (bs, 1H, NH), 5.65 (d, 1H, $J_{9,10}=7.4$ Hz, H-10), 4.88 (dd, 1H, $J_{8,9}=5.8$ Hz, H-9), 4.65 (dd, 1H, $J_{7a,7b}=13.6$ Hz, $J_{7a,8}=2.9$ Hz, H-7a), 4.49 (m, 1H, H-8), 4.33 (dd, 1H, $J_{7b,8}<1$, H-7b), 3.82 (s, 3H, OCH_3), 1.66, 1.42 (each s, each 3H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 183.2 (C-2), 168.9 (C-4), 165.4 (C=O), 160.0–114.3 (Ar), 110.3 (CCH_3), 85.5 (C-5), 73.7 (C-9), 72.8 (C-8), 71.1 (C-10), 63.3 (C-7), 55.3 (OCH_3), 27.5, 25.8 ($2CH_3$). HRCIMS *m/z* calcd for $C_{24}H_{24}O_7N_2S$ ($[M+H]^+$): 485.1383, found: 485.1377.

4.9.2. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro-[4.4]nonane (27α). *Method III*. $x=5$ min bubbling NH_3. $T=$ room temperature. $t=15$ min. Column chromatography: Et_2O/petroleum ether 1:2. Yield: 0.064 g, 74% (amorphous solid). $[\alpha]_D^{25}$ −68 (*c* 1.2). IR $\nu_{max.}$ 3288, 2992, 2944, 1771, 1717, 1507, 1385, 1275 and 1099 cm^{-1}. ^1H

NMR (500 MHz, $CDCl_3$, δ ppm, J Hz): δ 8.76 (bs, 1H, NH), 8.09–7.43 (m, 6H, Ar+NH), 4.92 (dd, 1H, $J_{2,3}=2.5$ Hz, H-3), 4.88 (d, 1H, $J_{3,4}=6.1$ Hz, H-4), 4.60 (td, 1H, H-2), 4.55 (dd, 1H, $J_{2,CH2a}=5.6$ Hz, CH_2a), 4.50 (dd, 1H, $^2J_{H,H}=11.7$ Hz, $J_{2,CH2b}=6.5$ Hz, CH_2b), 1.61, 1.38 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 181.2 (C-7), 171.2 (C-9), 166.1 (C=O), 133.4–127.6 (Ar), 114.9 (*C*CH_3), 94.7 (C-5), 82.8 (C-2), 82.2 (C-3), 80.7 (C-4), 64.3 (CH_2OBz), 26.6, 24.8 ($2CH_3$). Anal. Calcd for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.40. Found: C, 53.67; H, 5.19; N, 7.01.

4.9.3. (2*R*,3*R*,4*R*,5*S*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (27β). *Method III*. $x=5$ min bubbling NH_3. $T=$ room temperature. $t=15$ min. Column chromatography: Et_2O/petroleum ether 1:2. Yield: 0.017 g, 20% (amorphous solid). $[\alpha]_D^{25}$ −160 (*c* 0.6). IR: $\nu_{max.}$ 3229, 2928, 1777, 1717, 1593, 1379, 1265, 1103 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, (ppm, *J* Hz): (8.42 (bs, 1H, NH), 8.03–7.49 (m, 5H, Ar), 7.48 Method, 1H, NH), 4.93 (d, 1H, $J_{3,4}=6.4$ Hz, H-4), 4.90 (dd, 1H, $J_{2,3}=2.0$ Hz, H-3), 4.88 (m, 1H, H-2), 4.63 (dd, 1H, $^2J_{H,H}=12.4$ Hz, $J_{2,CH2a}=4.3$ Hz, CH_2a), 4.47 (dd,1H, $J_{2,CH2b}=3.3$ Hz, CH_2b), 1.62, 1.35 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (180.8 (C-7), 168.1 (C-9), 166.0 (C=O), 133.8–128.4 (Ar), 116.5 (*C*CH_3), 95.8 (C-5), 86.2 (C-4), 83.4 (C-2), 81.3 (C-3), 64.7 (CH_2OBz), 25.0, 24.9 ($2CH_3$). HREIMS *m*/*z* calcd for $C_{17}H_{18}O_6N_2S$ ($[M]^+$): 378.0886, found: 378.0889.

4.9.4. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-8-methyl-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (28α). *Method IV*. Column chromatography: Et_2O/petroleum ether 1:9. Yield: 0.057 g, 64%. $[\alpha]_D^{26}$ −82 (*c* 1.1). IR: ν_{max}. 2988, 2942, 1757, 1717, 1489, 1375, 1275 and 1099 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, (ppm, *J* Hz): (8.09–7.43 (m, 5H, Ar), 7.41 (sb, 1H, NH), 4.92 (dd, 1H, $J_{2,3}=1.8$ Hz, H-3), 4.83 (d, 1H, $J_{3,4}=6.0$ Hz, H-4), 4.60–4.48 (m, 3H, H-2, CH_2OBz), 3.23 (s, 3H, NCH_3), 1.68, 1.37 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (183.5 (C-7), 170.9 (C-9), 166.0 (C=O), 133.1–128.3 (Ar), 114.7 (*C*CH_3), 92.8 (C-5), 82.5 (C-2), 82.2 (C-3), 80.7 (C-4), 64.3 (CH_2OBz), 27.3, 26.5, 24.7 ($3CH_3$). HRCIMS *m*/*z* calcd for $C_{18}H_{21}O_6N_2S$ ($[M+H]^+$): 393.1120, found: 393.1114.

4.9.5. (2*R*,3*R*,4R,5*S*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-8-methyl-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (28β). *Method IV*. Column chromatography: Et_2O/petroleum ether 1:9.Yield: 0.019 g, 21% (amorphous solid). $[\alpha]_D^{24}$ −132 (*c* 1.1). IR: ν_{max} 3308, 2982, 2941, 1763, 1724, 1489, 1275 and 1097 cm^{-1}.^{1}H NMR (500 MHz, $CDCl_3$, *(* ppm, *J* Hz): *(* 8.05–7.49 (m, 5H, Ar), 7.43 (bs, 1H, NH), 4.92–4.87 (m, 3H, H-2, H-3, H-4), 4.63 (dd, 1H, $J_{2,H}=4.3$ Hz, C*H*HOBz), 4.46 (dd, 1H, $^2J_{H,H}=12.4$ Hz, $J_{2,H}=3.2$ Hz, CH*H*OBz), 3.23 (s, 3H, NCH_3), 1.64, 1.34 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (183.3 (C-7), 168.0 (C-9), 165.9 (C=O), 133.7–128.3 (Ar), 116.4 (*C*CH_3), 93.8 (C-5), 86.1 (C-4), 83.1*, 81.3* (C-2, C-3), 64.7 (CH_2OBz), 27.4, 25.1, 24.9 ($3CH_3$). HRCIMS *m*/*z* calcd for $C_{18}H_{21}O_6N_2S$ ($[M+H]^+$): 393.1120, found: 393.1122.

4.9.6. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-8-dodecyl-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (29α). *Method III*. $x=0.047$ mg, 10.25 mmol. $T=$ room temperature. $t=30$ min. Column chromatography: Et_2O/petroleum ether 1:9–1:5 gradient. Yield: 0.075 g, 60% (syrup). $[\alpha]_D^{25}$ −64 (*c* 0.9). IR: ν_{max} 2924, 2853, 1751, 1724, 1474, 1273 and 1101 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, (ppm, *J* Hz): (8.09–7.42 (m, 5H, Ar), 7.27 Method, 1H, NH), 4.92 (dd, 1H, $J_{2,3}=2.0$ Hz, H-3), 4.82 (d, 1H, $J_{3,4}=6.0$ Hz, H-4), 4.60–4.48 (m, 3H, H-2, CH_2OBz), 3.76 (t, 2H, $^3J_{H,H}=7.5$ Hz, NCH_2), 1.65–1.25 (m, 26H, $(CH_2)_{10}$, $2CH_3$), 0.87 (t, 3H, $^3J_{H,H}=6.3$ Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, (ppm): (183.2 (C-7), 171.0 (C-9), 165.9 (C=O), 133.1–128.3 (Ar), 114.6 (*C*CH_3), 92.4 (C-5), 82.4 (C-2), 82.3 (C-3), 80.6 (C-4), 64.2 (CH_2OBz), 41.2 (NCH_2), 31.7, 29.4 (for 2C), 29.3, 29.23, 29.1, 28.9, 27.4, 26.5 (for 2C), 24.7, 22.5 ($(CH_2)_{10}$, $2CH_3$), 13.9 (CH_3). Anal. Calcd for $C_{29}H_{42}O_6N_2S$: C, 63.71; H, 7.74; N, 5.12, found C, 63.68; H, 7.84; N, 5.14.

4.9.7. (2*R*,3*R*,4*R*,5*S*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-8-dodecyl-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (29β). *Method III*. $x=0.047$ mg, 10.25 mmol. $T=$ room temperature. $t=30$ min. Column chromatography: Et_2O/petroleum ether 1:9–1:5 gradient. Yield: 0.021 g, 17% (syrup). $[\alpha]_D^{26}$ −102 (*c* 1.0). IR: $\nu_{max.}$ 3295, 2926, 2855, 1763, 1723, 1599, 1489, 1273 and 1101 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, *(* ppm, *J* Hz): 8.07–7.48 (m, 5H, Ar), 7.32 (bs, 1H, NH), 4.92–4.85 (m, 3H, H-2, H-3, H-4), 4.62 (dd, 1H, $J_{2,H}=3.9$ Hz, C*H*HOBz), 4.48 (dd, 1H, $J_{2,H}=3.1$ Hz, $^2J_{H,H}=12.3$ Hz, CH*H*OBz), 3.77 (m, 2H, NCH_2), 1.63–0.85 (m, 26H, $(CH_2)_{10}$, $2CH_3$), 0.88 (t, 3H, $^3J_{H,H}=6.5$ Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$, (ppm): (183.1 (C-7), 168.2 (C-9), 165.9 (C=O), 133.7–128.3 (Ar), 116.5 (*C*CH_3), 93.6 (C-5), 86.1 (C-4), 83.0*, 81.3* (C-2, C-3), 64.7 (CH_2OBz), 41.3 (NCH_2), 31.8, 29.5 (for 2C), 29.4, 29.37, 29.2, 29.1, 27.5, 26.5, 25.1, 24.9, 22.6 ($(CH_2)_{10}$, $2CH_3$), 14.0 (t, 3H, CH_3). HRCIMS *m*/*z* Calcd for $C_{29}H_{43}N_2O_6S$ ($[M+H]^+$): 547.2842, found: 547.2835.

4.9.8. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-9-oxo-8-phenyl-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (30α). *Method III*. $x=23$ μl, 0.25 mmol. $T=40$ °C. $t=3$ h. Column chromatography: toluene/AcOEt 100:1–50:1 gradient. Yield: 0.076 g, 73% (amorphous solid). $[\alpha]_D^{24}$ −73 (*c* 1.0). IR: ν_{max}. 2987, 1763, 1719, 1497, 1452, 1230 and 1071 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.09–7.31 (m, 11H, Ar, NH), 4.99 (d, 1H, $J_{3,4}=6.1$ Hz, H-4), 4.97 (dd, 1H, $J_{2,3}=2.3$ Hz, H-3), 4.65 (td, 1H, H-2), 4.58 (dd, 1H, $J_{2,H}=5.7$ Hz, C*H*HOBz), 4.54 (dd, 1H, $J_{2,H}=6.5$ Hz, $^2J_{H,H}=11.8$ Hz, CH*H*OBz), 1.65, 1.42 (each s, each 3H, $2CH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ ppm): δ 182.8 (C-7), 170.4 (C-9), 166.0 (C=O), 133.2–127.9 (Ar), 114.9 (*C*CH_3), 93.0 (C-5), 82.8 (C-2), 82.4 (C-3), 80.9 (C-4), 64.3 (CH_2OBz), 26.6, 24.8 ($2CH_3$). Anal. Calcd for $C_{23}H_{22}O_6N_2S$: C, 60.78; H, 4.88; N, 6.16. Found: C, 60.85; H, 5.01; N, 6.11.

4.9.9. (2*R*,3*R*,4*R*,5*S*)-2-Benzoyloxymethyl-3,4-dimethylmethylenedioxy-9-oxo-8-phenyl-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (30β). *Method III*. $x=23$ μl, 0.25 mmol. $T=40$ °C. $t=3$ h. Column chromatography:

Toluene: AcOEt 100:1–50:1 gradient. Yield: 0.022 g, 21% (amorphous solid). $[\alpha]_D^{24}$ −138 (*c* 0.9). IR: $\nu_{max.}$ 3291, 2936, 1775, 1593, 1489, 1271 and 1103 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, (ppm, *J* Hz): (8.06–7.30 (m, 10H, Ar), 7.76 (bs, 1H, NH), 5.00 (d, 1H, $J_{3,4}=6.1$ Hz, H-4), 4.93 (m, 2H, H-2, H-3), 4.66 (dd, 1H, $J_{2,H}=3.9$ Hz, $^2J_{H,H}=12.3$ Hz, C*H*HOBz), 4.51 (dd, 1H, $J_{2,H}=2.9$ Hz, CH*H*OBz), 1.61, 1.36 (each s, each 3H, $2CH_3$). ^{13}C NMR: (125.7 MHz, $CDCl_3$, (ppm): (182.7 (C-7), 167.5 (C-9), 166.0 (C=O), 133.7–128.2 (Ar), 116.7 (*C*CH_3), 94.1 (C-5), 86.3 (C-4), 83.1*, 81.2* (C-2, C-3), 64.6 (CH_2OBz), 25.1, 25.0 ($2CH_3$). HRCIMS *m/z* calcd for $C_{23}H_{23}O_6N_2S$ ($[M+H]^+$): 455.1277, found: 455.1272.

4.10. (5*R*,8*R*,9*R*,10*S*)-10-Benzoyloxy-8,9-dihydroxy-4-oxo-3-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thioxo-6-oxa-1,3-diazaspiro[4.5]decane (33)

To a solution of 22α (100 mg, 0.14 mmol) in CH_3CN: H_2O 9:1 (3 mL), DDQ (7.5 mg, 0.033 mmol) was added. The mixture was stirred at 45 °C for 36 h. The solution was concentrated to dryness and the residue was purified by column chromatography (Et_2O). Yield: 0.078 g, 83% (amorphous solid). $[\alpha]_D^{25}$ −29 (*c* 0.1). IR: ν_{max} 2945, 1755, 1732, 1599, 1489, 1377, 1240 and 1103 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ ppm, *J* Hz): δ 8.72 (bs, 1H, NH), 7.89–7.32 (m, 5H, Ar), 5.98 (t, 1H, $J_{2',3'}=9.3$ Hz, H-2′), 5.80 (d, 1H, $J_{1',2'}=9.5$ Hz, H-1′), 5.75 (d, 1H, $J_{9,10}=9.7$ Hz, H-10), 5.31 (t, 1H, $J_{3',4'}=9.5$ Hz, H-3′), 5.26 (t, 1H, H-4′), 4.71 (dd, 1H, $J_{8,9}=2.9$ Hz, H-9), 4.54 (d, 1H, $J_{7a,7b}=12.7$ Hz, H-7a), 4.35 (dd, 1H, $J_{5',6'a}=4.2$ Hz, H-6′a), 4.24 (m, 1H, H-8), 4.18 (dd, 1H, $J_{5',6'b}=2.3$ Hz, $J_{6'a,6'b}=12.5$ Hz, H-6′b), 4.14 (d, 1H, H-7b), 3.78 (dt, 1H, $J_{4',5'}=9.6$ Hz, H-5′), 2.05, 2.04, 2.03, 1.94 (each s, each 3H, $4CH_3$), 1.68 (bs, 2H, 2OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): δ 182.1 (C-2), 170.6, 169.9, 169.4, 169.3, 167.4, 166.7 (6C=O), 134.0–127.8 (Ar), 84.5 (C-5), 80.9 (C-1′), 74.3 (C-5′), 73.0 (C-3′), 71.5 (C-10), 68.9*, 68.8* (C-8, C-9), 67.8 (C-4′), 67.2 (C-2′), 65.8 (C-7), 61.7 (C-6′), 20.5, 20.4 (parta 2C), 20.1 ($4CH_3$). HRCIMS *m/z* calcd for $C_{28}H_{33}O_{15}N_2S$ ($[M+H]^+$): 669.1602, found: 669.1594.

4.11. (2*R*,3*R*,4*R*,5*R*)-2-Benzoyloxymethyl-3,4-dihydroxy-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (35)

A solution of **27α** (55 mg, 0.145 mmol) in TFA:H_2O 2:3 (5 mL) was stirred at room temperature for 1 h. The solution was concentrated to dryness and the residual amount of acid was eliminated by repeated evaporations with toluene. The residue was purified by column chromatography (EtOAc/petroleum ether 1:2). Yield: 0.045 g, 91%. $[\alpha]_D^{20}$ −14 (*c* 1.0, MeOH). IR: ν_{max}. 3237, 2926, 1765, 1717, 1599, 1505, 1379, 1279 and 1103 cm^{-1}. ^1H NMR (500 MHz, MeOD, δ ppm, *J* Hz): δ 8.11–7.45 (m, 5H, Ar), 4.49 (dd, 1H, $^2J_{H,H}=13.4$ Hz, $J_{2,H}=5.0$ Hz, C*H*HOBz), 4.40 (m, 2H, H-2, CH*H*OBz), 4.34 (dd, 1H, $J_{2,3}=3.8$ Hz, H-3), 4.32 (d, 1H, $J_{3,4}=4.8$ Hz, H-4). ^{13}C NMR (125.7 MHz, MeOD, δ ppm): δ 185.0 (C-7), 174.9, 167.8 (2C=O), 134.4–129.6 (Ar), 95.5 (C-5), 83.7 (C-2), 74.6*, 73.2* (C-3, C-4), 65.4 (CH_2OBz). HRFABMS *m/z* calcd for $C_{14}H_{14}N_2O_6SNa$ ($[M+Na]^+$): 361.0470, found: 361.0478.

4.12. Preparation of 34 and 36(α+β)

To a solution of **33** (for **34**) ó **35** [for **36**(α+β)] (*x* mg, 0.15 mmol) in MeOH (1 mL), another solution of NaMeO 1 M in MeOH (1 mL) was added. The solution was stirred for 1 h at room temperature, neutralized with Dowex®, filtered, and concentrated to dryness. The residue was purified by HPLC (reversed-phase).

4.12.1. (5*R*,8*R*,9*R*,10*S*)-4-Oxo-8,9,10-trihydroxy-3-(β-D-glucopyranosyl)-2-thioxo-6-oxa-1,3-diazaspiro[4.5]-decane (34).

x=100 mg. HPLC: MeOH/H_2O 70: 1. Yield 0.037 g, 85%. $[\alpha]_D^{22}$ −32 (c 0.5, MeOH). IR: ν_{max}. 3308, 2893, 1763, 1491, 1379, 1103, 1071 cm^{-1}. ^1H NMR 500 MHz, D_2O, δ ppm, *J* Hz): δ 5.66 (d, 1H, $J_{1',2'}=9.6$ Hz, H-1′), 4.39 (dd, 1H, $^2J_{H,H}=13.0$ Hz, $J_{7a,8}<1$, H-7a), 4.34 (t, 1H, $J_{2',3'}=9.4$ Hz, H-2′), 4.28 (dd, 1H, $J_{8,9}=3.3$ Hz, H-9), 4.05 (m, 1H, H-8), 3.95 (d, 1H, $J_{9,10}=10.1$ Hz, H-10), 3.87 (dd, 1H, $J_{7b,8}=2.0$ Hz, H-7b), 3.83 (dd, 1H, $J_{5',6'a}=1.7$ Hz, H-6a), 3.69 (dd, $^2J_{H,H}=12.5$ Hz, $J_{5',6'b}=4.4$ Hz, H-6′b), 3.49 (m, 3H, H-3′, H-4′, H-5′). ^{13}C NMR (125.7 MHz, D_2O, δ ppm): δ 185.6 (C-7), 172.0 (C-9), 88.0 (C-5), 84.2 (C-1′), 79.8, 77.8, 70.5, 70.3 (C-3′, C-4′, C-5′, C-10), 69.3, 69.2 (C-8, C-2′), 67.7 (C-7), 61.6 (C-6′). HRFABMS calcd for $C_{13}H_{21}N_2O_{10}S$: 397.0917, found: 397.0891.

4.12.2. (2*R*,3*R*,4*R*,5*R* and *S*)-2-Hydroxymethyl-3,4-dihydroxy-9-oxo-7-thioxo-1-oxa-6,8-diazaspiro[4.4]nonane (36 α+β).

x=50 mg. HPLC: AcOEt/MeOH 10:1. Diastereoisomers in C-5 ratio (*R*:*S*): 6:1. Yield: 29 mg, 87%. The spectroscopic data were coincident with those reported[16c] in the literature.

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Efficient synthesis of enynes by tetraphosphine–palladium-catalysed reaction of vinyl bromides with terminal alkynes

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Abstract—Through the use of $[PdCl(C_3H_5)]_2$/*cis*,*cis*,*cis*-1,2,3,4-tetrakis(diphenylphosphinomethyl)cyclopentane as catalyst, a range of vinyl bromides undergoes Sonogashira cross-coupling reaction with a variety of alkynes, leading to the corresponding 1,3-enynes in good yields. The reaction tolerates several alkynes such as phenylacetylene, dec-1-yne, 2-methylbut-1-en-3-yne a range of alk-1-ynols, 3,3-diethoxyprop-1-yne and a propargyl amine. Higher reactions rates were observed in the presence of phenylacetylene, dec-1-yne, but-3-yn-1-ol, pent-4-yn-1-ol, 3,3-diethoxyprop-1-yne or 1,1-dipropyl-2-propynylamine than with propargyl alcohol, 3-methoxy-prop-1-yne or 2-methylbut-1-en-3-yne. This catalyst can be used at low loading even for reactions of sterically hindered vinyl bromides such as bromotriphenylethylene or 2-bromo-3-methyl-but-2-ene.

1. Introduction

Enynes are fundamental building blocks in organic synthesis. The palladium-catalysed so-called Sonogashira reaction is one of the most powerful methods for the synthesis of such compounds.[1–4] Cross-coupling palladium-catalysed reactions between aryl halides and alkynes have been largely described.[5–12] On the other hand, if the reactions between terminal acetylene and vinyl halides have been recognized since the mid-seventies,[13–14] their applications usually need harsh reaction conditions and high catalyst loadings. The most widely used catalysts for this reaction are $Pd(PPh_3)_4$ or $PdCl_2(PPh_3)_2$, associated with copper(I) iodide.[15–28] But these catalysts are not very efficient in terms of the ratio substrate/catalyst and 5–10% catalyst had to be used.[15–22] With these catalysts, several reactions conditions have been tested in order to improve the yields. For example, 1-trialkylsilyl-1-alkynes can be coupled directly with vinyl triflates using 1% $Pd(PPh_3)_4$ as catalyst in the presence of Bu_4NF and AgI.[23] Mori et al. have reported the coupling of terminal alkynes with vinyl bromides catalyzed by 1% $Pd(PPh_3)_4$ using Bu_4NF as additive.[24] Polymethylhydrosiloxane in association with $PdCl_2(PPh_3)_2$ can be used to promote the reaction between *E*-β-bromostyrene and 2-methyl-3-butyn-2-ol using 5% catalyst in the presence of CsF at room temperature.[25] Some results were reported in ionic liquids using 5% catalyst.[26] This reaction has also been performed in aqueous media using water soluble sulfonated triphenylphosphine ligand for the coupling of vinyl iodides.[27] A few other ligands have also been tested for this coupling. An *N*-heterocyclic carbene palladium catalyst has shown efficiency comparable to $Pd(PPh_3)_4$.[28] With an imidazolium carbene ligand good results were obtained for the coupling of 1-bromocyclohex-1-ene using 3% of palladium catalyst.[29] Alami et al. obtained high yields of adducts using 5% of $PdCl_2(PhCN)_2$ catalyst without added ligand at room temperature for the coupling reaction between vinyl chloride and alkynes.[30] The reaction between vinyl iodides and terminal alkynes can also be performed in aqueous media using potassium fluoride, palladium submicron powder, cuprous iodide and PPh_3.[31] Despite these recent advances, there still remained a need for a general protocol using low-catalyst loading for Sonogashira reactions in the presence of vinyl halides. Moreover, the efficiency of tetraphosphine ligands for the cross-coupling of vinyl halides with alkynes has not been reported.

2. Results and discussion

In order to find stable and efficient palladium catalysts, we have prepared the tetrapodal phosphine ligand, *cis*,*cis*,*cis*-1,2,3,4-tetrakis(diphenylphosphinomethyl) cyclopentane or Tedicyp (Fig. 1).[32] We have already reported the results

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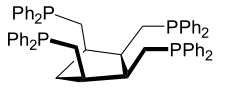


Tedicyp

Figure 1.

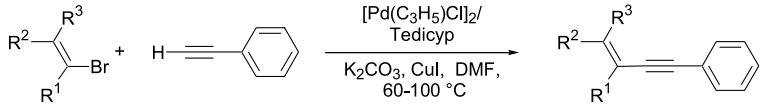
obtained in allylic substitution,[32] in Heck reaction,[33] in Suzuki cross-coupling[34] and in Sonogashira reaction[35–40] using Tedicyp as ligand. For example, we obtained a turnover number (TON) of 2,800,000 for the coupling of 3,5-bis(trifluoromethyl)bromobenzene with phenylacetylene.[35] We have also recently reported the Sonogashira coupling of sterically congested aryl bromides,[36] of heteroaryl halides,[37] of a range of aryl chlorides with as little as 0.01% catalyst without addition of co-catalysts,[38] and also the reactivity of several alkynols[39] or propargyl amines.[40] Here, we wish to report on the efficiency of Tedicyp ligand for the reaction of vinyl bromides with terminal alkynes such as phenylacetylene, dec-1-yne, 2-methylbut-1-en-3-yne, a range of alk-1-ynols, 3,3-diethoxyprop-1-yne and a propargyl amine.

For this study, based on our previous results,[35–40] DMF was chosen as the solvent and potassium carbonate as the base. The reactions were performed at 60–100 °C under argon in the presence of a ratio 1:2 of $[Pd(C_3H_5)Cl]_2$/Tedicyp as catalyst and 5% copper(I) iodide as co-catalyst. At higher temperatures (120–140 °C), lower yields were obtained due to partial polymerization of the substrates and products. The dimerisation of the alkynes was also detected in some cases. In order to obtain high conversions of the aryl bromides we have used 2 equiv of alkyne for all the reactions, however, most of the reactions should proceed with 1.2–1.5 equiv of alkyne. Some decomposition of the products was observed for long time reactions, so the reactions were stopped at 20 h.

First, we tried to couple phenylacetylene with a range of vinyl bromides (Scheme 1, Table 1). As expected, the reaction of β-bromostyrene with phenylacetylene proceeds nicely. The reaction can be performed with as little as 0.0001% catalyst (TON: 520,000) (Table 1, entry 3). The same reaction performed with dppe as ligand (Table 1, entry 2), led to the coupling adduct **1** in 12% yield in the presence of 0.001% catalyst (TON: 12,000). With a mixture of *Z* and *E* 1-bromoprop-1-ene (ratio *Z*/*E*: 45/55) a good yield was also obtained for a ratio S/C of 10,000 (Table 1, entries 4 and 5). A selectivity of 95% in favour of *E* isomer was obtained using 0.01% catalyst (Table 1, entry 5). This high selectivity in *E* isomer probably comes partially from the higher reactivity of (*E*)-1-bromoprop-1-ene[41] and also from partial isomerisation of (*Z*)-1-bromoprop-1-ene into (*E*)-1-bromoprop-1-ene under basic conditions.[42]

Then, we studied the reactivity of three α-substituted vinyl bromides. 2-Bromoprop-1-ene, 2-bromobut-1-ene and 3-bromobut-3-en-1-ol led to the coupling 1,3-enynes **3–5** in good yields using 0.01% catalyst (Table 1, entries 6–9). The highest TON was obtained with 2-bromobut-1-ene, with a yield of 48% for a ratio substrate/catalyst of 50,000 (Table 1, entry 8). The synthesis of tetrasubstituted alkenes was also possible by Sonogashira cross-coupling reactions with this catalyst. Sterically hindered substrates bromotriphenylethylene or 2-bromo-3-methylbut-2-ene reacts cleanly with phenylacetylene. For the reactions with these substrates TONs of 3300 and 6000 were obtained, respectively, (Table 1, entries 10–12).

Having demonstrated that a variety of vinyl bromides can be efficiently cross-coupled with phenylacetylene, we have investigated the scope of this reaction using dec-1-yne and 2-methylbut-1-en-3-yne (Scheme 2, Table 2), and also with



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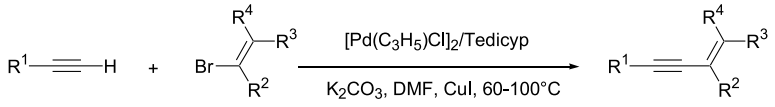
Scheme 1.

Table 1. Cross-coupling reactions of vinyl bromides with phenylacetylene (Scheme 1)

Entry	Vinyl bromide	Ratio substrate/catalyst	Temperature (°C)	Product number	Ratio *Z* (**a**)/*E* (**b**)	Yield (%)[a]
1	β-Bromostyrene[b]	100,000	100	**1a**,**b**	10/90	100[c]
2	β-Bromostyrene[b]	100,000	100	**1a**,**b**	10/90	12[c,d]
3	β-Bromostyrene[b]	1000,000	100	**1a**,**b**	10/90	52
4	1-Bromoprop-1-ene[e]	1000	60	**2a**,**b**	05/95	100[c]
5	1-Bromoprop-1-ene[e]	10,000	60	**2a**,**b**	05/95	85
6	2-Bromoprop-1-ene	10,000	60	**3**	—	75
7	2-Bromobut-1-ene	10,000	80	**4**	—	100[c]
8	2-Bromobut-1-ene	50,000	80	**4**	—	48
9	3-Bromobut-3-en-1-ol	10,000	80	**5**	—	85
10	2-Bromo-3-methyl-but-2-ene	10,000	80	**6**	—	60
11	Bromotriphenylethylene	1000	100	**7**	—	86
12	Bromotriphenylethylene	10,000	100	**7**	—	33[c]

[a] Conditions: catalyst: $[ClPd(C_3H_5)]_2$/Tedicyp = 1:2, vinyl bromide (1 equiv), phenylacetylene (2 equiv), K_2CO_3 (2 equiv), CuI (0.05 equiv), DMF, 20 h, isolated yields.
[b] β-Bromostyrene was a mixture of *Z* and *E* isomers, ratio *Z*/*E*: 10/90.
[c] GC and NMR yield.
[d] Reaction performed with dppe (ratio Pd/dppe: 1:2).
[e] 1-Bromoprop-1-ene was a mixture of *Z* and *E* isomers, ratio *Z*/*E*: 45/55.



Scheme 2.

Table 2. Cross-coupling reactions of vinyl bromides with dec-1-yne and 2-methylbut-1-en-3-yne (Scheme 2)

Entry	Vinyl bromide	Alkyne	Ratio substrate/catalyst	Temperature (°C)	Product number	Ratio *Z* (**a**)/*E* (**b**)	Yield (%)[a]
1	β-Bromostyrene[b]	Dec-1-yne	10,000	100	**8a**,**b**	10/90	90
2	Bromotriphenylethylene	Dec-1-yne	100	100	**9**	—	97
3	2-Bromobut-1-ene	Dec-1-yne	1000	80	**10**	—	99
4	2-Bromo-3-methylbut-2-ene	Dec-1-yne	100	80	**11**	—	89
5	2-Bromo-3-methylbut-2-ene	Dec-1-yne	1000	80	**11**	—	31[c]
6	1-Bromoprop-1-ene[d]	Dec-1-yne	100	60	**12a**,**b**	13/87	100[c]
7	1-Bromoprop-1-ene[d]	Dec-1-yne	1000	60	**12a**,**b**	05/95	65
8	2-Bromoprop-1-ene	Dec-1-yne	100	60	**13**	—	100[c]
9	2-Bromoprop-1-ene	Dec-1-yne	1000	60	**13**	—	51
10	β-Bromostyrene	2-Methylbut-1-en-3-yne	100	80	**14a**,**b**	10/90	59[e]
11	Bromotriphenylethylene	2-Methylbut-1-en-3-yne	50	80	**15**	—	60[e]

[a] Conditions: catalyst: $[ClPd(C_3H_5)]_2$/Tedicyp = 1:2, vinyl bromide (1 equiv), alkyne (2 equiv), K_2CO_3 (2 equiv), CuI (0.05 equiv), DMF, 20 h.
[b] β-Bromostyrene was a mixture of *Z* and *E* isomers, ratio *Z*/*E*: 10/90.
[c] GC and NMR yield.
[d] 1-Bromoprop-1-ene was a mixture of *Z* and *E* isomers, ratio *Z*/*E*: 45/55.
[e] Reaction performed in autoclave.

several functionalized alkynes: propargyl alcohol, 3-methoxyprop-1-yne, but-1-yn-3-ol, but-3-yn-1-ol, pent-4-yn-1-ol, 3,3-diethoxyprop-1-yne and 1,1-dipropyl-2-propynylamine (Scheme 2, Table 3).

2-Bromobut-1-ene, 1-bromoprop-1-ene and 2-bromoprop-1-ene were efficiently cross-coupled with dec-1-yne using 0.1% catalyst (Table 2, entries 3, 7, 9). The best result with dec-1-yne was obtained using β-bromostyrene (TON of 9000) (Table 2, entry 1). Sterically congested substrates, bromotriphenylethylene (entry 2), and 2-bromo-3-methylbut-2-ene (Table 2, entry 4) led to the corresponding enynes **9** and **11** in good yields but 1% catalyst were used. The reactions performed with the enyne: 2-methylbut-1-en-3-yne were much slower. This might be due to the coordination of the olefinic bond to palladium. With β-bromostyrene and bromotriphenylethylene the reactions had to be performed using 1–2% catalyst in order to obtain satisfactory yields of adducts **14** and **15** (Table 2, entries 10 and 11).

Next, we studied the reactivity of functionalized alkynes (Table 3). Several reactions were performed using a range of alkyn-1-ol derivatives. With alkynols, relatively low TONs were observed in comparison to those obtained with phenylacetylene or dec-1-yne. In the presence of the most reactive vinyl bromide: β-bromostyrene, the coupling reaction with propargyl alcohol was successful using 0.1% catalyst (Table 3, entry 2). With bromotriphenylethylene 1% catalyst had to be used to obtain product **17** in 74% yield (Table 3, entry 3). A similar reactivity was obtained using the protected propargyl alcohol: 3-methoxyprop-1-yne (Table 3, entries 6–8). Using but-1-yn-3-ol and but-3-yn-1-ol, the reaction with β-bromostyrene or 2-bromobut-1-ene, gave the desired products **22**–**24** and **26** using 0.1–0.01% catalyst (Table 3, entries 9–13 and 16). Slower reactions were observed with bromotriphenylethylene, 3-bromobut-3-en-1-ol and 2-bromo-3-methyl-but-2-ene (Table 3, entries 14, 15, 17 and 18).

We also performed a few reactions with pent-4-yn-1-ol (Table 3, entries 19–26). Better yields were obtained using the same amount of catalyst than with but-3-yn-1-ol. β-Bromostyrene, 1-bromoprop-1-ene and 2-bromoprop-1-ene reacts in satisfactory yields with a ratio substrate/catalyst of 10,000 (Table 3, entries 20, 24 and 26). We had already observed with aryl bromides a similar trend for the reactivity of alkynols: pent-4-yn-1-ol > but-3-yn-1-ol > propargyl alcohol.[39] (*E*)-3-Methylpent-2-en-4-yn-1-ol reacts cleanly with β-bromostyrene or 2-bromobut-1-ene to give the corresponding dienyne derivatives **33** and **34** (Table 3, entries 27 and 28). The Tedicyp–palladium system also provides an efficient catalyst for the coupling of vinyl bromides with propiolaldehyde diethyl acetal (Table 3, entries 29–35). With this alkyne, a very high TON of 72,000 was obtained for the coupling with β-bromostyrene. Finally, we were pleased to observe that 1,1-dipropyl-2-propynylamine with one of the less reactive vinyl bromide: 2-bromo-3-methylbut-2-ene gave **39** with a high TON of 6300 (Table 3, entries 36–37).

In conclusion, in the presence of the Tedicyp–palladium catalyst, Sonogashira reactions of several vinyl bromides, including sterically demanding ones, with a wide variety of

Table 3. Cross-coupling reactions of vinyl bromides with alkynols, 3,3-diethoxyprop-1-yne and a propargylamine (Scheme 2)

Entry	Vinyl bromide	Alkyne	Ratio substrate/catalyst	Temperature (°C)	Ratio *Z* (**a**)/*E* (**b**)	Product number	Yield (%)[a]
1	β-Bromostyrene[b]	Propargyl alcohol	100	100	10/90	**16a**,**b**	80
2	β-Bromostyrene[b]	Propargyl alcohol	1000	100	10/90	**16a**,**b**	40[c]
3	Bromotriphenylethylene	Propargyl alcohol	100	100	—	**17**	74
4	2-Bromobut-1-ene	Propargyl alcohol	100	80	—	**18**	96
5	2-Bromo-3-methylbut-2-ene	Propargyl alcohol	50	80	—	**19**	98
6	β-Bromostyrene[b]	3-Methoxyprop-1-yne	100	100	10/90	**20a**,**b**	100[c]
7	β-Bromostyrene[b]	3-Methoxyprop-1-yne	1000	100	10/90	**20a**,**b**	75
8	Bromotriphenylethylene	3-Methoxyprop-1-yne	100	100	—	**21**	74
9	β-Bromostyrene[b]	But-1-yn-3-ol	1000	100	10/90	**22a**,**b**	98
10	2-Bromobut-1-ene	But-1-yn-3-ol	100	80	—	**23**	100[c]
11	2-Bromobut-1-ene	But-1-yn-3-ol	1000	80	—	**23**	56
12	β-Bromostyrene[b]	But-3-yn-1-ol	1000	100	10/90	**24a**,**b**	97
13	β-Bromostyrene[b]	But-3-yn-1-ol	10,000	100	10/90	**24a**,**b**	29[c]
14	Bromotriphenylethylene	But-3-yn-1-ol	100	100	—	**25**	78
15	Bromotriphenylethylene	But-3-yn-1-ol	1000	100	—	**25**	20[c]
16	2-Bromobut-1-ene	But-3-yn-1-ol	1000	80	—	**26**	83
17	3-Bromobut-3-en-1-ol	But-3-yn-1-ol	100	80	—	**27**	96
18	2-Bromo-3-methylbut-2-ene	But-3-yn-1-ol	100	80	—	**28**	85
19	β-Bromostyrene[b]	Pent-4-yn-1-ol	1000	100	10/90	**29a**,**b**	95
20	β-Bromostyrene[b]	Pent-4-yn-1-ol	10,000	100	10/90	**29a**,**b**	62[c]
21	Bromotriphenylethylene	Pent-4-yn-1-ol	100	100	—	**30**	98
22	Bromotriphenylethylene	Pent-4-yn-1-ol	1000	100	—	**30**	25[c]
23	1-Bromoprop-1-ene[d]	Pent-4-yn-1-ol	1000	60	40/60	**31a**,**b**	98
24	1-Bromoprop-1-ene[d]	Pent-4-yn-1-ol	10,000	60	04/96	**31a**,**b**	37[c]
25	2-Bromoprop-1-ene	Pent-4-yn-1-ol	1000	60	—	**32**	90
26	2-Bromoprop-1-ene	Pent-4-yn-1-ol	10,000	60	—	**32**	28[c]
27	β-Bromostyrene[b]	(*E*)-3-Methylpent-2-en-4-yn-1-ol	500	100	10/90	**33a**,**b**	88
28	2-Bromobut-1-ene	(*E*)-3-Methylpent-2-en-4-yn-1-ol	100	80	—	**34**	80
29	β-Bromostyrene[b]	3,3-Diethoxyprop-1-yne	100,000	100	10/90	**35a**,**b**	72
30	Bromotriphenylethylene	3,3-Diethoxyprop-1-yne	100	100	—	**36**	95
31	Bromotriphenylethylene	3,3-Diethoxyprop-1-yne	1000	100	—	**36**	22[c]
32	2-Bromobut-1-ene	3,3-Diethoxyprop-1-yne	100	80	—	**37**	100[c]
33	2-Bromobut-1-ene	3,3-Diethoxyprop-1-yne	1000	80	—	**37**	70
34	2-Bromoprop-1-ene	3,3-Diethoxyprop-1-yne	100	60	—	**38**	100[c]
35	2-Bromoprop-1-ene	3,3-Diethoxyprop-1-yne	1000	60	—	**38**	68
36	2-Bromo-3-methylbut-2-ene	1,1-Dipropyl-2-propynylamine	1000	80	—	**39**	100[c]
37	2-Bromo-3-methylbut-2-ene	1,1-Dipropyl-2-propynylamine	10,000	80	—	**39**	63

[a] Conditions: catalyst: $[ClPd(C_3H_5)]_2$/Tedicyp=1:2, vinyl bromide (1 equiv), alkyne (2 equiv), K_2CO_3 (2 equiv), CuI (0.05 equiv), DMF, 20 h.
[b] β-Bromostyrene was a mixture of *Z* and *E* isomers, ratio *Z*/*E*: 10/90.
[c] GC and NMR yield.
[d] 1-Bromoprop-1-ene was a mixture of *Z* and *E* isomers, ratio *Z*/*E*: 45/55.

terminal alkynes can be performed with as little as 0.1–0.0001% with the most reactive vinyl bromides and several alkynes. The highest TONs were obtained with phenylacetylene, dec-1-yne, but-3-yn-1-ol, pent-4-yn-1-ol, 3,3-diethoxyprop-1-yne or 1,1-dipropyl-2-propynylamine. The reactions performed with propargyl alcohol, 3-methoxyprop-1-yne or 2-methylbut-1-en-3-yne required larger amounts of catalyst. The most reactive vinyl bromide was β-bromostyrene, but α-substituted vinyl bromides such as 1-bromoprop-1-ene or 2-bromobut-1-ene also gave the coupling adducts in high TONs. Furthermore, this catalyst can be used at low loading even for the reactions of sterically hindered vinyl bromides such as bromotriphenylethylene or 2-bromo-3-methylbut-2-ene. We believe that this system compares favourably with other catalysts that have been reported for this process.

3. Experimental

General remarks. All reactions were run under argon in Schlenk tubes using vacuum lines. DMF analytical grade was not distilled before use. Commercial potassium carbonate (99+), CuI (98%), vinyl bromides and terminal alkynes were used without purification. The reactions were followed by GC and NMR for high boiling point substrates and by GC for low boiling point substrates. ^1H and ^{13}C spectrum were recorded with a Bruker 300 MHz spectrometer in $CDCl_3$ solutions. GC/MS were recorded with a Varian Saturn 2100T spectrometer. Chemical shift are reported in ppm relative to $CDCl_3$ (7.25 for ^1H NMR and 77.0 for ^{13}C NMR). Flash chromatographies were performed on silica gel (230–400 mesh). GC and NMR yields in the tables are conversions of the vinyl halides into the product calculated with GC and ^1H NMR spectrum of the crude mixtures.

3.1. Preparation of the Pd–Tedicyp catalyst

An oven-dried 40 mL Schlenk tube equipped with a magnetic stirring bar under argon atmosphere, was charged with $[Pd(C_3H_5)Cl]_2$ (30 mg, 81 mmol) and Tedicyp (140 mg, 162 mmol). Ten millilitre of anhydrous DMF were added, then the solution was stirred at room temperature for 10 min. The appropriate catalyst concentration was obtained by successive dilutions. ^{31}P NMR (162 MHz, $CDCl_3$) δ 25 (w=80 Hz), 19.4 (w=110 Hz).

3.2. General procedure

In a typical experiment, the vinyl halide (1 mmol), terminal alkynes (2 mmol), CuI (0.05 mmol, 0.01 g) and K_2CO_3 (0.276 g, 2 mmol) were dissolved in DMF (3 mL) under an argon atmosphere. The prepared Pd–Tedicyp catalyst complex (see tables) was then transferred to the reaction flask via cannula. The reaction mixture was stirred at the appropriate temperature for 20 h. Then, the solution was diluted with H_2O (2 mL), and the product was extracted three times with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by silica gel column chromatography.

3.2.1. (*Z*)-1,4-Diphenylbut-1-en-3-yne (1a) and (*E*)-1,4-diphenylbut-1-en-3-yne (1b). From β-bromostyrene (0.128 mL, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), products **1a/1b** (10/90) were obtained in 52% (0.106 g) yield. *Compound* **1a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.43 (m, 2H), 7.35–7.32 (m, 2H), 7.28–7.20 (m, 6H), 6.62 (d, J=12.0 Hz, 1H), 5.87 (d, J=12.0 Hz, 1H). *Compound* **1b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.43 (m, 2H), 7.35–7.32 (m, 2H), 7.28–7.20 (m, 6H), 7.03 (d, J=16.2 Hz, 1H), 6.36 (d, J=16.2 Hz, 1H).

3.2.2. (*Z*)-1-Phenylpent-3-en-1-yne (2a) and (*E*)-1-phenylpent-3-en-1-yne (2b). From 1-bromoprop-1-ene (0.085 mL, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), products **2a/2b** (05/95) were obtained in 85% (0.121 g) yield. *Compound* **2a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.40 (m, 2H), 7.36–7.30 (m, 3H), 6.05 (dq, J=10.6, 6.8 Hz, 1H), 5.71 (dq, J=10.6, 1.7 Hz, 1H), 1.98 (dd, J=6.8, 1.7 Hz, 3H). *Compound* **2b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.40 (m, 2H), 7.33–7.28 (m, 3H), 6.25 (dq, J=15.8, 6.8 Hz, 1H), 5.71 (dq, J=15.8, 1.7 Hz, 1H), 1.84 (dd, J=6.8, 1.7 Hz, 3H).

3.2.3. 3-Methyl-1-phenylbut-3-en-1-yne (3). From 2-bromoprop-1-ene (0.088 mL, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), product **3** was obtained in 75% (0.107 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48–7.46 (m, 2H), 7.33–7.28 (m, 3H), 5.43 (s, 1H), 5.33 (s, 1H), 2.02 (s, 3H).

3.2.4. 2-Ethyl-4-phenylbut-1-en-3-yne (4). From 2-bromobut-1-ene (0.102 mL, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), product **4** was obtained in 48% (0.075 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.42 (m, 2H), 7.34–7.28 (m, 3H), 5.38 (s, 1H), 5.28 (s, 1H), 2.27 (q, J=7.1 Hz, 2H), 1.15 (t, J=7.1 Hz, 3H).

3.2.5. 3-Methylene-5-phenylpent-4-yn-1-ol (5). From 3-bromobut-3-en-1-ol (0.099 mL, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), product **5** was obtained in 85% (0.146 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.40 (m, 2H), 7.32–7.28 (m, 3H), 5.44 (s, 1H), 5.33 (s, 1H), 3.85 (t, J=6.2 Hz, 2H), 2.44 (t, J=6.2 Hz, 2H).

3.2.6. 2,3-Dimethyl-5-phenylpent-2-en-4-yne (6). From 2-bromo-3-methylbut-2-ene (0.151 mL, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), product **6** was obtained in 60% (0.102 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.40 (m, 2H), 7.34–7.28 (m, 3H), 2.00 (s, 3H), 1.87 (s, 3H), 1.77 (s, 3H).

3.2.7. 1,1,2,4-Tetraphenylbut-1-en-3-yne (7). From bromotriphenylethylene (0.335 g, 1 mmol) and phenylacetylene (0.220 mL, 2 mmol), product **7** was obtained in 86% (0.307 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.65 (m, 2H), 7.45–7.40 (m, 6H), 7.31–7.16 (m, 10H), 7.13–7.11 (m, 2H).

3.2.8. (*Z*)-1-Phenyldodec-1-en-3-yne (8a) and (*E*)-1-phenyldodec-1-en-3-yne (8b). From β-bromostyrene (0.128 mL, 1 mmol) and dec-1-yne (0.361 mL, 2 mmol), products **8a/8b** (10/90) were obtained in 90% (0.216 g) yield. *Compound* **8a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.30 (m, 5H), 6.61 (d, J=12.1 Hz, 1H), 5.88 (d, J=12.1 Hz, 1H), 2.40 (t, J=6.9 Hz, 2H), 1.64–1.55 (m, 2H), 1.46–1.32 (m, 10H), 0.90–0.87 (m, 3H). *Compound* **8b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.30 (m, 5H), 6.90 (d, J=16.2 Hz, 1H), 6.18 (d, J=16.2 Hz, 1H), 2.40 (t, J=6.9 Hz, 2H), 1.64–1.55 (m, 2H), 1.46–1.32 (m, 10H), 0.90–0.87 (m, 3H).

3.2.9. 1,1,2-Triphenyldodec-1-en-3-yne (9). From bromotriphenylethylene (0.335 g, 1 mmol) and dec-1-yne (0.361 mL, 2 mmol), product **9** was obtained in 97% (0.381 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.42 (m, 2H), 7.26–7.20 (m, 5H), 7.10–7.00 (m, 6H), 6.91 (m, 2H), 2.19 (t, J=6.8 Hz, 2H), 1.38–1.19 (m, 12H), 0.87–0.83 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0, 142.8, 141.4, 139.6, 130.6, 130.0, 128.4, 127.9, 127.8, 127.7, 127.3, 127.1, 126.8, 121.5, 91.1, 78.9, 32.5, 30.1, 29.4, 29.2, 29.2, 23.1, 18.2, 14.0; MS (70 eV); *m/z* (%) 392 ($M^{+\cdot}$, 100); $C_{30}H_{32}$: calcd C 91.78, H 8.22. Found C 91.89, H 8.31.

3.2.10. 2-Ethyldodec-1-en-3-yne (10). From 2-bromobut-1-ene (0.102 mL, 1 mmol) and dec-1-yne (0.361 mL, 2 mmol), product **10** was obtained in 99% (0.190 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.19 (s, 1H), 5.13 (s, 1H), 2.30 (t, J=6.8 Hz, 2H), 2.16 (q, J=7.1 Hz, 2H), 1.55–1.50 (m, 2H), 1.42–1.39 (m, 2H), 1.30 (br s, 8H), 1.08 (t, J=7.1 Hz, 3H), 0.88 (t, J=6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.8, 118.3, 90.2, 80.9, 31.8, 30.7, 29.2, 29.1, 28.8, 28.8, 22.6, 19.7, 14.1, 12.8; MS (70 eV); *m/z* (%) 192 ($M^{+\cdot}$, 1), 79 (100); $C_{14}H_{24}$: calcd C 87.42, H 12.58. Found C 87.28, H 12.34.

3.2.11. 2,3-Dimethyltridec-2-en-4-yne (11). From 2-bromo-3-methyl-but-2-ene (0.151 mL, 1 mmol) and dec-1-yne (0.361 mL, 2 mmol), product **11** was obtained in 89% (0.183 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (t, J=6.8 Hz, 2H), 1.90 (s, 3H), 1.77 (s, 3H), 1.70 (s, 3H), 1.55–1.50 (m, 2H), 1.42–1.39 (m, 2H), 1.26 (br s, 8H), 0.87 (t, J=6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 111.9, 91.3, 82.3, 31.8, 29.2, 29.1, 29.1, 28.9, 23.3, 22.6, 19.7, 19.5, 18.9, 14.1; MS (70 eV); *m/z* (%) 206 ($M^{+\cdot}$, 100), 107 (100); $C_{15}H_{26}$: calcd C 87.30, H 12.70. Found C 87.20, H 12.67.

3.2.12. (*Z*)-Tridecen-4-yne (12a) and (*E*)-tridecen-4-yne (12b). From 1-bromoprop-1-ene (0.085 mL, 1 mmol) and dec-1-yne (0.361 mL, 2 mmol), products **12a/12b** (05/95)

were obtained in 65% (0.116 g) yield. *Compound* **12a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.86 (dq, J=10.4, 6.6 Hz, 1H), 5.35 (dq, J=10.4, 1.7 Hz, 1H), 2.25 (t, J=6.7 Hz, 2H), 1.87 (dd, J=6.6, 1.7 Hz, 3H), 1.55–1.50 (m, 2H), 1.42–1.39 (m, 2H), 1.30 (br s, 8H), 0.88 (t, J=6.8 Hz, 3H). *Compound* **12b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.02 (dq, J=15.7, 6.8 Hz, 1H), 5.45 (dq, J=15.7, 1.7 Hz, 1H), 2.25 (t, J=6.7 Hz, 2H), 1.73 (dd, J=6.8, 1.7 Hz, 3H), 1.55–1.50 (m, 2H), 1.42–1.39 (m, 2H), 1.30 (br s, 8H), 0.88 (t, J=6.8 Hz, 3H).

3.2.13. 2-Methyldodec-1-en-3-yne (13). From 2-bromoprop-1-ene (0.088 mL, 1 mmol) and dec-1-yne (0.361 mL, 2 mmol), product **13** was obtained in 51% (0.091 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.18 (s, 1H), 5.11 (s, 1H), 2.28 (t, J=6.8 Hz, 2H), 1.86 (s, 3H), 1.54–1.47 (m, 2H), 1.40–1.27 (m, 10H), 0.88 (t, J=6.6 Hz, 3H).

3.2.14. (*Z*)-5-Methyl-1-phenyl-1,5-hexadien-3-yne (14a) and (*E*)-5-methyl-1-phenyl-1,5-hexadien-3-yne (14b). From β-bromostyrene (0.128 mL, 1 mmol) and 2-methylbut-1-en-3-yne (0.190 mL, 2 mmol), products **14a/14b** (10/90) were obtained in 59% (0.099 g) yield. *Compound* **14a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.28 (m, 5H), 6.68 (d, J=12.1 Hz, 1H), 5.86 (d, J=12.1 Hz, 1H), 5.40 (s, 1H), 5.34 (s, 1H), 2.03 (s, 3H). **14b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.28 (m, 5H), 6.99 (d, J=16.2 Hz, 1H), 6.33 (d, J=16.2 Hz, 1H), 5.40 (s, 1H), 5.31 (s, 1H), 2.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.1, 136.3, 128.7, 128.5, 128.1, 126.2, 121.8, 108.1, 92.9, 87.9, 23.4; $C_{13}H_{12}$: calcd C 92.81, H 7.19. Found C 92.68, H 7.30.

3.2.15. 5-Methyl-1,1,2-triphenylhexa-1,5-dien-3-yne (15). From bromotriphenylethylene (0.335 g, 1 mmol) and 2-methylbut-1-en-3-yne (0.190 mL, 2 mmol), product **15** was obtained in 60% (0.192 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.42 (m, 2H), 7.26–7.20 (m, 5H), 7.10–7.00 (m, 6H), 6.91 (m, 2H), 5.19 (s, 1H), 5.17 (s, 1H), 1.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0, 142.8, 141.4, 139.6, 130.6, 131.7, 130.0, 128.4, 128.0, 127.8, 127.7, 127.3, 127.1, 126.8, 121.8, 121.5, 91.2, 90.9, 22.1; MS (70 eV); *m*/*z* (%): 320 ($M^{+\cdot}$, 100); $C_{25}H_{20}$: calcd C 93.71, H 6.29. Found C 93.54, H 6.38.

3.2.16. (*Z*)-5-Phenylpent-4-en-2-yn-1-ol (16a) and (*E*)-5-phenylpent-4-en-2-yn-1-ol (16b). From β-bromostyrene (0.128 mL, 1 mmol) and propargyl alcohol (0.116 mL, 2 mmol), products **16a/16b** (10/90) were obtained in 80% (0.126 g) yield. *Compound* **16a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.26 (m, 5H), 6.60 (d, J=12.1 Hz, 1H), 5.86 (d, J=12.1 Hz, 1H), 4.44 (s, 2H), 2.25 (s, 1H). *Compound* **16b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.26 (m, 5H), 6.96 (d, J=16.5 Hz, 1H), 6.17 (d, J=16.5 Hz, 1H), 4.44 (s, 2H), 2.25 (s, 1H).

3.2.17. 1,1,2-Triphenylpent-1-en-3-yn-5-ol (17). From bromotriphenylethylene (0.335 g, 1 mmol) and propargyl alcohol (0.116 mL, 2 mmol), product **17** was obtained in 74% (0.230 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53–7.50 (m, 2H), 7.34–7.30 (m, 5H), 7.16–7.10 (m, 6H), 7.01–6.99 (m, 2H), 4.45 (s, 2H), 2.24 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6, 142.9, 141.3, 139.6, 130.5, 130.1, 128.4, 128.0, 127.8, 127.7, 127.3, 127.1, 126.8, 121.5, 91.1, 84.7, 51.0; MS (70 eV); *m*/*z* (%) 310 ($M^{+\cdot}$, 100); $C_{23}H_{18}O$: calcd C 89.00, H 5.85. Found C 88.90, H 5.62.

3.2.18. 4-Ethylpent-4-en-2-yn-1-ol (18). From 2-bromobut-1-ene (0.102 mL, 1 mmol) and propargyl alcohol (0.116 mL, 2 mmol), product **18** was obtained in 96% (0.106 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (s, 1H), 5.21 (s, 1H), 4.36 (s, 2H), 2.14 (q, J=7.5 Hz, 2H), 1.06 (t, J=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.6, 120.3, 87.0, 85.6, 51.3, 30.1, 12.7; $C_7H_{10}O$: calcd C 76.33, H 9.15. Found C 76.52, H 9.27.

3.2.19. 4,5-Dimethylhex-4-en-2-yn-1-ol (19). From 2-bromo-3-methylbut-2-ene (0.151 mL, 1 mmol) and propargyl alcohol (0.116 mL, 2 mmol), product **19** was obtained in 98% (0.122 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) 4.42 (s, 2H), 1.92 (s, 3H), 1.79 (s, 3H), 1.74 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.8, 111.0, 88.3, 87.6, 51.8, 23.5, 19.9, 18.5.

3.2.20. (*Z*)-1-Methoxy-5-phenylpent-4-en-2-yne (20a) and (*E*)-1-methoxy-5-phenylpent-4-en-2-yne (20b). From β-bromostyrene (0.128 mL, 1 mmol) and 3-methoxyprop-1-yne (0.169 mL, 2 mmol), products **20a/20b** (10/90) were obtained in 75% (0.129 g) yield. *Compound* **20a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.26 (m, 5H), 6.60 (d, J=12.1 Hz, 1H), 5.86 (d, J=12.1 Hz, 1H), 4.00 (s, 2H), 3.10 (s, 3H). *Compound* **20b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.26 (m, 5H), 6.96 (d, J=16.5 Hz, 1H), 6.17 (d, J=16.5 Hz, 1H), 4.00 (s, 2H), 3.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.1, 136.1, 128.8, 128.3, 126.2, 111.3, 91.1, 84.7, 61.0, 52.8; MS (70 eV); *m*/*z* (%): 172 ($M^{+\cdot}$, 65), 128 (100); $C_{12}H_{12}O$: calcd C 83.69, H 7.02. Found C 83.91, H 7.14.

3.2.21. 5-Methoxy-1,1,2-triphenylpent-1-en-3-yne (21). From bromotriphenylethylene (0.335 g, 1 mmol) and 3-methoxyprop-1-yne (0.169 mL, 2 mmol), product **21** was obtained in 74% (0.240 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53–7.50 (m, 2H), 7.34–7.30 (m, 5H), 7.16–7.10 (m, 6H), 7.01–6.99 (m, 2H), 4.15 (s, 2H), 3.19 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0, 142.8, 141.4, 139.6, 130.6, 130.0, 128.4, 128.0, 127.8, 127.7, 127.3, 127.1, 126.8, 121.5, 91.1, 85.6, 60.3, 52.8; MS (70 eV); *m*/*z* (%): 324 ($M^{+\cdot}$, 100); $C_{24}H_{20}O$: calcd C 88.85, H 6.21. Found C 88.72, H 6.10.

3.2.22. (*Z*)-1-Phenylhex-1-en-3-yn-5-ol (22a) and (*E*)-1-phenylhex-1-en-3-yn-5-ol (22b). From β-bromostyrene (0.128 mL, 1 mmol) and but-1-yn-3-ol (0.157 mL, 2 mmol), products **22a/22b** (10/90) were obtained in 98% (0.168 g) yield. *Compound* **22a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.29 (m, 5H), 6.69 (d, J=12.1 Hz, 1H), 5.72 (d, J=12.1 Hz, 1H), 4.70 (q, J=6.4 Hz, 1H), 2.06 (s, 1H), 1.50 (d, J=6.4 Hz, 3H). *Compound* **22b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.29 (m, 5H), 6.94 (d, J=16.1 Hz, 1H), 6.15 (d, J=16.1 Hz, 1H), 4.70 (q, J=6.4 Hz, 1H), 2.06 (s, 1H), 1.50 (d, J=6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.7, 136.1, 128.7, 128.6, 126.2, 107.4, 93.1, 83.2, 58.9, 24.3; $C_{12}H_{12}O$: calcd C 83.69, H 7.02. Found C 83.87, H 7.18.

3.2.23. 2-Ethylhex-1-en-3-yn-5-ol (23). From 2-bromobut-1-ene (0.102 mL, 1 mmol) and but-1-yn-3-ol (0.157 mL, 2 mmol), product **23** was obtained in 56% (0.069 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (s, 1H), 5.22 (s, 1H), 4.65 (q, J=6.4 Hz, 1H), 2.14 (q, J=7.5 Hz, 2H), 1.88 (s, 1H), 1.47 (d, J=6.4 Hz, 3H), 1.07 (t, J=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.6, 120.2, 90.7, 84.4, 58.8, 30.2, 24.4, 12.7; $C_8H_{12}O$: calcd C 77.38, H 9.74. Found C 77.54, H 9.57.

3.2.24. (*Z*)-1-Phenylhex-1-en-3-yn-5-ol (24a) and (*E*)-1-phenylhex-1-en-3-yn-5-ol (24b). From β-bromostyrene (0.128 mL, 1 mmol) and but-3-yn-1-ol (0.151 mL, 2 mmol), products **24a/24b** (10/90) were obtained in 97% (0.167 g) yield. *Compound* **24a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.24 (m, 5H), 6.62 (d, J=12.0 Hz, 1H), 5.87 (d, J=12.0 Hz, 1H), 3.76 (t, J=6.3 Hz, 2H), 2.64 (t, J=6.3 Hz, 2H), 2.44 (s, 1H). **24b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.24 (m, 5H), 6.90 (d, J=16.4 Hz, 1H), 6.14 (d, J=16.4 Hz, 1H), 3.76 (t, J=6.3 Hz, 2H), 2.64 (t, J=6.3 Hz, 2H), 2.44 (s, 1H).

3.2.25. 1,1,2-Triphenylhex-1-en-3-yn-5-ol (25). From bromotriphenylethylene (0.335 g, 1 mmol) and but-3-yn-1-ol (0.151 mL, 2 mmol), product **25** was obtained in 78% (0.253 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.37 (m, 2H), 7.35–7.28 (m, 5H), 7.17–7.10 (m, 6H), 6.98 (m, 2H), 3.55 (t, J=5.8 Hz, 2H), 2.50 (t, J=5.8 Hz, 2H), 1.50 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0, 142.8, 141.4, 139.6, 130.6, 130.0, 129.4, 128.0, 127.8, 127.7, 127.3, 127.1, 126.8, 121.5, 93.5, 83.4, 61.8, 21.2; MS (70 eV); *m/z* (%): 324 ($M^{+\cdot}$, 100), 293 (10), 215 (14); $C_{24}H_{20}O$: calcd C 88.85, H 6.21. Found C 88.74, H 6.35.

3.2.26. 2-Ethylhex-1-en-3-yn-5-ol (26). From 2-bromobut-1-ene (0.102 mL, 1 mmol) and but-3-yn-1-ol (0.151 mL, 2 mmol), product **26** was obtained in 83% (0.103 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.22 (s, 1H), 5.16 (s, 1H), 3.71 (t, J=6.2 Hz, 2H), 2.58 (t, J=6.2 Hz, 2H), 2.12 (q, J=7.1 Hz, 2H), 1.06 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.1, 119.4, 85.9, 82.9, 61.1, 30.5, 23.7, 12.8; MS (70 eV); *m/z* (%): 124 ($M^{+\cdot}$, 69), 79 (100); $C_8H_{12}O$: calcd C 77.38, H 9.74. Found C 77.50, H 9.70.

3.2.27. 5-Methylenhept-3-yn-1,7-diol (27). From 3-bromobut-3-en-1-ol (0.099 mL, 1 mmol) and but-3-yn-1-ol (0.151 mL, 2 mmol), product **27** was obtained in 96% (0.134 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (s, 1H), 5.23 (s, 1H), 3.75 (t, J=6.3 Hz, 2H), 3.70 (t, J=6.2 Hz, 2H), 2.53 (t, J=6.3 Hz, 2H), 2.35 (t, J=6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.6, 123.4, 87.7, 82.5, 61.4, 61.4, 40.9, 24.0; MS (70 eV); *m/z* (%): 140 ($M^{+\cdot}$, 3), 91 (100); $C_8H_{12}O_2$: calcd C 68.54, H 8.63. Found C 68.61, H 8.80.

3.2.28. 2,3-Dimethylhept-2-en-4-yn-7-ol (28). From 2-bromo-3-methylbut-2-ene (0.151 mL, 1 mmol) and but-3-yn-1-ol (0.151 mL, 2 mmol), product **28** was obtained in 85% (0.117 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75 (t, J=6.2 Hz, 2H), 2.69 (t, J=6.2 Hz, 2H), 1.89 (s, 3H), 1.76 (s, 3H), 1.70 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.2, 111.4, 86.9, 84.4, 61.4, 23.9, 23.4, 19.7, 18.8; MS (70 eV); *m/z* (%): 138 ($M^{+\cdot}$, 100); $C_9H_{14}O$: calcd C 78.21, H 10.21. Found C 78.04, H 10.34.

3.2.29. (*Z*)-1-Phenylhept-1-en-3-yn-7-ol (29a) and (*E*)-1-phenylhept-1-en-3-yn-7-ol (29b). From β-bromostyrene (0.128 mL, 1 mmol) and pent-4-yn-1-ol (1.86 mL, 20 mmol), products **29a/29b** (10/90) were obtained in 95% (0.177 g) yield. *Compound* **29a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 6.65 (d, J=11.9 Hz, 1H), 5.87 (d, J=11.9 Hz, 1H), 3.76 (t, J=6.4 Hz, 2H), 2.26 (t, J=6.3 Hz, 2H), 1.86 (tt, J=6.4, 6.3 Hz, 2H). *Compound* **29b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 6.90 (d, J=16.2 Hz, 1H), 6.14 (d, J=16.2 Hz, 1H), 3.76 (t, J=6.4 Hz, 2H), 2.26 (t, J=6.3 Hz, 2H), 1.86 (tt, J=6.4, 6.3 Hz, 2H).

3.2.30. 1,1,2-Triphenylhept-1-en-3-yn-7-ol (30). From bromotriphenylethylene (0.335 g, 1 mmol) and pent-4-yn-1-ol (1.86 mL, 20 mmol), product **30** was obtained in 98% (0.331 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.47 (m, 2H), 7.34–7.26 (m, 5H), 7.13–7.09 (m, 6H), 6.98 (m, 2H), 3.75 (t, J=6.4 Hz, 2H), 2.26 (t, J=6.3 Hz, 2H), 1.86 (tt, J=6.4, 6.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.7, 143.0, 141.4, 139.7, 130.9, 130.2, 129.8, 127.8, 127.7, 127.6, 127.4, 127.0, 126.8, 121.8, 93.3, 83.5, 61.4, 30.8, 16.1; MS (70 eV); *m/z* (%): 338 ($M^{+\cdot}$, 100); $C_{25}H_{22}O$: calcd C 88.72, H 6.55. Found C 88.87, H 6.41.

3.2.31. (*Z*)-Oct-6-en-4-yn-1-ol (31a) and (*E*)-oct-6-en-4-yn-1-ol (31b). From 1-bromoprop-1-ene (0.085 mL, 1 mmol) and pent-4-yn-1-ol (1.86 mL, 20 mmol), products **31a/31b** (40/60) were obtained in 98% (0.122 g) yield. *Compound* **31a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.88 (dq, J=10.8, 6.8 Hz, 1H), 5.42 (dtq, J=10.8, 1.9, 1.7 Hz, 1H), 3.75 (t, J=6.2 Hz, 2H), 2.46 (td, J=6.9, 1.9 Hz, 2H), 1.75 (m, 5H). *Compound* **31b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (dq, J=15.9, 6.8 Hz, 1H), 5.44 (dtq, J=15.9, 2.1, 1.7 Hz, 1H), 3.75 (t, J=6.2 Hz, 2H), 2.46 (td, J=6.8, 2.1 Hz, 2H), 1.82 (m, 5H).

3.2.32. 2-Methylhept-1-en-3-yn-7-ol (32). From 2-bromoprop-1-ene (0.088 mL, 1 mmol) and pent-4-yn-1-ol (1.86 mL, 20 mmol), product **32** was obtained in 90% (0.112 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.20 (s, 1H), 5.14 (s, 1H), 3.76 (t, J=6.3 Hz, 2H), 2.43 (t, J=7.0 Hz, 2H), 1.86 (s, 3H), 1.76 (tt, J=7.0, 6.3 Hz, 2H).

3.2.33. (*E,Z*)-3-Methyl-7-phenylhepta-2,6-dien-4-yn-1-ol (33a) and (*E,E*)-3-methyl-7-phenylhepta-2,6-dien-4-yn-1-ol (33b). From β-bromostyrene (0.128 mL, 1 mmol) and (*E*)-3-methylpent-2-en-4-yn-1-ol (0.192 g, 2 mmol), product **33a/33b** (10/90) were obtained in 88% (0.175 g) yield. *Compound* **33a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.24 (m, 5H), 6.63 (d, J=11.8 Hz, 1H), 6.04 (tq, J=6.8, 1.3 Hz, 1H), 5.80 (d, J=11.8 Hz, 1H), 4.24 (d, J=6.8 Hz, 2H), 1.88 (d, J=1.3 Hz, 3H). *Compound* **33b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.24 (m, 5H), 6.93 (d, J=16.0 Hz, 1H), 6.27 (d, J=16.0 Hz, 1H), 6.04 (tq, J=6.8, 1.3 Hz, 1H), 4.24 (d, J=6.8 Hz, 2H), 1.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.0, 136.2, 135.5, 128.7, 128.5, 126.2, 120.8, 108.0, 93.9, 87.2, 59.0, 17.5; $C_{14}H_{14}O$: calcd C 84.81, H 7.12. Found C 84.70, H 7.07.

3.2.34. (*E*)-6-Ethyl-3-methylhepta-2,6-dien-4-yn-1-ol (34). From 2-bromobut-1-ene (0.102 mL, 1 mmol) and (*E*)-3-methylpent-2-en-4-yn-1-ol (0.192 g, 2 mmol), product **34** was obtained in 80% (0.120 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.97 (tq, J=6.8, 1.3 Hz, 1H), 5.26 (s, 1H), 5.20 (s, 1H), 4.20 (d, J=6.8 Hz, 2H), 2.18 (q, J=7.6 Hz, 2H), 1.84 (d, J=1.3 Hz, 3H), 1.08 (t, J=7.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.2, 133.1, 120.8, 119.8, 91.3, 88.1, 59.1, 30.3, 17.5, 12.8; $C_{10}H_{14}O$: calcd C 79.96, H 9.39. Found C 79.87, H 9.50.

3.2.35. (*Z*)-5,5-Diethoxy-1-phenylpent-1-en-3-yne (35a) and (*E*)-5,5-diethoxy-1-phenylpent-1-en-3-yne (35b). From β-bromostyrene (0.128 mL, 1 mmol) and 3,3-diethoxyprop-1-yne (0.287 mL, 2 mmol), products **35a/35b** (10/90) were obtained in 72% (0.166 g) yield. *Compound* **35a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.24 (m, 5H), 6.69 (d, J=12.1 Hz, 1H), 5.80 (d, J=12.1 Hz, 1H), 5.29 (s, 1H), 3.80 (m, 2H), 3.65 (m, 2H), 1.25 (dd, J=7.2, 7.0 Hz, 6H). *Compound* **35b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.29 (m, 5H), 7.01 (d, J=16.2 Hz, 1H), 6.17 (d, J=16.2 Hz, 1H), 5.48 (s, 1H), 3.76 (dq, J=9.5, 7.0 Hz, 2H), 3.61 (dq, J=9.5, 7.2 Hz, 2H), 1.25 (dd, J=7.2, 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.7, 135.8, 128.8, 128.6, 126.3, 106.7, 91.7, 86.3, 84.4, 60.8, 15.0; $C_{15}H_{18}O_2$: calcd C 78.23, H 7.88. Found C 78.46, H 7.91.

3.2.36. 5,5-Diethoxy-1,1,2-triphenylpent-1-en-3-yne (36). From bromotriphenylethylene (0.335 g, 1 mmol) and 3,3-diethoxyprop-1-yne (0.287 mL, 2 mmol), product **36** was obtained in 95% (0.363 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.44 (m, 2H), 7.33–7.30 (m, 2H), 7.29–7.26 (m, 3H), 7.16–7.08 (m, 6H), 7.03–6.99 (m, 2H), 5.30 (s, 1H), 3.48 (m, 4H), 1.15 (dd, J=7.2, 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.8, 143.0, 141.5, 139.5, 131.3, 130.6, 130.4, 128.3, 128.2, 128.2, 128.1, 127.8, 127.5, 120.9, 92.2, 88.3, 87.7, 61.1, 15.5; $C_{27}H_{26}O_2$: calcd C 84.78, H 6.85. Found C 84.61, H 7.01.

3.2.37. 5,5-Diethoxy-2-ethylpent-1-en-3-yne (37). From 2-bromo-but-1-ene (0.102 mL, 1 mmol) and 3,3-diethoxyprop-1-yne (0.287 mL, 2 mmol), product **37** was obtained in 70% (0.127 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (s, 1H), 5.34 (s, 1H), 5.25 (s, 1H), 3.73 (dq, J=9.4, 7.2 Hz, 2H), 3.58 (dq, J=9.4, 7.0 Hz, 2H), 2.16 (q, J=7.5 Hz, 2H), 1.22 (dd, J=7.2, 7.0 Hz, 6H), 1.06 (t, J=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.0, 121.4, 91.6, 85.6, 84.0, 60.7, 29.9, 15.0, 12.6; $C_{11}H_{18}O_2$: calcd C 72.49, H 9.95. Found C 72.40, H 9.82.

3.2.38. 5,5-Diethoxy-2-methylpent-1-en-3-yne (38). From 2-bromoprop-1-ene (0.088 mL, 1 mmol) and 3,3-diethoxyprop-1-yne (0.287 mL, 2 mmol), product **38** was obtained in 68% (0.114 g) yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.37 (s, 1H), 5.36 (s, 1H), 5.27 (s, 1H), 3.75 (dq, J=9.6, 7.2 Hz, 2H), 3.58 (dq, J=9.6, 7.0 Hz, 2H), 1.89 (s, 3H), 1.24 (dd, J=7.2, 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.7, 123.3, 91.8, 86.4, 83.3, 60.8, 23.1, 15.1.

3.2.39. (2,3-Dimethylhex-2-en-4-yn-6-yl)dipropylamine (39). From 2-bromo-3-methylbut-2-ene (0.151 mL, 1 mmol) and 1,1-dipropyl-2-propynylamine (0.349 mL, 1 mmol) product **39** was obtained in 63% (0.131 g) yield. Thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.55 (s, 2H), 2.43 (t, J=7.6 Hz, 4H), 1.92 (s, 3H), 1.79 (s, 3H), 1.72 (s, 3H), 1.48 (tq, J=7.6, 7.3 Hz, 4H), 0.89 (t, J=7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.9, 111.6, 86.7, 85.4, 55.8, 42.6, 23.5, 20.7, 19.7, 18.9, 11.9; MS (70 eV); *m/z* (%): 208 ($M^{+\cdot}$, 18), 178 (100); $C_{14}H_{25}N$: calcd C 81.09, H 12.15. Found C 80.90, H 12.28.

Registry No.: **1a**, 13343-78-7; **1b**, 13343-79-8; **2**, 21979-82-8; **3**, 10469-89-3; **4**, 732284-08-1; **5**, 124475-73-6; **6a**, 31552-04-2; **6b**, 31552-03-1; **7**, 1463-04-3; **8a**, 845749-45-3; **8b**, 220185-68-2; **12a**, 171781-58-1; **12b**, 243870-53-3; **13**, 71313-54-7; **16a**, 374897-65-1; **16b**, 103606-73-1; **19**, 6822-09-9; **20a**, 845749-46-4; **22a**, 180787-32-0; **24**, 53081-56-4; **29a**, 114092-63-6; **29b**, 114092-61-4; **31**, 258819-00-0; **32**, 69719-47-7; **38**, 32365-41-6.

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Synthesis of novel 1-methyl-1*H*-pyridazino[3,4-*b*]indoles

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Cordially dedicated to Professor András Lipták on the occasion of his 70th birthday.

Abstract—New synthetic pathways have been elaborated to 1-methyl-1*H*-pyridazino[3,4-*b*]indoles starting from halopyridazin-3(2*H*)-ones. Suzuki cross-coupling reaction of chloro, iodo, dichloro, and dibromo substituted pyridazin-3(2*H*)-ones with 2-pivaloylaminophenylboronic acid followed by hydrolysis of the amide and subsequent ring closure via condensation gave fused indoles. Some of these compounds showed biological activity as antitrypanosomal agents.

1. Introduction

As a continuation of our earlier investigations, novel efficient pathways were developed for substituted 1-methyl-1*H*-pyridazino[3,4-*b*]indoles starting from halopyridazin-3(2*H*)-ones.[1] The area of these methylated pyridazino-fused indoles seemed particularly interesting as we have noticed some structural similarity between neocryptolepine (**A**) and the 1-methyl-1*H*-pyridazino[3,4-*b*]indole (**B**) ring system (Fig. 1).

Naturally occurring tetracyclic indolo[3,2-*b*]quinoline alkaloid cryptolepine as well as its [2,3-*b*] fused isomer neocryptolepine, isolated from a decoction of the root of *Cryptolepis sanguinolenta*,[2a,b] showed antitrypanosomal and antiplasmodial activity and have been used as lead compounds for new therapeutic agents.[2c] Introduction of halogen or nitro substituents has resulted in more active and/or more selective antiplasmodial agents.[3] Importantly, the 'debenzo' derivative of cryptolepine, that is, 1-methyl-δ-carboline, showed a much better selectivity index (cytotoxicity/antiplasmodial activity) than cryptolepine itself.[4] This finding prompted us to make efforts to synthetize substituted 1-methyl-1*H*-pyridazino[3,4-*b*]indoles and to investigate their antiplasmodial and antitrypanosomal activity.

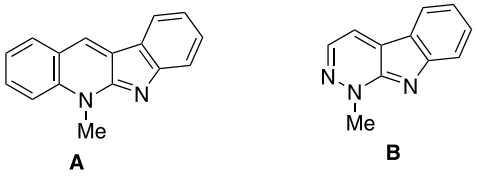

Figure 1. The structural similarity of neocryptolepine (**A**) and 1-methyl-1*H*-pyridazino[3,4-*b*]indole (**B**) ring systems.

2. Discussion

Earlier we found that 2-substituted 4,5-dichloropyridazin-3(2*H*)-ones undergo non-selective Suzuki cross-coupling reaction with arylboronic acids under classical Suzuki conditions resulting in a mixture of mono- and diaryl-substituted pyridazin-3(2*H*)-ones.[5] Recently, on 4,5-dichloro-2-methylpyridazin-3(2*H*)-one (**1**) a C-5 selectivity was observed in the coupling reaction with phenylboronic acid using $Pd(PEt_3)_2Cl_2$ as precatalyst and 1 M Na_2CO_3 as base in DMF.[6] Unfortunately, the general applicability of

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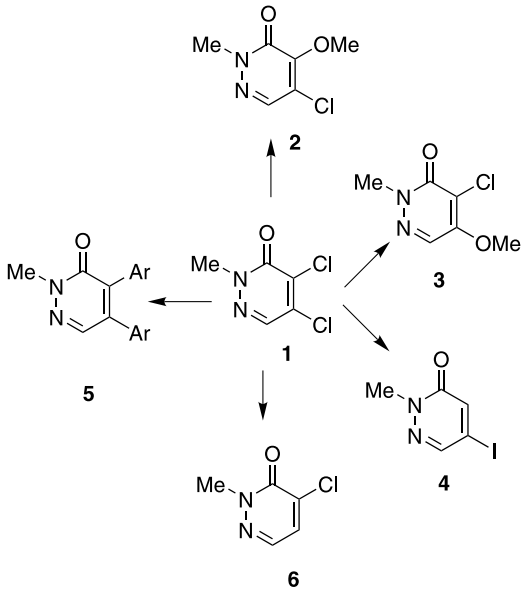
these optimized reaction conditions remain unknown. Alternatively, in order to achieve selective arylation we earlier introduced the strategy of 'provisionally masked functionalities' (PMFs).[1] For this purpose **1** was converted into chloro-methoxy substituted pyridazin-3(2*H*)-ones (**2**, **3**)[7] by nucleophilic substitution and into 5-iodo-2-methyl-pyridazin-3(2*H*)-one (**4**)[8] by halogen exchange followed by hydrodeiodination (Scheme 1).



Scheme 1.

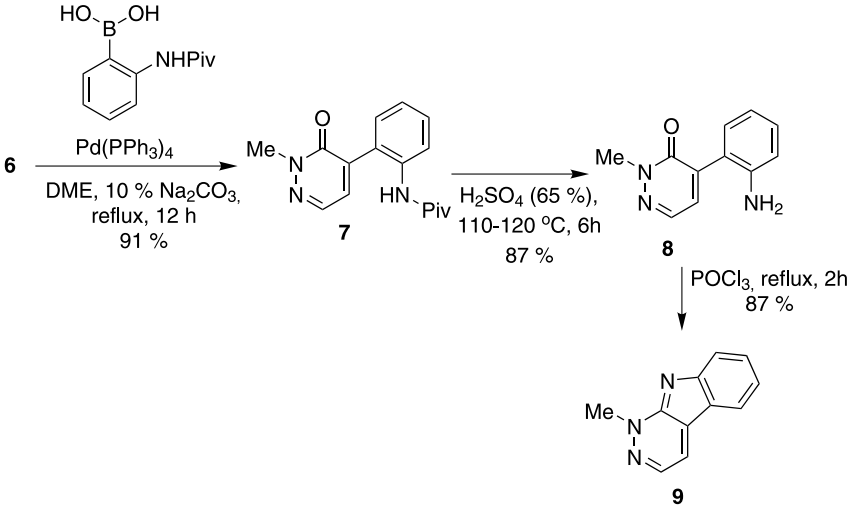
In this paper our efforts towards the synthesis of substituted 1-methyl-1*H*-pyridazino[3,4-*b*]indoles starting from (substituted) mono- and dihalopyridazin-3(2*H*)-ones are summarized. To the best of our knowledge, only very limited literature data are available for derivatives of the 1*H*-pyridazino[3,4-*b*]indole ring system.[9]

First, we attempted the synthesis of the unsubstituted 1-methyl-1*H*-pyridazino[3,4-*b*]indole (**9**) starting from 4-chloro-2-methylpyridazine-3(2*H*)-one (**6**). Compound **6**[10a] was synthesized from **1** via reaction with hydrazine followed by hydrodehydrazination of 4-chloro-5-hydrazino-2-methyl-pyridazin-3(2*H*)-one with $CuSO_4$, a procedure that has successfully been applied for the analogous demethyl derivative.[10b] This compound reacted with 2-pivaloylaminophenylboronic acid under the Gronowitz reaction conditions,[11] that is, in a mixture of dimethoxyethane and 10% aqueous sodium carbonate solution using tetrakis(triphenylphosphine)palladium as catalyst. The cross-coupling reaction afforded the pivaloyl protected compound **7**[12] in high yield (91%). Compound **7** was hydrolyzed to the aminophenyl derivative **8** under reaction conditions previously reported by us.[13] This compound—due to the proximity of the amino and oxo functions—underwent smooth condensation reaction in boiling phosphoryl chloride yielding the desired 1-methyl-1*H*-pyridazino[3,4-*b*]indole (**9**) as red crystals (Scheme 2).[13]

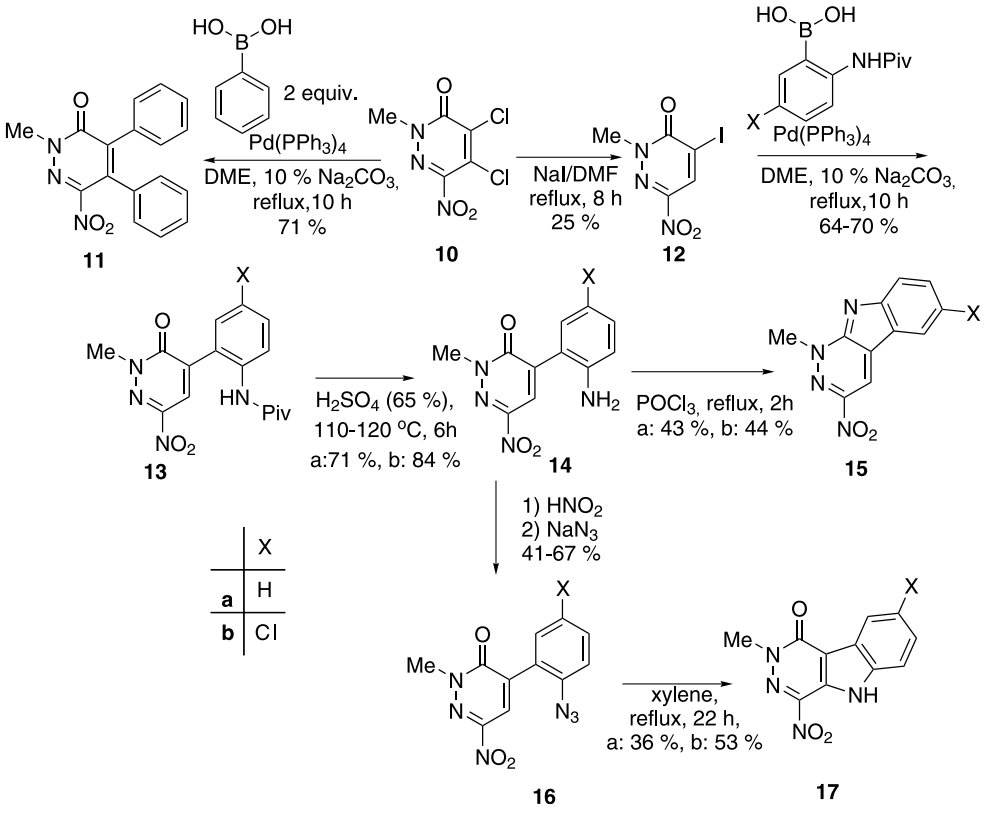
4,5-Dichloro-2-methylnitropyridazin-3(2*H*)-one (**10**) was synthesized from **1** by a nitration reaction.[14] Also on this substrate we observed that cross-coupling reaction with phenylboronic acid gave some diphenyl-substituted pyridazin-3(2*H*)-one (**11**) indicating the non-selective nature of the reaction. In order to realize the desired C-4 selective phenylation we used 4-iodo-2-methyl-6-nitropyridazin-3(2*H*)-one (**12**) which can be simply prepared from 4,5-dichloro-2-methyl-6-nitropyridazin-3(2*H*)-one (**10**) by reaction with sodium iodide in refluxing DMF.[8b]

When **12** was subjected to cross-coupling with 2-pivaloylaminophenylboronic acid and 5-chloro-2-pivaloylaminophenylboronic acid, 2-methyl-4-(2-pivaloylaminophenyl)-6-nitropyridazin-3(2*H*)-one (**13a**) and 2-methyl-4-(5-chloro-2-pivaloylaminophenyl)-6-nitropyridazin-3(2*H*)-one (**13b**), respectively, were obtained in good yield (Scheme 3). After hydrolytic removal of the pivaloyl protecting group the corresponding anilino compounds (**14a**,**b**) were obtained. These compounds were cyclized by the procedure described above for the synthesis of derivative **9** to yield 1-methyl-3-nitro-1*H*-pyridazino[3,4-*b*]indole (**15a**) and 6-chloro-1-methyl-3-nitro-1*H*-pyridazino[3,4-*b*]indole (**15b**).

Interestingly, 2-methyl-2,5-dihydro-1*H*-pyridazino[4,5-*b*]indol-1-ones (**17a**,**b**) could also be prepared starting



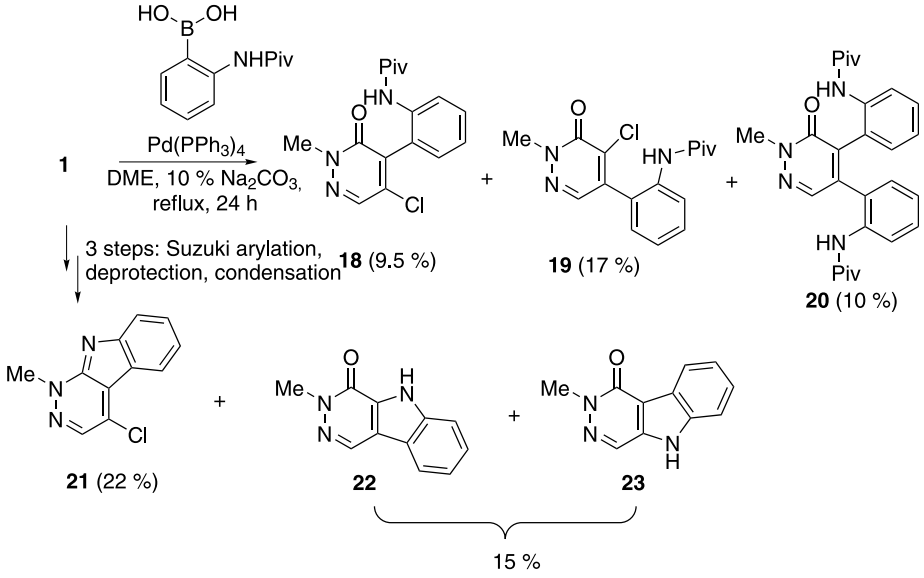
Scheme 2.



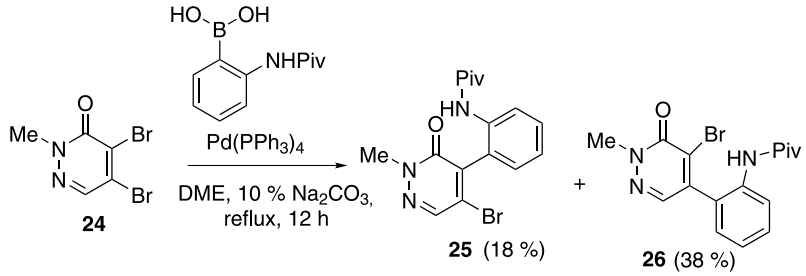
Scheme 3.

from **14a**,**b** using our earlier developed method for the synthesis of isomeric 3-methyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indol-4-ones:[15] (i) first azides **16a**,**b** were prepared from the corresponding anilines **14a**,**b** (ii) upon heating of these compounds in xylene the desired ring closed products (**17a**,**b**) were obtained via formation of an electrophilic nitrene. It is remarkable that the pyrrole ring formation occurs so smoothly taking into account the electron deficient nature of C-5 of **16a**,**b**. This is due to the fact that C-5 is part of an α,β-unsaturated lactam system, and the inductive effect of the nitro group.

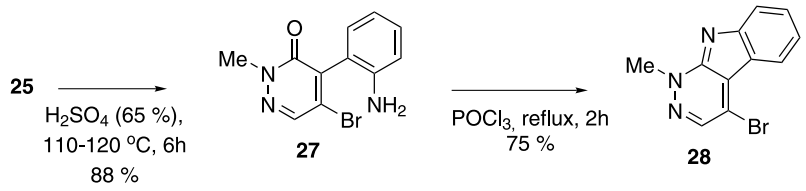
From a medicinal chemistry point of view, synthesis of derivatives having halogen substituents on the pyridazine ring are of interest and further efforts on 4,5-dihalo-2-methylpyridazin-3(2*H*)-one substrates seemed desirable. Therefore, we tried to follow a reaction pathway for the synthesis of 1*H*-pyridazino[3,4-*b*]indole **21** starting from **1** (Scheme 4). However, as could be expected Suzuki arylation of **1** with 2-pivaloylaminophenylboronic acid was not selective. After tedious column chromatography on silicagel some fractions of pure compounds **18**, **19** and **20** could be obtained. Structures of regioisomers **18** and **19**



Scheme 4.



Scheme 5.



Scheme 6.

were proven by COSY, HETCOR and long-range HETCOR measurements.

Because of the purification problems experienced with the reaction mixture from the Suzuki reaction on **1**, the crude reaction mixture (i.e., the mixture of **18**, **19**, and **20**) was subjected to two subsequent reaction steps (deprotection and ring closure) without isolation of the respective intermediates. Although the final reaction mixture was fairly complex, separation of 4-chloro-1-methyl-1*H*-pyridazino[3,4-*b*]indole (**21**) by chromatography was greatly facilitated by its bright orange-red colour. The overall yield of the three steps was 22%.

Furthermore, two unexpected ring closure products (**22** and **23**) were also detected and a mixture of these pyridazinoindoles was isolated in 15%. The ^{1}H NMR spectrum (H-6 signals of the pyridazin-3(2*H*)-one moiety) of this mixture indicated that the ratio of **22** and **23** was 4:1, respectively. Both regioisomers could easily be identified since these compounds (**22** and **23**) have already previously been synthesized by us via an independent route,[15] and their NMR-spectra were therefore available for comparison. Their formation can be rationalized by intramolecular nucleophilic substitution (addition–elimination reaction) of the corresponding anilines formed by deprotection of **18** and **19**.

In contrast to the difficulties experienced with the dichloro compound **1**, more favourable results have been obtained with the analoguous 4,5-dibromo-2-methylpyridazin-3(2*H*)-one **24**.[16] Coupling of **24** with 2-pivaloylaminophenylboronic acid under the same condition as applied with the synthesis of **7**, **13**, and **18** yielded a mixture of two regioisomeric aryl-bromopyridazin-3(2*H*)-ones (**25** and **26**) which, could be easily separated by a simple treatment with diethyl ether. When diethylether was added precipitation of only one of the two isomers namely 4-bromo-2-methyl-5-(2-pivaloyl-aminophenyl)pyridazin-3(2*H*)-one (**26**) occured. Chromatography on silicagel of the mother liquor yielded regioisomeric 5-bromo-2-methyl-4-(2-pivaloyl-aminophenyl)pyridazin-3(2*H*)-one (**25**) in pure form (Scheme 5). Its structure was identified by HETCOR and long-range HETCOR measurements (a long-range coupling of both H-6 and H-6′[12] with the quaternary C-4 was observed).

The further transformation of **25** was carried out in a similar way as used for the synthesis of **9**, **15**, and **21**. Thus, hydrolysis of the pivaloyl group of **25** gave the anilino substituted derivative **27** in good yield (Scheme 6). This compound smoothly underwent condensation reaction in boiling phosphoryl chloride yielding 4-bromo-1-methyl-1*H*-pyridazino[3,4-*b*]indole (**28**) as bright orange crystals.

Compounds **9**, **15a**, **21** and **28** were selected for biological tests. Antiprotozoal activities against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Plasmodium falciparum* (chloroquine-resistant), and cytotoxicity on human L6 cells, are listed in Table 1. Compound

Table 1. Antitrypanosomal, antileishmanial, antiplasmodial and cytotoxic (L6 cells) activity (IC_{50}, μg/ml)

	Trypanosoma brucei rhodesiense	*Trypanosoma cruzi*	*Leishmania donovani* (axenic amastigotes)	*Plasmodium falciparum* K1	*Cytotoxicity* (L6 cells)
28	0.83±0.10	8.2±1.2	15.87±1.05	3.22±0.95	4.80±0.75
15a	2.47±0.11	0.24±0.05	6.1±3.0	4.49±0.10	29.5±4.2
21	0.20±0.01	23.4	0.71[a]	>5	2.41±0.50
9	26.1	>90	>30	>5	nt[b]
Positive control[c]	0.0023	0.39	0.25	0.065	0.003

[a] Antileishmanial activity of compounds showing an $IC_{50}<1$ μg/ml in the assay on axenic amastigotes is confirmed in an assay on infected macrophages. However, for **21** this determination was not possible due to cytotoxicity on the host cells.
[b] nt, not tested.
[c] Positive controls used were: Melarsoprol for *T. b. rhodesiense*, benznidazole for *T. cruzi*, miltefosine for *Leishmania donovani*, chloroquine for *P. falciparum* and podophyllotoxin for L-6 cells.

9 shows no significant biological activity in the assays used. Introduction of a nitro-substituent in position 3, as in **15a**, results in a pronounced increase of the antitrypanosomal activity, especially against *T. cruzi* ($IC_{50}<1$ μg/ml). Introduction of a halo-substituent in position 4 on the other hand, as in **21** or **28**, leads to a high antitrypanosomal activity against *T. b. rhodesiense* ($IC_{50}<1$ μg/ml). In case of the chloro-derivative **21** also antileishmanial activity is observed against *L. donovani* axenic amastigotes. In an assay in infected macrophages, however, the antileishmanial activity could not be confirmed. From this limited and preliminary structure–activity relationship study it appears that this class of compounds deserves further attention as potential antitrypanosomal agents.

3. Conclusion

The obtained results reveal that the easily accessible 2-methyl-4,5-dichloropyridazin-3(2*H*)-ones (**1**, **10**) can serve as suitable precursors for the synthesis of 4-monoarylated pyridazin-3(2*H*)-ones by using Suzuki arylation in two ways: (i) via the 2-methyl-4-chloropyridazin-3(2*H*)-one (**6**) and (ii) via the 4-iodo-2-methyl-6-nitropyridazin-3(2*H*)-one (**12**). When 2-methyl-4,5-dihalopyridazin-3(2*H*)-ones (**1**, **10**, **24**) are directly used in Suzuki arylation reactions under Gronowitz conditions, using a small excess of arylboronic acid, at least a mixture of two monoarylated pyridazin-3(2*H*)-ones is obtained (also often diarylated pyridazin-3(2*H*)-one is formed). If an *ortho* amino group is present on the phenyl ring in position 4 of the pyridazin-3(2*H*)-one cyclization can take place upon heating with $POCl_3$ yielding 1-methyl-1*H*-pyridazino[3,4-*b*]indole derivatives. This reaction pathway represents a new approach to the parent tricyclic ring system. The antiprotozoal screening results show that 1-methyl-1*H*-pyridazino[3,4-*b*]indoles deserve further attention as potential antitrypanosomal agents.

4. Experimental

Melting points were determined on a Büchi apparatus and are uncorrected. The IR data were obtained with a Thermo Nicolet AVATAR 320 FT-IR or a Bruker Vector 22 spectrometer. The NMR spectra were recorded on a Varian spectrometer (200 or 400 MHz for ^1H and 50 or 100 MHz for ^{13}C) and a Bruker Avance-500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C). For mass-spectrometric analysis, samples were dissolved in CH_3OH containing 0.1% formic acid and diluted to a concentration of approximately 10^{-5} mol/L. One micro-litre injection was directed to the mass spectrometer at a flow rate of 5 μL/min (CH_3OH, 0.1% formic acid), using a CapLC HPLC system (Waters, Millford). Accurate mass data were acquired on a quadrupole-time-of-flight mass spectrometer (Q-Tof-II, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Cone voltage (approx. 35 V) and capillary voltage (approx. 3.3 kV) were optimized on one compound and used for all others. For the determination of the accurate mass of the molecular ion $[M+H]^+$, a solution of polyethylene glycol 300 in CH_3OH/H_2O with 1 mmol ammonium acetate, was added just before the mass spectrometer (at a rate of 1 μL/min) to the mobile phase. The calculated masses of PEG $[M+H]^+$ and $[M+NH_4]^+$ ions were used as lock mass.

Antiprotozoal evaluation and determination of cytotoxicity was carried out as described before.[17] Only compounds showing $IC_{50}<1$ μg/ml were tested in duplicate or triplicate (mean ± SD).

4.1. General procedure for the synthesis of arylpyridazin-3(2*H*)-ones (7, 13a, 13b, 18, 19, 20, 25, 26)

A mixture of the appropriate halogen substituted 2-methylpyridazin-3(2*H*)-one (2 mmol, **1**: 0.358 g, **6**: 0.289 g, **12**: 0.562 g, **24**: 0.536 g) and tetrakis(triphenylphosphine)palladium(0) (0.10 mmol, 0.116 g) in dimethoxyethane (12 mL) was stirred for 30 min at room temperature under argon. Then, 2-pivaloylaminophenylboronic acid (2.4 mmol, 0.532 g) or 5-chloro-2-pivaloylaminophenylboronic acid (2.4 mmol, 0.612 g) and aqueous sodium carbonate solution (4 mL, 10%) was added and the mixture was refluxed (oil bath temp. 97 °C) under an argon atmosphere for 6–24 h. The reaction mixture was cooled and poured onto ice-water (20 mL) and extracted with chloroform (3×20 mL). The combined organic fractions were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The purification of the crude reaction mixtures is indicated below.

4.1.1. 2-Methyl-4-(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (7). The evaporated crude product was purified by column chromatography (eluent: EtOAc–CH_2Cl_2 1:9).

Yield: 0.525 g, 92%; mp: 142–144 °C; IR (KBr) ν_{max}: 3290, 2962, 1666, 1638, 1606, 1590, 1512, 1476, 1448, 1400, 1368, 1296, 1270, 1240, 1170, 876, 754, 610 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 9.20 (br s, 1H, NH), 7.92 (d, 1H, $J=$ 4.2 Hz, H-6), 7.78 (d, 1H, $J=8.0$ Hz, H-3′ or H-6′), 7.47 (m, 1H, H-4′ or H-5′), 7.31 (d, $J=4.2$ Hz, H-5), 7.24 (m, 2H, H-3′ or H-6′, H′-4 or H-5′), 3.95 (s, 3H, CH_3), 1.22 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$, 100 MHz): 177.4, 160.8, 140.8, 137.0, 136.8, 131.9, 130.6, 130.1, 128.5, 126.3, 125.4, 41.3, 39.4, 27.5. MS (ESI): 185, 202; HRMS (ESI) Calcd for $C_{16}H_{19}N_3O_2$ 285.1447, found: 285.1459.

4.1.2. 2-Methyl-6-nitro-4-(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (13a). Recrystallization from acetonitrile yielded 0.462 g of product (70%); mp 187–188 °C; IR (KBr) ν_{max}: 3568, 3424, 3242, 2966, 2934, 2870, 1682, 1638, 1580, 1534, 1516, 1476, 1442, 1360, 922, 788, 750 cm^{-1}; δ_H ($CDCl_3$ 200 MHz): 8.60 (s, 1H, NH), 8.19 (s, 1H, H-5), 7.75 (m, 1H, H-3′ or H-6′), 7.58–7.49 (m, 1H, H-4′ or H-5′), 7.32–7.26 (m, 2H, H-4′ or H-5′, H-3′ or H-6′), 4.04 (s, 3H, CH_3), 1.21 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$ 50 MHz): 171.3 ($OCC(CH_3)_3$), 160.4 (C-3), 146.2 (C-6), 142.7 (C-2′), 136.9 (C-4), 131.4 and 130.8 (C-4′,6′), 127.5 (C-1′), 127.0, 126.1, 125.9 (C-5, 3′,5′), 42.3 (CH_3), 39.5 ($C(CH_3)_3$), 27.5 ($C(CH_3)_3$). Anal. Calcd for $C_{16}H_{18}N_4O_4$ (330.34): C, 58.17; H, 5.49; N, 16.96. Found: C, 58.12; H, 5.48; N, 17.02. MS (ESI): 146, 185, 233, 234, 261; HRMS (ESI) Calcd for $C_{16}H_{18}N_4O_4$ $[M+H]^+$331,1406, found 331,1407.

4.1.3. 4-(5-Chloro-2-pivaloylaminophenyl)-2-methyl-6-nitropyridazin-3(2*H*)-one (13b). Recrystallization from acetonitrile yielded 0.467 g of product (64%); mp 180–181 °C; IR (KBr) ν_{max}: 3568, 3436, 3274, 2964, 2932, 2872, 1682, 1644, 1586, 1500, 1480, 1402, 1362, 928, 788, 754 cm^{-1}: δ_H ($CDCl_3$ 200 MHz): 8.53 (s, 1H, NH), 8.19 (s, 1H, H-5), 7.71 (d, 1H, $J_{3',4'}=8.6$ Hz, H-3′), 7.48 (dd, $J_{3',4'}=8.6$ Hz, $J_{4',6'}=2.4$ Hz, 1H, H-4′), 7.31 (d, $J_{4',6'}=2.4$ Hz, 1H, H-6′), 4.04 (s, 3H, CH_3), 1.20 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$ 50 MHz): 177.3 ($OCC(CH_3)_3$), 160.1 (C-3), 146.0 (C-6), 141.1 (C-2′), 135.6 (C-4), 131.3 (C-5′), 131.2 and 130.3 (C-4′, and −6′), 128.8 (C-1′), 128.2 and 126.2 (C-3′, and -5), 42.3 (CH_3), 39.5 ($C(CH_3)_3$), 27.4 ($C(CH_3)_3$). HRMS (ESI) Calcd for $C_{16}H_{18}ClN_4O_4$ $[M+H]^+$365.1017, found 365.1024.

4.1.4. 5-Chloro-2-methyl-4-(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (18). The crude reaction mixture was purified by flash column chromatography on silica using a CH_2Cl_2–MeOH 100:0.5 mixture as the eluent.

Yield: 0.063 g, 9.8%; mp: 120–124 °C; IR (KBr) ν_{max}: 3487, 3335, 2967, 1670, 1637, 1605, 1515, 1486, 1447, 1370, 1295, 1255, 1210, 1170, 1016, 957, 779, 752, 743, 712, 635 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.16 (br s, 1H, NH), 7.91 (s, 1H, H-6), 7.80 (dd, 1H, $J=8.7$, 1.1 Hz, 1H, H-3′ or H-6′), 7.45 (ddd, 1H, $J=7.8$, 7.5, 1.7 Hz, H-4′ or H-5′), 7.31 (dd, 1H, $J=7.8$, 1.7 Hz, H-3′ or H-6′), 7.23 (ddd, 1H, $J=8.7$, 7.6, 1.2 Hz, H-4′ or H-5′), 3.87 (s, 3H, CH_3), 1.18 (s, 9H, C-$(CH_3)_3$); δ_C ($CDCl_3$, 100 MHz):176.9, 159.4, 138.0, 137.5, 136.9, 136.8, 130.8, 130.4, 126.1, 125.2, 124.9, 40.9, 39.4, 27.4. MS (ESI): 322, 320, 304, 284, 228, 200, 57; HRMS (ESI) Calcd for $C_{16}H_{19}ClN_3O_2$ $[M+H]^+$320.1166, found 320.1158.

4.1.5. 4-Chloro-2-methyl-5-(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (19). The crude reaction mixture was purified by flash column chromatography on silica using a CH_2Cl_2–MeOH 100:0.5 mixture as the eluent.

Yield: 0.110 g, 17%; mp: >150 °C (decomposition); IR (KBr) ν_{max}: 3340, 1682, 1646, 1579, 1520, 1479, 1442, 1284, 1156, 1025, 873, 768, 755, 728, 674 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.80 (dd, 1H, $J=8.7$, 1.2 Hz, H-3′ or H-6′), 7.69 (s, 1H, H-6), 7.52 (br s, 1H, NH), 7.50 (dd, 1H, $J=7.7$, 7.6 Hz, H-4′ or H-5′), 7.32 (ddd, 1H, $J=8.7$, 7.6, 1.2 Hz, H-4′ or H-5′), 7.24 (dd, 1H, $J=7.7$, 1.3 Hz, H-3′ or H-6′), 3.83 (s, 3H, CH_3), 1.18 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$, 100 MHz): 176.9, 157.3, 140.1, 137.1, 134.8, 133.7, 130.6, 128.9, 127.4, 125.9, 125.6, 41.0, 39.4, 27.4. MS (ESI): 322, 320, 304, 302, 246, 238, 236, 200, 85, 58, 57; HRMS (ESI) Calcd for $C_{16}H_{19}ClN_3O_2$ $[M+H]^+$320.1166, found: 320.1156.

4.1.6. 2-Methyl-4,5-bis(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (20). The crude reaction mixture was purified by flash column chromatography on silica using a CH_2Cl_2–MeOH 100:0.5 mixture as the eluent.

Yield: 0.095 g, 10%; mp: >215 °C (decomposition); IR (KBr) ν_{max}: 3325, 2962, 2926, 1675, 1616, 1601, 1576, 1505, 1479, 1444, 1368, 1298, 1262, 1161, 1019, 771, 747 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.44 (br s, 1H, N–H), 7.82 (s, 1H, H-6), 7.65 (br d, $J=8.1$ Hz, 1H), 7.59 (br d, $J=8.2$ Hz, 1H), 7.36 (br s, 1H, N–H), 7.24 (br m, 2H), 7.05 (br d, $J=7.6$ Hz, 1H), 6.95 (br t, $J=7.1$ Hz, 1H), 6.89 (br t, $J=7.6$ Hz, 1H), 6.75 (br d, $J=7.5$ Hz, 1H), 3.94 (s, 3H, CH_3), 1.29 (s, 9H, $C(CH_3)_3$), 1.21 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$, 100 MHz): 177.4, 176.7, 160.6, 141.6, 138.2, 137.9, 137.7, 134.9, 131.6, 130.9, 129.8, 128.6, 128.2, 127.0, 126.3, 125.5, 125.3, 124.7, 41.0, 39.6, 39.3, 27.6, 27.5. MS (ESI): 377, 359, 304, 303, 293, 57; HRMS (ESI) Calcd for $C_{27}H_{33}N_4O_3$ $[M+H]^+$461.2553, found: 461.2534.

4.1.7. 5-Bromo-2-methyl-4-(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (25). The crude reaction mixture was suspended with diethyl ether (5 mL), the white precipitated solid was removed by filtration. This etheral mother liquor was subjected to column chromatography (silica, eluent: chloroform-methanol 100:1 mixture) and the product was recrystallized from an ether–hexane mixture.

Yield: 0.131 g, 18%; mp: 114–116 °C; IR (KBr) ν_{max}: 3338, 3065, 3032, 2966, 1674, 1639, 1604, 1507, 1486, 1463, 1435, 930, 756 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.06 (br s, 1H, NH), 8.02 (s, 1H, H-6), 7.79 (dd, $J=8.2$, 1.1 Hz, 1H, H-3′), 7.45 (ddd, $J=7.8$, 7.5, 1.5 Hz, 1H, H-4′), 7.30 (dd, $J=7.8$, 1.5 Hz, 1H, H-6′), 7.24 (ddd, $J=7.8$, 7.6, 1.2 Hz, 1H, H-5′), 3.82 (s, 3H, CH_3), 1.18 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$, 100 MHz):176.8 (CO), 159.0 (C-3), 139.6 (C-4), 139.4 (C-6), 136.4 (C-2′), 130.6, 130.4 (C-5′,6′), 129.2 (C-5), 127.1 (C-1′), 125.9 (C-3′), 124.9 (C-4′) 40.8 (CH_3), 39.4 ($C(CH_3)_3$), 27.4 ($C(CH_3)_3$). Anal. Calcd for $C_{16}H_{18}BrN_3O_2$ (364.24): C, 52.76; H, 4.98; N, 11.54. Found: C, 52.65; H, 5.12; N, 11.45.

4.1.8. 4-Bromo-2-methyl-5-(2-pivaloylaminophenyl)pyridazin-3(2*H*)-one (26). The crude reaction mixture was suspended with diethyl ether (5 mL) and the white precipitated product was filtered off and recrystallized from acetonitrile.

Yield: 0.277 g, 38%; mp: 181–186 °C; IR (KBr): ν_{max}: 3340, 3048, 2975, 1682, 1643, 1579, 1519, 1441, 1282, 1155, 769, 755, 644, cm^{-1}; δ_H ($CDCl_3$, 200 MHz): 7.78 (dd, $J=8.1$, 1.2 Hz, 1H, H-3′ or H-6′), 7.61 (s, 1H, H-6), 7.50 (dd, $J=8.1$, 7.3 Hz, 1H, H-4′ or H-5′), 7.34 (ddd, $J=8.1$, 7.5, 1.2 Hz, 1H, H-4′ or H-5′), 7.25 (br s, 1H, NH), 7.22 (dd, $J=7.5$, 1.8 Hz, 1H, H-3′ or H-6′), 3.89 (s, 3H, CH_3), 1.17 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$, 50 MHz): 176.7, 157.4, 143.4, 136.8, 134.8, 134.2, 130.5, 128.4, 127.6, 125.9, 125.1, 41.3, 39.4, 27.3. Anal. Calcd for $C_{16}H_{18}BrN_3O_2$ (364.24): C, 52.76; H, 4.98; N, 11.54. Found: C, 52.88; H, 5.08; N, 11.65.

4.1.9. 2-Methyl-6-nitro-4,5-diphenylpyridazin-3(2*H*)-one (11). 4,5-Dichloro-2-methyl-6-nitropyridazin-3(2*H*)-one (**10**, 17.50 mmol, 3.92 g) and tetrakis(triphenylphosphine)-palladium(0) (0.88 mmol, 1.01 g) as catalyst were dissolved in anhydrous toluene (90 mL) and the mixture was stirred at room temperature under argon for 30 min. Phenylboronic acid (36.0 mmol, 4.39 g) and a solution of sodium carbonate (2 M, 35 mL) were then added and the mixture was refluxed for 10 h. The cold mixture was poured onto ice-water (200 mL), extracted with chloroform (3× 120 mL), and dried over anhydrous sodium sulphate.

Evaporation of the organic layer gave a crude product which was suspended in diethyl ether. The precipitated yellow crystals were filtered off and recrystallized from ethanol.

Yield: 3.83 g (12.4 mmol), 71%; mp 190.5–191 °C; IR (KBr) ν_{max}: 1662, 1592, 1540, 1445, 1372, 1332, 876, 744, 698 cm^{-1}; δ_H ($CDCl_3$ 200 MHz): 7.24–6.98 m, 10H, Ar-H), 3.86 (s, 3H, CH_3); δ_C ($CDCl_3$ 50 MHz): 159.5 (C-3), 149.2 (C-6), 141.2, 135.5, 131.3, 130.9 (C-4,-5,-1′,-1″), 129.9–127.8 (C-2′,3′,4′,5′,6′,2″,3″,4″,5″,6″), 40.91 (CH_3). Anal. Calcd for $C_{17}H_{13}N_3O_3$ (307.30): C, 66.44; H, 4.26; N, 13.67. Found: C, 66.60; H, 4.16; N, 13.72. MS (ESI): 170, 247; HRMS (ESI) Calcd for $C_{17}H_{13}N_3O_3$ $[M+H]^+$308.1035, found 308.1034.

4.2. General procedure for the preparation of aminophenyl substituded pyridazin-3(2*H*)-ones by hydrolysis (8, 14a, 14b, 27)

A mixture of the appropriate pivaloylaminophenyl pyridazinone (2 mmol, **7**: 0.57 g, **13a**: 0.66 g, **13b**: 0.73 g, **25**: 0.72 g) and sulphuric acid (65%, 10 mL) was heated at 110–120 °C for 6 h. The reaction mixture was cooled down to c. and diluted with water (50 mL) and the pH of the mixture was adjusted to 8 by addition of aqueous (25%) ammonia. It was extracted with dichloromethane (3×40 mL), and the organic layer was dried over Na_2SO_4 and evaporated. The crude product was suspended with ether (10 mL) and the yellow or orange crystals were filtered off.

4.2.1. 4-(2-Aminophenyl)-2-methylpyridazin-3(2*H*)-one (8). Yield: 0.35 g, 87%; mp: 123–126 °C; IR (KBr) ν_{max}: 3442, 3346, 3086, 3042, 2986, 2946, 2892, 1646, 1610, 1568, 1492, 1452, 1402, 1364, 1342, 1310, 1286, 1240, 1156, 1142, 1120, 1002, 872, 788, 752, 616, 594, 542, 520, 470 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.83 (d, 1H, J=4.2 Hz, H-6), 7.27 (d, 1H, J=4.2 Hz, H-5), 7.23–7.10 (m, 2H, H-3′, H-4′), 6.86–6.74 (m, 2H, H-5′, H-6′), 4.73 (br s, 2H, NH_2), 3.90 (s, 3H, CH_3); δ_C ($CDCl_3$, 100 MHz): 160.2, 146.6, 141.7, 136.2, 131.1 (2C), 130.6, 121.8, 119.0, 117.8, 41.1. MS (ESI): 114, 128, 130, 185; HRMS (ESI) Calcd for $C_{11}H_{12}N_3O$ $[M+H]^+$202.0980, found: 202.0988.

4.2.2. 4-(2-Aminophenyl)-2-methyl-6-nitropyridazin-3(2*H*)-one (14a). Yield: 0.35 g, 71%; mp 217–218 °C (acetonitrile); IR (KBr) ν_{max}: 3446, 3366, 1648, 1624, 1598, 1586, 1566, 1524, 1488, 1356, 1114, 758 cm^{-1}; δ_H (DMSO-d_6, 500 MHz): 8.11 (s, 1H, H-5), 7.13 (t, 1H, $J_{3',4'}=J_{4',5'}$=7.0 Hz, H-4′), 7.10 (d, 1H, $J_{5',6'}$=8.0 Hz, H-6′), 6.74 (d, 1H, $J_{3',4'}$=7.0 Hz, H-3′), 6.62 (dd, 1H, $J_{5',6'}$= 8.0 Hz, $J_{4',5'}$=7.0 Hz, H-5′), 5.16 (s, 2H, NH_2), 3.82 (s, 3H, CH_3); δ_C (DMSO-d_6, 125 MHz): 159.2 (C-3), 146.6 (C-2′), 145.8 (C-6), 140.0 (C-4), 130.3 (C-4′), 130.2 (C-6′), 125.0 (C-5), 117.4 (C-1′), 115.7 (C-5′), 115.6 (C-3′), 41.3 (CH_3). MS (ESI): 115, 142, 144, 170, 201, 247; HRMS (ESI) Calcd for $C_{11}H_{11}N_4O_3$ $[M+H]^+$ 247.0831, found 247.0820.

4.2.3. 4-(2-Amino-5-chlorophenyl)-2-methyl-6-nitropyridazin-3(2*H*)-one (14b). Yield: 0.47 g, 84%; mp 249–249.5 °C (acetonitrile); IR (KBr) ν_{max}: 3420, 3362, 3068, 1654, 1628, 1582, 1526, 1486, 1360, 1260, 834 cm^{-1}; δ_H (DMSO-d_6, 500 MHz): 8.17 (s, 1H, H-5), 7.16 (dd, 1H, $J_{3',4'}$=8.6 Hz, $J_{4',6'}$=2.2 Hz, H-4′), 7.12 (d, 1H, $J_{4',6'}$= 2.2 Hz, H-6′), 6.75 (d, 1H, $J_{3',4'}$=8.6 Hz, H-3′), 5.35 (s, 2H, NH_2), 3.81 (s, 3H, N–H_3); δ_C (DMSO-d_6, 125 MHz): 159.0 (C-3), 145.7 (C-6), 145.6 (C-2′), 138.3 (C-4), 129.9 (C-4′), 129.3 (C-6′), 125.5 (C-5), 118.7 (C-5′), 118.3 (C-1′), 116.9 (C-3′), 41.3 (CH_3). Anal. Calcd for $C_{11}H_9ClN_4O_3$ (280.67): C, 47.07; H, 3.23; N, 19.96; Cl, 12.63. Found: C, 46.94; H, 3.08; N, 20.00; Cl, 12.69. MS (ESI): 176, 178, 204; HRMS (ESI) Calcd for $C_{11}H_9ClN_4O_3$ $[M+H]^+$281.0437, found 281.0433.

4.2.4. 4-(2-Aminophenyl)-5-bromo-2-methylpyridazin-3(2*H*)-one (27). Yield: 0.49 g, 88%; mp 173–179 °C (ethanol); IR (KBr) ν_{max}: 3425, 3344, 1647, 1604, 1491, 1453, 1307, 926, 757 cm^{-1}; δ_H ($CDCl_3$, 200 MHz): 8.00 (s, 1H, H-6), 7.25 (ddd, 1H, J=7.4, 6.0, 1.6 Hz, H-4′), 7.08 (dd, J=8.0, 1.4 Hz 1H, H-3′), 6.85 (ddd, 1H, J=7.6, 7.2, 1.0 Hz, H-5′), 6.82 (d, 1H, J=8.0 Hz H-6′), 3.82 (s, 3H, CH_3), 3.82 (s, 2H, NH_2); δ_C ($CDCl_3$, 50 MHz): 158.3, 144.7, 138.7, 135.0, 130.5, 130.4, 129.0, 118.7, 117.3, 115.6, 40.6. Anal. Calcd for $C_{11}H_{10}BrN_3O$ (280.12): C, 47.16; H, 3.60; N, 15.00. Found: C, 47.04; H, 3.72; N, 15.11.

4.3. General procedure for ring closure reaction by phosphoryl chloride (9, 15a, 15b, 28)

A mixture of the appropriate aminophenyl compound (1 mmol, **8**: 0.21 g, **14a**: 0.25 g, **14b**: 0.28 g, **27**: 0.28 g) and phosphoryl chloride (10 mL) was refluxed at 110 °C for 2 h. The reaction mixture was evaporated, the residue was mixed with ice-cool water (50 mL) and the pH of the mixture was adjusted to 8 by addition of aqueous (25%) ammonia. The mixture was extracted with dichloromethane (3×20 mL) and the extract was dried over Na_2SO_4. Evaporation of the solvent gave a crude product which was suspended with ether (5 mL). The red precipitated crystals were filtered off to give the ring closed product.

4.3.1. 1-Methyl-1*H*-pyridazino[3,4-*b*]indole (9). Yield: 0.130 g; 71%; mp:172–173 °C; IR (KBr) ν_{max}: 3446, 1630, 1600, 1540, 1492, 1462, 1436, 1416, 1342, 1322, 1262, 1200, 1128, 1108, 1040, 1010, 998, 872, 862, 776, 762, 738 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.34 (d, 1H, J= 4.6 Hz, H-3), 8.13–8.09 (m, 2H, H-4 and H-8) 7.84 (d, 1H, J=8.2 Hz, H-5), 7.68 (ddd, 1H, J=8.2, 7.2, 1.2 Hz, H-6), 7.27 (ddd, 1H, J=8.2, 7.2, 1.2 Hz, H-7), 4.54 (s, 3H, CH_3); δ_C ($CDCl_3$, 100 MHz): 155.9, 153.2, 133.9, 133.2, 131.4, 122.4, 121.1, 119.7, 118.5, 118.4, 42.7. MS (ESI): 114, 128, 130, 140, 142, 169, 184; HRMS (ESI) Calcd for $C_{11}H_{10}N_3$ $[M+H]^+$184.0875, found 184.0867.

4.3.2. 1-Methyl-3-nitro-1*H*-pyridazino[3,4-*b*]indole (15a). Yield: 0.10 g (0.44 mmol), 43%; mp 209–210 °C; IR (KBr) ν_{max}: 3430, 2924, 1652, 1622, 1554, 1528, 1508, 1356, 1330, 1296, 764 cm^{-1}; δ_H (DMSO-d_6, 400 MHz): 9.49 (s, 1H, H-4), 8.52 (dd, 1H, $J_{5,6}$=6.8 Hz, $J_{5,7}$=1.0 Hz, H-5), 7.80 (dd, 1H, $J_{7,8}$=7.0 Hz, $J_{6,8}$=1.0 Hz, H-8), 7.75 (td, 1H, $J_{7,8}=J_{6,7}$=7.0 Hz, $J_{5,7}$=1.0 Hz H-7), 7.38 (ddd, 1H, $J_{6,7}$=7.0 Hz, $J_{5,6}$=6.8 Hz, $J_{6,8}$=1.0 Hz H-6), 4.55 (s, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz): 157.0 (C-9a), 152.7 (C-3), 145.6 (C-8a), 134.2 (C-4a), 132.4 (C-7), 124.3 (C-5), 122.9 (C-4b), 121.3 (C-6), 119.1 (C-8), 115.3 (C-4), 43.5 (CH_3). MS (ESI): 229, 183, 156; HRMS (ESI) Calcd for $C_{11}H_9N_4O_2$ $[M+H]^+$229.0726, found 229.0714.

4.3.3. 6-Chloro-1-methyl-3-nitro-1*H*-pyridazino[3,4-*b*]indole (15b). Yield: 0.115 g, 44%); mp 224–225 °C; IR (KBr) ν_{max}: 3068, 1556, 1502, 1432, 1352, 1332, 1296, 832, 690 cm^{-1}; δ_H (DMSO-d_6, 200 MHz): 9.49 (s, 1H, H-4), 8.59 (d, 1H, $J_{5,7}=3.0$ Hz, H-5), 7.78 (d, 1H, $J_{7,8}=7.5$ Hz, H-8), 7.71 (dd, 1H, $J_{7,8}=7.5$ Hz, $J_{5,7}=3.0$ Hz, H-7), 4.51 (s, 3H, CH_3); δ_C (DMSO-d_6, 50 MHz): 155.2, 152.8 (C-3, 9a), 145.5 (C-8a), 133.2 (C-6), 132.0 (C-4), 125.2, 123.7 (C-4a, 4b), 123.5, 120.4, 116.3 (C-5, 7, 8), 43.4 (CH_3). MS (ESI): 263, 217, 190; HRMS (ESI) Calcd for $C_{11}H_8ClN_4O_2$ $[M+H]^+$263.0336, found 263.0328.

4.3.4. 4-Bromo-1-methyl-1*H*-pyridazino[3,4-*b*]indole (28). Yield: 0.196 g, 75%; mp: 130–133 °C; IR (KBr) ν_{max}: 3045, 2942, 1627, 1598, 1536, 1495, 1463, 1438, 1405, 1338, 1262, 1117, 1093, 968, 756, 727 cm^{-1}; δ_H ($CDCl_3$, 200 MHz): 8.38 (dd, 1H, $J=7.7$, 0.9 Hz, H-8), 8.25 (s, 1H, H-3), 7.84 (dd, 1H, $J=8.2$, 0.8 Hz, H-5), 7.71 (dd, 1H, $J=8.2$, 6.9 Hz, H-6), 7.33 (dd, 1H, $J=7.7$, 6.9 Hz, H-7), 4.50 (s, 3H, CH_3), δ_C ($CDCl_3$, 50 MHz): 155.5, 153.1, 134.5, 132.0, 131.9, 129.9, 124.4, 121.1 120.7, 118.6, 42.8. MS (ESI): 218, 203, 155, 154; Anal. Calcd for $C_{11}H_8BrN_3$ (262.11): C, 50.41; H, 3.08; N, 16.03. Found: C, 50.54; H, 3.12; N, 15.91.

4.3.5. Procedure for the synthesis of 4-chloro-1-methyl-1*H*-pyridazino[3,4-*b*]indole (21). Reaction of **1** with 2-pivaloylaminophenylboronic acid was carried out as described above. Column chromatography of the crude reaction product was omitted and, instead, the mixture was lyophilized during 3 h. Subsequently 40% H_2SO_4 (5 mL) was added and this mixture was refluxed (temperature of the oil bath: 110 °C) overnight (17 h). Then, the reaction was cooled to rt and 20 mL H_2O was added. Concentrated aqueous ammonium hydroxide was added until pH=8, and the mixture was extracted with dichloromethane (3× 30 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. After this manipulation, $POCl_3$ (3 mL) was added and the mixture was refluxed (temperature of the oil bath: 117 °C) for 3 h. After cooling down, $POCl_3$ was removed by evaporation under reduced pressure, and ice-water (20 mL) was added. The mixture was adjusted to pH=8 using concentrated aqueous ammonium hydroxide and was extracted with dichloromethane (3×30 mL). The combined organic extract was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude reaction mixture was purified by flash column chromatography on silica using a EtOAc–EtOEt 4:1 mixture as the eluent.

Yield: 0.099 g, 22%; mp: 102–104 °C; IR (KBr) ν_{max}: 2924, 2854, 1626, 1598, 1536, 1493, 1462, 1438, 1399, 1336, 1261, 1238, 1226, 1210, 1197, 1120, 1093, 967, 758, 744, 730, 638 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.37 (ddd, 1H, $J=$ 7.9, 1.2, 0.8 Hz, H-8), 8.22 (s, 1H, H-3), 7.83 (ddd, 1H, $J=$ 8.2, 0.9, 0.7 Hz, H-5), 7.70 (ddd, 1H, $J=8.2$, 7.2, 1.2 Hz, H-6), 7.32 (ddd, 1H, $J=7.9$, 7.1, 0.9 Hz, H-7), 4.49 (s, 3H, CH_3), δ_C ($CDCl_3$, 100 MHz): 156.0, 153.2, 134.4, 132.1, 129.9, 129.5, 124.5, 121.3, 120.7, 118.8, 42.8. MS (ESI): 218, 203, 155, 154; HRMS (ESI) Calcd for $C_{11}H_9ClN_3$ $[M+H]^+$218.0485, found: 218.0483.

4.4. General procedure for the azidation of aminopyridazin-3(2H)-ones 14a,b

The appropriate aminopyridazin-3(2*H*)-one (5.0 mmol) was dissolved in 37% hydrochloric acid (100 mL) and was cooled to 0 °C with stirring. Aqueous sodium nitrite solution (0.73 g (10.62 mmol) of sodium nitrite in 27 mL of water) was added dropwise at such a rate that the temperature of the reaction mixture did not exceed 5 °C. The mixture was stirred at this temperature for 1.5 h.

A solution of sodium azide (0.664 g, 10.62 mmol) and anhydrous sodium acetate (5.744 g, 70.02 mmol) in water (24 mL) was then added at 0–5 °C and the mixture was stirred for an additional 1 h at this temperature. Then the mixture was neutralized with a saturated solution of sodium carbonate and extracted with dichloromethane (3× 100 mL). The organic layer was evaporated under reduced pressure (without heating) and the residue was suspended with diethyl ether to yield red-brown crystals which were filtered off. The product decomposed on air and was therefore stored under an argon atmosphere in a refrigerator.

4.4.1. 4-(2-Azidophenyl)-2-methyl-6-nitropyridazin-3(2*H*)-one (16a). Starting from **14a** (0.29 g, 1.18 mmol), 0.13 g of the crude product (0.48 mmol, 41%) was obtained; R_f (chloroform–ethyl acetate 95:5): 0.67; IR (KBr) ν_{max}: 3420, 2924, 2140, 2100, 1668, 1586, 1572, 1520, 1490, 1360, 1300, 830, 784, 768 cm^{-1}; δ_H ($CDCl_3$, 500 MHz): 8.16 (s, 1H, H-5), 7.53 (t, 1H, $J_{3',4'}=J_{4',5'}=7.8$ Hz, H-4′), 7.47 (d, 1H, $J_{5',6'}=7.5$ Hz, H-6′), 7.31 (d, 1H, $J_{3',4'}=$ 7.8 Hz, H-3′), 7.26 (dd, 1H, $J_{4',5'}=7.8$ Hz, $J_{5',6'}=7.5$ Hz, H-5′), 3.98 (s, 3H, CH_3); δ_C ($CDCl_3$, 125 MHz): 159.2 (C-3), 145.1 (C-6), 139.0 (C-4), 138.5 (C-2′), 131.5 (C-4′), 130.9 (C-6′), 125.2 (C-5), 124.9 (C-5′), 124.2 (C-1′), 118.9 (C-3′), 41.8 (CH_3).

4.4.2. 4-(2-Azido-5-chlorophenyl)-2-methyl-6-nitropyridazin-3(2*H*)-one (16b). Starting from **14b** (0.68 g, 2.42 mmol) 0.50 g of the crude product (1.63 mmol, 67%) was obtained; R_f (chloroform–ethyl acetate 95:5): 0.69; IR (KBr) ν_{max}: 3420, 2926, 2142, 1674, 1582, 1522, 1484, 1358, 1346, 1314, 826, 784, 760 cm^{-1}; δ_H ($CDCl_3$, 200 MHz): 8.16 (s, 1H, H-5), 7.48 (m, 1H, H-4′), 7.46 (m, 1H, H-6′), 7.25 (m, 1H, H-3′), 3.97 (s, 3H, CH_3); δ_C ($CDCl_3$, 50 MHz): 158.8 (C-3), 145.2 (C-6), 137.5 (C-4), 137.1 (C-2′), 131.4 (C-4′), 130.8 (C-6′), 130.3 (C-5′), 125.6 (C-5), 125.4 (C-1′), 120.1 (C-3′), 41.9 (CH_3).

4.5. General procedure for ring closure reaction of 16a,b

A solution of the appropriate azide (1.00 mmol) in dry xylene (5 mL) was refluxed for 22 h and the solvent was then removed under reduced pressure. The residue was purified by column chromatography and recrystallization from ethanol yielded pale yellow crystals.

4.5.1. 2-Methyl-4-nitro-2,5-dihydro-1*H*-pyridazino[4,5-*b*]indol-1-one (17a). Compound **16a** (0.09 g, 0.33 mmol) gave the ring closed title compound **17a** (0.03 g, 0.12 mmol, 36%); mp 314–316 °C; R_f (chloroform–ethyl acetate 95:5): 0.33; IR (KBr) ν_{max}: 3276, 1654, 1592, 1544, 1436, 1342, 1322, 760 cm^{-1}; δ_H (DMSO-d_6, 500 MHz): 12.65 (s, 1H,

NH), 8.23 (d, 1H, $J_{8,9}$=7.8 Hz, H-9), 7.82 (d, 1H, $J_{6,7}$=8.3 Hz, H-6), 7.59 (t, 1H, $J_{7,8}$=7.7 Hz, H-7), 7.42 (t, 1H, $J_{7,8}$=7.7 Hz, $J_{8,9}$=7.8 Hz, H-8), 3.92 (s, 3H, CH_3); δ_C (DMSO-d_6, 125 MHz): 158.0 (C-1), 139.0 (C-5a), 136.9 (C-4), 129.1 (C-4a), 127.5 (C-7), 122.6 (C-8), 121.9 (C-9a), 121.2 (C-9), 113.5 (C-6), 112.7 (C-9b), 40.7 (CH_3). MS (ESI): 245, 199, 169, 144; HRMS (ESI) Calcd for $C_{11}H_9N_4O_3$ $[M+H]^+$ 245.0675, found 245.0675.

4.5.2. 8-Chloro-2-methyl-4-nitro-2,5-dihydro-1*H*-pyridazino[4,5-*b*]indol-1-one (17b). Compound **16b** (0.25 g, 0.81 mmol) gave the ring closed title compound **17b** (0.12 g, 0.43 mmol, 53%); mp 354–354.5 °C; R_f (chloroform–ethyl acetate 95:5): 0.48; IR (KBr) ν_{max}: 3258, 1652, 1590, 1550, 1440, 1338, 1232, 800 cm^{-1}; δ_H (DMSO-d_6, 500 MHz): 8.15 (d, 1H, $J_{7,9}$=2.1 Hz, H-9), 7.81 (d, 1H, $J_{6,7}$=8.8 Hz, H-6), 7.61 (dd, 1H, $J_{6,7}$=8.8 Hz, $J_{7,9}$=2.1 Hz, H-7), 3.91 (s, 3H, CH_3); δ_C (DMSO-d_6, 125 MHz): 157.5 (C-1), 137.4 (C-5a), 136.5 (C-4), 129.7 (C-4a), 127.2 (C-7), 126.8 (C-8), 122.7 (C-9a), 119.8 (C-9), 114.8 (C-6), 111.7 (C-9b), 39.5 (CH_3). MS (ESI): 279, 233, 207, 203, 198, 152, 149, 121, 57; HRMS (ESI) Calcd for $C_{11}H_8ClN_4O_3$ $[M+H]^+$ 279.0285, found 279.0298.

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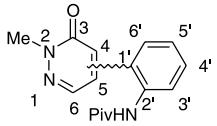
12. Although IUPAC nomenclature is used preferentially, in this paper compounds **7**, **13a**, **13b**, **18**, **19**, **20**, **25** and **26** are regarded as 2-pivaloylaminophenyl substituted pyridazin-3(2*H*)-ones rather than as 2-substituted *N*-phenylpivaloylamides. In this way the names and NMR assignments of these compounds can more easily be compared with those of the corresponding 2-aminophenyl- and 2-azidophenylpyridazin-3(2*H*)-ones.



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Stereoselective synthesis of amino acid-derived β-lactams. Experimental evidence for TADDOL as a memory of chirality enhancer

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Abstract—In model experiments, concerning the modulation of memory of chirality during the intramolecular alkylation of *N*-(*p*-methoxy)benzyl-*N*-chloroacetyl-Phe-O^{t}Bu derivative to the corresponding β-lactam, TADDOL was selected from a panel of different phase-transfer catalysts as the best chiral additive (ee up to 82%). We have demonstrated here that the degree and sign of the cyclization selectivity was virtually independent on TADDOL configuration, providing experimental proof that in this reaction this additive works as a memory of chirality enhancer and not as a real catalyst. The effect of TADDOL is strongly dependent on the starting amino acid derivative, the increase of selectivity being only observed for those with aromatic side chains.

1. Introduction

In recent years, seminal research from various laboratories has confirmed the memory of chirality phenomenon as a conceptually novel approach for asymmetric induction.[1] Memory of chirality occurs in processes where an initial stereogenic center is destroyed during the generation of the corresponding reactive intermediate, but this intermediate is able to 'remember' the configuration of its precursor to transfer the chirality to the final compound, without using any external chiral source. This intriguing phenomenon has been observed in a variety of reactions, involving different reactive intermediates, such as carbanions, carbenium cations, and different types of radicals. Except for a few examples,[2] most reactions occurring with memory of chirality involve chemical transformations at the α-carbon of α-amino acid derivatives. Thus, this type of sp^3–sp^3 transfer of chirality has made an important contribution to the stereochemical course of some α-*C*-substitution reactions[3–17] and different photocyclization processes.[18–22] Among them, the intermolecular α-alkylation of a number of α-amino acid derivatives, extensively studied by Fuji and Kawabata group, proceeded with a high degree of asymmetric induction.[3–8] Similarly, the intramolecular version of this process allowed the enantioselective preparation of valuable azacyclic amino acid derivatives.[9,10] The enantioselectivity in these reactions was attributed to the formation of chiral nonracemic enolates, with restricted rotation around the C–N axis. On the other hand, the sequential deprotonation/alkylation of amino acid-derived 1,4-benzodiazepine-2-ones, reported by Carlier and co-workers, permitted the direct preparation of the corresponding C-3-quaternary derivatives with high enantiomeric purity.[11,12] In this case, a conformationally chiral enolate was assumed to be the origin of the observed asymmetric induction.[12] Stoodley's group found that the intramolecular aldol cyclization of 1-(3-oxobutyryl) derivatives of Pro, and their 4-oxa and 4-thia analogues, as well as other related cyclization of thioproline derivatives, afforded the corresponding bicyclic derivatives with complete retention of the configuration.[13–15]

In this context, we have reported that memory of chirality applies in the base-promoted cyclization of some *N*-benzyl-*N*-chloroacetyl amino acids **1** to the corresponding 1,4,4-trisubstituted 2-azetidinones **2**.[23,24] In this asymmetric

Keywords: Memory of chirality; Enantioselective synthesis; β-Lactams; TADDOL.
Abbreviations: BEMP, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro1,3,2- diazaphosphorine; BINOL, 1,1′-bi-2-naphtol; BTPP, *tert*-butylimino-tri(pyrrolidino)phosphorane; DCM, dichloromethane; MeCN, acetonitrile; TADDOL, 2,3-*O*-isopropylidene-1,1,4,4-tetraphenyl-L-threitol.
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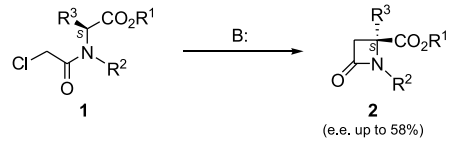
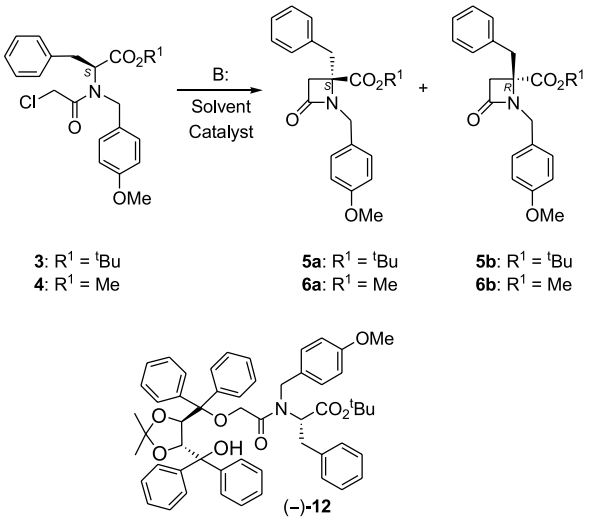
α-alkylation reaction, for which it was demonstrated the importance of the reaction conditions[25] and of the amino acid substituents,[26] only moderate enantioselectivities were obtained (ee up to 58%).

Alternatively, the asymmetric phase-transfer alkylation of amino acid aldimines is a powerful tool for the construction of a wide range of natural and unnatural α-amino acids, including α,α-dialkyl derivatives.[27] Up to three generations of cynchona-derived catalysts have been successfully exploited in this transformation.[28–31] Further research efforts in this field led to the development of C_2 symmetric quaternary ammonium salts,[32] tartaric acid derivatives (TADDOL),[33,34] aminophenols (NOBIN),[35] and chiral salen-metal complexes[36] as excellent catalysts to stereoselectively prepare α-amino acids (Scheme 1).



Scheme 1.

Considering that the generation of the quaternary stereogenic center in compounds **2** occurs through the intramolecular alkylation of an α-amino acid, it would be attractive to investigate whether the use of the above indicated phase-transfer catalysts could serve to get and/or to improve the stereoselectivity in the synthesis of β-lactams **2**. Moreover, in those cases where the azetidinone derivatives are obtained in an enantioselective manner due to memory of chirality, this study could give an idea if the memory event might be modulated using such chiral catalysts. In this paper, we describe the first experimental evidence for TADDOL as a helpful additive to improve memory of chirality during the synthesis of our amino acid-derived β-lactams.



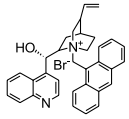
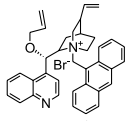
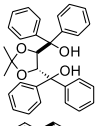
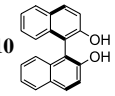
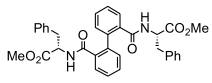
Scheme 2.

2. Results and discussions

2.1. Effect of different phase-transfer catalysts on the cyclization of Phe derivatives

To explore the usefulness of different phase-transfer catalysts in the synthesis of the amino acid-derived β-lactams **2**, we studied the base-assisted cyclization of the Phe-O^{t}Bu derivative **3** in the presence of the two cinchonidine related compounds (−)-**7** and (−)-**8**, (−)-TADDOL (**9**), (−)-BINOL (**10**), and the biphenyl catalyst **11** (Scheme 2, Table 1).

Table 1. Influence of the phase-transfer catalyst on the selectivity of the cyclization of L-Phe-O^{t}Bu derivative **3** (MeCN, BTPP)

Entry	Catalyst (10%)	Yield (%)[a]	**5a**:**5b** er[b]	ee (config.)
1	—	73	76:24	52 (*S*)
2	(−)-**7** 	85	74:26	48 (*S*)
3	(−)-**8** 	69	70:30	40 (*S*)
4	(−)-**9** 	71	85:15	70 (*S*)
5	(−)-**10** 	79	77:23	54 (*S*)
6	(−)-**11** 	55	76:24	52 (*S*)

[a] Isolated yield.
[b] Measured by chiral HPLC (Column: OL-389, hexane–acetone (96/4), 1.5 mL/min).

Chiral additives **7**–**9** were selected by their outstanding results in the synthesis of optically pure amino acids,[28–31,33,34] while BINOL has become among the most widely used ligands for both stoichiometric and catalytic asymmetric reaction, including different types of C–C bond formation.[37] On the other hand, the L-Phe-containing biphenyl derivative **11** has recently been introduced as an effective catalyst for the stereoselective preparation of cyanhydrines and the addition of diethylzinc to aldehydes.[38]

As shown in Table 1, the cinchonidine derivatives and the biphenyl compound **11** proved to be ineffective for improving the enantioselectivity obtained in the formation of compound **5**, compared to the reaction without chiral additive. In fact, a 4 and 12% drop in the *S*-ee was observed after the addition of catalysts **7** and **8**, respectively. It is well documented that the cinchona-derived catalysts preferably work at low temperatures, but in our case the reaction should be carried out above 0 °C to proceed. Only a marginal increase in the asymmetric induction was achieved in the reaction with (−)-BINOL, while the tartaric acid derivative (−)-TADDOL (**9**), that operates on a wider

Table 2. Influence of (−)-TADDOL on the selectivity of the base-promoted cyclization of compounds **3** and **4**

Entry	R[1]	Base	Solvent	Additive (10% mol)	Yield (%)[a]	**a**:**b** er[b]	ee (*S*)
1	^{t}Bu	BTPP	DCM	—	58	74:26	48
2	^{t}Bu	BTPP	DCM	(−)-**9**	73	82:18	64
3	^{t}Bu	BEMP	DCM	—	65	75:25	50
4(*)	^{t}Bu	BEMP	DCM	(−)-**9**	70	77:23	56
5(*)	^{t}Bu	BTPP	MeCN	—	73	76:24	52
6(*)	^{t}Bu	BTPP	MeCN	(−)-**9**	71	85:15	70
7(*)	^{t}Bu	BEMP	MeCN	—	81	76:24	52
8	^{t}Bu	BEMP	MeCN	(−)-**9**	76	81:19	62
9	Me	BTPP	DCM	—	65	68:32	36
10	Me	BTPP	DCM	(−)-**9**	83	80:20	60
11 (*)	Me	BEMP	DCM	—	69	68:32	36
12	Me	BEMP	DCM	(−)-**9**	83	70:30	46
13	Me	BTPP	MeCN	—	68	67:33	34
14	Me	BTPP	MeCN	(−)-**9**	87	85:15	70
15	Me	BEMP	MeCN	—	59	69:31	38
16(*)	Me	BEMP	MeCN	(−)-**9**	72	79:21	58

(*) Repeated experiences for the regression analysis.
[a] Isolated 2-azetidinone **5** or **6**.
[b] Measured by chiral HPLC (Ref. 26).

temperature range, was able to produce an important increase in the enantioselectivity of the final β-lactam (Δee, 18%). Although these improved enantioselectivities could in principle be due to double asymmetric induction, experiments with (±)-TADDOL and (+)TADDOL strongly argue against this explanation (vide infra). Based on these results, further research efforts were concentrated on studying TADDOL, which showed the best potential.

2.2. Effect of TADDOL on the cyclization of Phe derivatives

We have previously shown that the enantioselectivity of the cyclization of *N*-chloroacetyl-L-Phe esters is highly dependent on the base and solvent used[25] and also on the nature of the ester group.[26] A realistic study on the influence of the chiral additive TADDOL in this enantioselective reaction can not ignore these other factors to choose the model experiments to evaluate. Consequently, we decided to investigate the effect of (−)-TADDOL on the cyclization of the L-Phe *tert*-butyl and methyl ester derivatives **3** and **4** (Scheme 2), in the presence of BTPP or BEMP as base[39] and DCM or MeCN as solvent.

In order to know the influence of each variable, a careful experimental study was undertaken using a classical factorial experimental design[40] by selection of the appropriate 16 reactions for the four desired variables: the nature of the ester (x_1), the base (x_2), the presence of catalyst (x_3) and the solvent (x_4), being the ee the measured response.[41] The experiments and the results obtained are collected in Table 2. The coefficients of the main effects (b_i) and those all second- (b_{ij}) and third-order (b_{ijk}) interactions were calculated, and those significant are gathered in Table 3. The inspection of these coefficients firstly shows that the four individual variables are statistically important and, secondly, that there are some noteworthy second-order interactions, such as additive/ester (x_3x_1), additive/base (x_3x_2), and additive/solvent (x_3x_4). These results demonstrate that description of the effect of (−)-TADDOL would not be completed without consideration of its interactions with these others variables.

The obtained coefficients indicate that x_3 (presence of the additive, $b_3=8.2$) is the factor that greatly affects enantioselectivity. Thus, the addition of a 10% mol of catalyst (−)-**9** to the reaction of the L-Phe derivatives **3** and **4** resulted always in an increase of the enantiocontrol. This enhancement is higher for L-Phe-OMe derivative **4** than for *tert*-butyl ester analogue **3**. This fact is a consequence of the ester influence ($b_1=4.7$) in the enantioselectivity, since in absence of TADDOL, the *tert*-butyl derivative **3** cyclizes in a higher ee than the corresponding methyl ester **4** (Table 2, compare entries 1, 3, 5, and 7 to entries 9, 11, 13, and 15). It seems that a maximum limit is reached in both cases in the presence of the chiral additive (ee ≈ 60% in DCM, and 70% in MeCN).

The role of the solvent (x_4) is also important, although less than x_3 and x_1, being the enantioselectivity in MeCN higher than in DCM. Nevertheless, its interaction with the additive (x_3x_4) is the minor factor, and no different tendencies were observed with or without (−)-**9**.

A rather surprising result is the fact that the base is less important than their corresponding interaction with the additive ($b_{32}>b_2$). Thus, in the presence of (−)-TADDOL, the degree of stereocontrol was reliant on the base (BTPP > BEMP), while without the chiral additive the influence of this variable was negligible. Taking into account that the pK_a of both bases is similar, it is possible to consider their difference in volume and, hence, a different interaction of each base with the additive.

Table 3. Significant coefficients of main effects and second-order interactions calculated with the factorial design (22 experiments)

Factor	—	x_1	x_2	x_3	x_4	x_3x_1	x_3x_2	x_3x_4
Coefficient	52.2	4.7	−1.7	8.2	2.3	−2.8	−3.0	1.5

Table 4. Importance of the configuration of Phe residue and TADDOL on the selectivity (BTPP)

Entry	Starting Phe	R[1]	Solvent	TADDOL (10% mol)	Yield (%)[a]	**a**:**b** er[b]	ee
1	D-**4**	Me	DCM	—	73	31:69	38 (*R*)
2	D-**4**	Me	DCM	(−)-**9**	75	19:81	62 (*R*)
3	D,L-**4**	Me	DCM	—	52	51:49	2 (*S*)
4	D,L-**4**	Me	DCM	(−)-**9**	71	51:49	2 (*S*)
5	L-**4**	Me	DCM	(+)-**9**	74	81:19	62 (*S*)
6	D-**4**	Me	DCM	(+)-**9**	85	20:80	60 (*R*)
7	D,L-**4**	Me	DCM	(+)-**9**	61	51:49	2 (*S*)
8	L-**4**	Me	DCM	(±)-**9**	72	79:21	58 (*S*)
9	L-**4**	Me	MeCN	(+)-**9**	71	88:12	76 (*S*)
10	L-**4**	Me	MeCN	(±)-**9**	60	86:14	72 (*S*)
11	L-**3**	^{t}Bu	DCM	(+)-**9**	78	78:22	56 (*S*)
12	L-**3**	^{t}Bu	DCM	(±)-**9**	79	77:23	54 (*S*)
13	L-**3**	^{t}Bu	MeCN	(+)-**9**	67	81:19	62 (*S*)
14	L-**3**	^{t}Bu	MeCN	(±)-**9**	81	79:21	58 (*S*)

[a] Yield of isolated product.
[b] Measured by chiral HPLC (Ref. 26).

2.3. Influence of Phe and TADDOL configurations

After the initial stimulating results, we next explored the consequence of the use of (−)-TADDOL in the cyclization of compounds **4** derived from D- and D,L-Phe-OMe (Table 4). As expected, in the absence of the chiral additive, the D-Phe derivative led to the main formation of the *R*-enriched β-lactam **6b** (entry 1), while the D,L-analogue afforded compound **6** in almost racemic form (entry 3). Both results are proofs that the memory of chirality phenomenon is responsible for the observed selectivity.

Cyclization of D-**4** in the presence of (−)-**9** gave a 24% rise in the 4*R* enantioselectivity. This increment in the ee, due to the addition of the chiral additive, was totally comparable to that obtained for the L-Phe-OMe analogue (in absolute value, see Table 2, entry 10). On the contrary, the addition of (−)-TADDOL to the reaction of the racemic D,L-**4** had no influence on the estereoselectivity, indicating that TADDOL is only effective when the chiral memory event is operative.

These intriguing results prompted us to study the influence of TADDOL configuration on the cyclization of compounds **3** and **4** (Table 4). The addition of a 10% mol of catalyst (+)-**9** to the reaction of the L-**4** derivative in DCM produced a slightly higher asymmetric induction, also favoring the 4*S* enantiomer **6a**, than that obtained upon addition of the enantiomeric catalyst (−)-**9** (compare entry 10 in Table 2 with entry 5 in Table 4). These outcomes were corroborated during cyclization of the corresponding D-Phe analogue in the presence of (−)-**9** and (+)-**9**, providing quite similar ees in favor of the *R*-enriched β-lactam **6** (Table 4, entries 2 and 6). More interestingly, an analogous improvement in selectivity was also achieved when racemic (±)-TADDOL was used as additive (entry 8), suggesting a minor role for the additive configuration. Cyclization of L-**4** in MeCN and in the presence of (+)-**9** gave a 6% higher increase in the ee value than reaction with (−)-**9**, while (±)-**9** led to an intermediate situation (compare entry 14 in Table 2 with entries 9 and 10 in Table 4). On the contrary to the cyclization of Phe-OMe derivatives **4**, the increase in selectivity during the cyclization of the L-Phe-O^{t}Bu derivative **3** was higher using (−)-**9** than its enantiomer or the racemic TADDOL, especially when acetonitrile was used as solvent. This confirms again the strong interrelationships of the reaction variables and seems to indicate that the alkyl group of the ester could be, to some extent, implicated in the interaction with the chiral additive.

The fact that, for a given starting material, (−)-**9**, (+)-**9** and (±)-**9** generated quite similar asymmetric inductions, both in percentage and sign, is indicative that these chiral additives are not simply acting as chiral catalysts, for if they were, matched and mismatched behavior would be expected. Instead, remarkably, these chiral additives, independent of configuration and enantiopurity, enhance the chirality transfer from the starting material to the product. This particular behavior of TADDOL was also reproduced by (−)- and (+)-BINOL in the cyclization of compound L-**3**, although the total increase in selectivity was much less important than with TADDOL (Δee (−)-BINOL, 2%; Δee (+)-BINOL, 8%).

2.4. Importance of the quantity of TADDOL and its structure

An increase of the (−)-**9** catalyst led to a significant enhancement in the ee value (up to 82%), but accompanied with lower yields of the β-lactam **5**, due to significant *O*-monoalkylation of (−)-**9** with the *N*-chloroacetyl derivative **3**, to give compound (−)-**12** (Table 5). This latter compound did not contribute in any extent to the high enantioselectivity observed in the reactions where it is produced, as subsequently demonstrated by using it as the only additive (Table 5, entry 5). In standard trials, the transformation of a 10% (−)-TADDOL into compound (−)-**12** was complete within 2 h, as quantified by HPLC. Therefore, when the cyclization of compound **3** in the presence of (−)-**9** was stopped after 2 h, the ee of the β-lactam **5** (80%, Table 5, entry 6) was very close to that obtained with an equimolecular amount of the additive, corroborating that catalyst (−)-**9** was the only enhancer of the memory of chirality. The formation of the *O*-alkylation product (−)-**12** could suggest that TADDOL, in the presence of the strong organic bases, could work as a chiral TADDOLate base, but the extent and trend of the

Table 5. Influence of the concentration of (−)-TADDOL on the selectivity of the BTPP-promoted cyclization of compound **3**

Entry	R^1	Solvent	Additive (% mol)	Yield (%)	**a**:**b** er[a]	ee
1	^{t}Bu	DCM	(−)-**9** (50)	60	85:15	70
2	^{t}Bu	DCM	(−)-**9** (100)	46	86:14	72
3	^{t}Bu	MeCN	(−)-**9** (50)	68	91:9	82
4	^{t}Bu	MeCN	(−)-**9** (100)	53	91:9	82
5	^{t}Bu	MeCN	(−)-**12** (10)	84	73:27	46
6	^{t}Bu	MeCN	(−)-**9** (10)[b]	47	90:10	80

[a] Measured by chiral HPLC (Ref. 26).
[b] Reaction processed after 2 h.

asymmetric induction promoted by (−)-**9** and (+)-**9** seems to discard this possibility.

Of further interest were the experiments performed with differently substituted TADDOLs, intended for establishing the importance of the OH and Ph groups of this catalyst on the selectivity (Table 6).

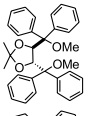
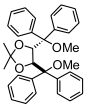
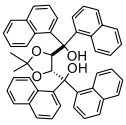
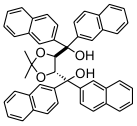
Dimethylated TADDOL derivatives (−)-**13** and (+)-**13** failed to induce any additional asymmetry over the usually obtained by the memory of chirality, demonstrating that the 1,4-hydroxyl groups were necessary features for the asymmetric inducing power of these catalysts. The incorporation of bulky 1-naphtyl substituents instead of the phenyl groups of TADDOL, as in (−)-**14**, resulted in a negligible increase of the selectivity with respect to the reaction without additive. In contrast, catalyst (−)-**15**, with a phenyl to 2-naphtyl interchange, was a quite efficient additive, yielding to similar ee values than (−)-**9** (Table 6, compare entries 1 and 2 with 9 and 10). The disparity between the behavior of 2-naphtyl (phenyl-like) and the 1-naphtyl additives could be attributed to a completely different spatial arrangement of the aromatic groups in these derivatives.[42] In fact, it has been described that TADDOLates bearing four 1-naphtyl groups often show a complete absence of catalytic activity due to high steric hindrance.[43] These results seem to suggest that the phenyl (or 2-naphtyl) groups of TADDOL could be implicated in direct interactions either with the starting amino acid or with the enolate intermediate.

Since the stereochemistry of TADDOL is not a determining factor for the observed selectivity, a structurally related achiral derivative, diphenylcarbinol **16**, was also explored as additive. However, the cyclization of compound **3** to **5** in the presence of diphenylcarbinol, far from amplify the asymmetric induction, led to a dramatic reduction in the selectivity, suggesting a role for the whole three-dimensional structure of TADDOL.

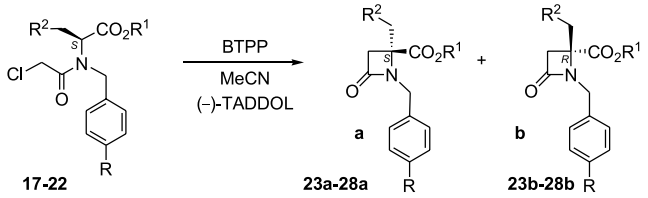
2.5. Effect of TADDOL on the cyclization of different amino acid derivatives

The capacity of TADDOL to enhance memory of chirality in the cyclization of other amino acid derivatives was next examined (Scheme 3).

Table 6. Influence of TADDOL structure on the selectivity of the BTPP-promoted cyclization of compound **3**

Entry	Solvent	Additive (10% mol)	Yield (%)[a]	**a**:**b** er[b]	ee (*S*)
1	MeCN	(−)-**9**	71	85:15	70
2	DCM	(−)-**9**	73	82:18	64
3	MeCN	(+)-**9**	67	81:19	62
4	DCM	(+)-**9**	79	78:22	56
5	MeCN	(−)-**13** 	79	75:25	50
6	MeCN	(+)-**13** 	79	75:25	50
7	MeCN	(−)-**14** 	65	79:21	58
8	DCM	(−)-**14**	73	76:24	52
9	MeCN	(−)-**15** 	70	83:17	66
10	DCM	(−)-**15**	73	79:21	58
11	MeCN	**16**	92	59:41	8

[a] Isolated compounds.
[b] Measured by chiral HPLC (Ref. 26).



17, 23: R = OMe, R^1 = Me, R^2 = Ph[4-OBzl(2,6-Cl)]
18, 24: R = OMe, R^1 = ^{t}Bu, R^2 = In(Boc)
19, 25: R = H, R^1 = ^{t}Bu, R^2 = In
20, 26: R = OMe, R^1 = Me, R^2 = $(CH_2)_2NHZ$
21, 27: R = OMe, R^1 = ^{t}Bu, R^2 = $CH(CH_2)_3$
22, 28: R = H, R^1 = ^{t}Bu, R^2 = H

Scheme 3.

As highlighted in Table 7, in addition to Phe derivatives, only Tyr and Trp analogues **17–19** were prone to the asymmetric influence of TADDOL. From these results, the improvement in the memory of chirality due to this chiral additive can be rationalized by postulating a transition-state model in which particular conformations of either the starting amino acid or the initially formed enolate, or both, would be fixed or stabilized through π–π interactions with the catalyst. This hypothetical transition state could explain the differences in the increase of selectivity between Phe Tyr and Trp derivatives [Δee due to TADDOL: 18–36% for Phe, 36% for Tyr, and 8% for Trp(Boc) and Trp], since the lower aromatic character of the indole ring in Trp could take account for the minor change in the asymmetric induction.[44] In agreement with the suggested importance of π–π interactions, TADDOL was ineffective for aliphatic amino acid derivatives, independently that the memory of chirality phenomenon existed previously (Leu, Orn) or not (Ala).

Preliminary molecular modeling studies have revealed that the starting amino acid derivative **4** was able to establish very similar interactions with both enantiomers of TADDOL (Fig. 1). Main contacts are characterized by an

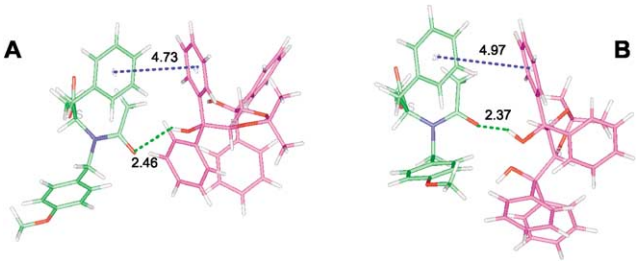


Figure 1. Low energy complexes between *N*-Pmb-*N*-chloroacetyl-L-Phe-OMe **4** and (−)-TADDOL (**A**), and (+)-TADDOL (**B**).

NCO–HO H-bond and an offset stacking interaction, with inclined ring moieties, between the phenyl ring of the Phe side chain and one aromatic group of TADDOL. The observed arrangements led to a correct positioning of the CH_2Cl substituent to allow the cyclization to take place. This mode of interaction is in good agreement with the capital importance of the free OH groups in TADDOL and the need for amino acid side chains that favor π–π contacts. Alternative scenarios, which postulate the interaction of TADDOL with the enolate intermediates, could not be discarded to participate in the production of selectivity.

3. Conclusions

In this paper, we have described the first examples of the TADDOL-promoted enhancement of the enantioselectivity due to memory of chirality. The degree of the selectivity improvement in the intramolecular α-alkylation of *N*-benzyl-*N*-chloroacetyl amino acid derivatives was dependent on the solvent and base used, and most importantly on the nature of the amino acid side chain, that should be aromatic. However, the extent and sign of the enantioselectivity in the β-lactam formation was practically independent on the configuration of TADDOL. This means that the chiral additive does not work as a true catalysts, but could be

Table 7. Effect of TADDOL in the cyclization of different amino acid derivatives (BTPP, MeCN)

Entry	Starting Compd.	Additive (10 mol %)	Final Compd.	Yield (%)[a]	**a**:**b** er[b]	ee (*S*)
1	**3**	—	**5**	73	76:24[c]	52
2	**3**	(−)-**9**	**5**	71	85:15[c]	70
3	**4**	—	**6**	68	67:33[d]	34
4	**4**	(−)-**9**	**6**	87	85:15[d]	70
5	**17**	—	**23**	68	63:37[e]	26
6	**17**	(−)-**9**	**23**	73	81:19[e]	62
7	**18**	—	**24**	89	71:29[f]	42
8	**18**	(−)-**9**	**24**	90	75:25[f]	50
9	**19**	—	**25**	24	65:35[f]	30
10	**19**	(−)-**9**	**25**	30	69:31[f]	38
11	**20**	—	**26**	75	55:45[g]	10
12	**20**	(−)-**9**	**26**	77	55:45[g]	10
13	**21**	—	**27**	42	78:22[h]	56
14	**21**	(−)-**9**	**27**	27	77:23[h]	54
15	**22**	—	**28**	60	50:50[h]	0
16	**22**	(−)-**9**	**28**	65	50:50[h]	0

[a] Yield of isolated compounds.
[b] Measured by chiral HPLC (column: OL-389).
[c] Hexane–acetone (96/4), 1.5 mL/min.
[d] Hexane–EtOH (95/5), 1 mL/min.
[e] Hexane–acetone–EtOH (95/4/1), 1.2 mL/min.
[f] Hexane–acetone (96/4), 1.2 mL/min.
[g] Hexane–EtOH (90/10), 1 mL/min.
[h] Measured by RP-HPLC from the corresponding dipeptide derivatives (Ref. 26).

considered like a memory of chirality enhancer. An hypothetical mechanism to rationalize the function of TADDOL in these reactions could involve the formation of complexes between the starting amino acid derivative, or its derived enolate intermediate, and the chiral additive, through H-bonds and π–π interactions.

In spite of the new insights into the memory phenomenon inferred from this paper, whether the use of other chiral catalysts, and even of non-chiral additives, could result in more general approaches to control memory of chirality events remains an elusive goal.

4. Experimental

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were from commercial sources. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of silica gel 60 F254. Silica gel 60 (230–400 mesh) was used for column chromatography. Analytical HPLC uses a Novapak C_{18} (3.9×150 mm, 4 μm) column, with a flow rate of 1 mL/min, using a tuneable UV detector set at 214 nm. Mixtures of MeCN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phase. ^{1}H NMR spectra were recorded with a spectrometer operating 300 MHz, using TMS as internal standard. ^{13}C NMR spectra were registered at 75 MHz. Electrospray mass spectra (positive mode) were recorded with a Hewlett Packard 1100SD spectrometer.

Chiral stationary phase was prepared by covalent bonding of a mixed cellulose derivative (10-undecenoate/3,5-dimethylphenylcarbamate) on allylsilica gel. This procedure was carried out by radical polymerization in the presence of AIBN, without solvent.[45]

Starting *N*-chloroacetyl derivatives **3**, **4**, and **16–21** were prepared as described.[24,26] Final β-lactams **5**, **6** and **22–27** showed the expected analytical and spectroscopic data.[24,26] Catalysts **8** and dimethylated TADDOL derivatives (−)-**13** and (+)-**13** were synthesized following previous reported procedures.[42,46] Compound **11** was a generous gift from Dr. Herradón. Catalysts (−)-**7**, (−)-TADDOL (**9**), (−)-BINOL (**10**), (−)-**14**, and (−)-**15** were obtained from commercial sources.

4.1. Synthesis of 2-azetidinones

General procedure. A solution of the corresponding chloroacetyl derivative (0.4 mmol) and the additive (normally 10% mol) in the appropriate solvent (1.5 ml) was treated, at room temperature and under Ar atmosphere, with the selected base (0. 6 mmol). The reaction was monitored by TLC or HPLC until complete disappearance of the starting material. Then, the solution was evaporated, dissolved in EtOAc, washed with H_2O, and dried over Na_2SO_4. After evaporation, the resulting residue was purified on a silica gel column. The obtained 2-azetidinone was directly evaluated by chiral HPLC, or transformed into the corresponding dipeptide derivatives, as previously described.[26]

4.1.1. (2*R*,3*R*,1′*S*)-2,3-*O*-Isopropyliden-1-*O*-[*N*-(*p*-methoxybenzyl)-*N*-[1′-*tert*-butoxycarbonyl-2′-(phenyl)-ethyl]-aminocarbonylmethyl-1,1,4,4-tetraphenyl-L-threitol (12). Eluent: EtOAc–hexane (1/6). Solid. Mp: 86–88 °C (EtOAc–hexane). HPLC: t_R=6.81 min. (A:B=80:20). ESI-MS: 870.4 $(M+Na)^+$. $[\alpha]_D$ −53.29 (*c* 0.85, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76–6.27 (m, 29H), 4.45 (d, 1H, *J*=8.0 Hz), 4.33 (m, 1H), 4.23 (d, 1H, *J*=8.0 Hz), 3.74 (d, 1H, *J*=14.5 Hz) 3.68 (m, 4H), 3.28 (d, 1H, *J*=16.6 Hz), 3.20 (m, 2H), 3.09 (d, 1H, *J*=14.5 Hz), 1.37 (s, 9H), 0.99 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.02, 168.53, 158.82, 146.68–108.17 (35C), 84.78, 84.70, 82.07, 81.72, 79.99, 63.30, 61.85, 55.19, 50.34, 35.12, 27.90, 27.04, 26.73. Anal. Calcd for $C_{54}H_{57}NO_8$: C, 76.48; H, 6.77; N, 1.65. Found: C, 76.35; H, 6.50; N, 1.44.

4.2. Factorial experimental design

The factors assumed to influence the reaction studied were chosen on the basis of prior knowledge of their weight on the enantioselectivity. They were: the nature of the ester (x_1), the base (x_2), the presence of catalyst (x_3) and the solvent (x_4), being the ee the measured response. For these four factors, at two levels, 16 experiments (2^4) are necessary to determinate the coefficients of the main effects and all the possible interactions between two or more factors. Six experiences, randomly selected (marked in Table 2 with an asterisk), were repeated in order to determine the experimental error that resulted to be 1.7% ee. The results of all reactions (22 experiments) were included in the regression analysis. The coefficients of main effects (b_i) and those of all second- (b_{ij}) and third-order (b_{ijk}) interactions were calculated in a first run. Then, only the variables with statistically significant coefficients (main effects and second-order x_3x_1, x_3x_2 and x_3x_4) were kept in the model in a second regression analysis to calculate the new coefficients. The fitting of this regression analysis was good (r=0.987) and the model error (2.1% ee) was not appreciably different from the experimental error, so we can be quite confident with the significance of the coefficients.

4.3. Molecular modeling

Complexes between TADDOL derivatives and different conformations of compound **4** were manually constructed. The 3D structures of (−)-TADDOL and (+)-TADDOL were taken from the Cambridge Structural Database (CSD). The resulting complexes were submitted to simulated annealing molecular dynamics calculations, using a constraint function to maintain the distance between the CO corresponding to compound **4** and one of the hydroxyl groups of TADDOL, and/or the distance between the center of the aromatic ring of the Phe side chain in compound **4** and the center of one of the aromatic rings of TADDOL. Moreover, the coordinates of TADDOL were kept fixed. The simulated annealing strategy consisted of 100 loops of slow cooling, each one leading to a low energy conformation. Each loop begins by fixing the temperature at 1000 K, followed by 5000 steps of molecular dynamics (MD). The temperature is then decreased in steps of 100 K every 50,000 steps, up till the temperature of the system corresponds approximately to 300 K. The final

conformation obtained at the end of this process was refined using a conjugate gradient algorithm, and after storage, was used to start a new simulation at high temperature with a slow cooling stage. This procedure produced samples of 100 energy-minimized conformations. From these samples, the lowest energy structures, within 3 Kcal/mol, that were able to explain the selectivity of the β-lactam formation were chosen. The molecular dynamic simulations were accomplished using the cff91 force field within the program package (Insight II, Discover, Homology, Biopolymer) from Molecular Simulations Inc. (San Diego, CA).

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Monoaza[5]helicenes. Part 2: Synthesis, characterisation and theoretical calculations

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Abstract—The synthesis of four different monoaza[5]helicenes is reported, to complete the whole series of these compounds, so that systematic studies on their properties can be carried out. They were fully characterised via NMR. A theoretical approach to explain why ring closure occurs to give the most crowded compound is reported, in comparison with earlier calculation methods.

1. Introduction

Photochemical cyclisation of stilbene derivatives is a well known method to obtain polycyclic molecules. In a previous paper,[1] which is part 1 of this work, we described the cyclisation of some ethylenic derivatives that allowed us to obtain a number of monoaza and diaza[5]helicenes. These molecules showed interesting properties depending on the position of the nitrogen in the helicene framework, like, for example, varying characteristic triplet lifetime or rate of racemisation.[1,2] Unfortunately, for different reasons, this synthetic approach appeared not to be of general application. In addition, among all possible products from ring closure reactions, helicene, the most crowded system, was obtained in all the cases we studied. Consequently, we were interested both in finding a way to obtain those aza[5]-helicenes that could not be synthesized with the route reported previously,[1] and also in applying theoretical considerations for the starting materials we used, to justify the photochemical cyclisation to give the helicene. In an earlier paper, we reported several different reasons why a single approach appeared not to be of general application,[3] and did not allow the formation of all the desired monoaza and diazahelicenes. We were interested in obtaining all the possible isomers because few aza[5]helicenes are known in the literature,[4–13] and there is no systematic study of their properties. This is despite the potential applications of helicenes that can be found, for example, in the fields of non-linear optics (NLO)[14] and circularly polarised luminescence.[15]

Indeed, the study we carried out on the already synthesized helicenes, showed a very long lifetime of the triplet state and a tendency to π–π stacking both in the solid state and for the azahelicenes by themselves or even for some metal complexes, allowing the build up of columnar system arranged in the space with a particular geometry, and with a control over chirality that may be valuable in designing new materials for optoelectronic applications.[16–20]

2. Results and discussion

2.1. Synthetic studies

The synthetic route we followed to obtain the azahelicenes was very simple and started from the synthesis of 1,2-disubstituted ethylenes (obtained through Wittig reaction from the appropriate aldehyde with the suitable phosphonium salt) that were afterwards cyclised photochemically to the desired helicenes as first described by Martin for the carbohelicene[21] and described in Scheme 1 for the synthesis of 4-aza[5]helicene.

Keywords: Helical structures; Helicenes; Molecular orbital calculations; Photochemistry.
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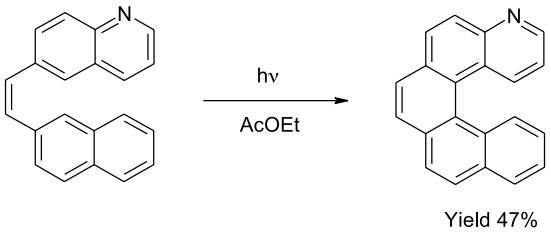
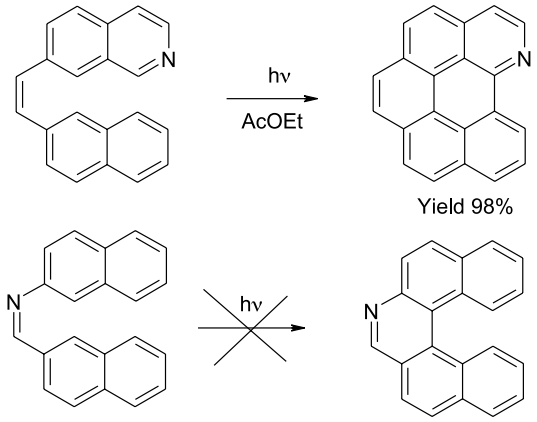


Scheme 1.



Scheme 2.

Following this synthetic approach, it was possible to synthesize nine different azahelicenes, namely 4-, 5-, 6-aza and 4,11-, 5,10-, 6,9-, 4,9-, 4,10- and 5,9-diaza[5]-helicenes. Other synthetic approaches were envisioned for the synthesis of carbo- and heterohelicenes,[22–26] but, in our opinion, when it is possible to apply the above route, this is the shortest, simplest and highest yielding. This approach, for different reasons, was not useful for obtaining the 2- and 7-aza derivatives (see Scheme 2), as well as for the preparation of the other monoazahelicenes. For example, we were not able to prepare 1-(2-naphthyl),2-(6-isoquinolyl) ethylene, the precursor for the 3-aza[5]-helicene, because 6-carboxaldehydeisoquinoline, to the best of our knowledge, is not reported in the literature, nor is 6-bromomethylisoquinoline, and we could not develop a synthetic strategy to obtain them. Also the preparation of 7-aza[5]helicene showed some difficulties, and attempts to repeat a published synthesis were unsuccessful.[12]

For these reasons, we decided to find a different approach to their synthesis, looking also to the possibility that it may open the route to obtain other diazahelicenes.

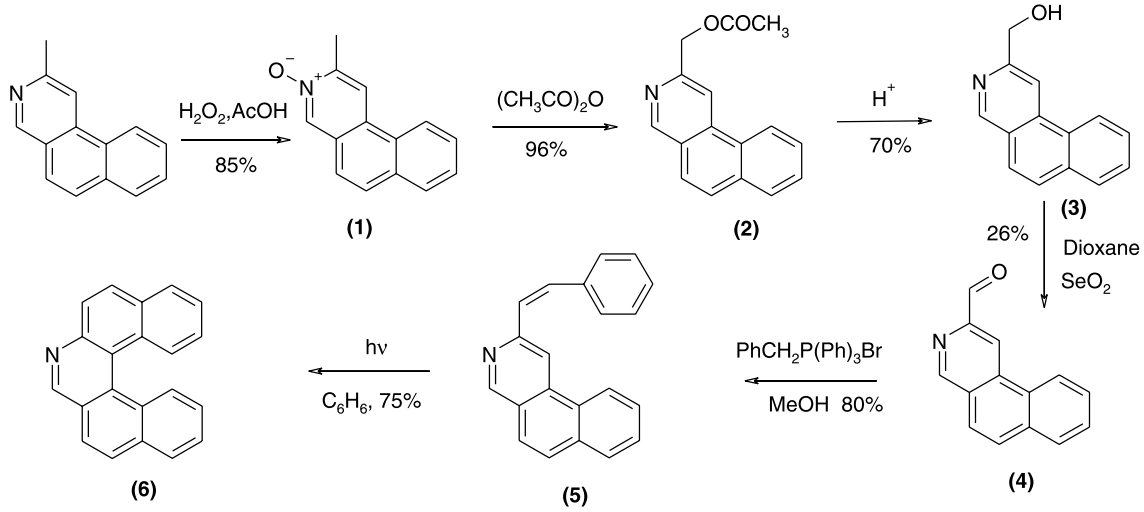
Since the photochemical ring closure had proven to be one of the simplest ways to obtain these compounds, we decided to study the photochemical ring closure of different ethylenic compounds, namely the compound carrying three *ortho*-condensed rings on one side of the ethene and one ring on the other side. The synthesis of these derivatives easily gave the desired helicenes, and eventually opened the route for the formation of some diaza[5]helicenes or of larger azahelicenes. We can anticipate that ring closure on the other side of the phenanthrene (or phenanthridine), giving a completely flat molecule with less strain, does not occur, at least in the case we studied.

The synthesis of the 2-methyl-benzo[*f*]isoquinoline[27] allowed to obtain the 7-aza[5]helicene (**6**) with a fair overall yield in few steps (Scheme 3).

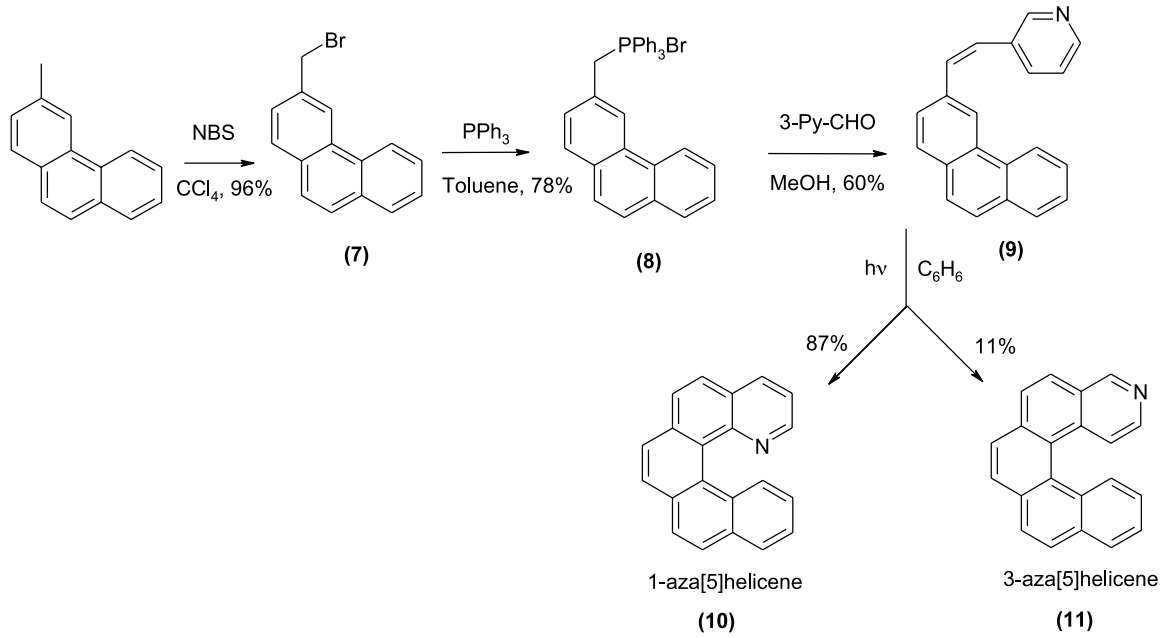
Despite the fact that generally the methyl group α to the heteroaromatic nitrogen is easily functionalised, in this phenanthridine it appeared to be rather unreactive. Transformation into the *N*-oxide allowed instead facile functionalisation through a rearrangement reaction. The other steps were rather obvious.

For the synthesis of 1- and 3-aza[5]helicenes two approaches are possible. The first starts by building an ethene derivative with a phenanthrene on one side and a 3-pyridine on the other. Its photochemical ring closure yielded two desired azahelicenes (**10**) and (**11**) in 9:1 ratio (Scheme 4).

The second synthetic approach may appear longer, because it was necessary to synthesize a starting phenanthridine



Scheme 3.



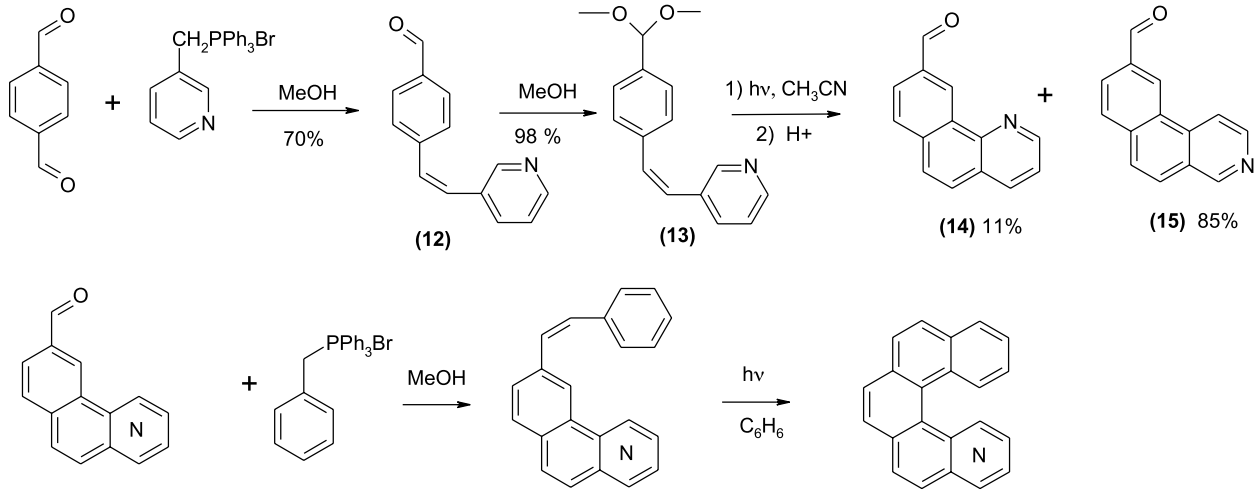
Scheme 4.

derivative. This was achieved through a Wittig reaction between terephthalaldehyde and the phosphonium salt of 3-bromomethylpyridine. With the use of an excess of terephthalaldehyde, it was possible to obtain the substitution of one aldehydic group only. Interestingly, the direct photolysis of this derivative did not give any ring closure, possibly because the $n\rightarrow\pi^*$ transition is lower in energy respect to the $\pi\rightarrow\pi^*$ transition. Confirmation of this hypothesis came from the transformation of the aldehyde to the corresponding dimethylketal. Irradiation of this derivative gave the two phenanthridines in very high overall yield (95%). Hydrolysis to their corresponding aldehydes was quantitative, and their separation was easily achieved (with an isomeric ratio of (**14**):(**15**) 1:8). The subsequent synthetic sequences to give (**10**) or (**11**) are illustrated in Scheme 5.

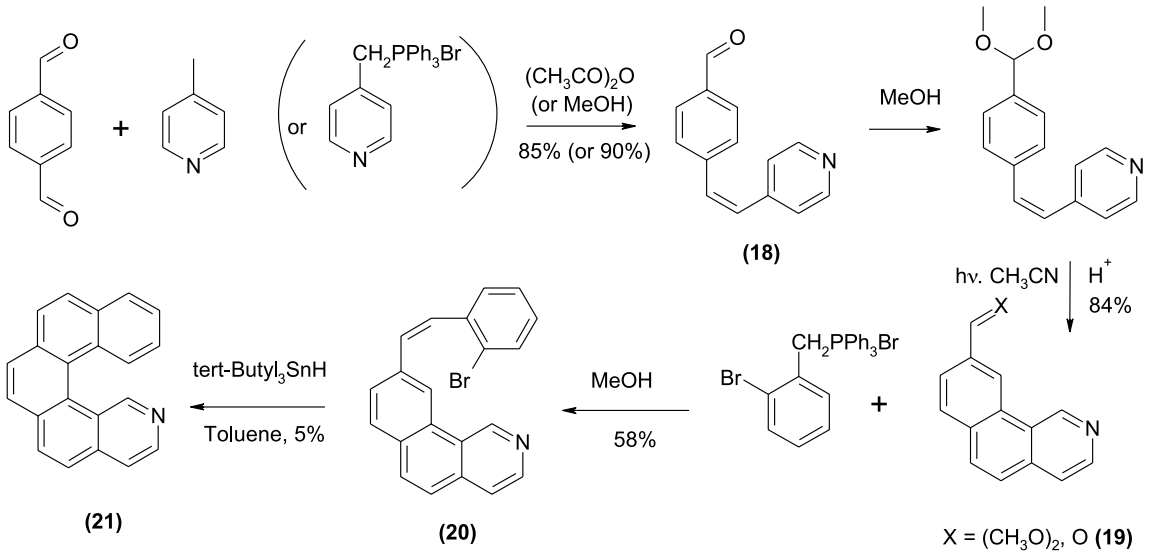
Since the yields were high in both cases, but the final amount of the two helicenes were different, one can choose the method depending on the helicene desired.

The synthesis of 2-aza[5]helicene was different, because, as we already stated, the photochemical ring closure of 7-(2-naphthalen-2-yl-vinyl)-isoquinoline yielded to the corresponding perylene, and the photochemical approach starting from either 4-(2-phenathrene-3-yl-vinyl)-pyridine or 9-styryl-benzo[*h*]isoquinoline gave the same result.

Since the last step cannot be a photochemical one, we designed another way to obtain the ring closure. This was obtained starting from benzo[*h*]isoquinoline-9-carbaldehyde (synthesised as illustrated in Scheme 6) and the 2-methylphosphonium salt of 1-bromobenzene. The



Scheme 5.



Scheme 6.

treatment of this derivative with (*tert*-butyl)$_3$SnH yielded the desired 2-aza[5]helicene (**21**). (Scheme 6).

2.2. Calculations

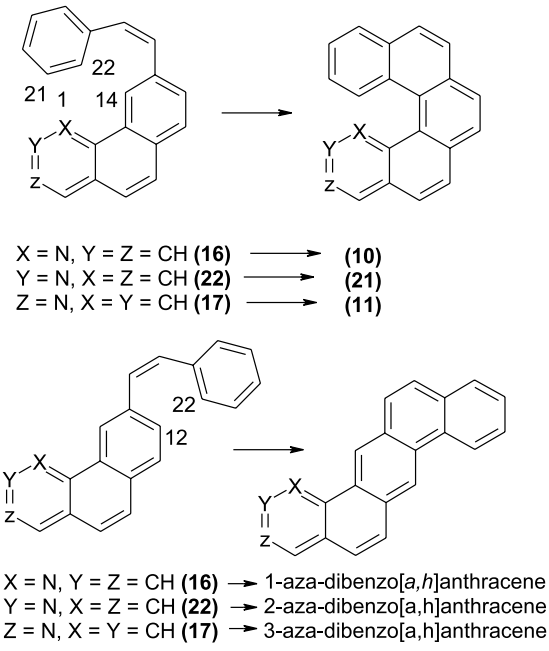
As pointed out in the first part of the paper, the photochemical cyclisation of these derivatives gave rise to the most crowded azahelicene rather that to the corresponding less strained dibenzo[*a*,*h*]anthracene (only the positions involved in the ring closure are highlighted in the scheme below for the sake of clarity).

Theoretical considerations and calculations on these mechanisms are already present in the literature. Some authors,[28,29] working in the context of the Hückel model, proposed a 'free valence' atomic index to characterize the reactivity of atoms in molecules, but generally they did not work together with experimental photochemists providing them with fresh experimental data. For these reasons, we decided to perform some calculations regarding the formation of the azahelicenes reported in this paper. Compounds (**16**), 9-styryl-benzo[*h*]isoquinoline (**22**) and (**17**) have been analysed by means of ab initio calculations using the package GAUSSIAN03.[30]

The ground-state geometry was determined by means of a DFT calculation with B3LYP functional and 6-31G basis set. Excited states have then been computed using the CIS method with the same basis set and geometry optimization has been carried out for the first excited state.

For each molecule two equilibrium configurations have been considered, the first one with a cis-geometry, close to the helicene structure (upper part in Scheme 7), while the second one with a trans-geometry close to the corresponding dibenzo[*a*,*h*]anthracene (lower part in Scheme 7).

In order to find an explanation for the different behaviour of the experiments for the production of helicenes with respect to other possible products, such as perilenes, (See Scheme 2) the value of an atomic index has been calculated by means



Scheme 7.

of the Natural Bond Orbital program included in the package GAUSSIAN03.[31]

In the present study, we have to take into account that the computational approach is different from a mere Hückel method and we need to define an atomic quantity F_A that mimics the 'free valence' atomic index. We introduce:

$$F_A = V_A - \sum_{B \neq A} b_{AB}^{(w)} \quad (1)$$

where V_A is the atomic valence returned by the NBO program as the sum of occupation numbers of the valence orbitals (as opposed to core orbitals) of atom A, $b_{AB}^{(w)}$ is the Wiberg bond index[32] of atoms A and B, and the sum is made over all the atoms B other than A.

Table 1. Differences of the '*F*' atomic indices in the excited state over the atoms of the scheme for the three structures (**16**), (**22**) and (**17**) (see Eq. 1 and discussion in the text)

	$F^*_{21}-F^*_1$	$F^*_{22}-F^*_{14}$	$F^*_{22}-F^*_{12}$
(**16**)	—	0.02078	0.01026
(**22**)	0.30355	0.01551	−0.00775
(**17**)	−0.03046	0.03432	0.00445

Table 2. Sums of the '*F*' atomic indices in the excited state over the atoms of the Scheme 7 for the three structures (**16**), (**22**) and (**17**) (see Eq. 1 and text)

	$(F^*_{21}+F^*_1)$	$(F^*_{22}+F^*_{14})$	$(F^*_{22}+F^*_{12})$
(**16**)	—	0.46762	0.50340
(**22**)	0.27981	0.48835	0.51153
(**17**)	0.61301	0.47542	0.50255

The above index has been calculated both in the ground state (F_A) and in the first excited state (F_A*).

In Table 1, we report the values of the differences $F^*_x-F^*_y$ for the positions considered in the above scheme; x and y indicate the reacting atoms, which have somewhat arbitrarily been chosen in the following order: x is on the benzene ring, y is on the phenanthridine system.

In Table 2, we report the sum $F^*_x+F^*_y$ for the reacting atoms x and y, as suggested in Ref. 29.

For compounds (**16**) and (**17**) the closure that yields the helicene is favoured with respect to the formation of the anthracene, and in accord to Table 1, this corresponds to the highest (positive) values for $F^*_x-F^*_y$. In the case of (**22**) however, the highest value for $F^*_x-F^*_y$ regards the closure between positions 21 and 1 suggesting that the perylene is formed first with this closure; the second highest value is calculated for positions 14 and 22. As matter of fact, even working at very low conversion, we were not able to detect any trace of the 2-aza[5]helicene that may close later to the perilene.

The calculations reported are for the cis conformers; we notice that the trans conformers have lower values for $F^*_x-F^*_y$, despite the fact that their ground state energies are slightly lower with the choice of the 6-31G basis set. Similar trends are found in Table 2, but the correlation with the observed reactivities of the studied molecules is not so immediate. Our obvious conclusion is that the first method seems to give better results for these indices.

Of course, more theoretical work should probably be done and further examples need to be considered to reach a definite conclusion; we also expect further support will

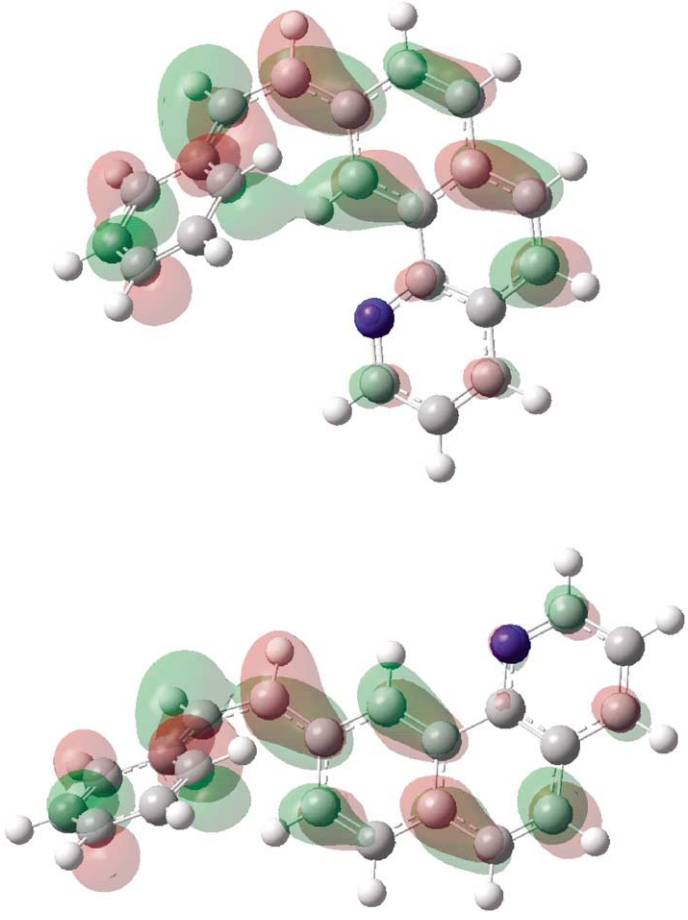


Figure 1. Isovalue surfaces of LUMO of the two conformers of (**16**) in the first excited state optimised geometries. The two colours refer to the two phases of the wavefunction.

come from future synthetic work. Also, due comparison with previous theoretical approaches in the literature[28,29] will be made in forthcoming work.

Further insight into this problem may be achieved by looking at the isovalue surface of LUMO orbital constructed with the aid of Gaussview package.[30] In Figure 1, we present the case of (**16**). A considerable superposition of the wavefunction for atoms 22 and 14 is observed, preparing compound (**16**) to react to helicene. In the single configuration interaction approach, the lower energy transition of both conformers has a fairly dominant contribution from HOMO–UMO, as expected, and its calculated dipole strength is higher than the dipole strength calculated for the two following transitions.

2.3. NMR

The assignment of the NMR spectra of the final helicenes was achieved by the combined use of spin–spin coupling constants in aromatic and heteroaromatic systems and H–H NOE, according to a general strategy already outlined in previous papers.[1,3]

In particular, we took advantage of the *J* coupling patterns within heteroaromatic bases to assign the signals in the N-containing part of the helicene. This approach can be conveniently used for the structural assessment of compounds (**10**), (**11**) and (**21**). Nevertheless, the assignment of the NMR spectrum of (**6**) deserves mention. Indeed, the position of the nitrogen atom in the molecular framework is such that the two naphthalene residues (C1–1 and C9–94) are in very similar chemical environments, making the discrimination of their ^{1}H NMR signals difficult. In this case, the assignment of H8 was the starting point, due to its diagnostic chemical shift. Selective irradiation of H8 gave 9.1% steady state NOE on H9. The large ^{3}J(H9–90) value (8.8 Hz) provided the assignment of the coupling partner H10. The signal assigned to H10 showed, by suitable resolution enhancement, a doublet of doublets multiplicity, with very small splitting (0.8 Hz) due to long range coupling with H14. The latter coupling is reported as 'zig zag' coupling in naphthalene derivatives.[33] This interpretation was confirmed by selective decoupling experiments. The unambiguous assignment of H14 (8.67 ppm) allowed us to assign the structurally related H1 (8.59 ppm). Steady state NOE difference spectra obtained after selective saturation of H1 and H14 allowed the assignment of the partially overlapped signals of H2 and H13. NOESY experiments allowed the assignment of H3 and H12, due to their dipolar contacts with already attributed H2 and H13. In turn, H3 and H12 gave cross peaks with the strongly overlapped H4 and H11 (8.01 and 8.02 ppm, respectively). The assignment of H5 was achieved via NOE with H4 and ^{5}J(H5–5)=0.8 Hz. The remaining doublet at 8.16 ppm was eventually assigned to H6.

3. Conclusions

The synthesis of four aza[5]helicenes is presented in order to complete the preparation of all the monoaza derivative of this class of molecules. The combined use of spin–spin coupling constants in aromatic and heteroaromatic systems and H–H NOE proved to be a general strategy for the elucidation of these structures. The Wiberg bond indexes seem to explain why the photochemical reactions bring to the most crowded of the two possible final molecules.

4. Experimental

All the solvents were distilled and dried before use. Benzaldehyde, terephthalaldeyde, bromomethylbenzene, 3-picoline, 3-pyridine aldehyde, 1-bromo,2-bromomethylbenzene, 2,2-dimethoxypropane, triphenylphosphine, azobisisobutyronitrile (AIBN),(*tert*-butyl)$_3$ tin hydride, acetic anhydride, SeO_2 and 1-bromo-pyrrolidine-2,5-dione (*N*-bromosuccinimide) are commercial products and were used without further purification. SeO_2 and Se (as product of the reactions) are poisonous and particular care must be used (well aerated hood and heavy gloves) when handled. NMR spectra were run either on Bruker ARX 400 or Bruker Avance 500 spectrometers operating at proton resonance frequencies of 400 and 500 MHz, respectively. The products were dissolved in $CDCl_3$ and tetramethylsilane (TMS) was added as reference. ^{1}H{^{1}H} NOE difference spectra and two-dimensional NOESY were carried out by using standard literature pulse sequences. Electron ionization mass spectra were recorded on a Finnigan MAT TSQ 70 instrument (70 ev, EI); the samples were introduced in the spectrometer source by direct probe insertion. Irradiations were carried out on a Rayonet RPR-100 photochemical reactor equipped with 16 interchangeable lamps irradiating either at 254, 300, or 350 nm as well in the visible range, dissolving the substance in the appropriate solvent. The ATR FT-IR spectra were recorded on a Avatar 370-Thermo.

4.1. Wittig reactions

General procedure. In a typical procedure in a flask containing a mixture of the aldehyde (1 mmol) and triphenyl-phosphonium bromide derivative (1 mmol) in methanol (15 mL), MeONa (0.065 g, 1.2 mmol) was added under stirring. The mixture was gently boiled for 3 h. After cooling, water was added, and the solution extracted many times with a large volume of CH_2Cl_2. After drying and solvent evacuation, the residue was chromatographed on silica gel. (Solvents), yield%, mp and physical properties are reported for each case.

4.2. Photochemical reactions

General procedure. The appropriate ethene derivative (1.0 mmol) was dissolved in benzene (150 mL) in a Pyrex vessel open to the air. The vessel was irradiated at 350 nm for a time ranging between 24 and 36 h. The irradiation was stopped when, and if, some tar begun to form. The solvent was removed under vacuum, and the residue was chromatographed on silica gel. R_f,(Solvents), yield%, mp, MS and NMR are reported for each case.

4.2.1. 2-Methylbenzo[*f*]isoquinoline. This compound was obtained as reported in the literature in 15% overall yield, mp 91–93 °C (lit. 93–95).[9]

4.2.2. 2-Methylbenzoisoquinoline *N*-oxide (1). The procedure used was adapted from the literature for the *N*-oxidation of the heterocyclic bases.[34] 2-Methylbenzoisoquinoline (1.00 g, 5.2 mmol) was dissolved in acetic acid (3 mL) and hydrogen peroxide 30% (5 mL) was added. The solution was warmed at 70 °C for 72 h. The solvent was evaporated and the residue was basified with a solution of $NaHCO_3$ and extracted with $CHCl_3$. After drying of the organic phase, the solvent was evacuated and the residue was triturated with diethyl ether to give (**1**) as a white solid in 85% yield, mp 145–147 °C, MS *m/z* 209 (M^+, 100%), 208 (78%), IR (cm^{-1}): 3384, 3038, 2918, 1600, 1454, 1317, 1237, 1180, 1162, 1000, 877, 803, 745, 618, ^1H NMR 2.75 (s, 3H, $1CH_3$), 7.52 (d, 1H, $J=9.0$ Hz, 1CH), 7.68 (m, 2H, 2CH), 7.78 (d, 1H, $J=9.0$ Hz, 1CH), 7.88 (dd, 1H, $J=7.0$, 2.0 Hz, 1CH), 8.36 (s, 1H, CH), 8.48 (dd, 1H, $J=7.0$, 2.0 Hz, 1CH), 8.84 (s, 1H, CH), Elemental analysis calcd(%) for $C_{14}H_{11}NO$ (209.2): C 80.36, H 5.30, N 6.69, found C 80.50, H 5.30, N 6.67.

4.2.3. Acetic acid benzo[*f*]isoquinolin-2-ylmethyl ester (2). Compound (**1**) (0.50 g, 2.4 mmol) was dissolved in acetic anhydride (8 mL) and refluxed for 24 h.[35] After cooling off the solution, the excess of acetic anhydride was eliminated under vacuum leaving an almost pure product. Compound (**2**) was obtained as brown oil in 96% yield, MS *m/z* 251 (M^+, 100%), IR (cm^{-1}): 2922, 1716, 1229, 1031, 877, 814, 743, ^1H NMR 2.22 (s, 3H, CH_3), 5.43 (s, 2H, CH_2), 7.72 (m, 2H, 2CH), 7.78 (d, 1H, $J=8.8$ Hz, 1CH), 7.82 (d, 1H, $J=8.8$ Hz, 1CH), 7.93 (m, 1H, 1CH), 8.43 (s, 1H, CH), 8.65 (m, 1H, 1CH), 9.22 (s, 1H, 1CH), elemental analysis calcd(%) for $C_{16}H_{13}NO_2$ (251.3): C 76.48, H 5.22, N 5.57, found C 76.66, H 5.23, N 5.56.

4.2.4. 2-Methylolbenzo[*f*]isoquinoline (3). Compound (**2**) (0.50 g, 1.99 mmol) was dissolved in concentrated hydrochloric acid, and the solution boiled for 18 h.[35] The solution was then cooled in an ice bath and basified with a solution of NaOH 2 M. The solution was extracted with diethyl ether. The organic layer was dried and the residue was chromatographed on silica gel, to give (**3**) (hexane/ethyl acetate 2:3) in 70% yield as a yellow solid, mp 106–108 °C, MS *m/z* 209 (M^+, 55%), 181 (100%), 152 (M^+, 53%), IR (cm^{-1}): 3031, 2938, 1740, 1457, 1375, 1228, 1098, 1054, 900, 823, 755, ^1H NMR 5.03 (s, 2H, CH_2), 7.71 (m, 2H, 2CH), 7.80 (d, 1H, $J=9.2$ Hz, 1CH), 7.83 (d, 1H, $J=9.2$ Hz, 1CH), 7.94 (dd, 1H, $J=9.1$, 1.9 Hz, 1CH), 8.43 (s, 1H, CH), 8.70 (dd, 1H, $J=9.1$, 1.9 Hz, 1CH), 9.22 (s, 1H, 1CH), elemental analysis calcd(%) for $C_{14}H_{11}NO$ (209.2): C 80.36, H 5.30, N 6.69, found C 80.09, H 5.32, N 6.67.

4.2.5. 2-Benzo[*f*]isoquinolinecarbaldehyde (4). The procedure used was adapted from the literature[36] for the oxidation of the heterocyclic alcohols. Compound (**3**) (0.50 g, 2.4 mmol) was dissolved in dioxane (15 mL) and SeO_2 (0.27 g, 2.4 mmol) was added. The resulting mixture was boiled for 3 h. After cooling, Se was filtered off, the solvent evacuated and the residue chromatographed on silica gel (hexane/ethyl acetate 2:3)to give (**4**) in 26% yield, and recovering unreacted (**3**) quantitatively. (**4**) is a yellow solid, mp 127–129 °C, MS *m/z* 207 (M^+, 52%), 179 (100%), 152 (46%), IR (cm^{-1}): 2843, 1691, 1402, 1116, 897, 821, 798, 734, 703, 600, elemental analysis calcd(%) for $C_{14}H_9NO$ (207.2): C 81.14, H 4.38, N 6.76, found C 81.35, H 4.37, N 6.79.

4.2.6. 2-Styrylbenzo[*f*]isoquinoline (5). For the preparation see the general procedure. Compound (**5**)(ethyl acetate) was obtained as a yellow solid in 80% yield, mp 147–149, MS *m/z* 281 (M^+, 39%), 280 (100%), IR (cm^{-1}): 2926, 1736, 1594, 1453, 1295, 974, 878, 825, 761, 600, ^1H NMR 7.30 (m, 2H, 2CH), 7.40 (m, 3H, 3CH), 7.43 (d, 1H, $J=16.1$ Hz, 1H), 7.66 (d, 2H, $J=7.0$ Hz, 2CH), 7.72 (m, 2H, 2CH), 7.88 (d, 1H, $J=16.1$ Hz, 1H),7.93 (m, 1H, 1CH), 8.42 (s, 1H, CH), 8.72 (m, 1H, CH), 9.23 (s, 1H, CH), elemental analysis calcd(%) for $C_{21}H_{15}N$ (281.4): C 89.65, H 5.37, N 4.98, found C 89.38, H 5.37, N 4.96.

4.2.7. 7-Aza[5]helicene (6). For the preparation see the general procedure.(**6**) was obtained as a yellow solid in yield 75%, mp 142–145 °C, MS *m/z* 279 (M^+, 54%), 278 (100%), IR (cm^{-1}): 2920, 818, 745, 715, 660, 614, 607, elemental analysis calcd(%) for $C_{21}H_{13}N$ (279.3): C 90.30, H 4.69, N 5.01, found C 90.08, H 4.70, N 5.02. NMR is reported in Table 3.

Table 3. NMR data for the aza[5]helicenes (**10**), (**21**), (**11**) and (**6**)

	(**10**)	(**21**)	(**11**)	(**6**)
H1	—	9.89s	8.28br d, $J=6.0$ Hz	8.59ddd, $J=0.8$, 1.2, 8.5 Hz
H2	8.70dd, $J=1.8$, 4.2 Hz	—	8.38br d, $J=6.0$ Hz	7.35ddd, $J=1.4$, 6.9, 8.5 Hz
H3	7.50dd, $J=4.2$, 8.1 Hz	8.56d, $J=5.6$ Hz	—	7.59ddd, $J=1.2$, 6.9, 8.0 Hz
H4	8.26dd, $J=1.8$, 8.1 Hz	7.70d, $J=5.6$ Hz	9.35br s	8.01dd, $J=1.4$, 8.0 Hz
H5	7.88d, $J=8.5$ Hz	8.08d, $J=8.6$ Hz	8.02m	8.10dd, $J=0.8$, 8.8 Hz
H6	7.98d, $J=8.5$ Hz	[a]	8.02m	8.10d, $J=8.8$ Hz
H7	7.99d, $J=8.3$ Hz[b]	[a]	7.99d, $J=8.3$ Hz[d]	—
H8	7.91d, $J=8.3$ Hz[b]	[a]	7.92d, $J=8.3$ Hz[d]	9.33s
H9	7.96d, $J=8.5$ Hz[c]	[a]	7.90d, $J=8.6$ Hz	7.99d, $J=8.8$ Hz
H10	7.86d, $J=8.5$ Hz[c]	[a]	7.90dd, $J=0.8$, 8.6 Hz	8.04dd, $J=0.8$, 8.8 Hz
H11	7.93dd, $J=1.6$, 8.0 Hz	7.97d, $J=8.2$ Hz	7.99d, $J=8.1$ Hz	8.02dd, $J=1.4$, 8.0 Hz
H12	7.54ddd, $J=1.5$, 6.8, 8.0 Hz	7.54t, $J=7.3$ Hz	7.58ddd, $J=1.2$, 6.9, 8.1 Hz	7.66ddd, $J=1.2$, 6.9, 8.0 Hz
H13	7.27ddd, $J=1.6$, 6.8, 8.3 Hz	7.36t, $J=7.3$ Hz	7.35ddd, $J=1.4$, 6.9, 8.4 Hz	7.39ddd, $J=1.6$, 6.9, 8.5 Hz
H14	8.05d, $J=8.3$ Hz	8.58d, $J=8.2$ Hz	8.46ddd, $J=0.8$, 1.2, 8.4 Hz	8.67ddd, $J=0.8$, 1.2, 8.5 Hz

[a] 7.97–7.84 ppm, m, H6+H7+H8+H9+H10.
[b,c,d] The assignment can be reversed.

4.2.8. 3-Bromomethylphenanthrene (7). The compound was prepared similarly to the procedure reported to obtain 9-bromomethylphenanthrene[37] by boiling 3-methylphenanthrene (1.0 g, 5.2 mmol) with *N*-bromosuccinimide (0.93 g, 5.2 mmol) and a few crystals of benzoylperoxide in CCl_4 for 4 h. (**7**) Was obtained as a white solid in 96% yield, mp 95–96 °C, MS *m/z* 271 (M^+, 8%), 270 (9%), 191 (100%), Elemental analysis calcd(%) for $C_{15}H_{11}Br$ (271.3): C 66.44, H 4.09, Br 29.47, found C 66.49, H 4.09, Br 29.51

4.2.9. 3-Phenanthrylmethyl-triphenyl-phosphonium-bromide (8). The compound was prepared from (**7**) (1.35 g, 5.0 mmol) and triphenylphosphine (1.31 g, 5.0 mmol) in boiling toluene (35 mL). The phosphonium salt precipitated and was filtered off at the end of the reaction. Compound (**8**) was obtained as a white solid in 78% yield, mp 270–272 °C dec, and was used directly without further characterisation.

4.2.10. 3-(2-Phenanthren-3-yl-vinyl)-pyridine (9). For the preparation see the general procedure. Compound (**9**) was obtained as orange solid in 60% yield, mp 110–112, MS *m/z* 281 (M^+, 71%), 280 (100%), ^{1}H NMR 7.15 (d, 2H, J=8.8 Hz, 2CH), 7.22 (d, 2H, J=8.6 Hz, 2CH), 7.40 (m, 4H, 4CH), 7.45 (m, 2H, 2CH), 7.65 (d, 1H, J=8.8 Hz, 1CH), 7.71 (d, 1H, J=8.8 Hz, 1CH), 8.42 (dd, 1H, J=7.8, 1.6 Hz, 1CH), 8.44 (s, 1H, 1CH), 8.48 (s, 1H, 1CH), elemental analysis calcd(%) for $C_{21}H_{15}N$ (281.4): C 89.65, H 5.37, N 4.98, found C 89.76, H 5.37, N 4.99.

4.2.11. 1-Aza[5]helicene (10) and 3-aza[5]helicene (11). For the preparation see the general procedure. Compound (**10**) (hexane/ethyl acetate 5:1) was obtained as a yellow solid in 87% yield, mp 164–165 °C, MS *m/z*: 279 (M^+, 30%), 278 (100%), IR (cm^{-1}): 2900, 2839, 1619, 1563, 1256, 842, 723, 686, 643, 615, elemental analysis calcd(%) for $C_{21}H_{13}N$ (279.3): C 90.30, H 4.69, N 5.01, found C 90.47, H 4.68, N 5.02 (**11**) (hexane/ethyl acetate 1:2) was obtained as yellow solid in 11% yield, mp 178–179 °C, MS *m/z* 279 (M^+, 52%), 278 (100%), IR (cm^{-1}): 3069, 2970, 2931, 2861, 1730, 1632, 1592, 1261, 1284, 846, 746, 654, 615, elemental analysis calcd(%) for $C_{21}H_{13}N$ (279.3): C 90.30, H 4.69, N 5.01, found C 89.97, H 4.69, N 5.00. NMR are reported in Table 3.

4.2.12. 4-(2-Pyridin-3-yl-vinyl)-benzaldehyde (12). For the preparation see the general procedure, the only difference was that the molar ratio terephthaldehyde/3-pyridinylphosphonium bromide was 5:1.[38,39] (**12**) (hexane/ethyl acetate 1:2) was obtained as orange oil in 70% yield, MS *m/z* 209 (M^+, 100%), 208 (51%), 180 (92%), 152 (43%), IR (cm^{-1}): 1759, 1577, 1398, 1238, 1200, 1064, 977, 829, ^{1}H NMR 6.69 (d, 1H, J=12.0 Hz, CH), 6.79 (d, 1H, J=12.0 Hz, CH), 7.16 (dd, 1H, J=7.8, 5.1 Hz, 1CH), 7.35 (d, 2H, J=8.2 Hz, 2CH), 7.49 (d, 1H, J=7.8 Hz, 1CH), 7.75 (d, 2H, J=8.2 Hz, 2CH), 8.43 (s and d, 2H, J=5.1 Hz, 2CH), 9.95 (s, 1H, 1CHO), elemental analysis calcd(%) for $C_{14}H_{11}NO$ (209.2): C 80.36, H 5.30, N 6.69, found C 80.64, H 5.31, N 6.72.

4.2.13. 4-(2-Pyridin-3-yl-vinyl)-benzaldehyde dimethoxy-acetal (13). Compound (**12**) (1.0 g, 4.8 mmol) and 2,2-dimethoxyacetone (10 mL, 80 mmol) were dissolved in MeOH (10 mL) and a few crystals of *p*-toluenesulfonic acid were added. The solution was boiled, distilling off the azeotrope of MeOH–acetone.[40] At the end of the reaction MeONa was added and the solvent evaporated. The residue was dissolved in CH_2Cl_2, washed with water and dried on Na_2SO_4. Compound (**13**) was obtained as a yellow oil in 98% yield and MS *m/z* 255 (M^+, 8%), 224 (100%); the product was used without further characterisation.

4.2.14. Benzo[*h*]quinoline-9-carbaldehyde (14) and benzo[*f*]isoquinoline-9-carbaldehyde (15). The irradiation was carried out in acetonitrile (200 mL) for 48 h at 254 nm. At the end of the irradiation, the solution was stirred with HCl 1 M for 2 h to hydrolyse the ketals. The organic layer was washed with water, dried and the solvent removed. The residue was chromatographed on silica gel obtaining (**14**) and (**15**). Compound (**14**) (hexane/ethyl acetate 1:1) was obtained as yellow solid in 11% yield, mp 110–112 °C, MS *m/z*: 207 (M^+, 100%), 206 (62%), 178 (91%), 150 (18%), IR (cm^{-1}): 2880, 2792, 1689, 1579, 1278, 1233, 1180, 1165, 880, 776, 702, 595, ^{1}H NMR 7.61 (dd, 1H, J=8.0, 4.6 Hz, 1CH), 7.87 (d not resolved, 2H, 2CH), 8.01 (d, 1H, J=8.3 Hz, 1CH), 8.21 (dd, 1H, J=8.3, 1.7 Hz, 1CH), 8.24 (dd, 1H, J=8.0, 1.7 Hz, 1CH), 9.08 (dd, 1H, J=4.6, 1.7 Hz, 1CH), 9.80 (s, 1H, 1CH), 10.34 (s, 1H, CHO), elemental analysis calcd(%) for $C_{14}H_9NO$ (207.2): C 81.14, H 4.38, N 6.76, found C 80.86, H 4.39, N 6.74. Compound (**15**) (hexane/ethyl acetate 1:1) was obtained as yellow solid in 85% yield, mp 154–157 °C, MS *m/z*: 207 (M^+, 100%), 206 (72%), 178 (23%), 151 (57%), IR (cm^{-1}): 3005, 1739, 1695, 1515, 1373, 1289, 1223, 1207, 1193, 844, 775, 745, 707, 615, 601, ^{1}H NMR 7.92 (d, 1H, J=8.9 Hz, 1CH), 7.99 (d, 1H, J=8.9 Hz, 1CH), 8.08 (d, 1H, J=8.3 Hz, 1CH), 8.21 (d, 1H, J=8.3 Hz, 1CH), 8.52 (d, 1H, J=5.8 Hz, 1CH), 8.86 (d, 1H, J=5.8 Hz, 1CH), 9.18 (s, 1H, 1CH), 9.32 (s, 1H, 1CH), 10.31 (s, 1H, CHO), elemental analysis calcd(%) for $C_{14}H_9NO$ (207.2) C 81.14, H 4.38, N 6.76, found C 81.43, H 4.38, N 6.70.

4.2.15. 9-Styryl-benzo[*h*]quinoline (16) and 9-styryl-benzo[*f*]isoquinoline (17). For the preparation see the general procedure. Compound (**16**) (hexane/ethyl acetate 2:3) was obtained as yellow solid in 69% yield, mp 92–95 °C, MS *m/z* 281 (M^+, 80%), 280 (100%), IR (cm^{-1}): 2992, 2900, 2725, 1485, 1427, 1377, 980, 962, 831, 761, 693, 615, ^{1}H NMR 7.29 (m, 1H, 1CH), 7.40 (m, 4H, 4CH), 7.53 (dd, 2H, J=8.0, 4.4 Hz, 2CH), 7.59 (dd, 2H, J=8.0, 1.8 Hz, 2CH), 7.66 (dd, 1H, J=8.8, 6.2 Hz, 1CH), 7.79 (d, 1H, J=8.8 Hz, 1CH), 7.90 (m, 2H, 2CH), 8.18 (dd, 1CH, J=8.0, 1.8 Hz, 1CH), 9.02 (dd, 1H, J=4.4, 1.8 Hz, 1CH), 9.38 (s, 1H, 1CH), elemental analysis calcd(%) for $C_{21}H_{15}N$ (281.4): C 89.65, H 5.37, N 4.98, found C 89.64, H 5.38, N 5.00. For the preparation see the general procedure. Compound (**17**) (hexane/ethyl acetate 1:3) was obtained as yellow solid in 92% yield, mp 180–182 °C, MS *m/z* 281 (M^+, 100%), 280 (64%), IR (cm^{-1}): 3077, 3054, 2954, 1562, 1430, 969, 835, 737, 696, 629, ^{1}H NMR 7.36 (d, 1H, J=7.3 Hz, 1CH), 7.42 (m, 2H, 2CH), 7.44 (d, 2H, J=7.3 Hz, 2CH), 7.61 (d, 2H, J=7.3 Hz, 2CH), 7.95 (d, 1H, J=8.8 Hz, 1CH), 8.06 (d, 1H, J=8.2 Hz, 1CH), 8.11 (d, 1H, J=8.8 Hz, 1CH), 8.15 (d, 1H, J=8.2 Hz, 1CH), 8.78 (s and d, J=6.4 Hz, 2H, 2CH), 8.94 (d, 1H, J=6.4 Hz, 1CH), 9.41 (s, 1H, CH), elemental analysis calcd(%) for $C_{21}H_{15}N$

(281.4): C 89.65, H 5.37, N 4.98, found C 89.45, H 5.36, N 4.96.

4.2.16. 1-Aza[5]helicene (10) and 3-aza[5]helicene (11). For the preparation see the general procedure. Compound (**10**) yield 80%, (**11**) yield 95%.

4.2.17. 1-(2-Pyridin-4-yl-vinyl)benzaldehyde (18). This compound may be synthesised in two equivalent ways: the first started from the phosphonium salt of 4-bromomethylpyridine with terephthalaldehyde under the same experimental conditions as (**12**) yield 90%, or by direct condensation of 4-methylpyridine (1.9 mL, 20 mmol) with terephthalaldehyde (5.4 g, 40 mmol) in acetic anhydride (20 mL) and boiling for 5 h (Yield 85%).[41] (**18**)(CH_2Cl_2/ethyl acetate 4:1) was obtained as yellow solid, mp 110–112 °C (lit. 113.5–115 °C), MS *m/z* 209 (M^+, 80%), 180 (100%), 152 (61%), IR (cm^{-1}): 1749, 1694, 1497, 1232, 1200, 965, 824, ^1H NMR 7.04 (d, 1H, $J=16.5$ Hz, 1CH), 7.29 (d, 1H, $J=16$, 5 Hz, 1CH), 7.37 (dd, 2H, $J=4.4$, 1.5 Hz, 2CH), 7.55 (m, 4H, 4CH), 8.58 (dd, 2H, $J=4.4$, 1.5 Hz, 2CH), 10.10 (s, 1H, CHO), elemental analysis calcd(%) for $C_{14}H_{11}NO$ (209.2): C 80.36, H 5.30, N 6.69, found C 80.17, H 5.31, N 6.72.

4.2.18. Benzo[*h*]isoquinoline-9-carbaldehyde (19). After transformation of compound (**18**) in the corresponding ketal, (for the procedure see compound (**13**)), the irradiation was carried out in acetonitrile (200 mL) for 48 h at 254 nm. At the end of the irradiation, the solution was stirred with HCl 1 M for 2 h to hydrolyse the ketal. The organic layer was washed with water, dried and the solvent removed. The residue was chromatographed on silica gel (hexane/ethyl acetate 1:3) obtaining (**19**) as a deliquescent yellow solid in 84% yield, mp 135–137 °C, MS *m/z* 207 (M^+, 100%), 178 (44%), 151 (33%), IR (cm^{-1}): 3360, 2903, 2835, 1684, 1602, 1423, 1387, 1298, 1206, 1168, 1046, 1006, 814, 785, ^1H NMR 7.90 (m, 2H, 2CH), 8.10 (m, 2H, 2CH), 8.21 (dd, 1H, $J=8.1$, 1.5 Hz, 1CH), 8.80 (d, 1H, $J=5.6$ Hz, 1CH), 9.31 (s, 1H, CH), 10.21 (s, 1H, 1CH), 10.33 (s, 1H, CHO), elemental analysis calcd(%) for $C_{14}H_9NO$ (207.2): C 81.14, H 4.38, N 6.76, found C 81.45, H 4.38, N 6.75.

4.2.19. 9-[2(2-Bromo-phenyl)vinyl]-benzo[*h*]isoquinoline (20). For the preparation see the general procedure, (**20**) (hexane/ethyl acetate 1:3) was obtained as yellow oil in 58% yield, mp>250 °C, MS *m/z* 361 ($M^+ + 1$, 40%), 360 (M^+, 38%), 280 (100%), 252 (24%), IR (cm^{-1}): 3073, 3029, 2900, 1573, 1428, 1243, 1015, 842, 761, 734, 661, ^1H NMR 6.80 (d, 1H, $J=11.9$ Hz, 1CH), 6.94 (d, 1H, $J=11.9$ Hz, 1CH), 7.15 (m, 4H, 4CH),7.74 (d, 1H, $J=8.2$ Hz, 1CH), 7.65 (d, 1H, $J=5.4$ Hz, 1CH), 7.71 (d, 1H, $J=8.8$ Hz, 1CH), 7.72 (d, 1H, $J=8.2$ Hz, 1CH),7.83 (d, 1H, $J=8.8$ Hz, 1CH), 8.55 (s, 1H, 1CH), 8.66 (d, 1H, $J=5.4$ Hz, 1CH), 9.62 (s, 1H, CH), elemental analysis calcd(%) for $C_{21}H_{14}BrN$ (360.3): C 70.02, H 3.92, Br 22.18, N 3.89, found C 70.14, H 3.90, Br 22.26, N 3.89.

4.2.20. 2-Aza[5]helicene (21). Compound (**20**) (0.25 g, 0.7 mol) (*tert*-butyl)$_3$SnH (0.23 mL, 0.85 mmol) and AIBN (0.10 g, 0.6 mmol) were dissolved in toluene (80 mL) and boiled for 18 h.[42] After cooling, an aqueous solution of KF was added and the two phases were stirred vigorously for an additional 8 h. The organic phase was separated, dried and evaporated. Compound (**19**) (hexane/ethyl acetate 2:3) was obtained as yellow solid in 5% yield, mp 152–154 °C, MS *m/z* 279 (M^+, 35%), 278 (100%), IR (cm^{-1}): 3197, 1633, 1600, 1454, 1085, 1039, 880, 802, 745, 639, elemental analysis calcd(%) for $C_{21}H_{13}N$ (279.3): C 90.30, H 4.69, N 5.01, found C 90.11, H 4.70, N 5.01. NMR is reported in Table 3.

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Synthesis and electrode properties of 19-membered azo- and azoxycrown ethers. Structure of dibenzo-19-azocrown-7

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Abstract—19-Membered azo- and azoxycrown ethers have been synthesized by reductive macrocyclization of respective bis-(nitrophenoxy)oxaalkanes. The behavior of these compounds as ionophores in ion-selective membrane electrodes has been studied. The structure of the 19-membered dibenzoazocrown ether has been determined.

1. Introduction

The chromogenic system of azo dyes consists of a chain of conjugated double bonds and azo group(s). An azo group inserted into conjugated system causes a strong increase of color intensity. Additionally azo compounds can exist in two isomeric forms (*Z* and *E*) that make the system even more interesting for physicochemical study especially if the azo group forms part of a macrocyclic system.

So far, three procedures for azocrown ether synthesis have been described. The first consists of alkylation of 2,2′-hydroxyazobenzene.[1] The second method utilizes reduction of bis(2-nitrophenoxy)-oxaalkanes with sodium or potassium stannite whereupon the azo bond is formed.[2] This procedure allowed two main macrocyclic products to be obtained with azo or azoxy groupings. According to this reductive macrocyclization method, 10-, 13-, and 16-membered azo and azoxycrown ethers have been synthesized.[3–5] Finally, the third procedure consists of nucleophilic substitution of difluoroazobenzene with alcoholates, thiolates or diamines.[6]

Preliminary studies have shown that 19-membered azo- and azoxycrown ethers could be prepared by reductive macrocyclization in a similar way to the 13- and 16-membered analogs.

The aim of this work was preparation of 19-membered azocrown ethers, identification of their stereoisomers, and presentation of their properties in ion-selective membrane electrodes.

2. Results and discussion

2.1. Synthesis

The presented synthesis of 19-membered azo- and azoxycrown ethers is a two-step reaction. 1,11-Bis(2-nitrophenoxy)-3,6,9-trioxaundecanes were obtained by reaction of 2-nitrophenol or its derivatives with 1,11-dichloro-3,6,9-trioxaundecane in dry dimethylformamide in the presence of anhydrous potassium carbonate (compounds **1–3**) (Scheme 1).

In the next step, nitroderivatives **1–3** were reduced in water–acetone with sodium or potassium stannite to produce 19-membered azo- and azoxycrown ethers. A remarkable sodium template effect for azo compound formation was noticed only in the case of reduction of compound **1**. If sodium hydroxide was used instead of potassium hydroxide the yield of compound **4** increased almost twice. The yield of macrocyclic products was lower than in the case of 13- and 16-membered compounds and more polymeric products were formed. In addition, the separation of *Z* and *E* isomers of 19-membered substituted azocrown ethers was troublesome because of rapid isomerization; in some cases, their geometry was ascribed considering significant differences in ^{1}H NMR spectra. Surprisingly, compound **4** in the solid state and in solutions in chloroform, methanol and acetone solution existed only in *E* form.

Keywords: Azocrown; Azoxycrown ethers; Ion-selective membrane; Thallium(I) selectivity.
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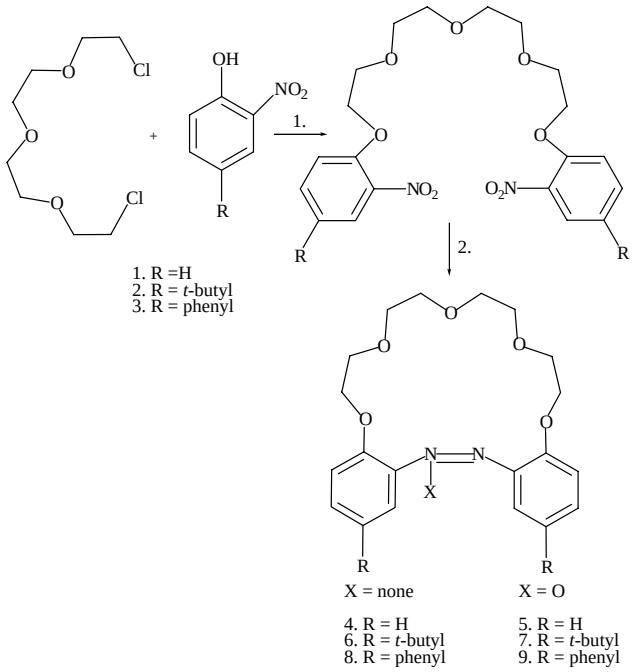

Scheme 1. Synthesis of 19-membered azo and azoxycrown ethers; (1) K_2CO_3, DMF, 120 °C; (2) NaOH or KOH, $SnCl_2$, water/acetone.

2.2. Membrane electrodes

The most frequently studied property of azo- and azoxycrown ethers is their behavior in ion-selective membrane electrodes. 13-Membered azocrown ethers are selective toward sodium cations[7] while 16-membered compounds are potassium selective[8] in membrane electrodes. We expected that 19-membered derivatives should be selective to larger cations. In a preliminary experiments the ion-selective membrane electrodes doped with 19-membered azo- and azoxycrown ethers were selective for the thallium(I) cation (Fig. 1).

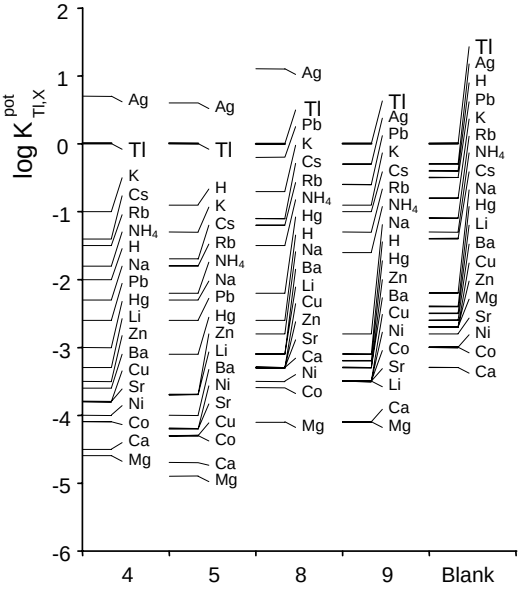

Figure 1. Diagram of log $K^{pot}_{Tl/X}$ values of ion-selective membrane electrode doped with azo- or azoxycrown ethers **4**, **5**, **8**, or **9** compared to blank electrode.

Compared to this cation, only the electrode response to Ag(I) was higher for ionophores **4**, **5**, and **8**. Furthermore, electrodes based on compounds **4** and **5** showed high thallium selectivity in the presence of many transition and heavy metal cations like Pb(II), Hg(II), Zn(II) and Ni(II), and alkali earth cations.

Thallium salts are extremely toxic. Thallium is obtained as a side product in sulfuric acid manufacture and during lead decontamination. Thallium can be delivered into human body from contaminated air, water or food. Thallium replaces potassium cations, thus causing deactivation of some enzymes. Therefore, thallium distribution has to be under strict control; the ion-selective membrane electrodes based on 19-membered azo- and azoxycrown ethers appeared as potentially useful for that purpose.

2.3. X-Ray structure

X-ray study reveals that in the crystal structure of **4** the asymmetric part of unit cell contains two crystallographically independent molecules labeled **A** and **B**, Figure 2. Both molecules are chemically equivalent and adopt *E* geometry with aromatic moieties in *trans*-positions around the –N═N– bond, but differ essentially in the conformation of their polyether chains.

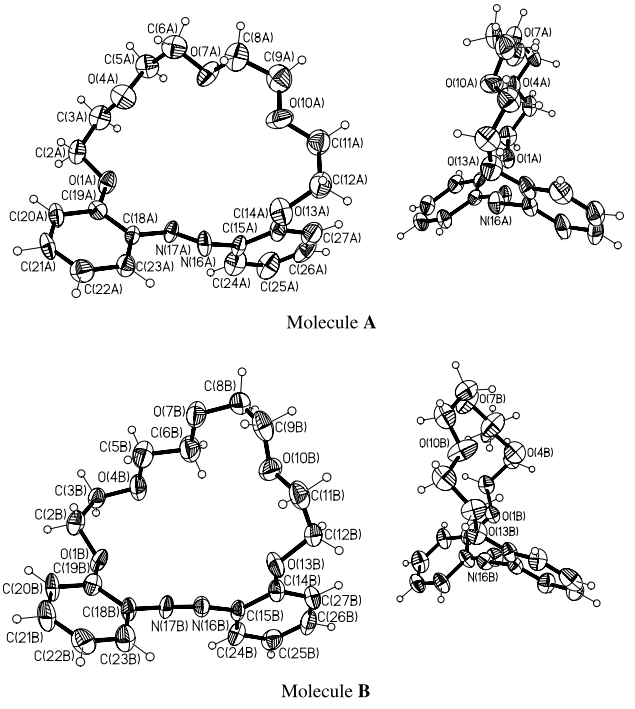

Figure 2. Top and side view of molecules **A** and **B** in the structure of **4** with atom numbering scheme.

The torsion angles around the C–C bonds in the polyethylene chain of **A** are *gauche*, and *anti* around C–O bonds, except C(11A)–C(12A)–O(13A)–C(14A)=74.5°, which is *gauche*, Table 1, and the conformation of the polyoxyethylene chain units may be described as: $a, a, -g, a, a, g, a, a, -g, a, a, g, g, a$, starting at the O(1A)–C(19A) bond. The sequence of two *gauche* torsion angles form corner fragment[9] at C(12A) atom

Table 1. Selected valence and torsion angles (°) for **4** and selected bond lengths for **4**

Angle	Molecule **A**	Molecule **B**	Angle	Molecule **A**	Molecule **B**
O(1)–C(2)–C(3)–O(4)	−67.9(8)	−58.5(9)	C(6)–O(7)–C(8)–C(9)	−161.2(8)	−85.8(11)
C(2)–C(3)–O(4)–C(5)	176.2(7)	−77.0(9)	O(7)–C(8)–C(9)–O(10)	−77.3(10)	72.2(11)
C(3)–O(4)–C(5)–C(6)	−175.6(7)	173.2(8)	C(8)–C(9)–O(10)–C(11)	170.4(7)	172.9(7)
O(4)–C(5)–C(6)–O(7)	74.6(8)	179.1(8)	C(9)–O(10)–C(11)–C(12)	172.1(8)	−164.2(7)
C(5)–C(6)–O(7)–C(8)	−177.0(7)	−177.1(8)	O(10)–C(11)–C(12)–O(13)	59.1(9)	−74.0(8)
C(11)–C(12)–O(13)–C(14)	74.5(10)	156.6(7)	N(16)–N(17)–C(18)–C(19)	160.0(7)	140.8(7)
C(12)–O(13)–C(14)–C(15)	−167.6(7)	−165.1(6)	N(17)–C(18)–C(19)–O(1)	−8.7(9)	−3.2(11)
O(13)–C(14)–C(15)–N(16)	−0.5(11)	−7.9(10)	C(18)–C(19)–O(1)–C(2)	−166.7(6)	−149.5(7)
C(14)–C(15)–N(16)–N(17)	137.1(7)	150.9(7)	C(19)–O(1)–C(2)–C(3)	153.9(6)	161.1(6)
C(15)–N(16)–N(17)–C(18)	170.7(6)	171.6(7)			
Bond	Molecule **A**	Molecule **B**	Bond	Molecule **A**	Molecule **B**
O(1)–C(19)	1.362(8)	1.357(9)	C(5)–O(4)–C(3)	113.7(6)	113.6(6)
O(1)–C(2)	1.437(9)	1.421(8)	O(4)–C(5)–C(6)	108.3(7)	108.6(7)
C(2)–C(3)	1.496(10)	1.483(10)	O(7)–C(6)–C(5)	111.9(8)	108.4(8)
C(3)–O(4)	1.397(9)	1.424(8)	C(6)–O(7)–C(8)	113.5(7)	115.3(7)
O(4)–C(5)	1.394(8)	1.389(10)	O(7)–C(8)–C(9)	112.3(8)	115.0(8)
C(5)–C(6)	1.471(10)	1.521(10)	O(10)–C(9)–C(8)	112.7(9)	113.1(8)
C(6)–O(7)	1.402(9)	1.350(10)	C(9)–O(10)–C(11)	112.6(8)	116.0(8)
O(7)–C(8)	1.403(9)	1.400(8)	O(10)–C(11)–C(12)	109.7(8)	107.1(7)
C(8)–C(9)	1.462(11)	1.449(11)	C(11)–C(12)–O(13)	111.7(7)	108.6(7)
C(9)–O(10)	1.332(10)	1.352(11)	C(14)–O(13)–C(12)	120.4(7)	116.0(6)
O(10)–C(11)	1.416(10)	1.457(10)	O(13)–C(14)–C(27)	124.0(9)	124.3(7)
C(11)–C(12)	1.442(11)	1.474(11)	O(13)–C(14)–C(15)	117.1(7)	116.4(7)
C(12)–O(13)	1.459(10)	1.441(8)	C(27)–C(14)–C(15)	118.8(9)	119.3(8)
O(13)–C(14)	1.366(10)	1.334(9)	C(24)–C(15)–C(14)	120.7(8)	120.6(7)
C(14)–C(27)	1.386(10)	1.375(10)	C(24)–C(15)–N(16)	123.3(8)	123.1(7)
C(14)–C(15)	1.400(11)	1.417(10)	C(14)–C(15)–N(16)	115.9(7)	115.9(8)
C(15)–C(24)	1.385(10)	1.359(10)	C(25)–C(24)–C(15)	119.7(9)	120.3(8)
C(15)–N(16)	1.416(9)	1.431(9)	C(24)–C(25)–C(26)	118.9(9)	118.2(8)
C(24)–C(25)	1.384(11)	1.396(10)	C(27)–C(26)–C(25)	121.9(9)	122.7(8)
C(25)–C(26)	1.385(12)	1.377(11)	C(26)–C(27)–C(14)	119.9(10)	118.9(8)
C(26)–C(27)	1.362(12)	1.376(11)	N(17)–N(16)–C(15)	113.3(6)	113.8(6)
N(16)–N(17)	1.236(7)	1.257(7)	N(16)–N(17)–C(18)	114.7(6)	112.3(6)
N(17)–C(18)	1.426(8)	1.425(9)	C(23)–C(18)–C(19)	119.1(7)	119.8(7)
C(18)–C(23)	1.393(9)	1.370(10)	C(23)–C(18)–N(17)	123.7(7)	122.4(6)
C(18)–C(19)	1.404(10)	1.401(9)	C(19)–C(18)–N(17)	117.0(7)	117.6(7)
C(19)–C(20)	1.373(9)	1.381(10)	O(1)–C(19)–C(20)	123.3(8)	123.7(7)
C(20)–C(21)	1.398(10)	1.370(11)	O(1)–C(19)–C(18)	116.8(7)	116.6(7)
C(21)–C(22)	1.333(11)	1.371(11)	C(20)–C(19)–C(18)	119.9(7)	119.7(8)
C(22)–C(23)	1.374(10)	1.387(10)	C(19)–C(20)–C(21)	119.1(8)	119.6(8)
C(19)–O(1)–C(2)	118.6(6)	117.5(6)	C(22)–C(21)–C(20)	121.0(8)	121.2(8)
O(1)–C(2)–C(3)	107.6(7)	108.5(6)	C(21)–C(22)–C(23)	121.2(8)	119.5(9)
O(4)–C(3)–C(2)	108.4(7)	115.0(6)	C(22)–C(23)–C(18)	119.5(8)	120.1(8)

of macrocycle **A**. In macrocycle **B** one of the torsion angles around C–C bond (O(4B)–C(5B)–C(6B)–O(7B)=179.1°) is *anti* creating sequence of three *anti* torsion angles in the polyether chain, which is unusual for azo-macrocycles. The conformation of the polyether chain starting from O(1B)–C(19B) is: *a*, *a*, −*g*, −*g*, *a*, *a*, *a*, −*g*, *g*, *a*, *a*, −*g*, *a*, *a*. The series of torsion angles points to the presence of two corner fragments in macrocycle **B**, at atoms C(3B) and C(8B). Torsion angles around N16–N17 and bond lengths equal 170.7(6) and 171.6(7)°, and 1.236(7) and 1.257(7) Å, respectively, for molecules **A** and **B** and had of common values, Table 1.

The heteroatoms of the macrocycle **A** cavity are roughly coplanar and deviate from their mean plane in the range −0.200(4) – 0.229(4) Å. In the case of macrocycle **B**, the cavity is non-planar and the heteroatoms deviate from the mean plane in the range from −0.761(5) to 0.480(4) Å. The benzene residues are located at different sides of the mean plane in both molecules. Despite conformational differences between the polyether chains, the dihedral angles between aromatic residues in molecules **A** and **B** adopt very similar values of 71.1(2) and 77.9(2)°, respectively. These angles essentially exceed the corresponding dihedral angles found in the relative small sized 10-membered[10] and 13-membered,[11] or bigger 21-membered[12] trans-isomers of azobenzocrown ethers bearing 2,2′-linked azobenzene moiety, where they are in the range of 0–40°. Interestingly, in the closest by size 20-membered azoazoxycrown[13] the dihedral angles between benzene residues of azo- and azoxybenzene moieties equal 73.5 and 76.4°, and agree well with the values reported here for the 19-membered azomacrocycle.

3. Experimental

All materials and solvents used for synthesis were of analytical reagent grade. Silica gel 60 (Merck) was used for column chromatography. Preparative TLC glass plates covered with Silica gel 60 F_{254} (Merck) were used for final separation of crown ethers. ^{1}H NMR spectra, all in $CDCl_3$, were taken on Varian instruments at 200 MHz and/or 500 MHz. In the case of *Z* or *E* isomers of azocrown ethers, the spectra were recorded immediately after

dissolution of crystals or oil. IR and mass spectra were recorded on AMD-604 and Genesis II (Mattson) apparatus, respectively. Additionally, purity and identity of macrocyclic compounds was established by elemental analysis taken on an EAGER 200 apparatus. The mp were uncorrected. 4-*t*-Butyl-2-nitrophenol and 4-phenyl-2-nitrophenol were obtained according to literature data.[9]

3.1. Membrane electrodes and potentiometric measurements

The preparation of membranes for ion-selective electrodes was described earlier in detail.[9] The standard composition of membranes was: ionophore 10 mg, potassium tetrakis-(4-chlorophenyl)borate 0.5 mg, poly(vinyl chloride) 50 mg, and 2-nitrophenyl octyl ether 0.1 mL.

3.2. X-Ray crystal structure determination

A single crystal of azocrown **4** was obtained by crystallization from hexane. The data were collected at room temperature on KUMA diffractometer using graphite-monochromated Mo $K\alpha$ radiation and were corrected for Lorentz and polarization effects. The structure was solved using direct methods and refined by full-matrix least squares on F^2.[14] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in geometrically calculated positions and refined using temperature factors 1.2 times those of their bonded carbon atoms. Crystal data for **4**: $C_{20}H_{24}N_2O_5$, $M_r=372.41$, monoclinic, $P2_1/n$, $a=14.976(3)$ Å, $b=14.024(3)$ Å, $c=18.577(4)$ Å, $\beta=97.71(3)°$, $V=3866(1)$ Å^3, $Z=8$, $D_c=1.280$ g/cm^3, $\mu=0.92$ cm^{-1}, $F(000)=1584$, $\theta_{max}=25.56°$($-18\le h\le 17$, $0\le k\le 17$, $0\le l\le 21$), reflections collected 7435, independent reflections 7203 ($R_{int}=0.0566$), goodness-of-fit on F^2 $S=1.068$. Final residuals (for 488 parameters) $R1=0.0633$, $wR2=0.1805$ for 2657 reflections with $I>2\sigma(I)$, and $R1=0.1724$, $wR2=0.2164$ for all data. Residual electron density: 0.432 and -0.244 eÅ^{-3}.

3.3. Syntheses.

3.3.1. 1,11-Bis(2-nitrophenoxy)-3,6,9-trioxaundecane (1). A mixture of 2-nitrophenol (6.9 g, 50 mmol), 1,11-dichloro-3,6,9-trioxaundecane (5.8 g, 25 mmol), anhydrous potassium carbonate (6.9 g, 50 mmol) and dimethylformamide (15 mL) was heated at 120 °C for 8 h. The mixture was poured into water and the product was extracted with chloroform. After evaporation of solvent, the residue was purified by gradient column chromatography using petroleum ether/chloroform solvent system. Yield of the pure oily product was 14.8 g (68%). Calculated for $C_{20}H_{24}N_2O_9$: [M+H peak] $m/z=437.1560$. Found: 437.1562. Anal. Calcd C 55.04, H 5.50, N 6.42. Found C 55.0, H 5.48, N 6.41; δ_H ($CDCl_3$, 200 MHz): 3.58–3.63 (4H, m); 3.67–3.71 (4H, m); 3.82–3.88 (4H, m); 4.18–4.23 (4H, m); 6.92–7.09 (4H, m); 7.42–7.51 (2H, m); 7.74 (2H, dd, $J_1=1.7$ Hz, $J_2=8.1$ Hz); Mass Spec (MeOH, ES+) *m/z* expected: 436.15. Found: 437.16 (M+H); IR (film) 2870, 1604, 1520, 1349, 1280, 1130, 936, 849, 746 (cm^{-1}).

3.3.2. 1,11-Bis(4-*tert*-butyl-2-nitrophenoxy)-3,6,9-trioxaundecane (2). Obtained analogously to **1** using: 4-*tert*-butyl-2-nitrophenol[9] (9.8 g, 50 mmol), 1,11-dichloro-3,6,9-trioxaundecane (5.8 g, 25 mmol), anhydrous potassium carbonate (6.9 g, 50 mmol) and dimethylformamide (15 mL). Yield of pure oily product was 16.5 g (60%). Calculated for $C_{28}H_{41}N_2O_9$: [M+H peak] $m/z=549.2812$. Found: 549.2810. Anal. Calcd C 61.31, H 7.30, N 5.11. Found C 61.28, H 7.31, N 5.10; δ_H ($CDCl_3$, 200 MHz): 1.31 (18H, s); 3.64–3.69 (4H, m); 3.73–3.78 (4H, m); 3.89 (4H, t, $J=4.6$ Hz); 4.24 (4H, t, $J=5.1$ Hz); 7.04 (2H, d, $J=8.8$ Hz); 7.52 (2H, dd, $J_1=4.8$ Hz, $J_2=8.8$ Hz); 7.82 (2H, d, $J=2.5$ Hz). Mass Spec (MeOH, ES+) *m/z* expected: 548.27. Found: 549.28 (M+H); IR (film) 2778, 1606, 1542, 1353, 1278, 1133, 1043, 938, 851, 746, 668 (cm^{-1}).

3.3.3. 1,11-Bis(4-phenyl-2-nitrophenoxy)-3,6,9-trioxaundecane (3). Obtained analogously to **1** using: 4-phenyl-2-nitrophenol[9] (10.8 g, 50 mmol), 1,11-dichloro-3,6,9-trioxaundecane (5.8 g, 25 mmol), anhydrous potassium carbonate (6.9 g, 50 mmol) and dimethylformamide (15 mL). Crude compound **3** was purified by column chromatography using chloroform as an eluent. Yield after crystallization from 2-propanol was 17.6 g (60%). Mp 80–82 °C, yellow crystals. Calculated for $C_{32}H_{33}N_2O_9$: [M+H peak] $m/z=589.2186$. Found: 549.2188. Anal. Calcd C 65.31, H 5.61, N 4.76. Found C 65.29, H 5.62, N 4.75; δ_H ($CDCl_3$, 200 MHz): 3.67–3.72 (4H, m); 3.76–3.81 (4H, m); 3.91–3.96 (4H, m); 4.28–4.33 (4H, m); 7.17 (2H, d, $J=8.7$ Hz); 7.36–7.56 (10H, m); 7.71 (2H, dd, $J_1=2.4$ Hz, $J_2=8.7$ Hz); 8.05 (2H, d, $J=2.3$ Hz). Mass Spec (MeOH, ES+) *m/z* expected: 588.21. Found: 589.22 (M+H); IR (film) 2783, 1607, 1524, 1356, 1279, 1132, 1044, 940, 850, 746, 664 (cm^{-1}).

3.3.4. Bis(benzo)-19-azocrown-7 (4) and bis(benzo)-19-azoxycrown-7 (5). *In the presence of potassium hydroxide*. Water (12 mL) was added dropwise to a vigorously stirred mixture of dinitroderivative **1** (1.3 g, 3 mmol), stannous chloride dihydrate (2.9 g, 13 mmol), potassium hydroxide (5.6 g) and acetone (15 mL). The mixture was additionally vigorously stirred at 50 °C for 5 h. Then the cooled mixture was diluted with water (10 mL) and extracted with chloroform (3×50 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was preliminarily chromatographed on a short silica gel column using methylene chloride at the beginning and then methylene chloride–acetone mixture (10/1) as eluents to remove polymers and diacetone alcohol. The eluate was evaporated and the residue was extracted with hot heptane to isolate a mixture of compounds **4** and **5** that finally were separated by preparative thin-layer chromatography. The azocrown ether was crystallized from heptane to obtain *E* isomer (56 mg, 5%), mp 114–116 °C. The azoxycrown ether was obtained as yellowish oil (112 mg, 10%).

In the presence of sodium hydroxide. The synthesis was performed analogously using an equivalent amount of sodium hydroxide. After the reaction was completed, the mixture was cooled and the precipitated sodium chloride was filtered off and washed with toluene (2×25 mL). The combined organic layers were dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. After separation of the crown ethers as above, the azocrown ether was crystallized from heptane to afford *E* isomer

(200 mg, 18%), mp 116 °C. The azoxycrown ether was obtained as yellowish oil (104 mg, 9%).

Azocrown **4**. Calculated for $C_{20}H_{25}N_2O_5$: [M+H peak] m/z=373.1763. Found: 373.1764. Anal. Calcd C 64.50, H 6.50, N 7.52. Found C 64.56, H 6.44, N 7.44; Mass Spec (MeOH, ES+) m/z expected: 372.17. Found: 373.18 (M+H); isomer *E*: δ_H ($CDCl_3$, 500 MHz): 3.45–3.47 (4H, m); 3.59–3.61 (4H, m); 4.40 (4H, t, J=4.4 Hz); 4.37 (4H, t, J=4.4 Hz); 7.02 (2H, d, J=7.8 Hz); 7.33 (2H, dd, J_1=1.5 Hz, J_2=7.8 Hz); 7.39 (2H, dt, J_1=1.5 Hz, J_2=7.32 Hz). IR (film), ν_{max} (cm^{-1}) 2924, 2872, 1589, 1482, 1452, 1285, 1242, 1124, 1046, 938, 753.

Azoxycrown **5**. Calculated for $C_{20}H_{25}N_2O_6$: [M+H peak] m/z=389.1713. Found: 389.1712. Anal. calcd C 61.84, H 6.23, N 7.21. Found C 61.90, H 6.23, N 7.18; δ_H ($CDCl_3$, 500 MHz): 3.59–3.62 (4H, m); 3.64–3.69 (4H, m); 3.90–3.93 (4H, m); 4.27–4.31 (4H, m); 7.04 (3H, m); 7.11 (1H, d, J=8.3 Hz); 7.32 (1H, dt, J_1=1.5 Hz, J_2=7.8 Hz); 7.42 (1H, dt, J_1=1.5 Hz, J_2=8.8 Hz); 7.63 (1H, dd, J_1=1.5 Hz, J_2=8.3 Hz); 8.05 (1H, dd, J_1=1.5 Hz, J_2=8.3 Hz); Mass Spec (MeOH, ES+) m/z expected: 388.16. Found: 389.17 (M+H); IR (film) 2957, 2917, 2874, 1600, 1521, 1486, 1453, 1351, 1261, 1107, 1056, 940, 850, 801,745, 672 (cm^{-1}).

3.3.5. Bis(4-*tert*-butylbenzo)-19-azocrown-7 (6) and bis(4-*tert*-butylbenzo)-19-azoxycrown-7 (7). Water (10 mL) was added drop by drop to vigorously stirred mixture of dinitroderivative **2** (1.1 g, 2 mmol), stannous chloride dihydrate (1.95 g, 8 mmol), sodium hydroxide (2.4 g) and acetone (8 mL). The mixture was additionally stirred at 65 °C for 4.5 h. The products were separated analogously to **4** and **5**, and finally purified by preparative thin-layer chromatography using chloroform as mobile phase. The azocrown ether crystallized in 'mass' as a mixture of isomers, 77 mg (8%), mp 128–129 °C. The azoxycrown ether was obtained as yellowish oil 100 mg (10%).

Azocrown **6**. Calculated for $C_{28}H_{41}N_2O_5$: [M+H peak] m/z=485.3015. Found: 485.3013. Anal. calcd C 69.40, H 8.32, N 5.78. Found C 68.95, H 8.27, N 5.74.

Azocrown **6**, isomer *Z*: δ_H ($CDCl_3$, 500 MHz): 1.15 (18H, s); 3.70–3.74 (8H, m); 3.86 (4H, t, J=4.9 Hz); 3.98 (4H, t, J=4.9 Hz); 6.80 (2H, d, J=8.8 Hz); 6.90 (2H, d, J=2.4 Hz); 7.15 (2H, dd, J_1=2.4 Hz, J_2=8.3 Hz).

Azocrown **6**, isomer *E*: δ_H ($CDCl_3$, 500 MHz): 1.35 (18H, m); 3.50–3.52 (4H, m); 3.62–3.64 (4H, m) 3.89 (4H, t, J=4.4 Hz); 4.33 (4H, t, J=4.4 Hz); 7.01 (2H, d, J=8.8 Hz); 7.40 (2H, dd, J_1=2.4 Hz, J_2=8.3 Hz); 7.42 (2H, d, J=2.4 Hz). The signals ascribed to this form were selected from spectrum of a mixture of isomers (around 75% *E*, and 25% *Z*). Mass Spec (MeOH, ES+) m/z expected: 484.29. Found: 485.30 (M+H); IR (mixture of isomers) (film), 2960, 2870, 1601, 1497, 1461, 1395, 1360, 1260, 1134, 1053, 992, 938, 894, 815, 755 (cm^{-1}).

Azoxycrown **7**. Calculated for $C_{28}H_{41}N_2O_6$: [M+H peak] m/z=501.2965. Found: 501.2967. Anal. calcd C 67.18, H 8.05, N 5.60. Found C 67.00, H 7.99, N 5.56; δ_H ($CDCl_3$, 500 MHz): 1.34 (9H, s); 1.36 (9H, s); 3.62–3.65 (4H, m); 3.67–3.69 (4H, m); 3.90–3.93 (4H, m); 4.26–4.29 (4H, m); 7.00 (1H, d, J=8.8 Hz); 7.04 (1H, d, J=8.8 Hz); 7.34 (1H, dd, J_1=2.4 Hz, J_2=8.8 Hz); 7.43 (1H, dd, J_1=2.4 Hz, J_2=8.3 Hz); 7.66 (1H, d, J=2.4 Hz); 8.03 (1H, d, J=2.4 Hz). Mass Spec (MeOH, ES+) m/z expected: 500.29. Found: 501.30 (M+H); IR (film) 2965, 2872, 1731, 1605, 1504, 1457, 1359, 1265, 1134, 942, 896, 819, 753 (cm^{-1}).

3.3.6. Bis(4-phenylbenzo)-19-azocrown-7 (6) and bis(4-phenylbenzo)-19-azoxycrown-7 (7). Water (8 mL) was dropwise added to a vigorously stirred mixture of dinitroderivative **3** (1.2 g, 2 mmol), stannous chloride dehydrate (1.95 g, 8 mmol), sodium hydroxide (2.4 g) and acetone (8 mL). The mixture was stirred at 55 °C additionally for 4 h. After work-up as above the azocrown ether was obtained as red oil, 83 mg (8%), mp 128–129 °C. The azoxycrown ether was obtained as yellowish oil (162 mg, 15%).

Azocrown **8**. Calculated for $C_{32}H_{33}N_2O_5$: [M+H peak] m/z=525.2395. Found: 529.2398. Anal. calcd C 73.28, H 6.11, N 5.34. Found C 73.01, H 8.22, N 5.70.

Azocrown **8**, isomer *Z*: δ_H ($CDCl_3$, 500 MHz): 3.73–3.75 (6H, m); 3.79–3.81 (2H, m) 3.85 (2H, t, J=4.5 Hz); 3.95 (2H, t, J=4.5 Hz); 4.19 (2H, t, J=4.5 Hz); 4.30 (2H, t, J=4.5 Hz); 6.95 (2H, d, J=8.8 Hz); 7.27 (2H, dd, J=2.2 Hz); 7.28–7.38 (10H, m); 7.67 (2H, dd, J_1=2.2 Hz, J_2=8.8 Hz); These signals ascribed to form *Z* were selected from spectrum of a mixture of isomers (around 50% *E*, and 50% *Z*).

Azocrown **8**, isomer *E*: δ_H ($CDCl_3$, 500 MHz): 3.49–3.50 (2H, m); 3.63–3.64 (2H, m); 3.71–3.73 3.85 (4H, m); 3.90–3.92 (4H, m); 4.06 (2H, t, J=4.8 Hz); 4.40 (2H, t, J=4.3 Hz); 7.11–7.15 (2H, m); 7.40–7.46 (6H, m); 7.48–7.52 (6H, m); 8.04 (2H, d, J=2.2 Hz). Signals ascribed to this form were selected from spectrum of a mixture of isomers (75% *E*, and 25% *Z*). Mass Spec (MeOH, ES+) m/z expected: 528.23. Found: 529.24 (M+H); IR (mixture of isomers) (film) 2920, 2867, 1602, 1516, 1480, 1356, 1273, 1128, 1057, 942, 828, 758, 698 (cm^{-1}).

Azoxycrown **9**. Calculated for $C_{32}H_{33}N_2O_6$: [M+H peak] m/z=541.2339. Found: 541.2335; (540.6) MS: m/e=540. Anal. calcd C 71.11, H 5.92, N 5.18. Found C 71.08, H 5.90, N 5.16; δ_H ($CDCl_3$, 500 MHz): 3.63–3.64 (4H, m); 3.69–3.71 (4H, m); 3.94–3.97 (4H, m); 4.33–4.36 (4H, m); 7.14 (1H, d, J=8.8 Hz); 7.20 (1H, d, J=8.8 Hz); 7.31–7.37 (2H, m); 7.43–7.46 (4H, m); 7.57 (1H, dd, J_1=2.4 Hz, J_2=8.8 Hz); 7.58–7.63 (4H, m); 7.66 (1H, dd, J_1=2.4 Hz, J_2=8.8 Hz); 7.91 (1H, d, J=2.0 Hz); 8.34 (1H, d, J=2.4 Hz). Mass Spec (MeOH, ES+) m/z expected: 540.23. Found: 541.23 (M+H); IR (film) 3016, 2925, 2872, 1606, 1517, 1478, 1456, 1278, 1136, 1055, 939, 890, 818, 757, 697 (cm^{-1}).

4. Supplementary data

Crystallographic data for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 247296. Copies of the data can be obtained free of charge from the

CCDC (12 Union Road, Cambridge CB2 1EZ, UK; Tel.: +44 1223 336 408; fax: +44 1223 336 003; e-mail:deposit@ccdc.cam.ac.uk; www:http://ccdc.cam.ac.uk).

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General and environmentally friendly synthesis of heterocyclic multidentate molecules based on microwave-assisted heating protocol

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Abstract—An efficient microwave heating methodology for the synthesis of heterocyclic multidentate molecules is reported. Each compound was obtained with high yield and purity in a few minutes from easily available starting materials such as amines, heteroaldehydes and *N*-hydroxymethyl pyrazoles or triazoles. In addition, this approach allows synthesis without any solvent or organic and inorganic by-products.

1. Introduction

A large number of amine and thiophene derivatives have shown real promise for the development of biologically active molecules and also in the area of coordination chemistry. Modular synthetic strategies, allowing structural modifications of a given scaffold, offer the ability to easily generate analogues or diversity.[1] For instance, several analogues of antergan[2] are reported in Figure 1. The substitution of antergan's phenyl rings with thiophenyl or pyridinyl moieties allowed optimization of the target ratio of antihistaminic properties/sedative side effects. In addition to drug discovery, new heterocyclic multidentate molecules are of interest as ligands for coordination chemistry and organometallic catalysis.[3] Thus, methods allowing modulation of electronic strength of donor atoms and steric hindrance of different substituants represent a key tool to prepare a large diversity of multichelating ligands or to elaborate a specific designed molecule.

Herein, we report a general approach for the synthesis of tripodal molecules based on a central nitrogen atom connecting three different functionalized arms (Fig. 2). This method, based on microwave-assisted heating, offers the opportunity (i) to easily change one, two or all building blocks R_3, *Z* and *Y*, (ii) to directly prepare these compounds in very high yield and purity and (iii) to perform the

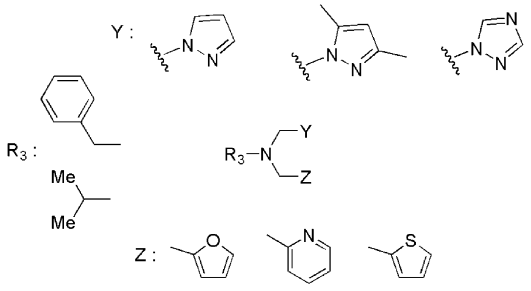

Figure 2. Tripodal molecules with three modular building blocks.

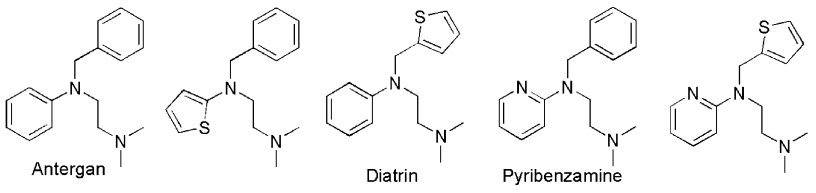

Figure 1. Antergan and analogues.

Keywords: Microwave; Amines; Heterocycles; Pyrazole; Triazole.
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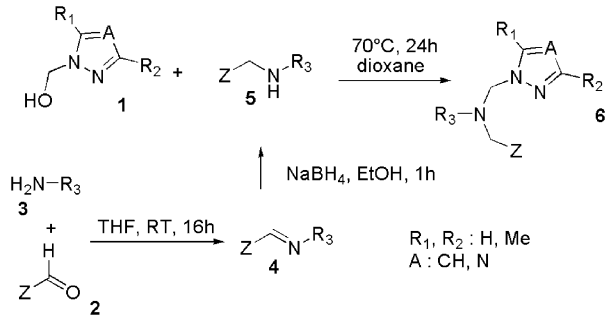
reactions rapidly without solvent and with only water as the by product.

We recently reported solution phase combinatorial access to a set of tripodal compounds.[4] This approach was based on the thermic condensation of 1-hydroxymethyl pyrazoles or 1-hydroxymethyl triazoles **1** with heterocyclic secondary amines **5** (Scheme 1). The later were easily obtained via reductive amination between the corresponding primary amines **3** and aldehydes **2**.



Scheme 1. Initial approach to synthesize tripodal compounds.

Long reaction times were necessary for both imine synthesis and coupling reactions, respectively, 16 and 24 h. This could represent a limitation for thermally sensitive starting reagents or expected products.

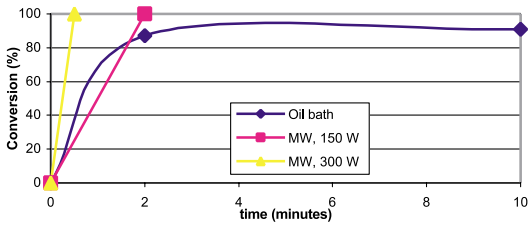
Microwave-assisted chemistry has attracted a considerable attention in recent years and has been applied successfully in various fields of organic chemistry with significant rate enhancement and higher product yields.[5] Therefore, we have investigated a microwave heating methodology in order to generalize the rapid access to tripodal molecules, in very high yield.

Our first study concerned the impact of a microwave-assisted methodology for preparation of imines **4a–f**. We first condensed 2-furaldehyde, 2-pyridylaldehyde or 2-thiophene carboxaldehyde with benzylamine or isopropylamine under microwave irradiation (automatic regulation to 80 °C) for 2 min. In each case, conversion is 100% (determined by ^{1}H NMR spectra). After filtration, the corresponding imines **4a–f** were obtained in high yield (Table 1).

Table 1. Synthesis of imines **4** using microwave heating

Entry	4	R_3	Z	Yield (%)
1	**4a**	Ph–CH_2	2-Furyl	98
2	**4b**	$(CH_3)_2CH$	2-Furyl	97
3	**4c**	Ph–CH_2	2-Pyridyl	98
4	**4d**	$(CH_3)_2CH$	2-Pyridyl	100
5	**4e**	Ph-CH_2	2-Thiophenyl	97
6	**4f**	$(CH_3)_2CH$	2-Thiophenyl	95

We have compared the thermal heating protocol with oil bath and two different power for the microwave irradiation for the amine **4e**, using the same technical conditions (substrates, size of vessels, solvent, temperature set, concentration) (Graph 1).



Graph 1. Synthesis of imine **4e** at 80 °C in *n*-heptane.

We observed that the reaction reached 88% conversion with the oil bath after 2 min and that longer reaction time did not improve this conversion. Purification step was then necessary to obtain pure compound **4e**. By contrast, the same heating time via microwave oven (power 150 W) led to complete conversion, **4e** was obtained in pure form without purification step. More interestingly, using a higher microwave power (300 W) the reaction is complete in 30 s.

We could explain this observation by the fact that this 'instantenous' increase of temperature is not possible with oil bath due to slow heat transfer from the wall of the vessel to the solution, even using a pre-heated oil bath.

The second step, that is, reduction of the imine by $NaBH_4$ was performed as already reported[4] and gave amines **5** with moderate to good yields. Hydroxyalkylation of pyrazole,[6] its 3,5-disubstituted derivatives or triazole[7] with formaldehyde afforded the other set of reagents **1**.

The impact of the microwave heating methodology was also applied to the coupling reactions between compounds **5** and **1**. The condensation reaction was performed in *n*-heptane, with $MgSO_4$ as drying agent, under microwave irradiation for 10 min (automatic regulation to 95 °C). A simple filtration and solvent evaporation under reduced pressure provided the tripodal molecules **6** with good yields (Table 2). In addition to several studies,[5] these observations validate the impact of a microwave-assisted reaction conditions. Therefore, we applied this method to the synthesis of new thiophene containing molecules **7a–f** (Fig. 3) with good to excellent yields (Table 2, entries 4–9). All derivatives **7** were obtained in very high purity without need of purification process. It should be noted that this microwave approach was crucial for the synthesis of these new thiophene containing molecules **7** as we observed significant amount of side products when heating in dioxane at 70 °C with oil bath was used.

For comparison, we have used an oil bath at 95 °C: **6a** and **7d** were obtained in quantitative yield after 3 h. After 10 min, the conversion were only 66% (**6a**) and 75% (**7d**) (Graph 2).

We checked that multi-grams synthesis of these new compounds **7a–f** was possible. The synthesis of 1.5 g of each derivative **7** was obtained without affecting yield or purity; the only limitation was the size of microwave vessels.

Interestingly, this microwave method allows also solvent free conditions and no use of drying agents. The new

Table 2. Synthesis of tripodal compounds **6–7** using microwave heating

Entry	6–7	A	R^1	R^2	R^3	Z	Yield (%)
1	**6a**	CH	H	H	Ph–CH_2	2-Furyl	99
2	**6b**	CH	Me	Me	$(CH_3)_2CH$	2-Furyl	97
3	**6c**	CH	H	H	Ph–CH_2	2-Pyridyl	82
New products							
4	**7a**	CH	H	H	Ph–CH_2	2-Thiophenyl	100
5	**7b**	CH	Me	Me	Ph–CH_2	2-Thiophenyl	100
6	**7c**	N	H	H	Ph–CH_2	2-Thiophenyl	81
7	**7d**	CH	H	H	$(CH_3)_2CH$	2-Thiophenyl	95
8	**7e**	CH	Me	Me	$(CH_3)_2CH$	2-Thiophenyl	91
9	**7f**	N	H	H	$(CH_3)_2CH$	2-Thiophenyl	87

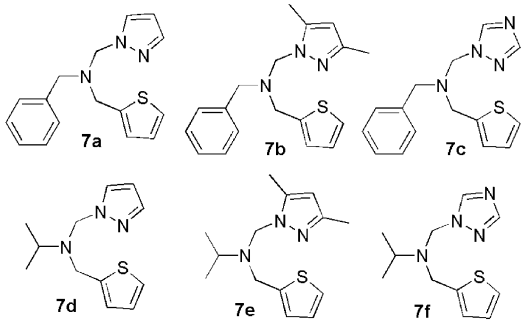
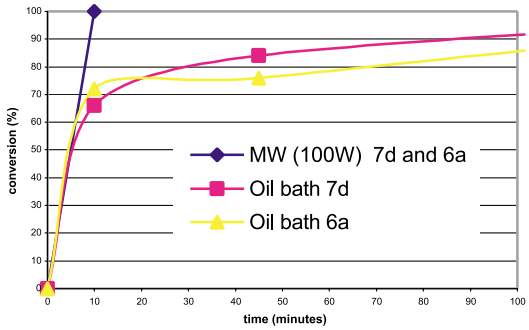


Figure 3. 3 New thiophene containing molecules **7a–f**.



Graph 2. Synthesis of **6e** and **7d** at 95 °C in *n*-heptane.

derivatives **7a–f** were synthesized in high yield and purity from mixing pure starting products **1** and **5** without any solvent or $MgSO_4$ (MW 100 W for 10 min at 95 °C). (We observed condensation of water at the top of the vessel (cold area by comparison with irradiated area) with derivatives **7** at the bottom of the flask). No filtration process was necessary as the drying agent $MgSO_4$ was not used. Suppression of solvents and waste (inorganic salts or organic side products) is a key point in the context of environmentally friendly processes.

In summary, we report an efficient access to tripodal compounds **6–7** using a microwave-assisted heating protocol. This approach allowed, easy modifications of different building blocks, higher yield and purity than an oil bath heating protocol and saving of time as no purification steps were necessary. These three aspects are key points in combinatorial chemistry to quickly generate large and diverse libraries of compounds without tedious work-up.

In addition, this microwave-assisted approach allowed synthesis of a diversity of tripodal molecules without any solvent. No inorganic or organic wastes were obtained as only water was generated and easily eliminated as side product. Regarding the diversity of commercially available primary and secondary amines, aldehydes, pyrazole or triazole derivatives, one could perform large libraries, even for thermo sensitive compounds, in short delays using automated microwave oven. Further developments on the synthesized thiophene series are currently in progress in the laboratory for potential applications in medicinal chemistry or in coordination chemistry as new ligands.

2. Experimental

Commercially available reagents from Aldrich or Acros were used without further purification. Solvents were dried by standard procedures. Reactions were performed using a CEM Discover (300 W) microwave oven controlled by Chemdriver Version 3.5.9 software. NMR spectra were recorded using a Brucker ARX 200 instrument operating at 200 MHz for ^1H spectra and a Brucker AC 300 P instrument operating at 50 MHz for ^{13}C spectra. *J* values were recorded in Hz, and multiplicities were expressed by usual conventions. HRMS were obtained with a VARIAN MAT311 (Centre Régional de Mesures Physiques de l'Ouest, Rennes, France). Microanalyses results were obtained via Service Centrale d'Analyse, CNRS, Vernaison, France.

2.1. Synthesis of imines 4, typical procedures

(a) *Under microwave heating conditions*. A mixture of carboxaldehyde (1 equiv), primary amine (1 equiv) *n*-heptane (4 mL) and magnesium sulfate were put in a microwave vessel. The sealed reactor was placed inside the microwave oven and irradiated for 30 s at 300 W. The temperature was set to 80 °C and the irradiation was automatically stopped at this temperature. After cooling to room temperature, the reaction mixture was filtrated and the solvent was removed under reduced pressure. The imine was obtained with good yields. Analytical data for amine **4a–d** were identical to those previously reported. (b) *Under conventional heating conditions*. A mixture of carboxaldehyde (1 equiv), primary amine (1 equiv), *n*-heptane (4 mL) and magnesium sulfate were put in an identical microwave vessel than (a). The sealed reactor was plunged into an oil bath pre-heated at 95 °C. In the reaction media, the temperature reached 80 °C and 1 mL aliquots were removed after 2, 10, 30, 60, 180 min. The aliquots were cooled to room temperature,

filtrated and the solvent was removed under reduced pressure.

2.1.1. *N*-Benzyl-*N*-(2-thiophenylmethyl)imine (4e). The typical procedure (a) with thiophene carboxaldehyde (0.94 mL, 10 mmol), benzylamine (1.09 mL) provided 1.99 g (99% yield) of pure thiophenyl imine as a pure yellow oil at room temperature. No purification was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.82 (s, 2H), 7.08–7.12 (m, 1H), 7.28–7.44 (m, 7H), 8.48 (s,1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 64.52, 127.12, 127.49, 128.61, 129.14, 130.78, 139.22, 142.58, 155.26. HRMS (EI) calcd for $M^{+\cdot}(C_{12}H_{11}NS)$: 201.0612, found 201.0610. Anal. Calcd for $C_{12}H_{11}NS$: C, 71.60; H, 5.51; N, 6.96; S, 15.93; found C, 71.41; H, 5.55; N, 7.10; S, 16.80.

2.1.2. *N*-Isopropyl-*N*-(2-thiophenylmethyl)amine (4f). The typical procedure (a) with thiophene carboxaldehyde (0.94 mL, 10 mmol), isopropylamine (0.94 mL) provided 1.35 g (88% yield) of thiophenyl imine, which contained less than 1% of unreacted thiophene carboxaldehyde. The crude product was directly engaged in the second step. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (d, 2H, $J=7$ Hz), 3.50 (hept, 1H, $J=6$ Hz), 7.05–7.08 (m, 1H), 7.28–7.38 (m, 2H), 8.39 (s, 1H).

2.1.3. Synthesis of amines 5, typical procedure. Sodium borohydride (1.1 equiv, 599 mg, 16.2 mmol) was slowly added to a solution of imine (1 equiv, 14.73 mmol) in EtOH (15 mL). The reaction mixture was stirred for 1 h at room temperature and solvent was totally removed under reduced pressure. The crude product was dissolved in diethyl ether (10 mL) and 3 mL of an aqueous solution of K_2CO_3 was added. The organic layer was dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to lead to thiophenyl amine with good yield. These products are instable and must be stored at low temperature and used within the few days following the synthesis.

2.1.4. *N*-Benzyl-*N*-(2-thiophenylmethyl)amine (5e). The typical procedure using phenyl-*N*-((thiophen-2-yl)methylene) methanamine (2.96 g) provided 2.93 g (98% yield, 96% total yield for the two steps) of pure secondary amine **5e**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73 (broad s, 1H), 3.88 (s, 2H), 4.04 (s, 2H), 6.98–7.01 (m, 2H), 7.02–7.40 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 47.61, 52.81, 124.43, 124.94, 126.67, 127.08, 128.28, 128.30, 140.07, 144.27. HRMS (EI) calcd for $M^{+\cdot}(C_{12}H_{13}NS)$: 203.0768, found 203.0766.

2.1.5. *N*-Isopropyl-*N*-(2-thiophenylmethyl)amine (5f). The typical procedure using *N*-((thiophen-2-yl)methylene)-propan-2-amine (2.25 g, crude) and a silica gel column chromatography (eluent Heptane/AcOEt/Et_3N 50:50:1) allowed a good purification and gave 1.92 g (84% yield, 77% total yield for the two steps) of pure product. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.10 (d, 6H, $J=7$ Hz), 1.39 (broad s, 1H), 2.90 (hept, 1H, $J=6$ Hz), 3.98 (s, 2H), 6.92–6.96 (m, 2H), 7.18 (d, 1H, $J=2$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.80, 45.88, 47.67, 124.14, 124.69, 126.61, 144.43. HRMS (EI) calcd for $M^{+\cdot}(C_8H_{13}NS)$: 155.0768, found 155.0769.

2.2. Coupling products 6–7, typical procedures

(a) *Using solvent*. In a sealed microwave reactor, a mixture of secondary amine (1 equiv, 0.49 mmol), pyrazole or triazole derivatives (1 equiv, 0.49 mmol), *n*-heptane (4 mL) and $MgSO_4$ (300 mg) were irradiated during 10 min. The temperature was set to 95 °C and automatically controlled by the software (Power: 100 W). After cooling to room temperature, the reaction mixture was filtrated, the solvent was eliminated under reduced pressure to lead to the corresponding tertiary amine with good yields and good purity. (b) *Without solvent*. In a sealed microwave reactor, a mixture of secondary amine (1 equiv, 0.49 mmol), pyrazole or triazole derivatives (1 equiv, 0.49 mmol) were irradiated during 10 min (Power: 100 W). After cooling to room temperature, water produced during the reaction was condensed at the top of the vessel.

2.2.1. *N*-Benzyl-*N*-(2-thiophenylmethyl)-*N*-((1*H*-pyrazol-1-yl)methyl)amine (7a). Typical procedure (b) with *N*-benzyl (thiophen-2-yl)methanamine (100 mg) and 1-hydroxymethyl pyrazole (48 mg) afforded the pure product with a quantitative yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.76 (s, 2H), 3.98 (s, 2H), 5.02 (s, 2H), 6.33 (s, 1H), 7.00–7.02 (m, 2H), 7.30–7.63 (3, 9H), 7.63 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.88, 55.22, 67.37, 105.26, 125.17, 126.03, 126.58, 127.37, 128.51, 128.75, 130.27, 138.35, 139.57, 142.83. HRMS (EI) calcd for $[M-C_3H_4N_2]^{+\cdot}$ ($C_{13}H_{13}NS$): 215.0768, found 215.0764. Anal. Calcd for $C_{16}H_{17}N_3S$: C, 67.81; H, 6.05; N, 14.83; found C, 67.78; H, 6.13; N, 14.83.

2.2.2. *N*-Benzyl-*N*-(2-thiophenylmethyl)-*N*-((3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)amine (7b). Typical procedure (b) with *N*-benzyl(thiophen-2-yl)methanamine (100 mg) and 1-hydroxymethyl-(3,5-dimethyl)-pyrazole (62 mg) afforded the pure product with a quantitative yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.09 (s, 3H), 2.26 (s, 3H), 3.75 (s, 2H), 3.96 (s, 2H), 4.82 (s, 2H), 5.82 (s, 1H), 6.96–6.98 (m, 2H), 7.27–7.43 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.00, 13.61, 50.52, 55.18, 65.54, 105.57, 124.88, 126.02, 126.51, 127.18, 128.32, 128.81, 138.54, 140.23, 142.67, 147.46. HRMS (EI) calcd for $[M-C_5H_8N_2]^{+\cdot}$ ($C_{13}H_{13}NS$): 215.0768, found 215.0777. Anal. Calcd for $C_{18}H_{21}N_3S$: C, 69.42; H, 6.80; N, 13.49; found C, 69.31; H, 6.93; N, 13.61.

2.2.3. *N*-Benzyl-*N*-(2-thiophenylmethyl)-*N*-((1*H*-1,2,4-triazol-1-yl)methyl)amine (7c). Typical procedure (b) with *N*-benzyl(thiophen-2-yl)methanamine (100 mg) and 1-hydroxymethyl triazole (48.50 mg) afforded the pure product with 81% yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.74 (s, 1H), 3.97 (s,1H), 5.04 (s, 1H), 6.97–7.02 (m, 2H), 7.29–7.43 (m, 6H), 7.99 (s, 1H), 8.02 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.93, 55.33, 64.91, 125.56, 126.40, 126.68, 127.67, 128.67, 128.74, 137.58, 141.90, 143.98, 152.03. HRMS (EI) calcd for $[M-C_2H_3N_3]^{+\cdot}$ ($C_{13}H_{13}NS$): 215.0768, found 215.0755. Anal. Calcd for $C_{15}H_{16}N_4S$: C, 63.35; H, 5.67; N, 19.70; found C, 63.15; H, 5.68; N, 18.76.

2.2.4. *N*-Isopropyl-*N*-(2-thiophenylmethyl)-*N*-((1*H*-pyrazol-1-yl)methyl)amine (7d). Typical procedure (b) with *N*-

((thiophen-2-yl)methyl)propan-2-amine (76 mg) and 1-hydroxymethyl pyrazole (48 mg) afforded the pure product with 95% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.03 (d, 6H, J=7 Hz), 3.19 (hept, 1H, J=6 Hz), 3.99 (s, 2H), 4.99 (s, 2H), 6.28 (t, 1H, J=2 Hz), 6.94–7.00 (m, 2H), 7.24 (d, 1H, J=2 Hz), 7.51–7.54 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.33, 46.05, 50.37, 66.58, 105.66, 124.85, 125.39, 126.58, 129.09, 138.98, 144.29. Anal. Calcd for $C_{18}H_{21}N_3S$: C, 61.24; H, 7.28; N, 17.85; found C, 60.84; H, 7.45; N, 17.99.

2.2.5. *N*-Isopropyl-*N*-(2-thiophenylmethyl)-*N*-((3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)amine (7e). Typical procedure (b) with *N*-((thiophen-2-yl)methyl)propan-2-amine (76 mg) and 1-hydroxymethyl-(3,5-dimethyl)-pyrazole (62 mg) afforded the pure product with 91% yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.09 (d, 6H, J=7 Hz), 2.20 (s, 3H), 2.28 (s, 3H), 3.12 (hept, 1H, J=6 Hz), 3.88 (s, 2H), 4.80 (s, 2H), 5.80 (s, 1H), 6.89–7.16 (m, 2H), 7.17 (d, 1H, J=2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.24, 13.48, 18.36, 46.07, 49.24, 63.98, 105.94, 124.37, 128.87, 126.36, 138.87, 145.05, 146.95. HRMS (EI) calcd for $[M-C_5H_7N_2–H]^{+\cdot}$ ($C_9H_{13}NS$): 167.0768, found 167.0774. Anal. Calcd for $C_{14}H_{21}N_3S$: C, 63.84; H, 8.04; N, 15.95; found C, 63.24; H, 8.19; N, 15.12.

2.2.6. *N*-Isopropyl-*N*-(2-thiophenylmethyl)-*N*-((1*H*-1,2,4-triazol-1-yl)methyl)amine (7f). Typical procedure (b) with *N*-((thiophen-2-yl)methyl)propan-2-amine (76 mg) and 1-hydroxymethyl triazole (48.50 mg) afforded the pure product with 85% yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.05 (d, 6H, J=7 Hz), 3.19 (hept, 1H, J=6 Hz), 3.99 (s, 2H), 5.04 (s, 2H), 6.93–6.99 (m, 2H), 7.25 (d, 1H, J=3 Hz), 7.92 (s, 1H), 8.12 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.64, 46.28, 51.13, 64.71, 125.26, 125.84, 126.68, 143.16, 143.20, 151.57. HRMS (EI) calcd for $[M-C_2H_3N_3]^+$ ($C_9H_{13}NS$): 167.0768, found 167.0758. Anal. Calcd for $C_{11}H_{16}N_4S$: C, 55.90; H, 6.82; N, 23.71; found C, 55.95; H, 7.01; N, 22.57.

2.2.7. *N*-Benzyl-*N*-(2-furylmethyl)-*N*-(1*H*-pyrazol-1-ylmethyl)amine (6a). Typical procedure with (b) *N*-((furan-2-yl)methyl)(phenyl)methanamine (91 mg) and 1-hydroxymethyl pyrazole (48 mg) afforded the pure product with a quantitative yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.75 (s, 2H), 3.80 (s, 2H), 5.01 (s, 2H), 6.34–6.38 (m, 3H), 7.30–7.46 (m, 7H), 7.64 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.6, 142.8, 140.0, 138.7, 130.6, 129.2, 128.7, 127.8, 110.7, 109.4, 105.8, 68.3, 55.8, 48.8. HRMS (EI) calcd for $C_{13}H_{13}NO$ $[M-C_3H_4N_2]^+$199.0997, found 199.099. Same analytical data than previously reported.[1]

2.2.8. *N*-Isopropyl-*N*-((2-furanylmethyl)-*N*-((3,5-dimethyl-1*H*-pyrazol-1-yl)methyl)amine (6b). Typical procedure (b) with *N*-((furan-2-yl)methyl)propan-2-amine (67 mg) and 1-hydroxymethyl-(3,5-dimethyl)-pyrazole (62 mg) afforded the pure product with 97% yield. No purification step was necessary ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.04 (d, 6H, J=7 Hz), 2.17 (s, 3H), 2.21 (s, 3H), 3.08 (hept, 1H, J=6 Hz), 3.70 (s, 2H), 4.80 (s, 2H), 5.78 (s, 1H), 6.18 (d, 1H, J=1 Hz), 6.30 (t, 1H, J=1 Hz), 7.35 (d, 1H, J=2 Hz). Same analytical data than previously reported.[1]

2.2.9. *N*-Benzyl-*N*-(2-pyridylmethyl)-*N*-(1*H*-pyrazol-1-ylmethyl)amine (6c). Typical procedure (b) with *N*-benzyl (pyridin-2-yl)methanamine (97 mg) and 1-hydroxymethyl pyrazole (48 mg) afforded the pure product with 82% yield. No purification step was necessary. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.75 (s, 2H), 3.88 (s, 2H), 5.00 (s, 2H), 6.29 (s, 1H), 7.16–7.62 (m, 10H), 8.57 (d, 1H, J=3 Hz). HRMS (EI) calcd for $C_{14}H_{14}N_2$ $[M-C_6H_6N]^+$210.1157, found 210.117. Same analytical data than previously reported.[1]

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Enantioselective total synthesis of (2*R*,3*R*,6*R*)-*N*-methyl-6-(deca-1′,3′,5′-trienyl)-3-methoxy-2-methylpiperidine, an insecticidal alkaloid

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Abstract—An insecticidal piperidine alkaloid, (2*R*,3*R*,6*R*)-*N*-methyl-6-(deca-1′,3′,5′-trienyl)-3-methoxy-2-methylpiperidine, was efficiently synthesized in a stereoselective manner starting from D-alanine. Chiral center at C-6 was controlled by hydrogenation of imine and side chain was introduced by Julia olefination. The absolute configuration of natural product was determined to be 2*R*, 3*R*, 6*R*.

1. Introduction

Natural alkaloids containing piperidine rings have many different varieties.[1] (2β,3β,6β)-*N*-Methyl-6-(deca-1′,3′,5′-trienyl)-3-methoxy-2-methylpiperidine (**1**) containing 2,3,6-trisubstituted piperidine ring was isolated from stem bark of *Microcos paniculata* L. (Tiliaceae) in 2001.[2] This compound shows moribund/toxic and growth-inhibitory effects[2] on the second instar larvae of the mosquito *Aedes aegypti* which mediates dengue fever. Dengue fever is epidemic mostly during and shortly after the rainy season in tropical and subtropical areas and 50–100 million cases of the infection occur each year.[3] Because there is no heroic drug for the dengue, which is mostly carried by the mosquito, a good compound showing insecticidal efficiency is necessary to prevent the dengue (Fig. 1).

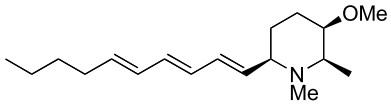

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Figure 1.

Although the relative configuration of compound **1** has been elucidated by NMR spectroscopic analysis, its absolute configuration remained unclear. Described herein is an efficient total synthesis of **1** to determine its absolute configuration.

2. Results and discussion

Our retrosynthetic strategy is shown in Scheme 1. The side chain unit bearing triene would be introduced by coupling reaction between **2** and **3**. Asymmetric center at C-6 of aldehyde **2** would be constructed by stereoselective hydrogenation of imine **4**, which has 2*R*-methyl and 3*R*-methoxy groups. Imine **4** would be synthesized from known alcohol **6**[4] via oxidation and intramolecular imine formation.

The synthesis of aldehyde unit is shown in Scheme 2. In the reported four-step sequence,[4] the readily available D-alanine was converted to *syn*-amino alcohol **6**. *O*-Methylation of hydroxy group under basic condition[5] was achieved by treating with one equivalent of sodium hydride and excess amount of methyl iodide to give alkene **7**. When more equivalents of sodium hydride were used, *N*-methylation also took place and it was difficult to separate the *O*-methylated and *N*,*O*-dimethylated compounds. The alkene **7** was oxidized with OsO_4 to the corresponding diol followed by preferential acylation of the primary hydroxy group to give mono pivalate **8**. PDC oxidation of the secondary hydroxyl group afforded the corresponding ketone **9** in excellent yield. *N*-Boc-cleavage with TFA resulted in a simultaneous cyclodehydration to form cyclic imine **10**. Catalytic hydrogenation of **10** stereoselectively

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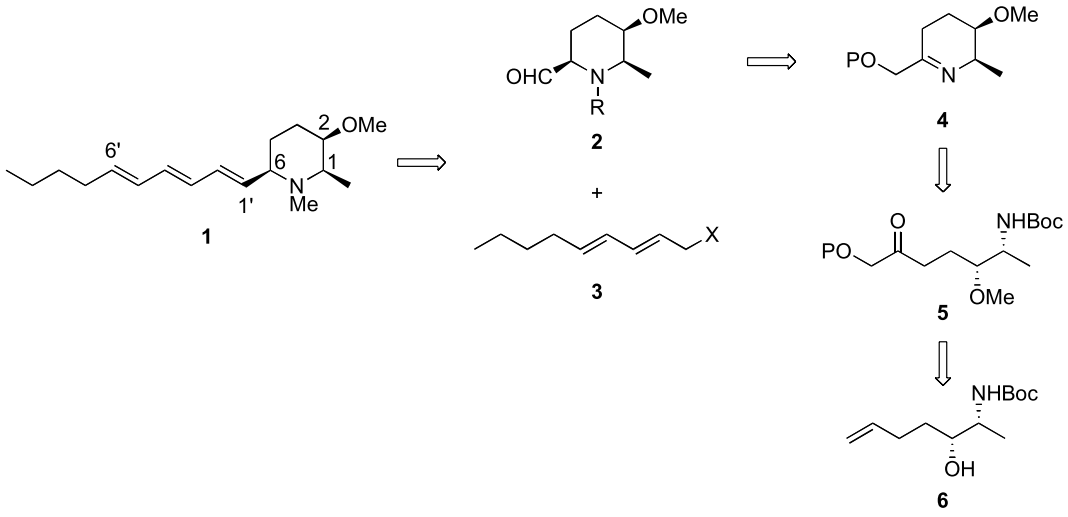
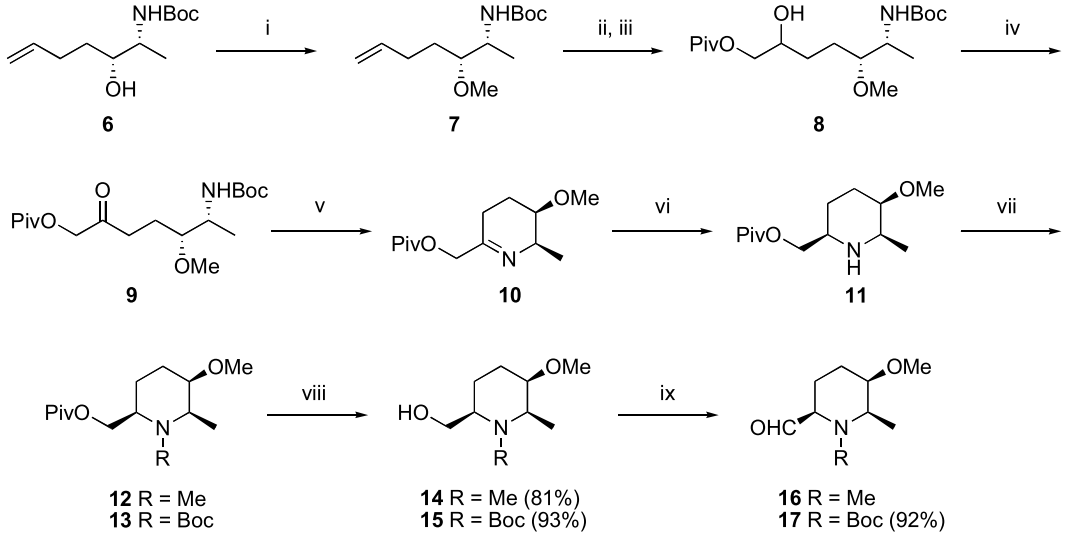


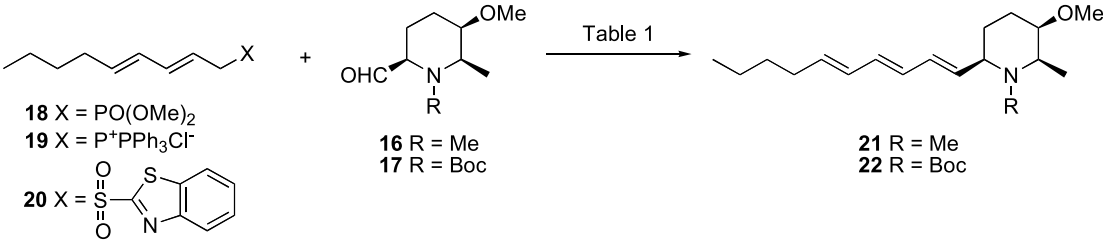
Scheme 1. Synthetic plan.



Scheme 2. Reagents and conditions. (i) NaH, MeI, THF, 63%, (ii) OsO_4, NMO, THF–acetone (1:1), (iii) Piv-Cl, pyridine, 0 °C, 2 steps 91%, (iv) PDC, 4A MS, CH_2Cl_2, 98%, (v) TFA, CH_2Cl_2, then $NaHCO_3$, (vi) Pd/C, H_2, MeOH, 2 steps 81%, (vii) (a) 35% HCHO, $NaBH_3CN$, CH_3CN, 63% for **12**, (b) $(Boc)_2O$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 99% for **13**, (viii) DIBAL, CH_2Cl_2, −78 °C, (ix) Swern oxidation.

afforded the saturated piperidine **11** as a single isomer. The stereochemistry of **11** was thought to be all *syn* configuration, because the catalyst could approach the imine **10** from the less hindered α-face of the molecule. The configuration was determined after conversion to final compound.

Reductive *N*-methylation[6] and removal of pivalate afforded **14** via **12**, and Swern oxidation[7] of the resulting alcohol provided coupling precursor **16**. Purification by silica gel chromatography of aldehyde **16** caused an epimerization to give a mixture of C-6 epimers in a ratio 1:1. We therefore employed crude **16** for the next reaction without purification. *N*-Boc-piperidine **17** as an alternative coupling precursor was also prepared from **11** via **13** and **15** in the same manner. In contrast with **16**, *N*-Boc-piperidine **17** was stable enough to be purified by silica gel column chromatography and was obtained as crystals in high yield.



Scheme 3.

Table 1

Entry	Aldehyde unit	Side chain unit	Additive	Base	Temperature (°C)	Yielf from **14** or **15** (%)	*E*:*Z*
1	**16**	**18**		*n*-BuLi	−78	Decomp.	1:1
2	**16**	**19**		*n*-BuLi	−78	19	1:1
3	**16**	**20**		LHMDS	−78	25	1:1
4	**16**	**20**		KHMDS	−78	32	1:1
5	**17**	**18**		*n*-BuLi	−78	Trace	1:1
6	**17**	**19**		*n*-BuLi	−78	42	1:1
7	**17**	**20**		LHMDS	−78	83	1:1
8	**17**	**20**	12-Crown-4	LHMDS	−78	80	1:1
9	**17**	**20**		KHMDS	−78	71	1:1
10	**17**	**20**	HMPA	KHMDS	−78	83	2:1
11	**17**	**20**	18-Crown-6	KHMDS	−78	78	3:1
12	**17**	**20**	18-Crown-6	KHMDS	−100	53	3:1

On the other hand, three types of side chain units (**18**–**20**) were prepared from *trans*,*trans*−2,4-nonadienol to investigate the diverse olefinations (Scheme 3). To find the optimal condition for highest yield and *E*/*Z* selectivity at C-1′, effects of substrats and bases were examined and the results are shown in Table 1. When *N*-methylpiperidine **16** was used as the aldehyde unit, both the yields (0–32%) and *E*/*Z* selectivities (1:1) were unsatisfactory (entry 1–4). It was supposed that unstable *N*-methyl piperidine **16** decomposed during the reaction, and therefore we decided to employ much stabler **17** to improve the yield and *E*/*Z* selectivity. Horner–Emmons reaction (entry 5) and Wittig reaction (entry 6) resulted in poor yield, while Julia reaction[8] gave coupling compound **22** in satisfactory yields (entry 7–12). Addition of 18-crown-6 improved the *E*/*Z* selectivity[9] and the condition of entry 11 provided the desired compound **22** in high yield and moderate *E*/*Z* selectivity. Since the *E*- and *Z*- products were inseparable at this stage, the mixture was used for further transformations and isomers were separated after the final step as described below.

Final steps are shown in Scheme 4. Removal of Boc group of **22** with TMSOTf and 2,6-lutidine[10] took place smoothly at room temperature, and subsequent reductive *N*-methylation[6] and chromatographic removal of *Z*-isomer afforded (2*R*,3*R*,6*R*)-*N*-Methyl-6-(deca-1′,3′,5′-trienyl)-3-methoxy-2-methylpiperidine **1** successfully: mp 52–53 °C, $[\alpha]_D^{15}$ +37.5 (*c* 1.0, $CHCl_3$), (lit.[1], mp 52–53 °C, $[\alpha]_D^{22}$ +29.2 ($CHCl_3$)).

The spectral data of synthetic **1** were identical with those reported for natural **1**. In addition, from the same positive sign of the specific rotations, it was shown that natural **1** have the same stereochemistry with our synthetic **1**, and the absolute configuration of natural compound was determined to be 2*R*, 3*R*, 6*R*.

3. Conclusion

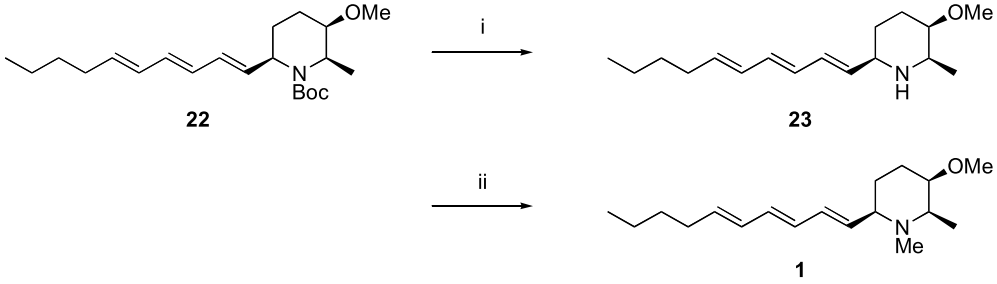
We achieved the enantioselective total synthesis of piperidine alkaloid **1** in good overall yield (18.3%) in 12 steps from **6** via stereoselective hydrogenation of imine and determined the absolute configuration of natural **1** as 2*R*, 3*R*, 6*R* by comparison of specific rotation. The strategy and the approach may be applicable to related analogues for further biological studies.

4. Experimental

4.1. General

Optical rotations were recorded with a JASCO DIP-1000 polarimeter. IR spectra were measured with a JASCO FT/IR-230 spectrophotometer. ^1H and ^{13}C NMR were recorded on JEOL JNM AL300 (300 MHz) and JEOL JNM LA 500 (500 MHz). Mass spectra were recorded on JEOL JMS-700T and JMS-SX102. Column chromatography was performed using Merck silica gel 60 (0.060–0.200 mm). TLC was carried out on Merck glass plates precoated with silica gel 60 F_{254} (0.25 mm). HPLC was performed using Shimadzu LC-6A. Melting points were measured on a Yanaco MP and were uncorrected.

4.1.1. (5*R*,6*R*)-6-[(*tert*-Butoxycarbonyl)amino]-5-methoxy-1-heptene (7). To a solution of **6** (5.0 g, 22 mmol) in THF were successively added 55% NaH (950 mg, 22 mmol) and MeI (11 ml, 175 mmol) at 0 °C under argon atmosphere. After being stirred for 1 h, 5% aqueous $KHSO_4$ solution was added to the reaction mixture at 0 °C and extracted with Et_2O. The combined organic layer was washed with 5% aqueous $Na_2S_2O_3$ solution and brine. The organic layer was dried over Na_2SO_4 and solvent



Scheme 4. Reagents and conditions. (i) TMSOTf, 2,6-lutidine, CH_2Cl_2, 84%, (ii) $NaBH_3CN$, HCHO, CH_3CN, 68%.

was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5/1) to afford **7** (3.3 g, 63%) as a colorless oil.

$[\alpha]_D^{26}$ +14.9 (*c* 1.0, MeOH). IR (film): ν = 1713, 1503, 1365, 1173, 911 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ = 1.15 (3H, d, *J* = 6.9 Hz, 7-H_3), 1.44 (9H, s, Boc), 1.60 (2H, m, 3-H_2), 2.13 (2H, q, *J* = 6.9 Hz, 4-H_2), 3.09 (1H, dt, *J* = 2.4, 6.9 Hz, 5-H), 3.41 (3H, s, OCH_3), 3.79 (1H, br m, 6-H), 4.65 (1H, br s, NH), 4.96 (1H, dd, *J* = 9.9, 1.8 Hz, 1-H_a), 5.03 (1H, dd, *J* = 17.4, 1.8 Hz, 1-H_b), 5.81 (1H, ddt, *J* = 17.4, 9.9, 9.6 Hz, 2-H). Anal. Calcd For $C_{13}H_{25}NO_3$: C, 64.16; H, 10.36; N, 5.76. Found: C, 64.02; H, 10.36; N, 5.56.

4.1.2. (5*R*,6*R*)-6-[(*tert*-Butoxycarbonyl)amino]-5-methoxy-1-(pivaloyloxy)-2-heptanol (8). To a stirred solution of **7** (1.0 g, 4.1 mmol) and *N*-methylmorpholine-*N*-oxide (50% w/w in water, 1.7 ml, 8.2 mmol) in acetone (10 ml) and THF (10 ml) was added a catalytic amount of OsO_4 (1% w/v in *t*-BuOH, 1.0 ml, 0.04 mmol) at room temperature. After being stirred for 2 h, a saturated aqueous Na_2SO_3 solution was added to the mixture and extracted with $CHCl_3$. The combined organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded the corresponding diol, which was taken to the next step without purification.

To the solution of crude diol in pyridine (20 ml) were added PivCl (0.6 ml, 4.9 mmol) at 0 °C under argon atmosphere. After being stirred for 40 min, a saturated aqueous $NaHCO_3$ solution was added to the mixture and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3/1) to afford **8** (1.4 g, 91%) as a colorless oil.

$[\alpha]_D^{26}$ +10.5 (*c* 1.0, MeOH). IR (film): ν = 1714, 1455, 1366, 1170, 756 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ = 1.15 (3H, d, *J* = 6.9 Hz, 7-H_3), 1.23 (9H, s, Piv), 1.44 (9H, s, Boc), 1.50–1.60 (4H, m, 3-H_2, 4-H_2), 3.09 (1H, br m, 5-H), 3.41 (3H, s, OCH_3), 3.82 (2H, br m, 2-H, 6-H), 4.02 (1H, m, 1-H_a), 4.10 (1H, m, 1-H_b), 4.63 (1H, br s, NH); ESI-HRMS *m*/*z* calcd for $C_{18}H_{36}NO_6$ $[M+H]^+$ 362.2543, found 362.2566.

4.1.3. (5*R*,6*R*)-6-[(*tert*-Butoxycarbonyl)amino]-5-methoxy-1-(pivaloyloxy)-2-heptanone (9). A solution of **8** (1.1 g, 3.0 mmol) in CH_2Cl_2 (20 ml) was added dropwise to a solution of PDC (1.7 g, 4.4 mmol) and MS 4A (1.6 g) in CH_2Cl_2 (10 ml), and the mixture was stirred for 12 h. The solvent was removed by evaporation and the residue was diluted with Et_2O. The mixture was filtered through a pad of Florisil and a filter cake was washed with Et_2O. Combined filtrate and washings were concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3/1) to afford **9** (1.0 g, 96%) as a colorless oil.

$[\alpha]_D^{26}$ +13.8 (*c* 1.0, MeOH). IR (film): ν = 1729, 1504, 1366, 1162, 756 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ = 1.14 (3H, d, *J* = 6.9 Hz, 7-H_3), 1.27 (9H, s, Piv), 1.44 (9H, s, Boc), 1.68–1.86 (2H, m, 4-H_2), 2.50 (1H, dt, *J* = 17.1, 7.5 Hz, 3-H_a), 2.53 (1H, dt, *J* = 17.1, 7.5 Hz, 3-H_b), 3.11 (1H, dt, *J* = 2.7, 6.6 Hz, 5-H), 3.39 (3H, s, OCH_3), 3.79 (1H, br m, 6-H), 4.63 (1H, br m, NH), 4.64 (2H, s, 1-H_2). Anal. Calcd For $C_{18}H_{33}NO_6$: C, 60.14; H, 9.25; N, 3.90. Found: C, 59.94; H, 9.21; N, 4.01.

4.1.4. (2*R*,3*R*,6*R*)-3-Methoxy-2-methyl-6-(pivaloyloxymethyl)piperidine (11). A solution of **9** (4.2 g, 12 mmol) in CH_2Cl_2 (40 ml) was treated with trifluoroacetic acid (40 ml) at 0 °C, followed by stirring at room temperature for 1 h. Excess acid was removed under reduced pressure and the residue was diluted with CH_2Cl_2. The mixture was cooled to 0 °C and saturated aqueous $NaHCO_3$ solution was added. Separated aqueous layer was extracted with CH_2Cl_2 and combined organic layer was washed with brine. After drying over Na_2SO_4, removal of the solvent under reduced pressure afforded the desired imine **10**, which was taken to the next step without purification.

ν = 2824, 1731, 1667, 1480 cm^{-1}.

To a solution of crude imine **10** in MeOH (60 ml) was added 5% Pd–C (310 mg) and the mixture was stirred under H_2 atmosphere for 12 h. The reaction mixture was filtered through a pad of Celite and a filter cake was washed with MeOH. The combined filtrate and washings were concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10/1) to afford **11** (2.3 g, 2 steps 81%) as a yellow oil.

$[\alpha]_D^{26}$ −19.2 (*c* 1.0, MeOH). IR (film): ν = 1730, 1480, 1365, 1154, 756 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ = 1.14 (3H, d, *J* = 6.6 Hz, 2-CH_3), 1.22 (9H, s, Piv), 1.84–1.88 (4H, br m, 4-H_2, 5-H_2), 2.78 (1H, dq, *J* = 1.8, 6.6 Hz, 2-H), 2.90 (1H, m, 6-H), 3.10 (1H, br s, 3-H), 3.34 (3H, s, 3-OCH_3), 4.00 (1H, dd, *J* = 6.3, 10.5 Hz, 1′-H_a), 4.02 (1H, dd, *J* = 6.3, 10.5 Hz, 1′-H_b); ESI-HRMS *m*/*z* calcd for $C_{13}H_{26}NO_3$ $[M+H]^+$ 244.1913, found 244.1883.

4.1.5. (2*R*,3*R*,6*R*)-3-Methoxy-1,2-dimethyl-6-(pivaloyloxymethyl)piperidine (12). To a solution of **11** (100 mg, 0.41 mmol) and HCHO (35% w/w in water, 500 µl, 6.5 mmol) in CH_3CN (7 ml) was added $NaBH_3CN$ (52 mg, 0.82 mmol) at 0 °C under argon atmosphere. The mixture was warmed to room temperature and stirred for 5 h at this temperature. The reaction mixture was poured into 10% aqueous NaOH solution and extracted with $CHCl_3$. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 19/1) to afford **12** (67 mg, 63%) as a yellow oil.

^1H NMR (300 MHz, $CDCl_3$); δ = 1.18 (3H, d, *J* = 6.9 Hz, 2-CH_3), 1.20 (9H, s, Piv), 1.30–1.41 (2H, m, 4-H_2), 1.55–1.67 (2H, m, 5-H_2), 2.01–2.09 (1H, dq, *J* = 3.3, 6.9 Hz, 2-H), 2.15–2.23 (1H, m, 6-H), 2.26 (3H, s, *N*-CH_3), 3.14 (1H, br s, 3-H), 3.32 (3H, s, 3-OCH_3), 4.00 (1H, dd, *J* = 5.1, 11.4 Hz, 1′-H_a), 4.26 (1H, dd, *J* = 5.1, 11.4 Hz, 1′-H_b)

4.1.6. (2*R*,3*R*,6*R*)-1-(*tert*-Butoxycarbonyl)-3-methoxy-2-methyl-6-(pivaloyloxymethyl)piperidine (13). To a solution of **11** (1.0 g, 4.1 mmol) in CH_2Cl_2 (100 ml) were

added $(Boc)_2O$ (1.0 ml, 4.5 mmol) and $(i\text{-}Pr)_2NEt$ (0.79 ml, 4.5 mmol) under argon atmosphere. After being stirred at 50 °C for 36 h, a saturated aqueous $NaHSO_4$ solution was added to the mixture and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane/ethyl acetate=3/1) to afford **13** (1.4 g, 99%) as a colorless oil.

$[\alpha]_D^{26}$ +22.7 (*c* 1.0, MeOH). IR (film): ν=1731, 1694, 1317, 1154, 769 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=1.04 (3H, d, J=6.9 Hz, 2-CH_3), 1.20 (9H, s, Piv), 1.47 (9H, s, Boc), 1.57–1.78 (4H, m, 4-H_2, 5-H_2), 3.30 (1H, ddd, J=11.1, 10.2, 6.0 Hz, 3-H), 3.36 (3H, s, 3-OCH_3), 3.97 (1H, br s, 2-H), 4.11 (1H, br t, J=9.9 Hz, 6-H), 4.31 (1H, br m, 1′-H_a), 4.53 (1H, br m, 1′-H_b). Anal. Calcd For $C_{18}H_{33}NO_5$: C, 62.95; H, 9.68; N, 4.08. Found: C, 62.70; H, 9.59; N, 4.19.

4.1.7. (2*R*,3*R*,6*R*)-6-Hydroxymethyl-3-methoxy-1,2-dimethylpiperidine (14). To a solution of **12** (47 mg, 0.18 mmol) in CH_2Cl_2 (3 ml) was added dropwise DIBALH (0.95 M in toluene, 1.9 ml, 1.8 mmol) at −78 °C under argon atmosphere. After being stirred at the same temperature for 2 h, a saturated aqueous potassium sodium tartrate solution was added to the mixture and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=10/1) to afford **14** (25 mg, 81%) as a colorless oil.

^1H NMR (300 MHz, $CDCl_3$); δ=1.22 (3H, d, J=6.9 Hz, 2-CH_3), 1.36–1.44 (2H, m, 4-H_2), 1.55–1.67 (2H, m, 5-H_2), 2.04–2.17 (1H, m, 2-H), 2.26 (3H, s, *N*-CH_3), 2.27–2.33 (1H, m, 6-H), 3.15 (1H, br s, 3-H), 3.32 (3H, s, 3-OCH_3), 3.46 (1H, dd, J=5.1, 11.4 Hz, 1′-H_a), 3.84 (1H, dd, J=5.1, 11.4 Hz, 1′-H_b).

4.1.8. (2*R*,3*R*,6*R*)-1-(*tert*-Butoxycarbonyl)-6-hydroxymethyl-3-methoxy-2-methylpiperidine (15). To a solution of **13** (1.2 g, 3.4 mmol) in CH_2Cl_2 (50 ml) was added dropwise DIBALH (0.95 M in toluene, 35 ml, 34 mmol) at −78 °C under argon atmosphere. After being stirred for 2 h at the same temperature, a saturated aqueous potassium sodium tartrate solution was added to the mixture and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=3/1) to afford **15** (0.80 g, 93%) as a colorless oil.

Mp 84–85 °C, $[\alpha]_D^{26}$ +22.8 (*c* 1.0, MeOH). IR (film): ν=1682, 1458, 1373, 1081, 990 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=1.08 (3H, d, J=6.9 Hz, 2-CH_3), 1.48 (9H, s, Boc), 1.63–1.86 (4H, m, 4-H_2, 5-H_2), 3.30 (1H, m, 3-H), 3.37 (3H, s, 3-OCH_3), 3.64 (2H, dd, J=7.5, 6.0 Hz, 1′-H_2), 4.21 (1H, m, 2-H), 4.50 (1H, m, 6-H). Anal. Calcd For $C_{13}H_{25}NO_4$: C, 60.21; H, 9.72; N, 5.40. Found: C, 60.44; H, 9.68; N, 5.40.

4.1.9. (2*R*,3*R*,6*R*)-1-(*tert*-Butoxycarbonyl)-6-formyl-3-methoxy-2-methylpiperidine (17). To a solution of oxalyl chloride (0.11 ml, 1.3 mmol) in CH_2Cl_2 (5 ml) was added dropwise DMSO (0.11 ml, 1.7 mmol) at −78 °C under argon atmosphere. After being stirred for 30 min, a solution of **15** (108 mg, 0.42 mmol) in CH_2Cl_2 (5 ml) was added to the reaction mixture over 30 min. The mixture was warmed to −50 °C and stirred for 4 h at the same temperature, followed by dropwise addition of Et_3N (0.47 ml, 3.3 mmol). The reaction mixture was poured into saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane/ethyl acetate=3/1) to afford **17** (98 mg, 92%) as colorless crystals.

Mp 45–46 °C, $[\alpha]_D^{26}$ +161.4 (*c* 1.0, $CHCl_3$). IR (film): ν=2977, 1731, 1693, 1399, 1170, 1018 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=0.97 (3H, d, J=6.9 Hz, 2-CH_3), 1.49 (9H, s, Boc), 1.26–1.74 (4H, m, 4-H_2, 5-H_2), 3.30 (1H, ddd, J=11.1, 10.2, 6.0 Hz, 3-H), 3.35 (3H, s, 3-OCH_3), 4.54 (2H, br m, 2-H, 6-H), 9.60 (1H, br, 6-CHO); ESI-HRMS *m*/*z* calcd for $C_{13}H_{24}NO_4$ $[M+H]^+$ 258.1705, found 258.1694.

4.1.10. 2-[(2′*E*,4′*E*)-Nona-2′,4′-dienylsulfonyl]benzothiazole (20). To a solution of *trans*,*trans*-2,4-nonadienol (100 mg, 0.71 mmol), 2-mercaptobenzothiazole (180 mg, 1.1 mmol), and triphenylphosphine (280 mg, 1.1 mmol) in THF (3 ml) was added dropwise diisopropyl azodicarboxylate (40% in toluene, 0.6 ml, 1.1 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h and the precipitate was removed by filtration through a pad of Celite to give a solution of the crude products, which was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=20/1) to afford thiazole (200 mg, 96%) as a colorless oil.

IR (film): ν=2955, 2924, 1457, 1427, 991 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=0.89 (3H, t, J=7.2 Hz, 9′-H_3), 1.35 (4H, m, 7′-H_2, 8′-H_2), 2.07 (2H, q, J=6.6 Hz, 6′-H_2), 4.04 (2H, d, J=6.6 Hz, 1′-H_2), 5.71 (2H, dt, J=14.8, 6.6 Hz, 2′-H, 5′-H), 6.02 (1H, dd, J=14.8, 10.4 Hz, 4′-H), 6.31 (1H, dd, J=14.8, 10.4 Hz, 3′-H), 7.29 (1H, t, J=6.9 Hz, Ar-H), 7.41 (1H, t, J=6.9 Hz, Ar-H), 7.75 (1H, d, J=6.9 Hz, Ar-H), 7.87 (1H, d, J=6.9 Hz, Ar-H). Anal. Calcd For $C_{16}H_{19}NS_2$: C, 66.39; H, 6.62; N, 4.84. Found: C, 66.17; H, 6.68; N, 4.82.

To a solution of thiazole (1.3 g, 4.3 mmol) in 95% ethanol (80 ml) was added dropwise a solution of ammonium heptamolybdate tetrahydrate (535 mg, 0.43 mmol) in hydrogen peroxide (34%, 4.3 ml, 43 mmol) at 0 °C. After being stirred for 5.5 h at room temperature, the reaction mixture was poured into water and then extracted with Et_2O. Ethereal solution was washed with 5% sodium thiosulfate, saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=5/1) to afford **20** (1.2 g, 89%) as a colorless oil.

IR (film): ν=2955, 2924, 1470, 1331, 1148, 991 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=0.87 (3H, t, J=7.2 Hz,

9′-H_3), 1.28 (4H, m, 7′-H_2, 8′-H_2), 2.05 (2H, q, J=6.6 Hz, 6′-H_2), 4.24 (2H, d, J=6.6 Hz, 1′-H_2), 5.51 (1H, dt, J=14.8, 6.6 Hz, 2′-H), 5.66 (1H, dt, J=14.8, 6.6 Hz, 5′-H), 5.99 (1H, dd, J=14.8, 10.4 Hz, 4′-H), 6.21 (1H, dd, J=14.8, 10.4 Hz, 3′-H), 7.59 (1H, t, J=6.9 Hz, Ar-H), 7.67 (1H, t, J=6.9 Hz, Ar-H), 8.01 (1H, d, J=6.9 Hz, Ar-H), 8.25 (1H, d, J=6.9 Hz, Ar-H); ESI-HRMS *m/z* calcd for $C_{16}H_{20}NO_2S_2$ $[M+H]^+$ 322.0936, found 322.0934.

4.1.11. (2*R*,3*R*,6*R*,1′*E*,3′*E*,5′*E*)-1-(*tert*-Butoxycarbonyl)-6-(deca-1′,3′,5′-trienyl)-3-methoxy-2-methylpiperidine (22). To a solution of **20** (71 mg, 0.22 mmol) and 18-crown-6 (59 mg, 0.22 mmol) in THF (3 ml) was added dropwise KHMDS (0.5 M in toluene, 0.4 ml, 0.19 mmol) at −78 °C under argon atmosphere. After being stirred for 30 min, a solution of **17** (38 mg, 0.15 mmol) in THF (3 ml) was added slowly to the reaction mixture and the mixture was stirred for 2 h at the same temperature. The reaction mixture was poured into aqueous saturated NH_4Cl solution and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=3/1) to afford **22** (45 mg, 85%) as a colorless oil.

$[\alpha]_D^{26}$ +24.3 (*c* 1.0, MeOH). IR (film): ν=2929, 2872, 1693, 1310, 1173, 996 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=0.89 (3H, t, J=6.6 Hz, 10′-H_3), 1.04 (3H, d, J=6.9 Hz, 2-CH_3), 1.26–1.36 (6H, m, 4-H_a, 5-H_a, 8′-H_2, 9′-H_2), 1.47 (9H, s, Boc), 1.88 (1H, m, 5-H_b), 1.91 (1H, m, 4-H_b), 2.08 (2H, br q, J=6.6 Hz, 7′-H_2), 3.31 (1H, ddd, J=11.1, 10.2, 6.0 Hz, 3-H), 3.37 (3H, s, 3-OCH_3), 4.54 (1H, m, 2-H), 4.70 (1H, br m, 6-H), 5.70 (2H, m, 1′-H, 6′-H), 6.11 (4H, m, 2′-H, 3′-H, 4′-H, 5′-H); ESI-HRMS *m/z* calcd for $C_{22}H_{38}NO_3$ $[M+H]^+$ 364.2852, found 364.2868.

4.1.12. (2*R*,3*R*,6*R*,1′*E*,3′*E*,5′*E*)-6-(Deca-1′,3′,5′-trienyl)-3-methoxy-2-methylpiperidine (23). To a solution of **22** (95 mg, 0.26 mmol) and 2,6-lutidine (120 μl, 1.1 mmol) in CH_2Cl_2 (2 ml) was added dropwise TMSOTf (71 μl, 0.39 mmol) at 0 °C under argon atmosphere. The mixture was warmed to room temperature and stirred for 2 h at the same temperature. The reaction mixture was poured into aqueous saturated $NaHCO_3$ solution and extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4 and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=5/1) to afford **23** (58 mg, 84%) as a yellow oil.

$[\alpha]_D^{26}$ −36.3 (*c* 1.0, $CHCl_3$). IR (film): ν=3014, 2930, 1462, 1098, 995 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$); δ=0.89 (3H, t, J=6.6 Hz, 10′-H_3), 1.16 (3H, d, J=6.9 Hz, 2-CH_3), 1.26–1.51 (7H, m, 4-H_a, 5-H_2, 8′-H_2, 9′-H_2), 2.00–2.18 (4H, m, 4-H_b, 2-H, 7′-H_2), 2.88 (1H, ddd, J=12.9, 6.3, 1.5 Hz, 6-H), 3.11 (1H, br s, 3-H), 3.35 (3H, s, 3-OCH_3), 5.69 (2H, m, 1′-H, 6′-H), 6.15 (4H, m, 2′-H, 3′-H, 4′-H, 5′-H); ESI-HRMS *m/z* calcd for $C_{17}H_{29}NO$ $[M]^+$ 263.2249, found 263.2246.

4.1.13. (2*R*,3*R*,6*R*,1′*E*,3′*E*,5′*E*)-6-(Deca-1′,3′,5′-trienyl)-3-methoxy-1,2-dimethylpiperidine (1). To a solution of **23** (60 mg, 0.23 mmol) and HCHO (35% w/w in water, 200 μl, 2.3 mmol) in CH_3CN (4 ml) was added $NaBH_3CN$ (43 mg, 0.68 mmol) at 0 °C under argon atmosphere. The mixture was warmed to room temperature and stirred for 2.5 h at this temperature. The reaction mixture was poured into aqueous 10% NaOH solution and extracted with $CHCl_3$. The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=10/1) to afford **1** (43 mg, 68%) as yellow crystals.

Mp 52–53 °C, $[\alpha]_D^{15}$ +37.5 (*c* 1.0, $CHCl_3$). IR (film): ν=3008, 2766, 1728, 1453, 1101, 992 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$); δ=0.88 (3H, t, J=7.5 Hz, 10′-H_3), 1.19 (3H, d, J=6.5 Hz, 2-CH_3), 1.27–1.39 (6H, m, 4-H_a, 5-H_a, 8′-H_2, 9′-H_2), 1.77 (1H, ddt, J=11.5, 4.5, 15.0 Hz, 5-H_b), 2.02–2.13 (4H, m, 4-H_b, 2-H, 7′-H_2), 2.14 (3H, s, 1-CH_3), 2.44 (1H, dt, J=11.0, 2.5 Hz, 6-H), 3.13 (1H, br s, 3-H), 3.32 (3H, s, 3-OCH_3), 5.67 (2H, m, 1′-H, 6′-H), 6.10 (4H, m, 2′-H, 3′-H, 4′-H, 5′-H); ESI-HRMS *m/z* calcd for $C_{18}H_{32}NO$ $[M+H]^+$ 278.2484, found 278.2472.

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Asymmetric synthesis of a diastereomer of the structure proposed for amphidinolide A and the determination of its absolute configuration

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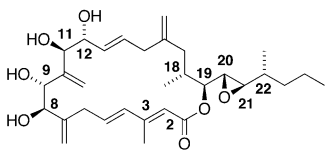
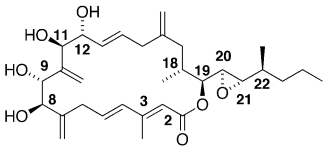
Abstract—An asymmetric synthesis of a diastereomer (**2**) of the structure (**1**) proposed for amphidinolide A, a cytotoxic macrolide from the cultured dinoflagellate *Amphidinium* sp., has been accomplished. The absolute configuration of amphidinolide A was established as **3** from comparison of NMR data, HPLC analysis, and $[\alpha]_D$ values of amphidinolide A, and comparison with the synthetic diastereomers **2** and **3**, the latter of which was synthesized previously by Trost's group.

Amphidinolide A is a cytotoxic 20-membered macrolide, isolated from the cultured dinoflagellate *Amphidinium* sp., which is a symbiont of the Okinawan marine flatworm *Amphiscolops* sp.[1a] The relative stereochemistry of the nine stereogenic centers in amphidinolide A was proposed to be **1** on the basis of extensive NMR experiments by our group.[1b] The unique structure and bioactivity of amphidinolide A have prompted studies of its total syntheses. First Pattenden,[2] and later Maleczka,[3] and Trost[4] accomplished total syntheses of the stereostructure (**1**) proposed for amphidinolide A, and indicated that the proposed stereostructure (**1**) was incorrect from comparison of the NMR data of their synthetic compounds with those reported for amphidinolide A. More recently, Trost's group achieved the syntheses of nine stereoisomers of the proposed stereostructure (**1**), and suggested that the diastereomer **3** may be the correct stereostructure of amphidinolide A.[4b,c,d]



1



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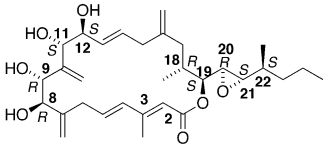

3

In our efforts to determine the correct stereostructure of amphidinolide A, we have re-examined the relative stereochemistry of amphidinolide A. Our re-examination of the ^1H and ^{13}C NMR data have indicated that the correct stereostructure of amphidinolide A could be either of the diastereomer **2** or **3**. Since the diastereomer **3** has been synthesized by Trost's group,[4b] we decided to synthesize the alternative diastereomer **2** and to compare the NMR data of the synthetic diastereomers **2** and **3** with those of naturally derived amphidinolide A. In this paper, we describe an asymmetric synthesis of the diastereomer **2**, and the determination of the absolute stereochemistry of amphidinolide A to be **3**.

Although earlier we reported previously the relative stereochemistry for C-19 and C-20 in natural amphidinolide A as 19,20-*threo* from NOESY correlations of H-19 to H-21, H-20 to H_2-17, and H-20 to H_3-30 (Fig. 1a), the proposed stereostructure **1** was not correct. Alternatively, it was elucidated to be 19,20-*erythro* from the NOESY correlations and the ^1H–^1H coupling constant (6.8 Hz) between H-19 and H-20 (Fig. 1b). On the other hand, the ^{13}C NMR data obtained for amphidinolide A were compared

Keywords: *Amphidinium sp*; Macrolide; Amphidinolide A; Absolute configuration.
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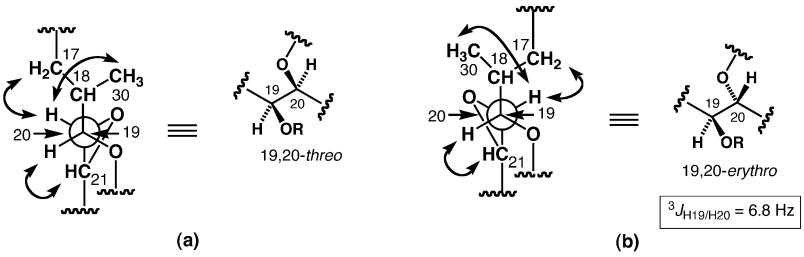
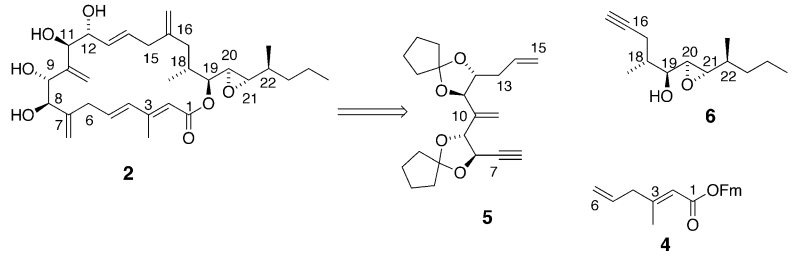


Figure 1. NOESY correlations and relative stereochemistry for C-17–C-21 segments in amphidinolide A based on the previous (a) and present (b) assignments.

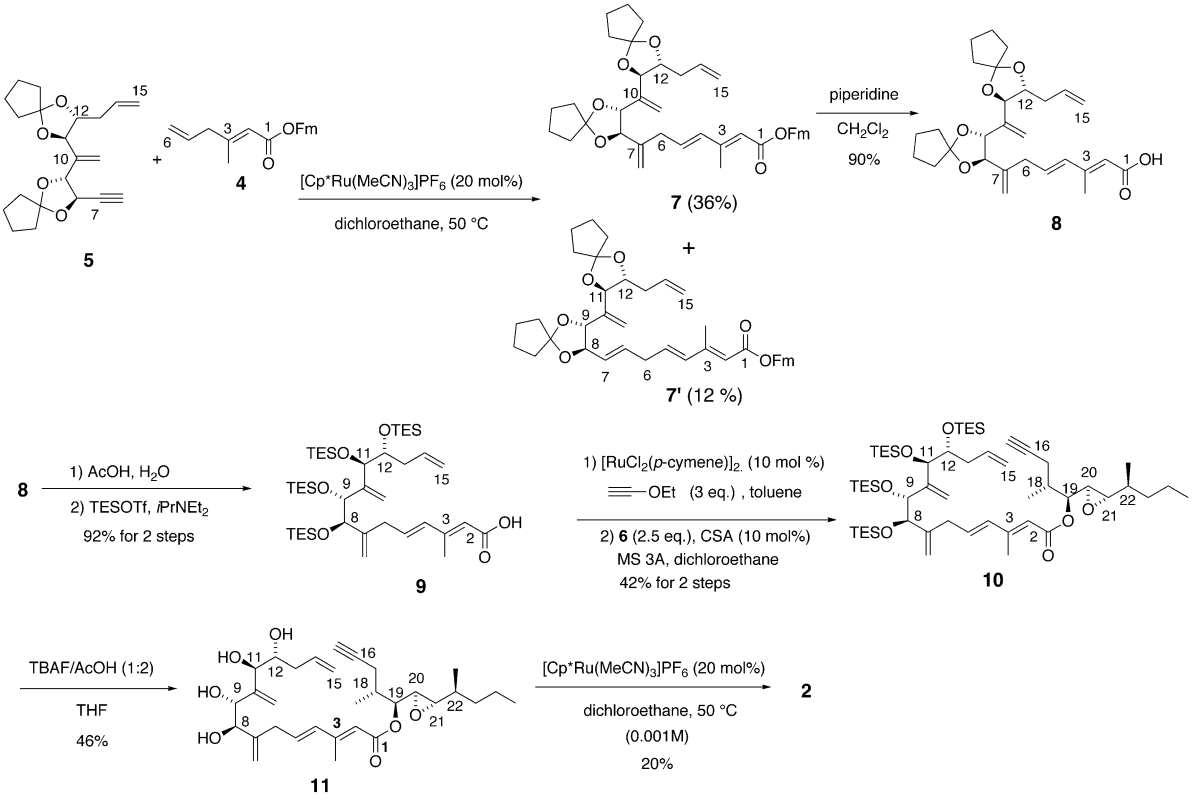
with those of the synthetic compound **1** reported by the Maleckzka group,[3] in which the difference ($\Delta+1.8$ ppm) in chemical shifts at C-12 was slightly larger than those ($|\Delta|<1.3$ ppm) of the other stereogenic centers.[5] The ^1H–^1H coupling constant ($\sim$0 Hz) between H-11 and H-12 indicated 11,12-*threo* as reported previously.[1b] These observations suggested that the possible stereostructure of amphidinolide A could be either diastereomer **2** or **3**.

Since Trost's group has recently reported a synthesis of the diastereomer **3**, we planned to synthesize the other possible diastereomer **2**, using the synthetic strategy by Trost[4b], but using Kita's esterification[6] and a ruthenium-catalyzed coupling reaction[7] (Scheme 1).

The alkyne **5**[4b] and the alkene **4**[4b] were prepared according to the same procedure as Trost's group. Treatment of the alkyne **5** and the alkene **4** with ruthenium catalyst provided **7** and its isomer **7′** (Scheme 2). After deprotection of the fluorenymethanol (Fm) group in **7** with piperidine, the protecting group of **8** was changed from ketal to TES ether to give the acid **9**.[8] Esterification of **9** with the alcohol **6**



Scheme 1.



Scheme 2.

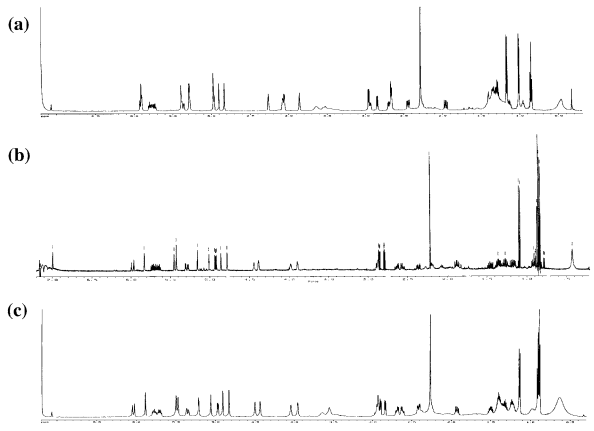


Figure 2. ^{1}H NMR profiles of synthetic diastereomers **2** (a) and **3** (c), and amphidinolide A (b) in C_6D_6.

using the modified method[9] of Kita[6] provided the desired ester **10** with no isomerization of any olefin moiety.[10] After removal of the TES group, intramolecular cyclo-isomerization of **11** under high dilution conditions, to form the C15–C16 bond, gave the desired diastereomer **2**.

The ^{1}H and ^{13}C NMR data for synthetic compound **2** were not coincident with those of amphidinolide A, whereas the NMR data for compound **3** synthesized by Trost's group were close to those of amphidinolide A (Fig. 2). Compounds **2** and **3**, and amphidinolide A were subjected to C_{18} HPLC [Mightysil RP-18, 4.6×250 mm; flow rate 1.0 mL/min: eluent; MeCN/H_2O (60:40); UV detection at 265 nm], and it was found that the retention time of amphidinolide A (t_R 16.5 mim) was identical with that of **3** (t_R 16.5 min) but not that of **2** (t_R 12.8 min) (Fig. 3). Thus, the relative stereostructure for amphidinolide A was assigned as **3**. The optical rotations of compounds **2** and **3**, and amphidinolide A were compared as follows; $[\alpha]_D^{21}$ −11° (*c* 0.6, $CHCl_3$) for **2**, $[\alpha]_D^{24}$ +56° (*c* 0.05, $CHCl_3$) for **3**, and $[\alpha]_D^{24}$ +46° (*c* 1.0, $CHCl_3$) for amphidinolide A. Therefore, it was concluded that the absolute configurations at the nine chiral centers of amphidinolide A were 8*R*, 9*R*, 11*S*, 12*S*, 18*R*, 19*S*, 20*R*, 21*S*, and 22*S*.

1. Experimental

1.1. General methods

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. The IR spectrum was taken on a JASCO FT/IR-5300 spectrometer. Proton and carbon NMR spectra were recorded on a Bruker 500 and/or 600 MHz and JEOL 400 MHz spectrometer. ESI mass spectra were obtained on a JEOL JMS-SX102A spectrometer.

1.1.1. 7-{3-[1-(3-Allyl-1,4-dioxa-spiro[4,4]non-2-yl)-vinyl]-1,4-dioxa-spiro[4,4]non-2-yl}-3-methyl-octa-2,4,7-trienoic acid 9*H*-fluoren-9-ylmethyl ester (7). A solution of alkyne **5**[4a] (208.4 mg, 0.605 mmol) and alkene **4**[4a] (916.5 mg, 3.01 mmol) in dichloroethane (DCE) (1.7 mL) was degassed by F. T. P. (Freeze-pump-thaw cycles), the reaction mixture was heated to 50 °C, and Cp*Ru $(MeCN)_3$-PF_6 (60.7 mg, 0.120 mmol) was added in one portion. After 3 h, the reaction mixture was purified by flash column chromatography on silica gel (10–15% Et_2O in petroleum ether) to give branched ester **7** (141.4 mg, 0.218 mmol, 36%) and linear ester **7′** (47.3 mg, 0.0729 mmol, 12%) as colorless oils.

Data for branched ester **7**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J=7.6 Hz, 2H), 7.62 (d, J=8.0 Hz, 2H), 7.41 (dd, J=7.6, 7.6 Hz, 2H), 7.32 (dd, J=8.0, 7.6 Hz, 2H), 6.16–6.23 (m, 2H), 5.81–5.89 (m, 2H), 5.42–5.49 (m, 2H), 5.24 (s, 1H), 5.09–5.14 (m, 2H), 5.01 (s, 1H), 4.39–4.43 (m, 3H), 4.26 (t, J=7.2 Hz, 1H), 4.20 (d, J=8.8 Hz, 1H), 4.06 (d, J=8.0 Hz, 1H), 3.89–3.94 (m, 1H), 3.05 (dd, J=16.4, 5.6 Hz, 1H), 2.92 (m, 1H), 2.43 (m, 1H), 2.25–2.31 (m, 4H), 1.67–1.90 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) 166.8, 152.7, 143.9, 143.3, 142.4, 141.2, 135.6, 134.1, 133.9, 127.6, 127.0, 125.0, 119.9, 119.0, 118.6, 117.9, 117.7, 117.4, 115.6, 84.0, 81.3, 79.8, 79.7, 65.8, 46.9, 37.7, 37.5, 37.4, 37.4, 36.4, 34.7, 23.6, 23.5, 23.5, 14.0; ESIMS *m/z* 480 (M−$C_{10}H_{16}O_2$).

1.1.2. 7-{3-[1-(3-Allyl-1,4-dioxa-spiro[4,4]non-2-yl)-vinyl]-1,4-dioxa-spiro[4,4]non-2-yl}-3-methyl-octa-2,4,7-trienoic acid (8). To a solution of branched ester **7** (170.3 mg, 0.262 mmol) in CH_2Cl_2 (6.9 mL) at 0 °C was added piperidine (0.86 mL, 8.7 mmol). After 2.5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with 0.2 M H_2SO_4, brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography on silica gel (hexane/EtOAc/MeOH, 30:1:2) gave acid **8** (111.2 mg, 90%) as a colorless oil.

Compound **8**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14–6.20 (m, 2H), 5.80–5.90 (m, 1H), 5.75 (s, 1H), 5.47 (s, 1H), 5.46 (s, 1H), 5.23 (s, 1H), 5.09–5.13 (m, 2H), 4.99 (s, 1H), 4.40 (d, J=8.4 Hz, 1H), 4.18 (d, J=8.4 Hz, 1H), 4.05 (d, J=8.0 Hz, 1H), 3.90 (td, J=7.4, 4.0 Hz, 1H), 3.02–3.07 (m, 1H), 2.87–2.92 (m, 1H), 2.38–2.43 (m, 1H), 2.30 (s, 3H), 2.24–2.33 (m, 1H), 1.64–1.91 (m, 17H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 154.4, 143.2, 142.3, 135.5, 134.7, 133.8, 119.0, 118.6, 117.7, 117.6, 117.3, 115.6, 84.0, 81.2, 79.7, 79.7, 37.6, 37.5, 37.4, 37.4, 36.3, 34.7, 23.6, 23.5, 23.5, 14.2; ESIMS *m/z* 493 (M+Na); $[\alpha]_D^{22}$ +37° (*c* 1.2, CH_2Cl_2).

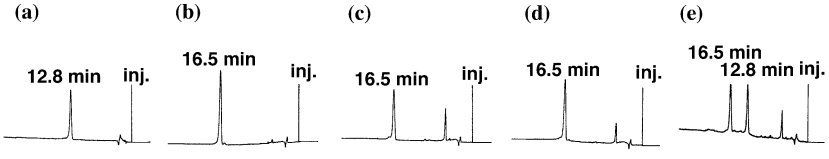


Figure 3. HPLC profiles of synthetic diastereomers **2** (a) and **3** (b), amphidinolide A (c), **3** and amphidinolide A (d), **2** and amphidinolide A (e).

1.1.3. 3-Methyl-7,10-dimethylene-8,9,11,12-tetrakis-triethylsilanyloxy-pentadeca-2,4,14-trienoic acid (9). To ketal **8** (111.2 mg, 0.236 mmol) at room temperature was added acetic acid (1.5 mL) and water (0.5 mL). The reaction mixture was heated to 40 °C for 24 h and concentrated to give the tetraol which was used in the next step without further purification. To a solution of the tetraol in THF (5.9 mL) at 0 °C was added *i*-Pr_2NEt (576 μL, 3.31 mmol) and TESOTf (530 μL, 2.36 mmol). The reaction mixture was stirred at 0 °C for 20 min, quenched with 1 M HCl (3.3 mL), stirred for 10 min, and diluted with ether (3 mL) and water (3 mL). The aqueous phase was extracted with ether and the combined organic extracts were washed with saturated KH_2PO_4, brine, dried over $MgSO_4$, and concentrated. Purification by flash column chromatography on silica gel (9% EtOAc in hexanes) gave silyl ether **9** (172.9 mg, 92%) as a colorless oil.

Compound **9**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.11–6.20 (m, 2H), 5.80–5.85 (m, 1H), 5.74 (s, 1H), 5.32 (s, 2H), 4.96–5.03 (m, 3H), 4.84 (s, 1H), 4.49 (d, J=4.0 Hz, 1H), 4.35 (d, J=3.5 Hz, 1H), 4.15 (d, J=4.0 Hz, 1H), 3.74 (ddd, J=8.2, 4.0, 3.5 Hz, 1H), 3.01 (dd, J=16.3, 5.5 Hz, 1H), 2.94 (dd, J=16.3, 6.5 Hz, 1H), 2.34–2.40 (m, 2H), 2.30 (s, 3H), 0.90–0.99 (m, 36H), 0.56–0.67 (m, 24H); ^{13}C NMR (125 MHz, $CDCl_3$) 171.9, 155.1, 148.4, 146.9, 137.1, 136.3, 135.0, 116.9, 116.1, 113.6, 113.5, 80.1, 76.8, 74.9, 74.7, 36.9, 36.6, 14.0, 7.1, 7.1, 7.0, 7.0, 5.5, 5.2, 4.9, 4.9; IR (film) ν_{max} 2955, 2874, 1686, 1609, 1092, and 742 cm^{-1}; HRESIMS calcd for $C_{42}H_{82}O_6Si_4Na$ *m/z* 817.5086, found *m/z* 817.5078; $[\alpha]_D^{22}+29°$ (*c* 1.0, $CHCl_3$).

1.1.4. 3-Methyl-7,10-dimethylene-8,9,11,12-tetrakis-triethylsilanyloxy-pentadeca-2,4,14-trienoic acid 2-methyl-1-[3-(1-methyl-butyl)-oxiranyl]-pent-4-ynyl ester (10). To a solution of acid **9** (17.7 mg, 22.3 μmol) in toluene (0.59 mL) at room temperature was added $[RuCl_2(p\text{-cymene})]_2$ (1.4 mg, 17.7 μmol) and a toluene (0.19 mL) solution of ethyl ethynyl ether (40 wt% in hexane, 16 μL, 66.8 μmol). The reaction mixture was stirred at room temperature for 3 h and concentrated under a stream of argon. A solution of epoxy alcohol **6** (11.7 mg, 55.6 μmol) in DCE (0.15 mL) was added via cannula followed by CSA (0.52 mg, 2.24 μmol) and MS3A (10 mg). The reaction mixture was stirred at room temperature for 2 h, filtered through silica gel, and concentrated. Purification by flash column chromatography on silica gel (5% EtOAc in hexane) gave ester **10** (9.3 mg, 42%) as a colorless oil.

Compound **10**. ^{1}H NMR (600 MHz, C_6D_6) δ 6.23–6.30 (m, 2H), 6.07 (dddd, J=17.0, 9.4, 7.7, 7.2 Hz, 1H), 5.89 (s, 1H), 5.70 (s, 1H), 5.67 (s, 1H), 5.31 (s, 1H), 5.25 (d, J=17.0 Hz, 1H), 5.15 (d, J=9.4 Hz, 1H), 5.07 (s, 1H), 4.95 (dd, J=7.1, 4.6 Hz, 1H), 4.78 (d, J=3.8 Hz, 1H), 4.62 (d, J=2.8 Hz, 1H), 4.41 (d, J=3.8 Hz, 1H), 4.02 (ddd, J=8.2, 3.9, 2.8 Hz, 1H), 3.16–3.23 (m, 2H), 2.89 (dd, J=7.6, 1.7 Hz, 1H), 2.75 (dd, J=7.1, 1.7 Hz, 1H), 2.70–2.73 (m, 1H), 2.47 (s, 3H), 2.33–2.40 (m, 3H), 2.12–2.20 (m, 1H), 1.82 (t, J=2.3 Hz, 1H), 1.18 (d, J=6.5 Hz, 3H), 1.09–1.32 (m, 41H), 0.99 (d, J=6.6 Hz, 3H), 0.74–0.87 (m, 27H); ^{13}C NMR (150 MHz, C_6D_6) δ 165.9, 153.4, 149.0, 147.5, 137.2, 135.8, 135.2, 118.1, 116.7, 114.2, 113.9, 82.3, 80.7, 77.4, 75.4, 75.3, 75.1, 70.2, 62.8, 57.0, 37.5, 37.2, 36.1, 35.9, 35.7, 22.7, 20.5, 17.3, 14.5, 14.4, 14.0, 7.5, 7.4, 7.4, 7.3, 6.0, 5.7, 5.5, 5.4; IR (neat) ν_{max} 2956, 2121, 1717, 1235, 1146 cm^{-1}; HRESIMS calcd for $C_{55}H_{102}O_7Si_4Na$ *m/z* 1009.6600, found *m/z* 1009.6638; $[\alpha]_D^{20}+41°$ (*c* 1.0, CH_2Cl_2).

1.1.5. 8,9,11,12-Tetrahydroxy-3-methyl-7,10-dimethylene-pentadeca-2,4,14-trienoic acid 2-methyl-1-[3-(1-methyl-butyl)-oxiranyl]-pent-4-ynyl ester (11). To a solution of silyl ether **10** (9.3 mg, 9.42 μmol) in THF (0.2 mL) at 0 °C was added a mixture of TBAF (190 μL, 1 M in THF, 190 μmol) and acetic acid (21.5 μL). The reaction mixture was stirred at room temperature for 24 h, diluted with water (3 mL), extracted with EtOAc (4 mL×3), washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography on silica gel (13–67% EtOAc in hexane) gave tetraol **11** (2.3 mg, 46%) as an amorphous solid.

Compound **11**. ^{1}H NMR (600 MHz, CD_3OD) δ 6.23–6.27 (m, 2H), 5.89 (dddd, J=17.2, 10.2, 7.0, 7.0 Hz, 1H), 5.78 (s, 1H), 5.38 (s, 1H), 5.34 (s, 1H), 5.20 (s, 1H), 5.10 (dd, J=17.2, 1.5 Hz, 1H), 5.05 (dd, J=10.2, 1.5 Hz, 1H), 4.97 (s, 1H), 4.69 (dd, J=6.6, 4.8 Hz, 1H), 4.16 (d, J=4.4 Hz, 1H), 4.14 (d, J=4.4 Hz, 1H), 3.98 (d, J=3.8 Hz, 1H), 3.70 (ddd, J=7.7, 3.8, 3.8 Hz, 1H), 3.06 (dd, J=16.4, 4.5 Hz, 1H), 2.96 (dd, J=16.4, 5.0 Hz, 1H), 2.87 (dd, J=6.7, 2.0 Hz, 1H), 2.71 (dd, J=7.4, 2.0 Hz, 1H), 2.17–2.38 (m, 5H), 2.29 (s, 3H), 2.07–2.11 (m, 1H), 1.22–1.32 (m, 5H), 1.13 (d, J=6.8 Hz, 3H), 0.99 (d, J=6.2 Hz, 3H), 0.85 (t, J=7.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.4, 155.8, 151.9, 149.4, 137.5, 137.5, 137.0, 119.1, 118.2, 115.0, 114.6, 83.6, 78.0, 76.7, 76.4, 75.5, 74.9, 71.9, 64.6, 59.0, 39.6, 38.4, 37.8, 37.6, 37.2, 22.9, 22.0, 18.4, 15.5, 15.4, 15.1; IR (KBr) ν_{max} 3437 (br), 2958, 2353, 1716, 1150 cm^{-1}; HRESIMS calcd for $C_{31}H_{46}O_7Na$ *m/z* 553.3141, found *m/z* 553.3138; $[\alpha]_D^{20}+6.7°$ (*c* 1.0, CH_2Cl_2).

1.1.6. Diastereomer 2. A solution of tetraol **11** (4.7 mg, 8.86 μmol) in DCE (8.9 mL) was degassed by F. T. P. The reaction mixture was heated to 50 °C and Cp*Ru $(MeCN)_3$-PF_6 (2.2 mg, 4.4 μmol) was added. After 7 h at 50 °C, the reaction mixture was filtered through silica gel, and concentrated. Purification by C_{18} HPLC (Mightysil RP-18 250–4.6 (5 mm); eluent, 45% CH_3CN aq.; flow rate, 1.0 mL/min; UV detection at 265 nm) afforded **2** (0.96 mg, 20%, t_R=40.0 min).

Compound **2**. ^{1}H NMR (600 MHz, C_6D_6) δ 5.91 (d, J=15.6 Hz, 1H), 5.91 (s, 1H), 5.72–5.81 (m, 2H), 5.39 (s, 1H), 5.35–5.39 (m, 1H), 5.29 (s, 1H), 5.28 (s, 1H), 4.97 (s, 1H), 4.96–4.97 (m, 1H), 4.90 (s, 1H), 4.83 (s, 1H), 4.26 (d, J=4.3 Hz, 1H), 4.08 (br s, 1H), 4.06 (d, J=4.3 Hz, 1H), 3.86 (br s, 1H), 2.96 (dd, J=7.1, 1.7 Hz, 1H), 2.93–2.97 (m, 1H), 2.85 (dd, J=7.3, 1.7 Hz, 1H), 2.67–2.71 (m, 1H), 2.66 (d, J=7.5 Hz, 2H), 2.44 (dd, J=14.1, 5.2 Hz, 1H), 2.24–2.38 (m, 1H), 2.28 (s, 3H), 1.96 (dd, J=14.1, 9.1 Hz, 1H), 1.27–1.42 (m, 5H), 1.17 (d, J=7.0 Hz, 3H), 1.01 (d, J=6.5 Hz, 3H), 0.86 (t, J=7.2 Hz, 3H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.16 (d, J=15.7 Hz, 1H), 6.08 (ddd, J=15.7, 7.0, 7.0 Hz, 1H), 5.77 (s, 1H), 5.69 (ddd, J=15.4, 7.6, 7.6 Hz, 1H), 5.48 (s, 1H), 5.42 (dd, J=15.4, 5.1 Hz, 1H), 5.39 (s, 1H), 5.33 (s, 1H), 5.20 (s, 1H), 4.87 (s, 1H), 4.79 (s, 1H), 4.63

(dd, $J=6.8$, 3.0 Hz, 1H), 4.38 (br s, 1H), 4.18 (br s, 1H), 4.14 (br s, 1H), 3.92 (br s, 1H), 3.21 (dd, $J=14.7$, 7.0 Hz, 1H), 2.98 (dd, $J=14.7$, 7.0 Hz, 1H), 2.88 (dd, $J=6.7$, 1.9 Hz, 1H), 2.71–2.76 (m, 2H), 2.66–2.71 (m, 2H), 2.50 (br s, 1H), 2.47 (br s, 1H), 2.25–2.29 (m, 2H), 2.25 (s, 3H), 2.12–2.17 (m, 1H), 1.89 (dd, $J=14.0$, 9.2 Hz, 1H), 1.29–1.36 (m, 5H), 1.06 (d, $J=7.0$ Hz, 3H), 1.00 (d, $J=6.4$ Hz, 3H), 0.88 (t, $J=7.0$ Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.8, 152.4, 147.0, 145.0, 144.9, 135.3, 134.9, 131.4, 130.5, 118.1, 116.1, 115.1, 113.1, 75.5, 75.4, 74.2, 73.5, 72.4, 62.3, 55.4, 40.0, 38.4, 36.3, 35.6, 35.3, 33.9, 20.1, 16.9, 14.9, 14.4, 14.2. ^{1}H NMR (600 MHz, CD_3OD) δ 6.30 (d, $J=15.7$ Hz, 1H), 6.21 (ddd, $J=15.7$, 7.1, 7.1 Hz, 1H), 5.84 (s, 1H), 5.56 (ddd, $J=15.2$, 6.7, 6.7 Hz, 1H), 5.41–5.44 (m, 1H), 5.43 (s, 1H), 5.38 (s, 1H), 5.27 (s, 1H), 5.13 (s, 1H), 4.86 (s, 1H), 4.77 (s, 1H), 4.61 (dd, $J=6.5$, 3.0 Hz, 1H), 4.22 (d, $J=3.2$ Hz, 1H), 4.11 (d, $J=3.2$ Hz, 1H), 4.05 (dd, $J=4.4$, 3.6 Hz, 1H), 3.87 (d, $J=3.6$ Hz, 1H), 3.24 (dd, $J=14.6$, 7.1 Hz, 1H), 2.97–3.00 (m, 1H), 2.96 (dd, $J=6.4$, 2.1 Hz, 1H), 2.71 (dd, $J=7.4$, 2.1 Hz, 1H), 2.68–2.71 (m, 1H), 2.60 (dd, $J=14.5$, 6.7 Hz, 1H), 2.34 (d, $J=13.9$, 6.0 Hz, 1H), 2.26 (s, 3H), 2.10–2.16 (m, 1H), 1.93 (dd, $J=13.9$, 8.0 Hz, 1H), 1.23–1.40 (m, 5H), 1.08 (d, $J=7.1$ Hz, 3H), 0.99 (d, $J=6.5$ Hz, 3H), 0.89 (t, $J=7.1$ Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.4, 154.8, 151.3, 148.4, 148.0, 138.2, 137.1, 133.9, 131.9, 120.1, 116.1, 114.1, 113.7, 77.3, 76.6, 76.1, 75.4, 73.6, 64.3, 57.6, 41.9, 40.6, 38.8, 37.7, 37.5, 36.4, 22.0, 18.2, 16.5, 15.4, 15.4; IR (KBr) ν_{max} 3423 (br), 2925, 1634, 1151 cm^{-1}; HRESIMS calcd for $C_{31}H_{46}O_7Na$ *m/z* 553.3141, found *m/z* 553.3145; $[\alpha]_D^{21}$ -11° (*c* 0.6, $CHCl_3$).

Acknowledgements

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10. Esterification under Keck and Mitsunobu conditions gave a complex mixture.

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Mechanistic studies of amination of ketenimines: change of rate-determining step by N-substituents through electronic effects

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Abstract—Vinylidenediamine intermediate was not found in amination reactions of *N*-*i*-propyl-*p*-substituted-phenylketenimines **3a**–**3e** with *n*-$BuNH_2$, but it was found in amination reactions of *N*-*p*-substituted-phenylphenylketenimines **1a**–**1c** with *n*-$BuNH_2$ by low-temperature ^1H NMR spectrometer, indicating that the rate-determining step is changed from the first step (C═N addition) to the second step (tautomerization) when N-substituent of the ketenimines is changed from *i*-propyl group to *p*-substituted-phenyl group. Amination reactions of ketenimines **5** and **10** in a solvent with $\varepsilon=35.9$ were designed to explore electronic effects of N-substituents on the amination reactions by means of ab initio calculations. Computation results at level of MP2/6-31+G*//HF/6-31+G* (Onsager model) show that significant electronic stabilization of the first transition state involving C═N addition by *N*-phenyl group is the major factor causing the change of the rate-determining step for the amination reactions.

1. Introduction

Chemistry of ketenes and ketenimines has been intensely studied for a century.[1] They are important reactive intermediates, which may occur as transients in many thermal and photochemical reactions.[1,2] There has been intense interest in their addition reactions, including cycloadditions,[3] nucleophilic additions,[2b,f,4] electrophilic additions,[5] and radical additions.[6] Both amination of ketenes[2f,4c–f] and hydration of ketenimines[2b,4h,i] generate the amide functional group, which is a repeating unit in peptides and other synthetic polymers like nylon.

There was a mechanistic controversy regarding where an initial addition occurs on ketenes and ketenimines when they reacts with amines.[2f,4c–f,7,8] Recent results[2f,4c–f,7] confirm that they are two-step reactions and involve an initial addition of an amine to C═O or C═N, followed by tautomerization. In the recent literatures regarding amination reactions of ketenimines[7] or ketenes,[2f,4d,e] intermediates of vinylidenediamines or enol amides were found, indicating that the rate-determining step is the second step involving tautomerization.

In our previous research, we found that reaction of *N*-phenylphenylketenimine **1a** with *n*-butylamine involves two steps including an initial addition to C═N, followed by tautomerization to give amidine **2a**.[7] The reaction runs fast at room temperature, so the metastable intermediate of vinylidenediamine was caught and identified by means of low-temperature proton NMR. In the low-temperature proton NMR experiment, it was found that the second step involving tautomerization is much slower than the first addition step.[7] On the other hand, when we changed N-substituent of the ketenimine from phenyl group to *i*-propyl group **3a** and did the same amination reaction, surprisingly no intermediate was found at all by means of the low-temperature proton NMR. In this article, we designed model reactions and used ab initio calculations to inspect how and why the rate-determining step of these amination reactions is changed. To our knowledge, this is the first example to demonstrate change of rate-determining step in the reactions of ketenimines and ketenes (Scheme 1).

2. Computational details

All the calculations reported here were performed with Gaussian98 program.[9] The Onsager self-consistent reaction field (SCRF) model has been used to monitor systems in a solvent with dielectric constant of 35.9 which is close to that of acetonitrile. The model treats the solvent as a continuum of uniform dielectric constant (the reaction field) and the solute is placed into a fixed spherical cavity of radius a_0 within the solvent. The radius a_0 of the cavity for each solute was evaluated based on its optimized structure in the gas

Keywords: Amination; Ketenimine; Mechanistic study.
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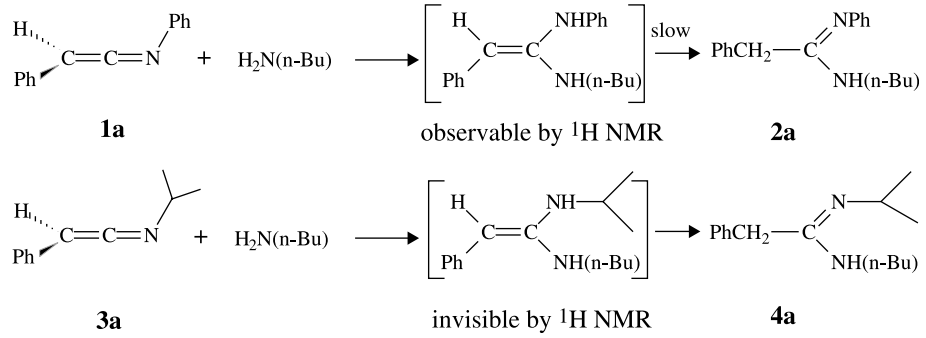


Scheme 1.

phase. SCRF geometry optimizations of **5–14** were carried out at level of HF/6-31+G* in solvent (ε=35.9) without any symmetry restriction. Many possible conformations have been optimized for each of these configurations, and the conformation with the lowest energy was chosen for each configuration. Their optimized structures are shown in Figure 1. An SCRF frequency calculation at each of SCRF optimized structures was run at the same level and analytical

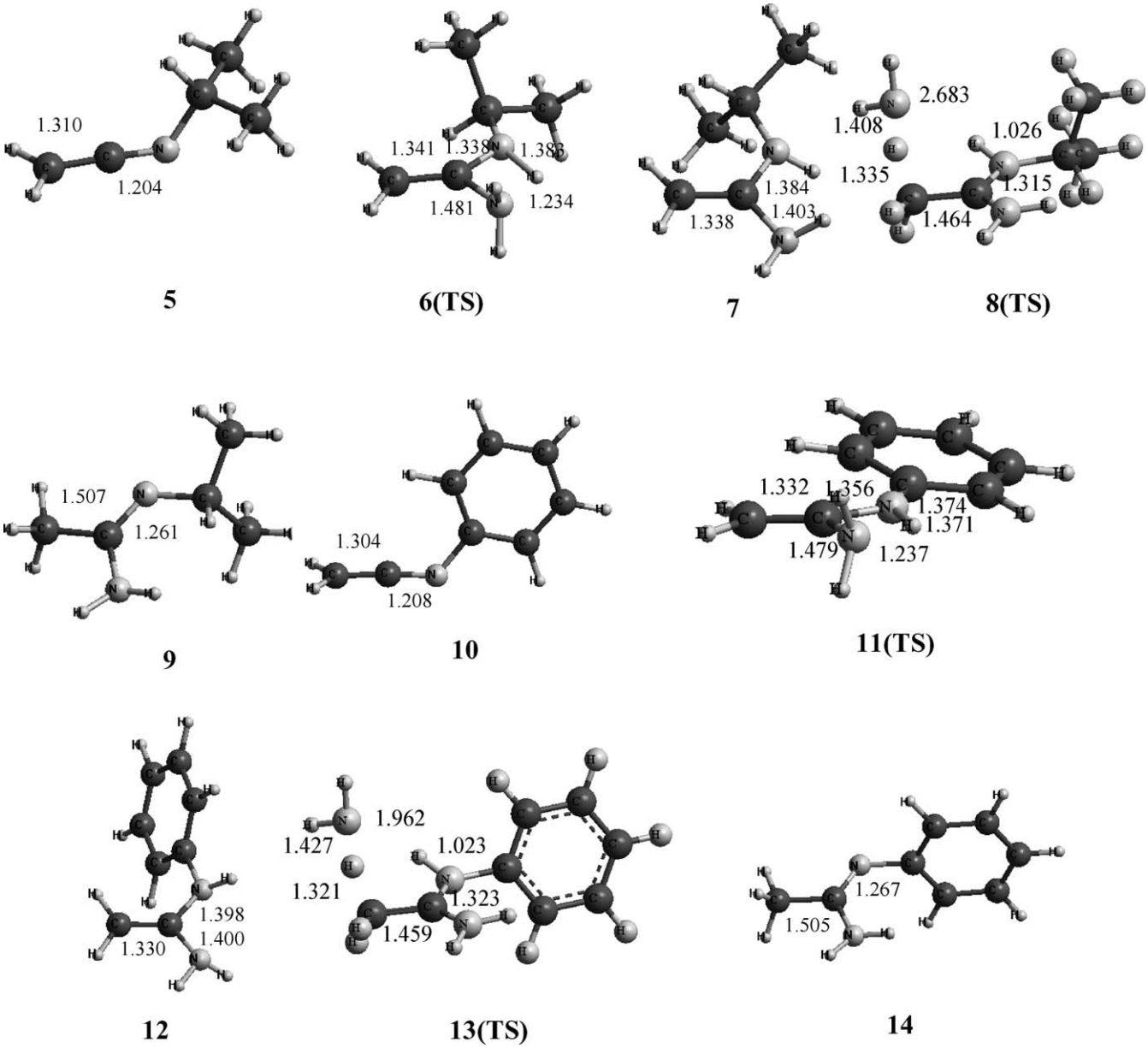


Figure 1. SCRF optimized structures of **5–14** at level of HF/6-31+G* (Onsager) in a solvent (ε=35.9). Bond lengths are in Å.

Table 1. Calculated energies (hartree), imaginary frequencies (cm^{-1}), and spherical cavity of radius a_0 (Å) of stationary points along amination of ketenimines in solvent ($\varepsilon=35.9$) at MP2/6-31+G*//HF/6-31+G* Level (Onsager model)

Stationary point	a_0	Energy	Imaginary frequency	Stationary point	a_0	Energy	Imaginary frequency
5	3.86	−249.66817		**10**	4.04	−362.47773	
6(**TS**)	4.29	−305.95331	−2109	**11**(**TS**)	4.66	−418.77114	−2115
7	4.06	−306.02672		**12**	4.41	−418.83987	
8(**TS**)	4.23	−362.31219	−1880	**13**(**TS**)	4.60	−475.12451	−1825
9	4.22	−306.05061		**14**	4.46	−418.86874	
NH_3	2.77	−56.33085					

vibration frequencies were calculated to determine the nature of the located stationary points. Thus, all the stationary points found were properly characterized by evaluation of the harmonic frequencies. The energies of all the stationary points were calculated at MP2/6-31+G* with scale zero-point vibration energies included, and population analyses of **5** and **10** were carried out at the same level. (Table 1) The scale factor of 0.9135 for zero-point vibration energies is used according to the literatures.[10] As shown in Table 2,[10] it was reported that the Onsager model at MP2/6-31+G* level gives the best prediction results for the energy difference between the *gauche* and *trans* conformers of dichloroethane in several solvents.[10] Therefore, the Onsager model at MP2/6-31+G* level was used in this research to predict the reaction mechanism of the amination of ketenimines.

Table 2. Predicted energy differences (ΔE) between the *gauche* and *trans* conformers of dichloroethane in four solvent environments

Medium	ΔE (kcal/mol)			
	Onsager		IPCM	Exp.
	HF/6-31+G*	MP2/6-31+G*	B3LYP/6-31+G*	
Gas phase	1.96	1.51	1.76	1.20
Cyclohexane	1.32	0.99	1.45	0.91
Pure liquid	0.50	0.29	0.90	0.31
Acetonitrile	0.30	0.13	0.73	0.15

3. Results and discussion

When we studied the amination of *N*-phenylphenylketenimine **1a** with *n*-butylamine in CD_3CN at −10 °C by proton NMR spectrometer, the vinylidenediamine intermediate was caught and we found that it involves two steps, C=N addition and tautomerization, and the second step is much slower than the first step.[7] The same reaction mechanism was found for amination of other *N*-*p*-substituted-phenyl-phenylketenimines **1b** and **1c** with *n*-$BuNH_2$ by monitoring the reactions with proton NMR spectrometer in CD_3CN at −10 °C Scheme 2). One representative example is shown in Figure 2.[7] On the other hand, reactions of *N*-*i*-propyl-*p*-substituted-phenylketenimines **3a**–**3e** with *n*-$BuNH_2$ in CD_3CN are much slower than those of **1a**–**1c** with *n*-butylamine, so they were monitored by proton NMR spectrometer at 10 °C Scheme 3). Surprisingly, there is no intermediate found for each of the reactions, and one representative example is shown in Figure 3. To find out this strange result, reactions of ketenimines **5** and **10** with NH_3 were monitored by ab initio calculations in the solvent with $\varepsilon=35.9$.

The major structure difference between **1a**–**1c** and **3a**–**3e** is N-substituent. To avoid inaccurate calculations due to too many atoms,[10] the investigated molecules need to be simplified, so ketenimines **5** and **10** were chosen as model substrates in order to inspect N-substituent effects on the amination reactions. Amination reactions of **5** and **10** with NH_3 were monitored at the MP2/6-31+G* level in the solvent with dielectric constant of 35.9.

In early studies, theoretical[8a,b] and kinetic studies[8c–e] of amination of ketenes were interpreted as involving initial addition to the C=C bond of ketenes. Later on, high-level ab initio calculations[4c,e] and kinetic studies[2f,4d–f,l–m] overthrew the previous mechanism and suggested the amination reactions proceed via amine addition across the C=O bond of ketenes, followed by tautomerization. Meanwhile, some labile intermediate of amide enols from amination of ketenes were caught by IR[2f,4e,l] and UV[4m] spectrometers, and a stable amide enol from the amination of a crowded diarylketene was even isolated.[4f] Similarly, the amination of ketenimines was found to proceed via amine addition across the C=N bond, followed by tautomerization, much easier than via amine addition across the C=C bond by high-level ab initio calculations in both gas phase and solution,[7] and the metastable intermediate of vinylidenediamine was caught and identified by means of low-temperature proton NMR spectrometer.[7] Therefore, the amination reactions of **5** and **10** were designed to involve the two steps, C=N addition and tautomerization (Scheme 4).

According to preliminary results of kinetic studies for the amination reactions of **1a**–**1c** and **3a**–**3e** with *n*-$BuNH_2$ in CH_3CN, C=N addition of these ketenimines involves one molecule of *n*-$BuNH_2$.[11] Therefore, C=N addition of **5** and **10** are designed to involve one molecule of NH_3. Because suprafacial 1,3-hydrogen shift is thermally forbidden,[12] tautomerization of vinylidenediamines **7** and **12** without catalyst is unlikely. Therefore, we designed tautomerization of **7** and **12** to be catalyzed by one molecule of NH_3, forming amidines **9** and **14**.

Calculated activation enthalpies, activation entropies, activation free energies, enthalpies, entropies, and free energies for the amination reactions of **5** and **10** with NH_3 are shown in Table 3. Both C=N addition of **5** and tautomerization of **7** are exothermic reactions, and activation free energy of the former is 5.1 kcal/mol more than that of the latter. This indicates that the first step involving C=N addition is a rate-determining step in the amination reaction of **5** with NH_3. That is the reason why there is no intermediate found by low-temperature proton NMR spectrometer in the amination reactions of **3a**–**3e** with

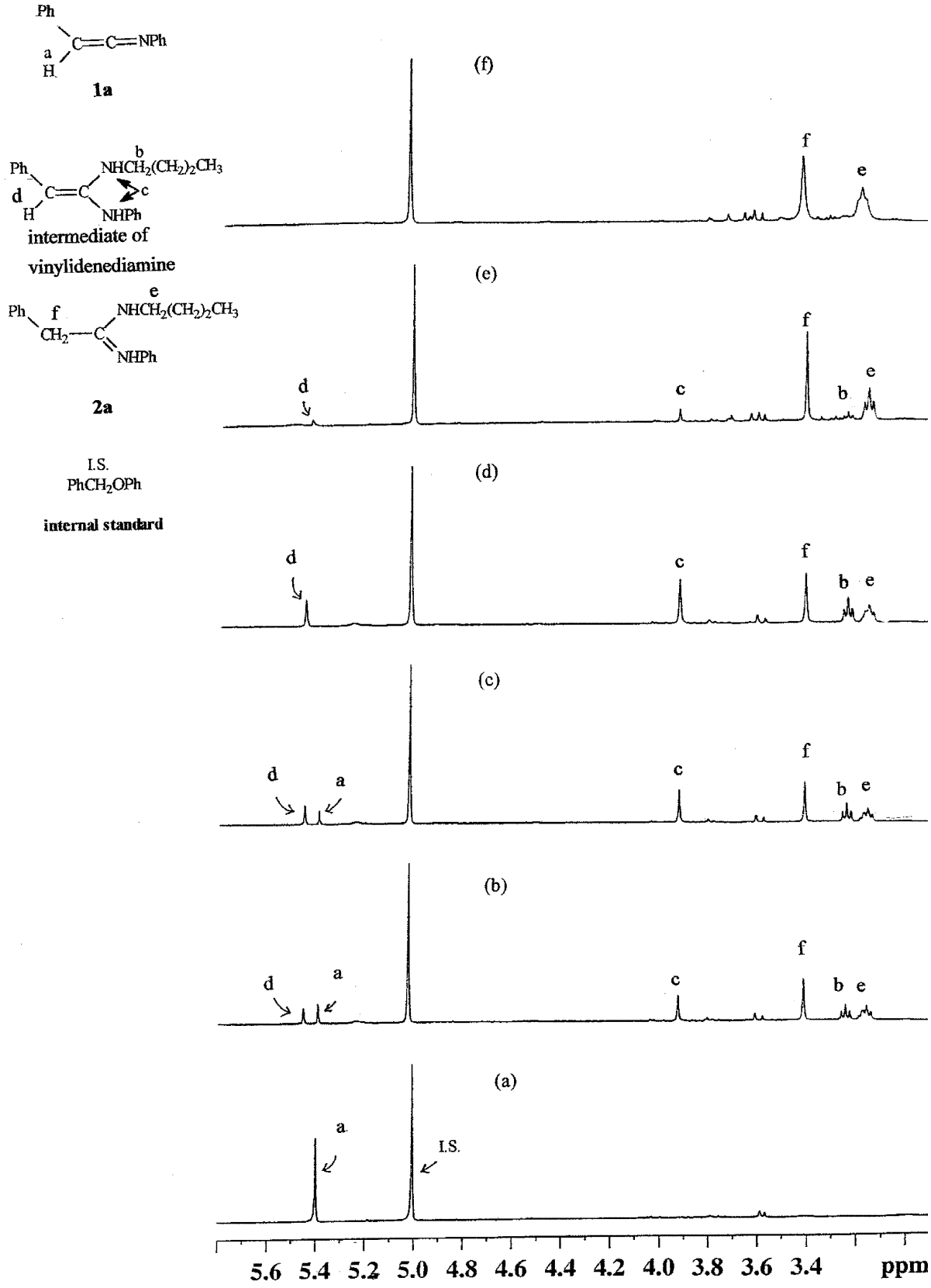


Figure 2. Part of ^{1}H NMR (CD_3CN) spectra for the reaction of **1a** with *n*-butylamine in the presence of an internal standard (benzyl phenyl ether) at −10 °C (a) before adding *n*-butylamine, (b) at 10 min after mixing the solution, (c) at 14 min, (d) at 45 min, (e) at 19 h, and (f) at 30 min after increasing the temperature to 25 °C.

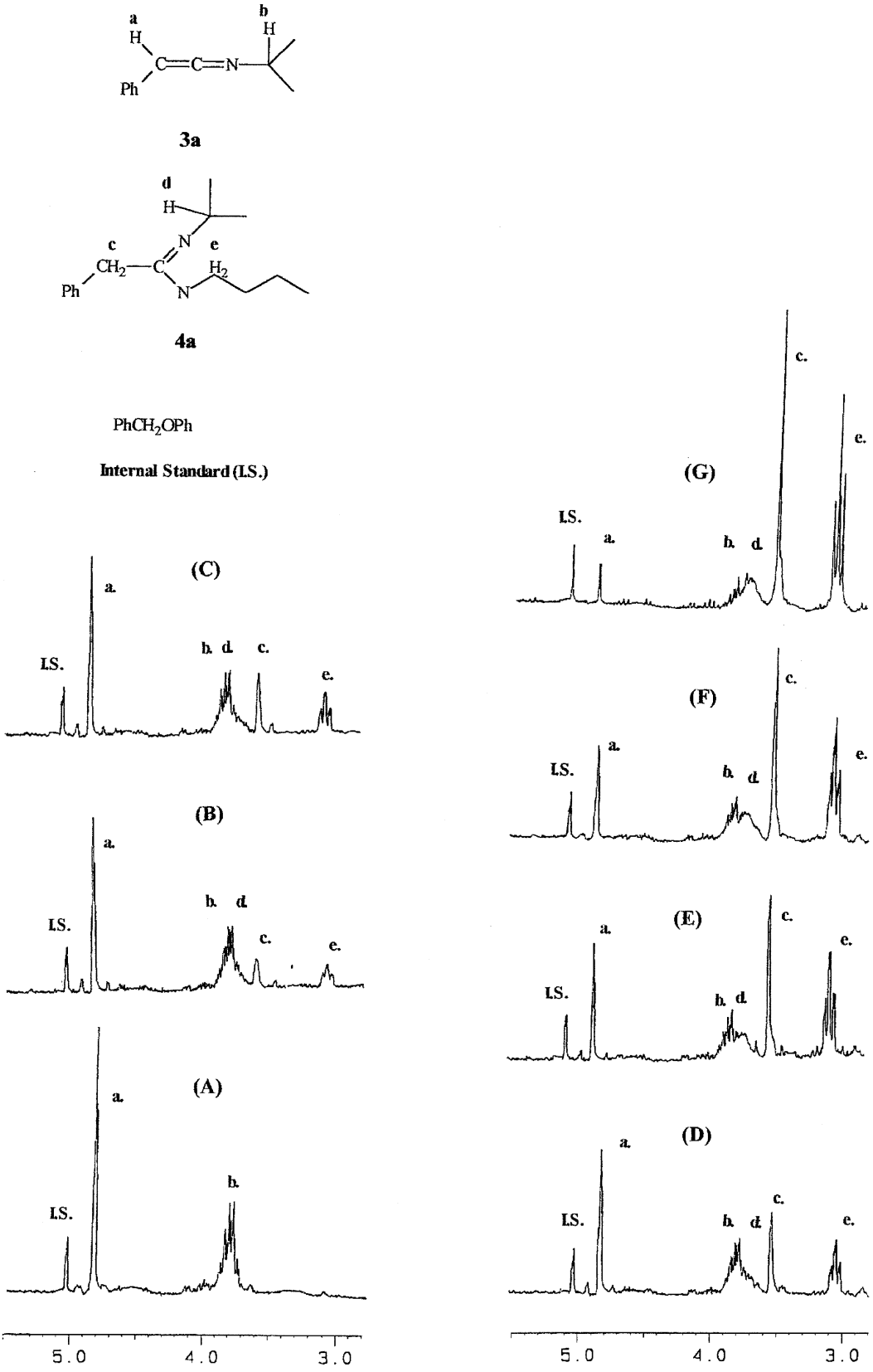
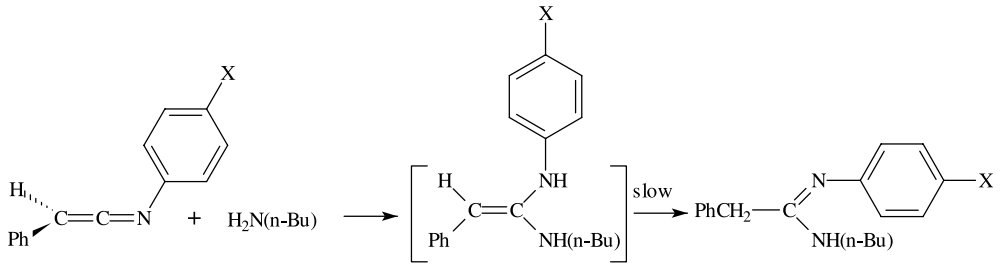
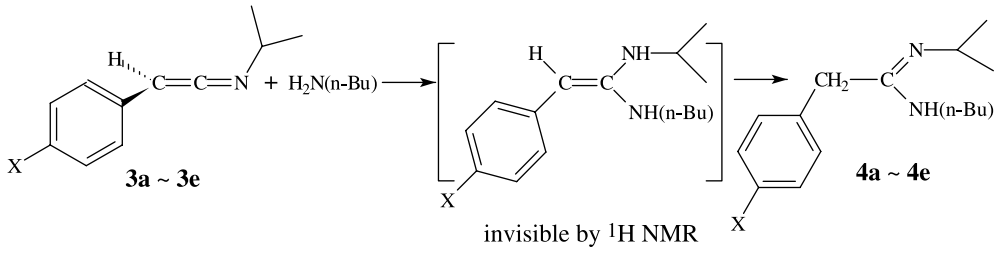


Figure 3. Part of ^{1}H NMR (CD_3CN) spectra for amination of **3a** with *n*-$BuNH_2$ in the presence of benzyl phenyl ether as an internal standard at 10 °C (A) before adding *n*-$BuNH_2$, (B) at 5 min after mixing the solution, (C) at 20 min, (D) at 40 min, (E) at 100 min, (F) at 130 min, (G) at 250 min.



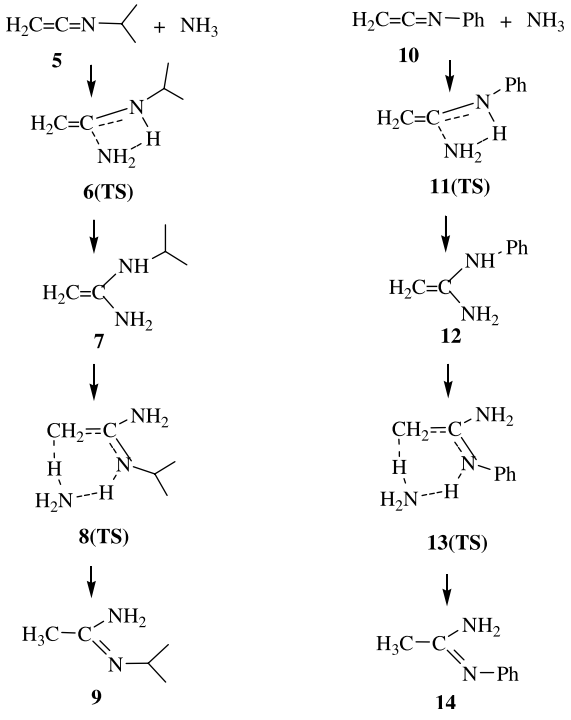
1a, **2a**: X = H ; **1b**, **2b** : X = OMe ; **1c**, **2c** : X = Br

Scheme 2.



3a, **4a**: X = H ; **3b**, **4b** : X = CH_3 ; **3c**, **4c** : X = OMe ; **3d**, **4d** : X = Cl ; **3e**, **4e** : X = NO_2

Scheme 3.



Scheme 4.

n-$BuNH_2$ in CD_3CN. On the other hand, both C═N addition of **10** and tautomerization of **12** are exothermic reactions, and activation free energy of the former is 0.33 kcal/mol less than that of the latter, indicating that the second step involving taumomerization becomes a rate-determining step in the amination reaction of **10** with NH_3. This is consistent with the experimental results that the metastable vinylidene-diamine intermediates were caught by low-temperature proton NMR spectrometer in the amination reactions of **1a**–**1c** with n-$BuNH_2$ in CD_3CN.

Activation free energy for tautomerization of **7** is very close to that for tautomerization of **12**, indicating that electronic effects of N-substituent make little difference in the tautomerization processes. In contrast, activation free energy for C═N addition of **5** with NH_3 is 4.69 kcal/mol more than that for C═N addition of **10** with NH_3, indicating that *N*-phenyl group strongly stabilizes the transition state in the C═N addition process of **10** with NH_3. This is the major reason why the rate-determining step is changed from the first step (C═N addition) to the second step (tautomerization) when N-substituent of the ketenimines is changed from *i*-propyl group **3a**–**3e** to *p*-substituted-phenyl group **1a**–**1c** in the amination reactions. Because steric effect of phenyl group is very close to that of *i*-propyl group,[13] it turns out that electronic effects, instead of steric effect, control the change of the rate-determining step in these amination reactions.

Table 3. Calculated activation enthalpies (kcal/mol), activation entropies (cal/mol K), activation free energies (kcal/mol), enthalpies (kcal/mol), entropies (cal/mol K), and free energies (kcal/mol) for amination of **5** and **10** in the solvent ($\varepsilon=35.9$) at MP2/6-31+G*//HF/6-31+G* level (Onsager model)

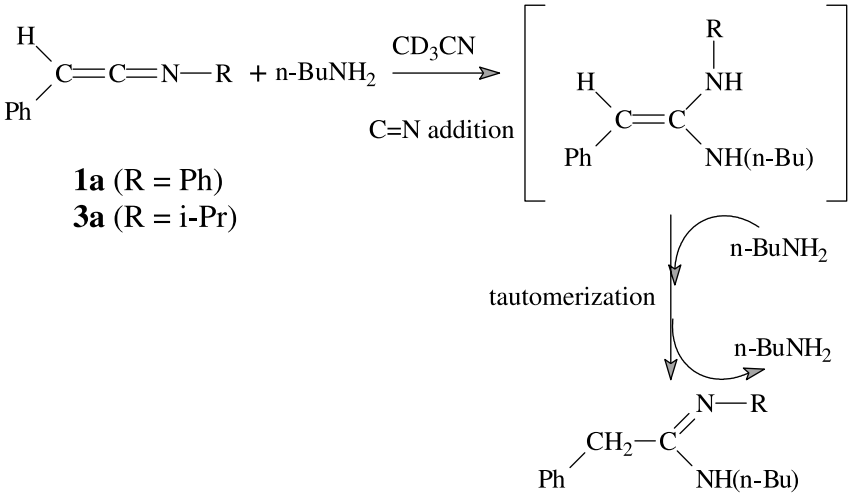
	$\Delta H^{\ddagger}$ (298 K)	$\Delta S^{\ddagger}$ (298 K)	$\Delta G^{\ddagger}$ (298 K)	ΔH (298 K)	ΔS (298 K)	ΔG (298 K)
Process: **5**+NH_3→**6**(**TS**)→**7**						
	28.25	−40.44	40.30	−13.85	−40.54	−1.77
Process: **7**+NH_3→**8**(**TS**)→**9**+NH_3						
	25.27	−33.31	35.20	−15.37	1.99	−15.96
Process: **10**+NH_3→**11**(**TS**)→**12**						
	23.46	−40.78	35.61	−16.07	−39.67	−4.25
Process: **12**+NH_3→**13**(**TS**)→**14**+NH_3						
	25.62	−34.61	35.94	−18.52	3.22	−19.48

Rate constants for the amination reactions of ketenimines **1a** and **3a** with *n*-$BuNH_2$ in CD_3CN were measured by means of low-temperature proton NMR spectrometer and their results are shown in Table 4. The rate constants of the first steps (C=N addition) for the amination of ketenimines **1a** and **3a** were measured by following disappearance of ketenimines. Their pseudo-first-order rate constants are $1.52\times10^{-3}\ s^{-1}$ (at −10 °C) for the amination of **1a** and $9.18\times10^{-5}\ s^{-1}$ (at 10 °C) for the amination of **3a**, and the corresponding second-order rate constants are $3.04\times10^{-3}\ M^{-1}\ s^{-1}$ (at −10 °C) and $1.84\times10^{-4}\ M^{-1}\ s^{-1}$ (at 10 °C), respectively, indicating that *N*-phenyl ketenimine makes the C=N addition step much faster during the amination reaction than *N*-*i*-propyl ketenimine does. These experimental results are well consistent with our computation results that *N*-phenyl group strongly stabilizes the transition state in the C=N addition process of amination of ketenimines.

The rate constant of the second step (tautomerization) for the amination of ketenimine **1a** was measured by following appearance of amidine product. The pseudo-first-order rate constant are $3.00\times10^{-5}\ s^{-1}$ (at −10 °C), and the corresponding second-order rate constants are $6.00\times10^{-5}\ M^{-1}\ s^{-1}$ (at −10 °C), indicating that the tautomerization is much slower than the corresponding C=N addition, resulting in accumulation of the vinylidenediamine intermediate. On the other hand, in the case of amination of ketenimine **3a**, the C=N addition step is very slow and presumably slower than the next tautomerization step, leading to no accumulation of the vinylidenediamine intermediate. All the experimental results are consistent with our computation results that the rate-determining step is changed from the first step (C=N addition) to the second step (tautomerization) when N-substituent of the ketenimines is changed from *i*-propyl group to phenyl group in the amination reactions.

Due to lack of special NMR sample-injection system, we had problem to inject CD_3CN solution of ketenimine into a thermostated NMR tube inside a NMR spectrometer, which contained CD_3CN solution of *n*-$BuNH_2$ and internal standard and had finished shimming. To compensate this deficiency, we added *n*-$BuNH_2$, CD_3CN, internal standard, and ketenimine together in a NMR tube at liquid nitrogen temperature, and let them warm up to a preset temperature inside a NMR spectrometer for further rate-constant measurement. As shown in Table 4, this method did provide

Table 4. Pseudo-first-order and second-order rate constants of the C=N addition and tautomerization for the amination reactions of ketenimines **1a** and **3a** with *n*-$BuNH_2$ in CD_3CN by means of proton NMR spectrometer[a]



R	Temperature	C=N Addition		Tautomerization	
		k_{obs} (s^{-1})	k_2 ($M^{-1}\ s^{-1}$)	k_{obs} (s^{-1})	k_2 ($M^{-1}\ s^{-1}$)
Ph	−10 °C	1.52×10^{-3}	3.04×10^{-3}	3.00×10^{-5}	6.00×10^{-5}
i-Pr	10 °C	9.18×10^{-5}	1.84×10^{-4}	—	—

[a] All rate constants were measured at least in duplicate with maximum deviations of ±5%.

acceptable results in distinguishing N-substituent effects for the amination of ketenimines. However, this method is not accurate enough to measure temperature and concentration effects on the rate constants and linear free energy relationship (LFER) for the amination of ketenimines. Therefore, we couldn't get experimental data of activation parameters for the amination of ketenimines, which may be used to fit our computational results. Nevertheless, literature data can be used for the comparison purpose. Base-catalyzed hydration of **3a** was reported to have observed first-order rate constant of 1.3×10^{-3} s^{-1} at pH=10 and 25 °C in water,[4i] which is faster than the amination of **3a** in CD_3CN ($k_{obs}=9.18\times10^{-5}$ s^{-1} at 10 °C). Presumably, it is because water concentration in the base-catalyzed hydration reaction is much more than *n*-$BuNH_2$ concentration in the amination reaction. The base-catalyzed hydration of **3a** was reported to have the experimental activation enthalpy of 15.2 kcal/mol,[4i] which is smaller than that for the amination of **5** in acetonitrile (calculated $\Delta H^{\ddagger}$(298 K) =28.25 kcal/mol). It was reported that water solvent is involved in the transition state of base-catalyzed hydration of **3a**,[4i] and it is likely that involvement of water solvent in the transition state reduces its activation enthalpy.

Calculated activation barriers for hydration of ketenimine with H_2O and $(H_2O)_2$ addition across C=N bond in solution by PCM model at MP2/6-31G(d,p) level were reported to be 44.5 and 27.0 kcal/mol, respectively.[14] Calculated activation barriers for amination of ketene with NH_3 and $NH_3\cdot H_2O$ addition across C=O bond in gas phase at MP2/6-31G* level were reported to be 30.03 and 2.86 kcal/mol, respectively.[4c] These two examples indicate that involvement of additional water molecule in the transition states significantly reduce activation barrier. Compared with the experimental rate constants for the amination reactions of ketenimines **1a** and **3a**, the calculated activation free energies for the amination of ketenimines **5** and **10** are somewhat big, indicating that additional water molecule is likely involved in the transition states of the amination of ketenimines **1a** and **3a** during the NMR studies. Nevertheless, this does not change the finding that *N*-phenyl group strongly stabilizes the transition state in the C=N addition process of amination of ketenimines.

LUMO of ketenimines lies in the ketenimine plane with a large coefficient on C_α, so amination of ketenimine involves an in-plane nucleophilic attack on the ketenimine LUMO at C_α.[7] This can be easily seen from the optimized transition structures **6**(**TS**) and **11**(**TS**). LUMO energies of **5** and **10** were calculated to be 1.95 and 1.83 eV at MP2/6-31+G* level in the solvent with dielectric constant of 35.9 (Onsager model), indicating that *N*-phenyl substituent decreases LUMO energy of ketenimine. According to PMO theory,[12,15] *N*-phenyl ketenimine **10** with low-lying LUMO is more reactive toward nucleophilic amines than *N*-*i*-propyl ketenimine **5**, and this is consistent with both our experimental and computational results.

In the optimized transition structure of **11**(**TS**), dihedral angle between *N*-phenyl substituent and ketenimine (or enamine) plane is 12.8°, and the *N*-phenyl group is conjugated with the enamine backbone, making electron delocalization go through these groups. Bond distances in these groups can be an evidence for this electron delocalization. It is very likely that this electron delocalization provides one of ways to stabilize **11**(**TS**) significantly.

4. Conclusion

The rate-determining step of the amination reactions of **3a–3e** is the first addition step, so the vinylidenediamine intermediate cannot be found naturally. In contrast, due to electronic stabilization of the transition state of the first C=N addition step by *N*-phenyl group, the rate-determining step of the amination reactions of **1a–1c** switches to the second tautomerization step, resulting in accumulation of the vinylidenediamine intermediate. Therefore, the vinylidenediamine intermediate can be caught in the amination reactions of **1a–1c**. *N*-phenyl substituent decreases LUMO energy of ketenimine, and that makes *N*-phenyl ketenimine more reactive toward nucleophilic amines than *N*-*i*-propyl ketenimine. The transition state **11**(**TS**) for the C=N addition of *N*-phenyl ketene **10** is significantly stabilized by electron delocalization through the system.

5. Experimental

5.1. General

Ketenimines **1a** is known and other ketenimines **1b–1c** and **3a–3e** were prepared by a known procedure.[4h,i,7]

5.2. General method to prepare ketenimines 1b–1c and 3a–3e

Phosphorus pentachloride (7 mmol) was added to a solution of *N*-substituted-*p*-substituted-phenylacetamide (7 mmol) in dry benzene (20 mL). The solution was refluxed for 1 h, and the benzene and by-product of phosphorus oxychloride were removed in high vacuum. The green-yellow residue was dissolved in dry ether (20 mL) and dry triethylamine (6 mL) was added to the solution to give an instant color change to red. The reaction was refluxed for 6 h. The solution was filtered to remove insoluble ammonium chloride under nitrogen atmosphere. After evaporation of the solvent and triethylamine, crude ketenimine was obtained as red oil, and it was purified by sublimation in high vacuum with finger temperature at −196 °C. The ketenimine was diluted with hexane for storage.

5.2.1. *N*-*p*-Methoxyphenylphenylketenimine (1b). Yield: 74%; ^{1}H NMR ($CDCl_3$) δ 3.83 (3H, s, OCH_3), 5.24 (1H, s, CH), 6.92 (2H, d, J=8.2 Hz, PhH), 7.19 (2H, d, J=8.2 Hz, PhH); 7.26–7.34 (5H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 55.5, 60.9, 114.6, 125.1, 125.2, 125.5, 128.9, 132.9, 133.0, 159.2, 189.4; IR (hexane) 2007 (C=C=N) cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{15}H_{13}NO$ 223.0997, found 223.0999. Anal. Calcd for $C_{15}H_{13}NO$: C 80.68, H 5.87, N 6.28. Found: C 80.70, H 5.81, N 6.22.

5.2.2. *N*-*p*-Bromophenylphenylketenimine (1c). Yield: 62%; ^{1}H NMR ($CDCl_3$) δ 5.30 (1H, s, CH), 7.10–7.53 (9H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 61.3, 121.3, 125.4,

125.6, 125.7, 129.0, 131.9, 132.6, 139.6, 191.9; IR (hexane) 2016 (C═C═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{14}H_{10}BrN$ 270.9996, found 270.9995. Anal. Calcd for $C_{14}H_{10}BrN$: C 61.99, H 3.72, N 5.17. Found: C 61.93, H 3.77, N 5.10.

5.2.3. *N*-*i*-Propylphenylketenimine (3a). Yield: 78%; ^1H NMR ($CDCl_3$) δ 1.35 (6H, d, $J=8.5$ Hz, CH_3), 3.85 (1H, m, CH), 4.82 (1H, d, $J=2.4$ Hz, CH), 7.08–7.30 (5H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 23.5, 54.6, 58.4, 124.4, 124.7, 128.7, 134.3, 184.7; IR (hexane) 2026 (C═C═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{11}H_{13}N$ 159.1048, found 159.1049. Anal. Calcd for $C_{11}H_{13}N$: C 82.96, H 8.23, N 8.80. Found: C 82.92, H 8.19, N 8.89.

5.2.4. *N*-*i*-Propyl-*p*-methylphenylketenimine (3b). Yield: 66%; ^1H NMR ($CDCl_3$) δ 1.33 (6H, d, $J=5.2$ Hz, CH_3), 2.30 (3H, s, CH_3), 3.83 (1H, m, CH), 4.79 (1H, d, $J=1.8$ Hz, CH), 7.00 (2H, d, $J=8.0$ Hz, PhH), 7.07 (2H, d, $J=8.0$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 21.4, 23.5, 54.7, 58.6, 124.7, 129.4, 130.9, 134.1, 185.8; IR (hexane) 2026 (C═C═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{12}H_{15}N$ 173.1204, found 173.1206. Anal. Calcd for $C_{12}H_{15}N$: C 83.18, H 8.73, N 8.09. Found: C 83.10, H 8.80, N 8.10.

5.2.5. *N*-*i*-Propyl-*p*-methoxyphenylketenimine (3c). Yield: 71%; ^1H NMR ($CDCl_3$) δ 1.30 (6H, d, $J=2.6$ Hz, CH_3), 3.80 (3H, s, OCH_3), 3.82 (1H, m, CH), 4.79 (1H, d, $J=2.0$ Hz, CH), 6.80 (2H, d, $J=5.0$ Hz, PhH), 7.00 (2H, d, $J=5.0$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 23.5, 54.7, 55.2, 58.3, 114.4, 121.1, 125.8, 157.0, 186.6; IR (hexane) 2026 (C═C═N) cm^{-1}; MS (EI) *m/z* 196 (30, M^+), 140 (45), 99 (100), 84 (25), 57 (70); HRMS (EI) *m/z* calcd for $C_{12}H_{15}NO$ 189.1154, found 189.1153. Anal. Calcd for $C_{12}H_{15}NO$: C 76.14, H 7.99, N 7.40. Found: C 76.10, H 8.18, N 7.35.

5.2.6. *N*-*i*-Propyl-*p*-chlorophenylketenimine (3d). Yield: 59%; ^1H NMR ($CDCl_3$) δ 1.30 (6H, d, $J=2.6$ Hz, CH_3), 3.85 (1H, m, CH), 4.76 (1H, d, $J=2.0$ Hz, CH), 7.03 (2H, d, $J=5.9$ Hz, PhH), 7.20 (2H, d, $J=5.9$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 23.5, 54.7, 58.0, 125.8, 129.1, 130.6, 132.7, 183.7; IR (hexane) 2026 (C═C═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{11}H_{12}ClN$ 193.0658, found 193.0656. Anal. Calcd for $C_{11}H_{12}ClN$: C 68.37, H 6.26, N 7.25. Found: C 68.40, H 6.19, N 7.31.

5.2.7. *N*-*i*-Propyl-*p*-nitrophenylketenimine (3e). Yield: 45%; ^1H NMR ($CDCl_3$) δ 1.33 (6H, d, $J=5.2$ Hz, CH_3), 3.88 (1H, m, CH), 4.76 (1H, d, $J=1.7$ Hz, CH), 7.06 (2H, d, $J=7.0$ Hz, PhH), 7.98 (2H, d, $J=7.0$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 23.3, 54.6, 57.7, 123.7, 129.9, 134.9, 143.8, 177.3; IR (hexane) 2032 (C═C═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{11}H_{12}NO_2$ 190.0868, found 190.0870. Anal. Calcd for $C_{11}H_{12}NO_2$: C 69.44, H 6.36, N 7.37. Found: C 69.51, H 6.42, N 7.39.

5.3. NMR study of amination of ketenimines 1b–1c and 3a–3e

A NMR tube filled with 1 mL of a CD_3CN solution of a ketenimine (0.01 mmol) and benzyl phenyl ether (0.033 mmol, serving as an internal standard with δ 5.0 ppm) was cooled by liquid nitrogen. Pure *n*-butylamine (50 μL, 0.5 mmol) was injected into the NMR tube through a rubber cap. The solution in the NMR tube was shaken in a cold bath and then put into the NMR spectrometer. Then the reaction was monitored by proton NMR spectrometer at −10 °C for **1b–1c** and 10 °C for **3a–3e** until all the ketenimine and most of intermediate (if it exists) were consumed, and then the temperature was raised to 25 °C. Monitored ^1H NMR spectra for amination of **1a–1c** look alike with vinylidenediamine intermediate involved and one representative of them was shown in Figure 2.[7] Monitored ^1H NMR spectra for the amination of **3a–3e** look alike without any detected intermediate and one representative of them is shown in Figure 3. Final products of amidines were identified as follows.

5.4. Product analysis for amination of 1b–1c and 3a–3e with *n*-$BuNH_2$

5.4.1. *N*-Butyl-*N′*-(*p*-methoxyphenyl)-2-phenylacetamidine (2b). ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, $J=7.2$ Hz, CH_3), 1.27–1.43 (4H, m, CH_2), 3.16 (2H, t, $J=7.0$ Hz, CH_2), 3.44 (2H, s, CH_2), 6.93–7.30 (9H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 13.77, 20.07, 31.17, 36.19, 41.02, 55.20, 114.21, 114.52, 125.14, 128.28, 128.43, 142.82, 143.88, 151.01, 158.53; IR (thin film) 1655 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{19}H_{24}N_2O$ 296.1889, found 296.1888.

5.4.2. *N*-Butyl-*N′*-(*p*-bromophenyl)-2-phenylacetamidine (2c). ^1H NMR ($CDCl_3$) δ 0.92 (3H, t, $J=7.2$ Hz, CH_3), 1.29–1.45 (4H, m, CH_2), 3.17 (2H, t, $J=6.8$ Hz, CH_2), 3.46 (2H, s, CH_2), 7.11–7.50 (9H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 13.70, 20.12, 31.07, 36.39, 41.25, 110.63, 116.29, 125.55, 128.30, 128.74, 131.36, 142.72, 145.04, 157.09; IR (thin film) 1651 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{18}H_{21}N_2$ 265.1705, found 265.1703.

5.4.3. *N*-Butyl-*N′*-*i*-propyl-2-phenylacetamidine (4a). ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, $J=7.2$ Hz, CH_3), 1.17 (6H, d, $J=6.4$ Hz, CH_3), 1.31–1.52 (4H, m, CH_2), 3.07 (2H, t, $J=6.8$ Hz, CH_2), 3.50 (2H, s, CH_2), 3.78 (1H, m, CH), 7.06–7.15 (5H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 13.71, 19.73, 20.11, 31.13, 36.25, 41.30, 46.02, 125.81, 128.02, 128.83, 142.51, 157.91; IR (thin film) 1663 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{15}H_{24}N_2$ 232.1939, found 232.1941.

5.4.4. *N*-Butyl-*N′*-*i*-propyl-2-(*p*-methylphenyl)acetamidine (4b). ^1H NMR ($CDCl_3$) δ 0.93 (3H, t, $J=7.2$ Hz, CH_3), 1.17 (6H, d, $J=6.4$ Hz, CH_3), 1.31–1.52 (4H, m, CH_2), 2.31 (3H, s, CH_3), 3.13 (2H, t, $J=7.0$ Hz, CH_2), 3.64 (2H, s, CH_2), 3.80 (1H, m, CH), 7.00 (2H, d, $J=8.0$ Hz, PhH), 7.07 (2H, d, $J=8.0$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 13.75, 20.10, 21.30, 32.34, 36.65, 41.01, 46.82, 126.23, 128.52, 134.73, 141.51, 153.91; IR (thin film) 1662 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{16}H_{26}N_2$ 246.2096, found 246.2095.

5.4.5. *N*-Butyl-*N′*-*i*-propyl-2-(*p*-methoxyphenyl)acetamidine (4c). ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, $J=7.2$ Hz, CH_3), 1.18 (6H, d, $J=6.5$ Hz, CH_3), 1.31–1.52 (4H, m, CH_2), 3.06 (2H, t, $J=7.0$ Hz, CH_2), 3.48 (2H, s, CH_2), 3.70 (3H, s, OCH_3), 3.80 (1H, m, CH), 6.80 (2H, d, $J=5.0$ Hz, PhH), 7.00 (2H, d, $J=5.0$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 13.95, 19.81, 20.30, 32.13, 36.95, 41.43, 46.71, 113.81,

128.82, 129.13, 154.51, 157.91; IR (thin film) 1661 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{16}H_{26}N_2O$ 262.2045, found 262.2044.

5.4.6. *N*-Butyl-*N′*-*i*-propyl-2-(*p*-chlorophenyl)acetamidine (4d). ^1H NMR ($CDCl_3$) δ 0.97 (3H, t, $J=7.2$ Hz, CH_3), 1.08 (6H, d, $J=6.4$ Hz, CH_3), 1.31–1.52 (4H, m, CH_2), 3.13 (2H, t, $J=7.1$ Hz, CH_2), 3.61 (2H, s, CH_2), 3.80 (1H, m, CH), 7.03 (2H, d, $J=5.9$ Hz, PhH), 7.20 (2H, d, $J=5.9$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 14.05, 19.80, 20.90, 31.83, 36.75, 41.22, 46.53, 128.71, 130.42, 131.11, 136.53, 153.62; IR (thin film) 1662 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{15}H_{23}ClN_2$ 266.1550, found 266.1549.

5.4.7. *N*-Butyl-*N′*-*i*-propyl-2-(*p*-nitrophenyl)acetamidine (4e). ^1H NMR ($CDCl_3$) δ 0.90 (3H, t, $J=7.2$ Hz, CH_3), 1.11 (6H, d, $J=6.5$ Hz, CH_3), 1.31–91.52 (4H, m, CH_2), 3.04 (2H, t, $J=7.0$ Hz, CH_2), 3.64 (2H, s, CH_2), 3.80 (1H, m, CH), 7.05 (2H, d, $J=7.0$ Hz, PhH), 7.95 (2H, d, $J=7.0$ Hz, PhH); ^{13}C NMR ($CDCl_3$) δ 14.55, 20.81, 20.98, 33.21, 37.05, 41.11, 46.57, 123.81, 129.82, 146.10, 147.26, 154.10; IR (thin film) 1660 (N–═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{15}H_{23}N_3O_2$ 277.1790, found 277.1787.

5.5. Rate-constant measurement for amination of ketenimines 1a and 3a

Setup for the rate-constant measurement is the same as the one shown in NMR study of amination of ketenimines **1b–1c** and **3a–3e**. The rate constant for the first step, C═N addition, of the amination of ketenimine **1a** was measured at −10 °C by following disappearance rate of characteristic resonance peak at δ 5.39 which is assigned vinyl proton of ketenimine **1a**. The rate constant for the second step, tautomerization, of the amination of ketenimine **1a** was measured at −10 °C after complete consumption of **1a** by following appearance rate of characteristic resonance absorption at δ 3.42 which is assigned methylene protons of the corresponding amidine product. The rate constant for the first step, C═N addition, of the amination of ketenimine **3a** was measured at 10 °C by following disappearance rate of characteristic resonance peak at δ 4.82 which is assigned vinyl proton of ketenimine **3a**. Integration of the characteristic resonance peaks is divided by that of the characteristic resonance peak at δ 5.0 of the internal standard. Combination of the ratios with the corresponding measured time generated first-order exponential decay or rise. The Sigma Plot software was used to fit the plots in order to get first-order rate constants. All rate constants were measured at least in duplicate with maximum deviations of $\pm$5%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.09.119

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11. Time scans for the amination reactions of **1a–e** and **3a–c** with excess of *n*-$BuNH_2$ in acetonitrile at both -10 or 20 °C were carried out by following decay of the ketenimines with UV spectrophotometer at $\lambda=260$ or 270 nm. They all showed a pseudo-first-order kinetics. A plot of k_{obs} for the pseudo-first-order kinetics versus [*n*-$BuNH_2$] is a straight line with a slope of k_2 for each of these amination reactions, indicating that the C=N addition of these amination reactions involves one molecule of *n*-$BuNH_2$.
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New cytotoxic sesquiterpenes from the red algae *Laurencia obtusa* and *Laurencia microcladia*

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Abstract—Three new sesquiterpenes (**1**–**3**), along with five known (**5**–**9**), were isolated from the organic extract of the red alga *Laurencia obtusa*, collected from the coastal rocks of Serifos in the Aegean Sea. A new dimeric sesquiterpene of the cyclolaurane-type (**4**) along with four previously reported (**7**, **10**–**12**) metabolites, were isolated from the extract of *Laurencia microcladia*, collected at Chios island in the North Aegean Sea. The structures and the relative stereochemistry of the compounds are proposed on the basis of their spectral data. The cytotoxicity of the isolated metabolites was evaluated against several cell lines including human tumor cell lines.

1. Introduction

Red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae) are some of the most prolific producers of secondary metabolites in the marine environment.[1,2] The chemistry of *Laurencia* species is a very interesting topic of research that never fails to offer the possibility of discovering interesting and novel structures, as well as biologically active metabolites.[3,4] Secondary metabolites from these algae are predominantly sesquiterpenes, diterpenes, triterpenes and C_{15}-acetogenins,[5–7] that usually are characterized by the presence of halogen atoms in their chemical formulas.[8] Although the function of them has not been completely clarified, it is suggested that they serve as feeding deterrents against marine herbivores.[9–11] Most species of *Laurencia* biosynthesize a characteristic metabolite that usually is not widely distributed within the genus.[12] Thus, secondary metabolite chemistry can serve as important tool for taxonomical studies in the genus *Laurencia* especially since many species appear morphologically very similar.[13]

As part of our ongoing program aiming at the isolation of biologically active compounds from marine organisms of the Greek seas,[14–16] we studied specimens of *Laurencia obtusa* (Hudson) J. V. Lamouroux and *Laurencia microcladia* Kützing, collected off the coasts of Serifos and Chios island, respectively. In this report, we describe the isolation and structure elucidation of three new metabolites (**1**–**3**) along with the known metabolites 7-hydroxylaurene (**5**), isolaurenisol (**6**), (*E*)-2-tridecyl-2-heptadecenal (**7**), perforenone A (**8**) and 3-epi-perforenone A (**9**), all of which were obtained from the non-polar fractions of the organic extract of *L. obtusa*. We also describe the isolation and structure elucidation of the new dimeric sesquiterpene of the cyclolaurane-type (**4**) along with laurinterol (**10**), the aromatic sesquiterpene **11** and bromolaurenisol (**12**) all of which were obtained from the organic extract of *L. microcladia*. Metabolite **7** was found in both *Laurencia* species (Fig. 1).

Assessment of cytotoxicity was performed on CHO cells (ovaries biopsy, Chinese hamster) and the following human tumor cell lines: K562 (a chronic myelogenous leukemia cell line), MCF7 (derived from a mammary adenocarcinoma), PC3 (derived from a prostate adenocarcinoma), HeLa (derived from cervix adenocarcinoma) and A431 (derived from epidermoid carcinoma). The cytotoxicity of metabolites **4**, **10**, **11** and **12** was also examined against the lung cancer cell lines A549 and NSCLC-N6.

Keywords: *Laurencia obtusa*; *Laurencia microcladia*; Sesquiterpenes; Cytotoxic activity.
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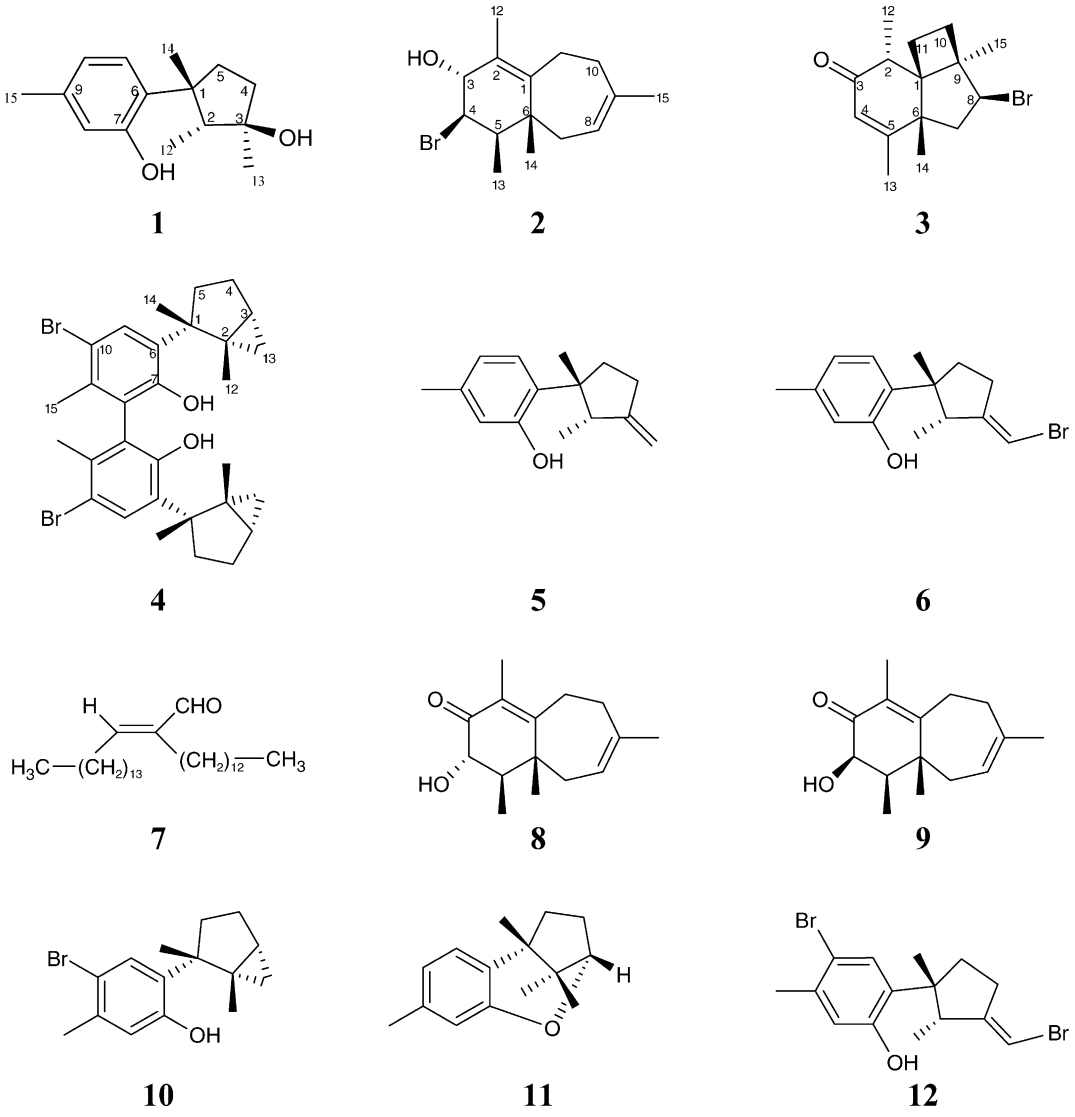

Figure 1. Metabolites isolated from *L. obtusa* (**1**–**3**, **5**–**9**) and *L. microcladia* (**4**, **7**, **10**–**12**).

2. Results and discussion

L. obtusa was collected from the island of Serifos and the CH_2Cl_2/MeOH extract of the freeze-dried alga was subjected to a series of vacuum column chromatography (VCC) on silica gel and normal phase high pressure liquid chromatography (HPLC), using mixtures of cyclohexane/EtOAc as mobile phase, to yield compounds **1**–**3** and **5**–**9** in pure form. *L. microcladia* was collected from the island of Chios and similar purification protocols led to the isolation of metabolites **4**, **7** and **10**–**12**.

Compound **1**, after HPLC purification, was isolated as a colorless oil. The molecular formula $C_{15}H_{22}O_2$ was deduced from HRFAB-MS data in combination with the NMR spectra (Tables 1 and 2). The ^{13}C NMR and DEPT experiments allowed the determination of five quaternary, four methine, two methylene and four methyl carbon atoms. The ^{1}H and ^{13}C NMR spectra displayed resonances for a secondary methyl ($\delta_{H/C}$ 0.76/7.4), one aromatic methyl ($\delta_{H/C}$ 2.24/21.0), two quaternary methyls ($\delta_{H/C}$ 1.32/20.6 and $\delta_{H/C}$ 1.38/23.1), three aromatic protons ($\delta_{H/C}$ 6.51/115.7, $\delta_{H/C}$ 6.62/120.6 and $\delta_{H/C}$ 6.96/124.8), one methine ($\delta_{H/C}$ 1.48/46.6) and two methylenes ($\delta_{H/C}$ 2.04, 1.76/37.3 and $\delta_{H/C}$ 1.63, 1.88/42.3). A strong IR absorption band at 3590 cm^{-1} and ^{13}C NMR signals at δ_C 153.0 (C-7) and δ_C 85.1 (C-3) indicated that both oxygen atoms were in hydroxyl groups. With five degrees of unsaturation, the structure was suggested to contain, besides the aromatic an additional five-membered ring. The correlation in the HMBC experiments, between H-14 (δ_H 1.32) with C-1 (δ_C 44.7) and the aromatic carbon C-6 (δ_C 127.4) confirmed the position of H-14 on C-1. The correlation of signals at δ_H 0.76 (H-12) with 44.7 (C-1), 46.6 (C-2) and 85.1 (C-3) as well as that at δ_H 1.38 (H-13) with 46.6 (C-2), 85.1 (C-3) and 37.3 (C-4) secured the position of the methyls H-12, H-13 on C-2, C-3, respectively. The relative stereochemistry of **1** was assigned on the basis of NOESY experiments. The strong NOE correlations between H-14/H-2, H-14/H-5β and H-12/H-13, H-12/H-5α, H-13/H-4α determined the stereochemistry at C-1, C-2 and C-3 (Fig. 2). In view of the above-mentioned data, metabolite **1** was named 3,7-dihydroxy-dihydrolaurene.

Table 1. ^{1}H NMR and ^{1}H–^{1}H COSY data of compounds **1–4**

No	**1**		**2**		**3**		**4**	
	^{1}H (δ)	COSY	^{1}H (δ)	COSY	^{1}H (δ)	COSY	^{1}H (δ)	COSY
1	—		—		—		—	
2	1.48 (q, 6.7)	H-12	—		2.58 (q, 7.0)	H-12	—	
3	—		4.15 (dd, 6.2, 5.8)	OH-C-3, H-4	—		1.09 (m)	H-13, H-4a
4	2.04 (−α) (m)	H-5α	4.48 (dd, 5.5, 3.4)	H-3, H-5	5.86 (s)		1.91 (−a) (m)	H-3, H-4b, H-5β
	1.76 (−β) (m)	H-5α, H-5β					1.61 (−b) (m)	H-4a, H-5α
5	1.63 (−α) (m)	H-4α, H-4β	1.96 (m)	H-4, H-13	—		1.23 (−α) (m)	H-4b, H-5β
	1.88 (−β) (m)	H-4β					2.18 (−β) (m)	H-4a, H-5α
6	—		—		—		—	
7	—		2.11 (−α) (m)	H-7β, H-8	2.24 (−α) (dd, 13.0, 13.0)	H-7β, H-8	—	
			2.00 (β) (m)	H-7α, H-8	2.44 (-β) (dd, 13.0, 6.0)	H-7α, H-8		
8	6.51 (br s)	H-10	5.28 (m)	H-7α, H-7β	3.72 (ddd, 13.0, 6.0, 1.0)	H-7α, H-7β	—	
9	—		—		—		—	
10	6.62 (d, 7.9)	H-11, H-8	2.02 (-α) (m)	H-10β, H-11α, H-11β	1.61 (-α) (ddd, 13.0, 6.0, 1.0)	H-10β, H-11α, H-11β	—	
			2.18 (-β) (m)	H-10α, H-11α, H-11β	2.13 (-β) (dd, 4.4, 2.4)	H-10α, H-11α, H-11β		
11	6.96 (d, 7.9)	H-10	2.40 (-α) (m)	H-10α, H-10β, H-11β	1.80 (-α) (m)	H-10α, H-10β, H-11β	7.80 (1H, s)	OH-C-7
			2.20 (-β) (m)	H-10α, H-10β, H-11α	1.95 (-β) (ddd, 13.0, 13.0, 4.4)	H-10α, H-10β, H-11α		
12	0.76 (d, 6.7)	H-2	1.80 (s)		1.45 (d, 6.8)	H-2	1.31 (3H, s)	
13	1.38 (s)		1.10 (d, 6.8)	H-5	1.88 (s)		0.53 (2H, m)	H-3
14	1.32 (s)		1.07 (s)		1.08 (s)		1.36 (3H, s)	
15	2.24 (s)		1.66 (s)		1.02 (s)		2.00 (3H, s)	
–OH			1.76 (m)	H-3			4.74 (s)	H-11

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm. *J* values in parentheses are in Hz.

Table 2. ^{13}C NMR and HMBC data of compounds **1–4**

No	**1**		**2**		**3**		**4**	
	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC
1	44.7	H-12, H-14	141.9	H-12, H-14	56.0	H-2	48.5	H-4b, H-11, H-12, H-14
2	46.6	H-12, H-13	124.6	H-12	42.1	H-12	29.4	H-4b, H-12, H-14
3	85.1	H-12, H-13	74.6		199.7	H-2, H-4, H-12	24.0	H-12
4	37.3	H-13	62.4		128.3		25.2	
5	42.3		39.9	H-13	162.0	H-4, H-13	35.2	H-14
6	127.4	H-14	45.5	H-14	50.2	H-14	135.1	H-14
7	153.0	H-8, H-11	39.4	H-8	43.7		151.9	OH-C-7, H-11
8	115.7		120.6		60.5		121.7	OH-C-7, H-11, H-15
9	137.1		138.4	H-8, H-15	50.0	H-2, H-15	134.6	OH-C-7, H-11, H-15
10	120.6		33.7		26.3		115.7	H-11, H-15
11	124.8		24.5		21.7		133.2	H-11
12	7.4		16.3		9.1		18.7	
13	23.1		14.0		18.9	H-4	16.1	H-12
14	20.6		22.0		16.3		22.3	
15	21.0	H-8, H-9, H-10	26.3	H-8	23.0		19.8	

All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm.

Compound **2** was purified by means of HPLC and was isolated as a pale yellow oil. Combination of its ^{13}C NMR data and HRFAB-MS measurements suggested a molecular formula of $C_{15}H_{23}OBr$. The LREI-MS peaks at *m/z* 298/300 [M^+], with relative intensities 1:1/Br^{79}:Br^{81}, indicated the presence of one bromine atom. The intense sharp absorption at ν_{max} 3647 cm^{-1} showed the presence of a hydroxyl functionality in the molecule. The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of 15 carbons corresponding to four quaternary, four methine, three methylene and four methyl carbon atoms. Among the carbons, one resonating at δ_C 74.6, was bonded to oxygen, one resonating at δ_C 62.4 was brominated and four were olefinic resonating at δ_C 141.9, 124.6, 138.4 and 120.6 ppm. Furthermore, the ^1H NMR spectrum revealed signals due to a halomethine proton at δ_H 4.48 (dd), a hydroxy methine at δ_H 4.15 (dd), one olefinic proton at δ_H 5.28 (m), one secondary methyl group at δ_H 1.10 (d), two vinyl methyl groups at δ_H 1.80 (s) and 1.66 (s) and one tertiary methyl group at δ_H 1.07 (s). With four degrees of unsaturation, the structure was suggested to contain, besides the two double bonds, two rings. All protonated carbons and their protons were assigned by the COSY and HMQC experiments. The structure elucidation was assisted by analyses of the HMBC experiments. The position of the conjugated double bond at $\delta_{1,2}$ was confirmed by correlations between H-12/C-1, H-12/C-2 and H-14/C-1. The position of the olefinic proton at C-8 was determined by correlations between H-8/C-15, H-8/C-9 and H-8/C-7. Moreover, the correlations between H-12 and C-2, H-13 and C-5, H-14 and C-6 and H-15 and C-9, confirmed the positions of the methyl groups. The stereochemical configuration of the asymmetric centers is proposed on the basis of NOE enhancements. The strong NOE correlations between H-3, H-13 and H-14 showed the *cis* orientation of H-3 and confirmed the axial configuration of H-13 and H-14. Moreover the NOE correlation between H-5 and the proton of the hydroxyl group supported the stereochemistry at C-3 and C-5 (Fig. 2). Comparison of spectral data (Tables 1 and 2) for **2** with literature values,[14] suggested that metabolite **2** possessed the skeleton of perforenol. Consequently, metabolite **2** was named perforenol B.

Compound **3** after HPLC purification, was isolated as a colorless oil. The HRFAB-MS measurements supported the molecular formula $C_{15}H_{21}OBr$ and the $[M+1]^+$ peaks at *m/z* 297 and 299 with equal intensities, revealed the presence of one bromine atom in the molecule. The presence of a carbonyl group was indicated by the strong IR absorption at 1716 cm^{-1}. The ^{13}C NMR spectrum of **3** (Table 2) exhibited signals for 15 carbons, with the multiplicities of the carbon signals determined from the DEPT spectrum as: five quaternary, three methine, three methylene and four methyl carbon atoms. Among the carbons, one was a carbonyl, resonating at δ_C 199.7, two were olefinic resonating at δ_C 162.0 and 128.3 and one was brominated resonating at δ_C 60.5. Furthermore, the ^1H NMR spectrum revealed signals due to a halomethine proton at δ_H 3.72 (ddd), an olefinic proton at δ_H 5.86 (s), a secondary methyl group at δ_H 1.45 (d), a vinyl methyl group at δ_H 1.88

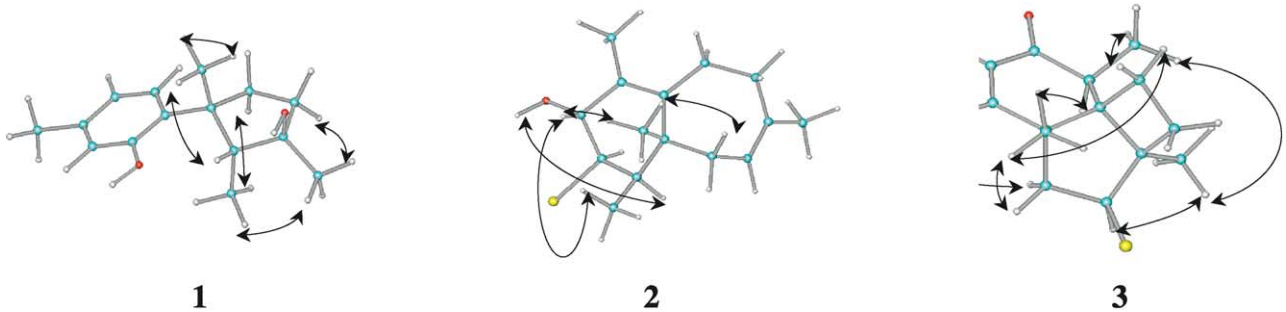

Figure 2. Stereochemical drawing and selected NOE correlations of metabolites **1–3**.

(s) and two tertiary methyl groups at δ_H 1.08 (s) and 1.02 (s). With an unsaturation degree of five, the structure was suggested to contain besides the carbonyl group, one double bond and three rings. Comparison of spectral data (Tables 1 and 2) with literature values suggested that metabolite **3** was related to a brominated sesquiterpene isolated earlier from *L. obtusa*.[14] Based on the correlations of carbonyl C-3 (δ_C 199.7) with H-2 (δ_H 2.58), H-4 (δ_H 5.86) and H-12 (δ_H 1.45) observed in the HMBC spectrum, the carbonyl group was placed on C-3. The position of the olefinic proton of the trisubstituted double bond at C-4 was determined from correlations between H-4/C-5, H-4/C-3 and H-4/C-13. The carbon bridge was positioned between carbons C-1 and C-9 on the basis of the correlation of H-2 (δ_H 2.58) with C-1 (δ_C 56.0) and C-9 (δ_C 50.0). Moreover the correlations of H-12 with C-2, H-13 with C-5, H-14 with C-6 and H-15 with C-9, confirmed the positions of the methyl groups. The relative stereochemistry of **3** was assigned using NOE experiments. The strong NOE correlation between H-2 and H-14 indicated the axial orientation of H-2 and H-14. The axial configuration of the methyl groups H-12, H-15 and the halomethine proton H-8 was supported by the NOE interactions between H-12/H-15 and H-15/H-8. Strong correlations of H-12 (δ_H 1.45) with H-11α (δ_H 1.80) and H-14 (δ_H 1.08) with H-11β (δ_H 1.95) suggested β configuration for C-1 and C-9 bridgehead carbons (Fig. 2). Consequently, the structure of metabolite **3** was established as (1*S**, 2*R**, 6*R**, 8*S**, 9*R**)-8-bromo-2,5,6,9-tetramethyltricyclo-[7.2.0.0^{1,6}]undec-4-en-3-one.

Compound **4** was purified by means of HPLC separation and was isolated as a colorless oil. Both ^{13}C NMR data and HRFAB-MS measurements supported the molecular formula of $C_{30}H_{36}O_2Br_2$. The LREI-MS showed $[M]^+$ peaks at *m/z* 586, 588, 590 with intensities 1.0/2.0/1.0, indicating the presence of two bromine atoms. The presence of a hydroxyl group was evident from the IR band at 3428 cm^{-1}. The ^{13}C NMR spectrum of **4** (Table 2) showed signals for 15 carbons, with the multiplicities of the carbon signals determined from the DEPT spectrum as: seven quaternary, two methine, three methylene and three methyl carbon atoms. The ^{1}H and ^{13}C NMR spectra displayed resonances for one aromatic proton ($\delta_{H/C}$ 7.80/133.2), an aromatic methyl group ($\delta_{H/C}$ 2.00/19.8), two tertiary methyl groups ($\delta_{H/C}$ 1.31/18.7 and 1.36/22.3), cyclopropyl protons ($\delta_{H/C}$ 0.53/16.1), one methine ($\delta_{H/C}$ 1.09/24.0), two methylenes ($\delta_{H/C}$ 1.91, 1.61/25.2 and 1.23, 2.18/35.2), one exchangeable proton (δ_H 4.74) and six aromatic carbons (δ_C 115.7, 121.7, 133.2, 134.6, 135.1, 151.9). In the ^{1}H–^{1}H NOESY spectrum of **4**, H-12 (δ_H 1.31) and H-14 (δ_H 1.36) protons showed correlations with H-5β (δ_H 2.18). Moreover, the aromatic proton H-11 at δ_H 7.80 showed correlation with H-13 (δ_H 0.53) and H-13 showed correlation with H-3 (δ_H 1.09). The spectral data of **4** were similar to laurinterol,[17,18] but on the basis of the ^{13}C NMR, the absence of the second aromatic proton and the molecular formula, compound **4** was suggested to be a dimeric sesquiterpene. Compound **4** is biogenetically derived by *ortho* coupling of the phenolic rings of two laurinterol molecules. The strong correlation of H-11 (δ_H 7.80) with C-1 (δ_C 48.5), C-6 (δ_C 135.1), C-10 (δ_C 115.7) and C-7 (δ_C 151.9), observed in HMBC, supported the *ortho* coupling.

Compound **5** was purified by HPLC as a colorless oil and identified by comparison of its NMR spectra with literature values as being 7-hydroxylaurene.[19] Metabolite **5** is reported for the first time as *L. obtusa* metabolite.

Compound **6** after purification by HPLC was isolated as a colorless oil and identified by comparison of its NMR spectra with literature values as being isolaurenisol.[20]

Compound **7** was isolated as a colorless oil following HPLC purification and identified on the basis of its NMR, MS spectra and comparison with previously reported data as being (*E*)-2-tridecyl-2-heptadecenal.[21] Compound **7** is an unexpected metabolite and is reported for the first time as *L. obtusa* and *L. microcladia* metabolite.

Compound **8** after purification by HPLC was isolated as a colorless oil and identified by comparison of its NMR spectra with literature values as being perforenone A.[22]

Compound **9** was isolated as a colorless oil following HPLC purification and identified on the basis of its NMR, MS spectra and comparison with previously reported data as being 3-epi-perforenone A.[1] This metabolite is isolated for the first time from *L. obtusa*.

Compound **10** was purified following chromatographic separations and was isolated in large amounts as a crystalline compound. It was identified by comparison of its NMR spectra with previously reported data as being laurinterol.[17] Laurinterol was found to be the major component of the extract and this is the first report of laurinterol from *L. microcladia*.

Compound **11** was isolated as a colorless oil following HPLC purification and was identified on the basis of its NMR, MS spectra and comparison with previously reported data.[23] This is the first report of **11** from *L. microcladia*.

Compound **12** after purification by HPLC was isolated as a colorless oil and identified by comparison of its NMR spectra with literature values as being bromolaurenisol.[24] Metabolite **12** is reported for the first time as a *L. microcladia* metabolite.

Metabolites **1–3** and **5–12** were evaluated for their cytotoxicity against five human tumor cell lines and CHO. The results of the cytotoxic activity of the tested-compounds after 48 h of incubation are given in Table 3. Compounds **2**, **5**, **7** and **10** were consistently more potent as cytotoxic agents for most of the cell lines tested whereas compounds **3**, **8** and **9** exhibited the lowest activity (IC_{50} more than 300 μM) against the K562 and the CHO cell lines. CHO, an epithelial-like cell line, showed the lowest sensitivity in the presence of all tested compounds with IC_{50} values consistently higher in all cases (Table 3).

Comparison of the activities exhibited by metabolites **2**, **8** and **9**, which posses many structural similarities, indicated that the presence of the bromine atom in the molecule, greatly enhanced the activity against all cell lines.

Table 3. In vitro cytotoxic activities of metabolites **1–3** and **5–12**

Compound	Cell line					
	K562	MCF7	PC3	HeLa	A431	CHO
	IC_{50} (μM)					
1	>300	201.7	182.3	121.3	176.4	234.7
2	67.4	28.2	54.8	50.9	73.2	80.2
3	>300	>300	126.2	111.3	137.1	>300
5	64.2	15.8	18.1	40.5	23.9	78.2
6	127.4	95.5	103.2	88.6	122.0	165.5
7	82.7	51.4	71.6	51.8	45.8	107.6
8	>300	>300	138.3	117.7	105.1	>300
9	>300	>300	144.4	154.2	151.9	>300
10	128.3	67.2	76.6	83.9	74.6	165.8
11	>200	>200	>200	>200	>200	>200
12	112.7	78.3	92.4	105.8	81.6	>200

For clarity, SEM values are not included in the table, but they were in all cases less than 5% of the mean.

The much higher activity of metabolite **5** compared to those of **1** and **6** indicates that the presence of *exo*-methylene moiety is essential for the promotion of the activity.

Metabolites **10** and **12** showed similar levels of activity which were higher than those expressed by **11**, indicating that the cyclopropyl ring or the trisubstituted double bond are not essential for the cytotoxicity of the molecule but, the absence of bromine or hydroxy functionalities can greatly reduce the activity.

Moreover, the cytotoxicity of compounds **4**, **10**, **11** and **12** was assayed against NSCLC-N6 and A549 lung cancer cell lines. Metabolites **4** and **11** showed only moderate cytotoxicity: $IC_{50}=153.5$ μM (A549) and 150.5 μM (NSCLC-N6), respectively. Metabolite **10** showed no activity against the cancer cell lines, whereas metabolite **12** showed stronger levels of activity with: $IC_{50}=26.5$ μM (NSCLC-N6). The lower activity observed for **10** compared to metabolite **11** indicated that the bromine on the aromatic ring is not significantly important for cytotoxicity expression. The presence of the trisubstituted double bond on the five-membered ring seems to greatly enhance the cytotoxicity of this class of compounds against the NSCLC-N6 cell line.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a Perkin-Elmer model 341 polarimeter and a 10 cm cell. UV spectra were determined in spectroscopic grade *n*-C_6H_{14} and CH_2Cl_2 on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained using a Paragon 500 Perkin-Elmer spectrophotometer. NMR spectra were recorded using a Bruker AC 200 and a Bruker DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard. The 2D experiments (^1H–^1H COSY, HMQC, HMBC, NOESY) were performed using standard Bruker microprograms. High-resolution mass spectra data were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, IN, USA. EIMS data were recorded on a Hewlett Packard 5973 Mass Selective Detector. VCC separation was performed with Kieselgel 60H (Merck), TLC were performed with Kieselgel 60 F_{254} aluminum support plates (Merck) and spots were detected with 15% H_2SO_4 in MeOH reagent. HPLC separation was conducted using a Pharmacia LKB 2248 model equipped with a refractive index detector RI GBC LC-1240 and a Spherisorb HPLC normal phase column, 25 cm×10 mm, S10W, 64,340 plates/m.

3.2. Plant material

L. obtusa was collected by hand at Koutalas bay, Serifos island in the Central Aegean Sea, Greece, at a depth of 0.5–1 m in August of 2001. *L. microcladia* was collected by hand at Vroulidia bay, Chios island in the North Aegean Sea, Greece, at a depth of 0.5–1 m in May of 2002. Specimens are kept at the Herbarium of the Laboratory of Pharmacognosy and Chemistry of Natural Products, University of Athens (*L. obtusa* ATPH/MO/150 and *L. microcladia* ATPH/MO/151).

3.3. Extraction and isolation

L. obtusa was initially freeze-dried (187.4 g dry weight) and then exhaustively extracted with mixtures of CH_2Cl_2–MeOH (3/1) at room temperature. The extract after evaporation of the solvents afforded a red-green oily residue (3.45 g). The crude extract was subjected to vacuum column chromatography (VCC) on silica gel, using cyclohexane with increasing amounts (10%) of EtOAc as mobile phase and finally MeOH. Fractions B (20% EtOAc in cyclohexane) (215.7 mg) and C (30% EtOAc in cyclohexane) (413.4 mg) were combined and further purified by VCC on silica gel using cyclohexane with increasing amounts (2%) of EtOAc as mobile phase. From fraction II (8% EtOAc) (142.1 mg), 44.8 mg were subjected to normal phase HPLC chromatography, using as mobile phase cyclohexane–EtOAc (97.5/2.5) to yield pure compounds **5** (2.4 mg) and **6** (1.3 mg). The rest 97.3 mg of fraction II were subjected to normal phase HPLC chromatography, using as mobile phase cyclohexane–EtOAc (99/1) to yield pure compounds **1** (9.0 mg) and **7** (3.4 mg). Fraction IV (12% EtOAC) (75.5 mg) was subjected to normal phase HPLC chromatography, using as mobile phase cyclohexane–EtOAc (95/5) to yield pure compounds **2** (5.2 mg), **3** (7.7 mg) and **9** (4.8 mg). Fraction V (14% EtOAc) (53 mg) was subjected to normal phase HPLC chromatography, using as mobile

phase *n*-hexane–EtOAc (90/10) to yield pure compound **8** (2.2 mg).

L. microcladia was initially freeze-dried (291.4 g dry weight) and then exhaustively extracted with mixtures of CH_2Cl_2–MeOH (3/1) at room temperature. The extract was concentrated to give a dark green residue (12.0 g), which later was subjected to vacuum column chromatography (VCC) on silica gel, using cyclohexane with increasing amounts (10%) of EtOAc as mobile phase and finally MeOH. Fraction D (30% EtOAc in cyclohexane) (2.8 g) was further purified by gravity column on silica gel using cyclohexane with increasing amounts (1%) of EtOAc as mobile phase. Fraction III (4% EtOAc) (4.4 mg) was subjected to normal phase HPLC chromatography, using as mobile phase *n*-hexane 100% to yield pure compound **4** (2.4 mg). Fraction IV (5% EtOAc) (420.0 mg) was subjected to normal phase HPLC chromatography, using as mobile phase *n*-hexane–EtOAc (99/1) to yield pure compounds **7** (0.5 mg), **10** (285.6 mg) and **11** (6.3 mg). Fraction V (6% EtOAc) (89.0 mg) was subjected to normal phase HPLC chromatography, using as mobile phase *n*-hexane–EtOAc (96/4) to yield pure compound **12** (11.3 mg).

3.3.1. Compound 1. Colorless oil; $[\alpha]_D^{20}$ −12.0 (*c* 0.15, CH_2Cl_2); UV $\lambda_{max}^{C_6H_{14}}$ (log ε): 203.8 (4.4), 231.0 (3.6), 287.0 (3.3) (nm); IR (CH_2Cl_2) ν_{max} 3590, 3097, 3027, 2987, 2930, 1500, 1449, 1260, 1161, 892, 873 cm^{-1}; HRFAB-MS (*m/z*): 235.1713 $[M+1]^+$ (calcd for $C_{15}H_{23}O_2$ 235.1703), $\Delta m/m=-42$ (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel. int.%): 216 $[M-H_2O]^+$ (71), 201 $[M-H_2O-H_3]^+$(100), 186 (37), 159 (55), 135 (9), 115 (13), 93 (17), 41 (8).

3.3.2. Compound 2. Pale yellow oil; $[\alpha]_D^{20}$ −36.7 (*c* 0.16, CH_2Cl_2); UV $\lambda_{max}^{C_6H_{14}}$ (log ε): 202.0 (3.7), 239.4 (3.1) (nm); IR (CH_2Cl_2) ν_{max} 3647, 3097, 3040, 2306, 1449, 1396, 1293, 884 cm^{-1}; HRFAB-MS (*m/z*): 299.1007 $[M+1]^+$ (calcd for $C_{15}H_{24}O^{79}Br$ 299.0933), $\Delta m/m=-247$ (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel. int.%): 298, 300 $[M]^+/[M+2]^+$ (5:5), 283, 285 (2:2), 269, 271 (16:16), 219 (53), 201 (68), 149 (100), 133 (79), 121 (47), 91 (37), 43 (21).

3.3.3. Compound 3. Colorless oil; $[\alpha]_D^{20}$ −40.4 (*c* 0.14 CH_2Cl_2); UV $\lambda_{max}^{C_6H_{14}}$ (log ε): 228.0 (4.1) (nm); IR (CH_2Cl_2) ν_{max} 3060, 2986, 1716, 1428, 1266, 1218, 892, 840 cm^{-1}; HRFAB-MS (*m/z*): 297.0849 $[M+1]^+$ (calcd for $C_{15}H_{22}O^{79}Br$ 297.0856), $\Delta m/m=23$ (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel. int.%): 297, 299 $[M+1]^+/[M+3]^+$ (8:8), 217 ($[M+1]^+-HBr$) (100), 199 (17), 189 (29), 131 (20), 107 (57), 93 (52), 67 (40), 39 (32).

3.3.4. Compound 4. Colorless oil; $[\alpha]_D^{20}$ −28.8 (*c* 0.06 CH_2Cl_2); UV $\lambda_{max}^{CH_2Cl_{14}}$ (log ε): 236.0 (3.3), 289 (3.0) (nm); IR (CH_2Cl_2) ν_{max} 3428, 2952, 2921, 2861, 1722, 1626, 1445 cm^{-1}; HRFAB-MS (*m/z*): 586.1082 $[M]^+$ (calcd for $C_{30}H_{36}O_2^{79}Br_2$ 586.1084), $\Delta m/m=3$ (ppm); NMR data ($CDCl_3$), see Tables 1 and 2; EIMS 70 eV, *m/z* (rel. int.%): 586, 588, 590 (30:60:30), 571, 573, 575 (31:62:31), 518, 520, 522 (52:100:51), 503, 505, 507 (28:55:30), 450, 452, 454 (13:26:13), 294, 296 (9:9), 279 (20), 109 (35), 67 (23).

3.4. Conditions of cell cultures

Cells were routinely grown as monolayer cell cultures in Dulbecco's Modified Eagle's Medium (containing 4.5 g glucose/l) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin in an environment of 37 °C, 85% humidity and 5% CO_2. Cells were passaged by trypsinisation 1–2 times a week to keep them in log phase. Chronic myelogenous leukaemia derived K-562 cells were grown as suspension culture in RPMI 1640 medium under the same conditions.

The NSCLC-N6 cell line,[25] derived from a human non-small-cell bronchopulmonary carcinoma (moderately differentiated, rarely keratinizing, classified as T2N0M0) was also used for evaluating the cytotoxicity of compounds. The cells were cultured in RPMI 1640 medium with 5% fetal calf serum. In these conditions, cell doubling time was 48 h. Cells used in all experiments never exceeded 35 passages.

3.5. Determination of cytotoxicity

Cells were seeded into 96-well plates (100 μl/well at a density of 1×10^5 cells/ml) and exposed to various concentrations of the compounds for 48 h. The cytotoxicity was determined with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay,[26] as previously modified.[27] Briefly, after incubation with the test compounds, MTT solution (5 mg/ml in PBS) was added (20 μl/well). Plates were further incubated for 4 h at 37 °C and the formazan crystals formed were dissolved by adding 100 μl/well of 0.1 N HCl in 2-propanol. Absorption was measured by an enzyme-linked immunosorbant assay (ELISA) reader at 545 nm, with reference filter at 690 nm. For each concentration at least nine wells were used from three separate experiments. One hundred microlitres of RPMI 1640 supplemented with the same amount of MTT solution and solvent was used as blank solution. Data obtained were presented as IC_{50} (μM), which is the concentration of the compound where $100\times(A_0-A)/A_0=50$. In this formula, A is the optical density of the wells after 48 h of exposure to test compound and A_0 is the optical density of the control wells.

Acknowledgements

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A new approach for the asymmetric syntheses of 2-*epi*-deoxoprosopinine and azasugar derivatives

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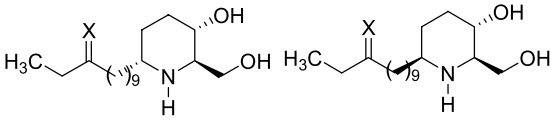
Dedicated to, Professor Ben-Li Huang on the occasion of his 80th birthday

Abstract—A new approach to 2-*epi*-deoxoprosopinine **11**, 1-deoxygulonojirimycin **7**, and L-gulono-1,5-lactam **9** was described. The C-2 hydroxymethyl group was introduced regioselectively using SmI_2 mediated coupling of (*S*)-3-silyloxyglutarimide **13b** with either chloromethyl benzyl ether **16a** or the Beau–Skrydstrup reagent **16b**, followed by debenzylation and highly *cis*-diastereoselective reductive deoxygenation. Adoption of the Savoi's chemoselective ring-opening alkylation method allowed a highly diastereoselective introduction of the lipid side chain of 2-*epi*-deoxoprosopinine **11** in a straightforward manner. Dehydration followed by highly *trans*-diastereoselective dihydroxylation led to polyoxygenated lactam derivative **27** as a key intermediate for the syntheses of **7** and **9**.

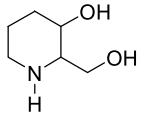
1. Introduction

2-Hydroxymethyl-3-piperidinol **1** is a salient structural feature found in two classes of natural products, namely, piperidine alkaloids[1] and azasugars (or iminosugars).[2] Several 2,3,6-trisubstituted piperidine alkaloids (e.g., prosopinine **2** and prosophylline **3**) have been isolated from the leaves of the West African savanna tree *Prosopis africana* Taub[3] and from the leaves of *Microcos philippinensis* (Perk) Burrett (*Tiliaceae*).[4] These alkaloids and their deoxygenated analogs (e.g., deoxoprosopinine **4** and deoxoprosophylline **5**) exhibit antibiotic, anesthetic, analgesic, and CNS stimulating properties, and are of considerable pharmaceutical interest.[5] Many synthetic approaches have thus been developed.[6,7] Moreover, many azasugars are inhibitors of glycosidases and related enzymes, showing therapeutic potential for the treatment of diseases related to metabolic disorders such as diabetes, cancer, AIDS, and viral infections. For example, 1-deoxymannojirimycin (DMJ, **6**) is a mannosidase inhibitor;[8] 1-deoxygulonojirimycin[9–12] (DGJ, **7**) is a potent and selective inhibitor of fucosidases,[12e–g] which was isolated from the back of *Angylocalyx pynaertii* (*Leguminosae*);[9] D-mannonolactam (**8**) is a powerful inhibitor of rat epididymal α-mannosidases, and of apricot β-glucosidase;[13] other δ-lactams have been shown to be glycosidase inhibitors.[14] As a result, the synthesis of azasugars and their synthetic analogs has attracted a great deal of attention.[12,15,16]

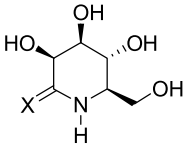


prosopinine **2.** X=O
deoxoprosopinine **4.** X=2H

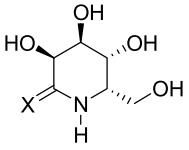
prosophylline **3.** X=O
deoxoprosophylline **5.** X=2H



1



1-deoxymannojirimycin **6.** X=2H
D-mannonolactam **8.** X=O



L-1-deoxygulonojirimycin **7.** X=2H
L-gulono-1,5-lactam **9.** X=O

As the part of our ongoing project aimed at the development of 3-hydroxyglutarimides-based synthetic methodology,[17] we wish to report herein a versatile approach to (2*S*,3*S*,6*R*)-2-*epi*-deoxoprosopinine **11**,[18] L-1-deoxygulonojirimycin **7**,[12] and L-gulono-1,5-lactam **9**.[16]

Keywords: Piperidine; Alkaloid; Hydroxymethylation; Diastereoselective reaction; Regioselective reaction; Building block.
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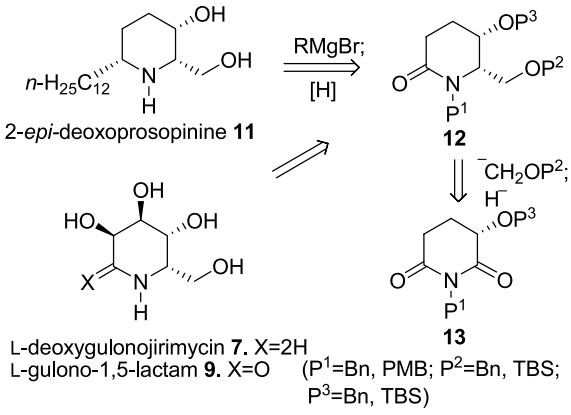
2. Results and discussion

As depicted retrosynthetically in Scheme 1, our approach featured, firstly, the use of protected (5*S*,6*S*)-5-hydroxy-6-hydroxymethyl-2-piperidinone **12** as a common intermediate to **11**, **9**, and **7**. The presence of the amide functionality would not only allow the introduction of the C-6 side chain of the *Prosopis* alkaloids as well as the two hydroxyl groups of L-gulono-1,5-lactam **9** and 1-deoxygulonojirimycin **7**, but also allow its transformation into cyclic amidine sugars, which demonstrated good to excellent inhibition toward glycosidases.[19] The second feature of the approach was the installation of the hydroxymethyl group by a stepwise regio- and diastereo-selective reductive hydroxymethylation procedure (**13**→**12**). The introduction of the C-6 side chain in the piperidine ring (**12**→**11**) by a straightforward method constituted the third feature of the approach.

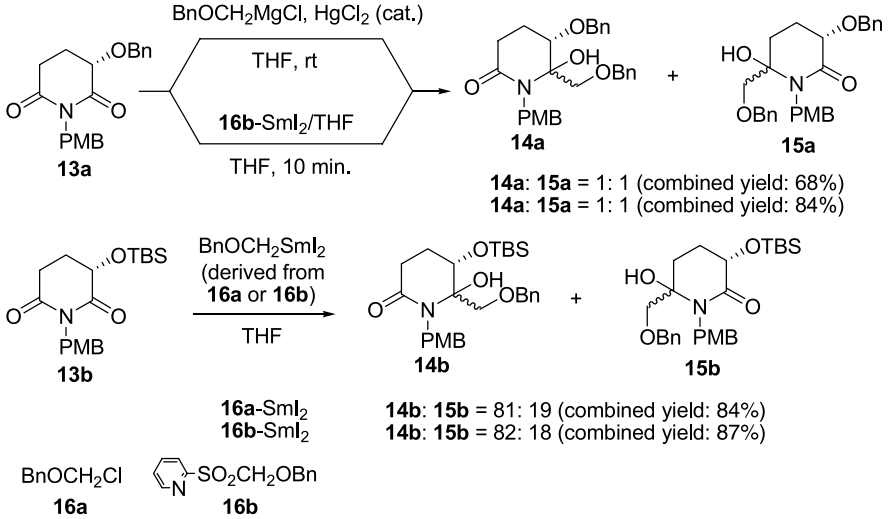


Scheme 1.

Our first task was the introduction of a hydroxymethyl group[20,21] to the C-2 position of protected 3-hydroxyglutarimides **13**. Although the stepwise reductive alkylation of **13a** with un-functionalized Grignard reagents has been demonstrated to proceed with high C-2 regioselectivity and high *trans*-diastereoselectivity,[17a,c] considerable difficulties were encountered in attempts to introduce a protected hydroxymethyl group in a regio- and diastereo-selective manner. For example, in the presence of a catalytic amount of mercury chloride(II),[22] addition of benzyloxymethyl magnesium chloride, derived from commercially available benzyl chloromethyl ether (**16a**) with **13a**, yielded two regioisomers **14a** and **15a** in disappointing 1:1 ratio (Scheme 2). Reaction of the Beau–Skrydstrup benzyloxymethylation reagent[23] **16b** with **13a** gave once again a 1:1 regioisomeric mixture, albeit in higher combined yield (84%).

After several unsuccessful attempts, including performing the reaction at lower temperature and using less reactive chloromethyl benzyl ether (**16a**)—SmI_2[20c–f] as a benzyloxymethylation system, we were delighted to find that the benzyloxymethylation of *O*-*tert*-butyldimethylsilyl protected 3-hydroxyglutarimide **13b** provided better results. Thus, when a 1:1.2 mixture of glutarimide **13b** and benzyl chloromethyl ether **16a** was treated with 3 molar equiv of a freshly prepared solution of SmI_2 (0.1 M in THF) at rt for 10 min, the C-2 addition product **14b** and the C-6 addition regioisomer **15b** were obtained in a ratio of 81:19 with a combined yield of 84%. Similar treatment of **13b** with the Beau–Skrydstrup reagent **16b** gave similar C-2/C-6 regioselectivity (82:18) and slightly higher combined yield (87%).

The observed protecting group effect (TBS vs Bn) on the regioselectivity of the SmI_2 mediated Barbier type benzyloxymethylation of **13a/13b** was contrary to the Grignard reagents addition to malimides, where the *O*-benzyl protected malimide gave excellent C-2 regioselectivity, whereas the *O*-TBS protected malimide gave nearly 1:1 C-2/C-5 regioselectivity.[24] The regioselectivity observed during the benzyloxymethylation of **13b** might be attributed to the oxyphilicity of the samarium(III) species, which formed a five-membered chelating structure, and polarized the Si–O bond, thus rending the Si/I interaction via a four-membered chelating structure plausible (Fig. 1). In such a manner, the steric hindrance of the TBS group was recompensed and consequently the C-2 carbonyl was activated.



Scheme 2.

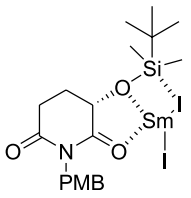
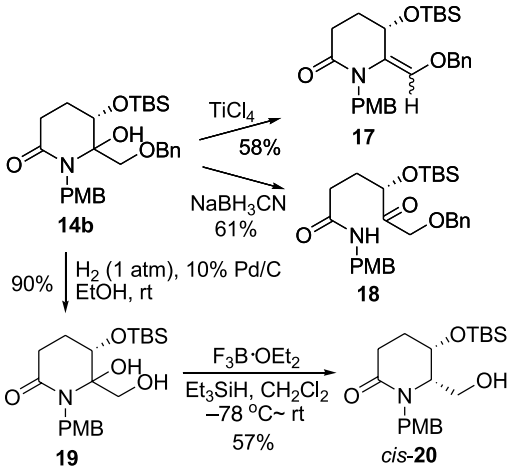


Figure 1.

With the *N*,*O*-acetal **14b** in hand, we then investigated its reductive deoxygenation. Much to our surprise, when **14b** was subjected to ionic hydrogenation conditions ($BF_3 \cdot OEt_2$, Et_3SiH, CH_2Cl_2, −78 °C to rt), only the starting material was recovered (Scheme 3). Even after heated to 40 °C for 3 days, only 5% of the desired product was isolated. Other ionic hydrogenation conditions led to either dehydrated product **17** ($TiCl_4$, Et_3SiH, −78 °C to rt)[25] or ring-opening product **18** ($NaBH_3CN$, at pH 3, rt).[26] It was envisioned that the failure to perform the reductive deoxygenation might be due to the steric hindrance of the *N*,*O*-acetal **14b**. To test this hypothesis, the benzyl group was cleaved (1 atm H_2, 10% Pd/C, rt, 8 h), and the resulted *N*,*O*-semiacetal **19** was subjected to $BF_3 \cdot OEt_2$ mediated Et_3SiH reduction. In such a manner, the desired product **20** was obtained in 57% yield, with a diastereoselectivity of 93:7. The stereochemistry of the major diastereomer **20** was assigned as *cis*[12,15,16,18,27] by comparing with the known (5*S*,6*R*)-*trans*-**20**.[28]



Scheme 3.

The stereochemical course of the reductive deoxygenation of **19** deserved comments. The fact that the reductive deoxygenation of a diastereomeric mixture of **19** led to preponderant formation of a diastereomer, implicating that the transformation involved an *N*-acyliminium intermediate[17] (**A**/**B**) (Fig. 2). Among the two possible conformers **A** and **B**, $A^{1,2}$ interaction and $A^{1,3}$ interaction[29] existed in the conformers **A** and **B**, respectively. Possible interactions between and *F*∼*Si* would compensate for the unfavorable $A^{1,2}$ interaction existed in **A**, rending thus conformer **A** the more favored one. Subsequent nucleophilic attack of a hydride was expected to take place from the axial direction owing to the stereoelectronic effects.[30] Thus, starting from the more stable conformer **A**, a hydride approached from the β-face of the ring, leading to the more stable chair conformer (*cis*-**20**).

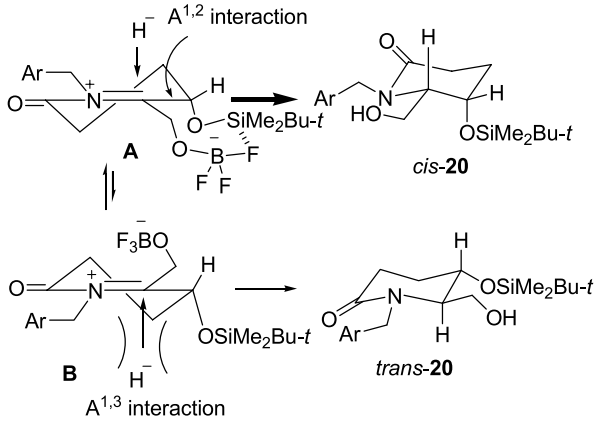
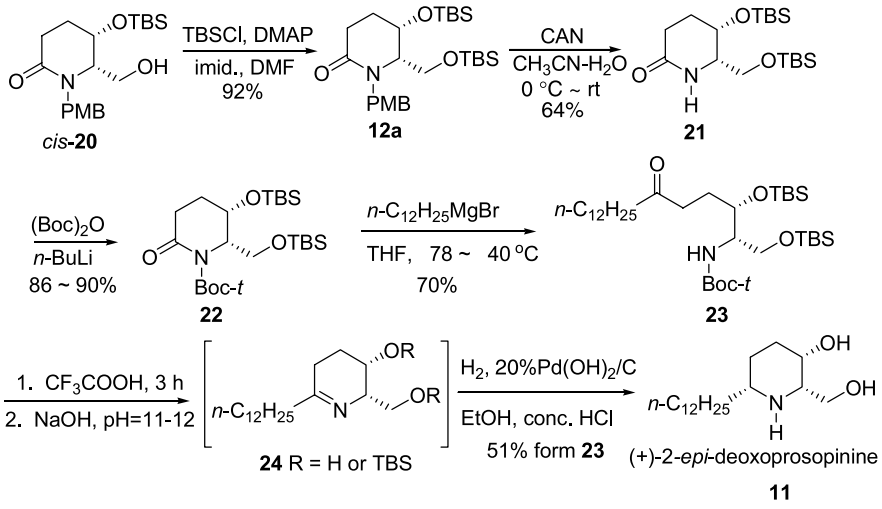


Figure 2.

The next stage for the synthesis of 2-*epi*-deoxoprosopinine **11** was the introduction of the C-6 side chain in a *cis*-diastereoselective manner. Most known methods for the *cis*-diastereoselective installation of a C-6 side chain in a 2-substituted piperidine ring involve α-amidoallylation of an appropriate piperidine *N*,*O*-acetal with allyltrimethylsilane, followed by multistep chain elongation manipulation.[7f–h] A direct method, however, was required to prepare the nucleophilic allylic silane C_{12} side chain in several steps.[7a] Keeping in mind that the 2,6-*cis*-stereochemistry was also a key structural feature found in prosophylline, micropine, cassine, spectaline, azmic acid, canavaline, and other 3-piperidinol alkaloids,[1] it was desirable to develop a flexible method enabling the diastereoselective introduction of different C-6 side chains in a straightforward manner.

In this context, Savoia's flexible organometallic ring-opening method[31] appeared to be promising. This turned out to be true, when *N*-Boc activated δ-lactam **22**, prepared from *cis*-**20** via successive *O*-silylation (TBSCl, DMAP, imid., DMF, rt, 12 h, 92%), CAN mediated cleavage of PMB group (CAN, MeCN/H_2O 9:1, 0 °C to rt, 4 h), and lactam activation ($(Boc)_2O$, *n*-BuLi, −78 °C, 0.5 h), was treated with a solution of *n*-$C_{12}H_{25}MgBr$ in THF at −78 °C for 3 h, then at −40 °C for 40 min, the desired ketone **23** was obtained in 70% yield based on the recovered starting material (81% conversion) (Scheme 4). *N*-Deprotection with TFA, followed by treating with a 30% NaOH solution led to cyclic imines **24** (containing partially desilylated products), which, without purification, was hydrogenated (H_2, 1 atm, 20% $Pd(OH)_2$, EtOH/concd HCl 10:1, rt, 30 h)[7j,s] under acidic conditions to provide (+)-2-*epi*-deoxoprosopinine **11** (51% overall yield from **23**) as a single diastereomer {$[\alpha]_D^{20}+3.0$ (*c* 0.6, CH_3OH); lit.[18] $[\alpha]_D^{26}+2.7$ (*c* 1.0, CH_3OH)}. The spectroscopic and physical data of the synthetic product were identical with those reported.[18] Noteworthy was that starting from the keto-amide **23**, the deprotection of *t*-Boc and two TBS protective groups, cyclisation, and hydrogenation were accomplished without chromatographic separation of the intermediates. The stereochemistry of the newly formed chiral center in **11** was ascertained by comparing with the known compound.[18]

As regarding the stereochemical course[71] of the reaction, in the light of the preponderant formation of the *cis*-isomer, it was reasonable to assume that among two possible



Scheme 4.

conformers **C** and **D** (Fig. 3), conformer **C** predominated over **D** due to an intramolecular hydrogen bond. The stereoelectronic controlled axial attack of hydrogen occured from the β-face of **C** leading to the desired product **11** with chair conformation, where the formation of intramolecular hydrogen bonds was possible.

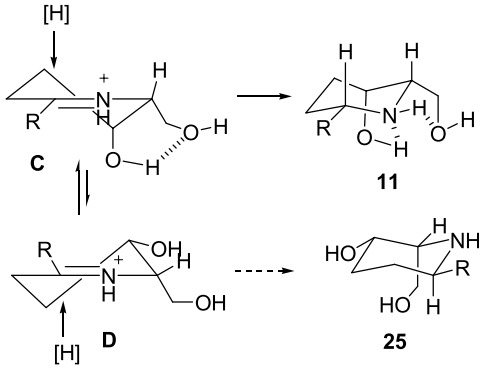
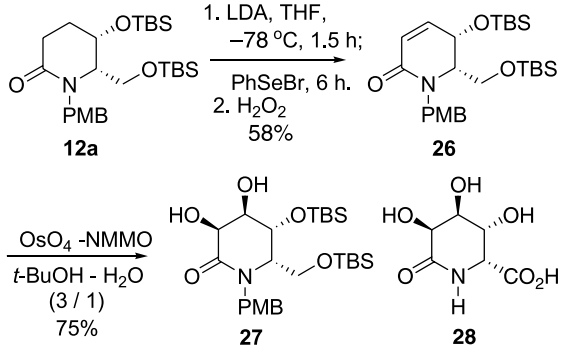


Figure 3.

Having accomplished the synthesis of (+)-2-*epi*-deoxoprosopinine, we then turned our attention to explore an entrance to **7** and **9**. Thus, successive treatment of lactam **12a** with LDA (THF, −78 °C) and phenylselenium bromide yielded the α-phenylselenide derivative of **12a**,[32] which without purification, was subjected to H_2O_2 oxidation in wet CH_2Cl_2 at rt to give directly **26** in 58% overall yield from **12a** (Scheme 5).



Scheme 5.

Diastereoselective dihydroxylation[11b,15d,33] of **26** was achieved by treating with OsO_4 (cat)-NMMO system in a mixed solvent system (*t*-BuOH-H_2O 3:1, rt, 3 h) to give **27** (yield: 75%) as the only isolable product. Lactam **27** can be easily converted, via deprotection and/or reduction, to azasugars **7**, **9** and other analogous, such as (2*R*,3*R*,4*S*,5*S*)-trihydroxypipecolic acid δ-lactam derivative **28**[2→b], according to the known procedures.[11b,12g,15a,15e,27b]

3. Conclusion

In summary, taking advantages of the multifunctionality of the TBS protected 3-hydroxyglutarimide **13b** and the protecting group effect, we were able to introduce both the C-2 hydroxymethyl group and the C-6 side chain in good regioselectivity (C-2/C-6 81:19) and in excellent diastereoselectivities (C-2/C-3 93:7; C-2/C-6 100:0). Using a Grignard reagent as a carbon nucleophile to introducing the C-6 side chain made this method flexible, this would find applications in the synthesis of other piperidine alkaloids.

4. Experimental

4.1. General methods

Melting points are uncorrected. Optical rotations were recorded on an automatic polarimeter. IR spectra were recorded on a FT-IR spectrophotometer. NMR spectra were recorded in $CDCl_3$ (^1H at 500 MHz and ^{13}C at 125 MHz), and chemical shifts were expressed in parts per million (δ) relative to internal Me_4Si. HRESIMS spectra were recorded on a FTMS apparatus. Silica gel (300–400 mesh) was used for column chromatography, eluting (unless otherwise stated) with ethyl acetate/petroleum ether (PE) (60–90 °C) mixture. Dichloromethane, DMF, and diisopropylamine were distilled over calcium hydride under N_2. Ether and THF were distilled over sodium benzophenone ketyl under N_2.

4.1.1. (5*S*,6*S*)-5-(*tert*-Butyldimethylsilyloxy)-6-(hydroxymethyl)-1-(4-methoxybenzyl)piperidin-2-one (20). *Method 1*. To a mixture of **13b** (1.452 g, 4.0 mmol)

and benzyloxymethyl chloride **16a** (60% purity, 1.2 mL, ca. 12 mmol) in anhydrous THF (10 mL) was quickly added a freshly prepared THF solution of SmI_2[34] (12 mmol, 120 mL) under an argon atmosphere at rt. After stirring for 45 min at rt, saturated NH_4Cl (10 mL) was added. The clear solution was poured into ether (200 mL), and the solid was washed successively with ether (30 mL×3). The combined organic layers were washed with saturated aqueous NH_4Cl (30 mL×3) and brine (30 mL×3), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **14b** (*C*-2 addition regioisomer, 1.319 g, yield: 67%) and **15b** (*C*-5 addition regioisomer, 304 mg) both as inseparable diastereomeric mixtures.

Method 2. Following the same procedure as described above, treatment of **13b** with the Beau–Skrydstrup reagent **16b**[23] for 10 min at rt gave **14b** and **15b** in 82:18 ratio with a combined yield of 87%.

Major diastereomer of **14b**. Colorless oil. $[\alpha]_D^{20}-5.6$ (*c* 1.0, $CHCl_3$). IR (film) *ν*: 3528, 3364, 2952, 2929, 1651, 1513, 1402, 1246, 1101 cm^{-1}. ^1H NMR (500 MHz, CD_3CN) *δ*: 7.42–7.20 (m, 7H, Ar-H), 6.82–6.78 (m, 2H, Ar-H), 4.64 (d, *J*=15.5 Hz, 1H, NCH_2), 4.49 (d, *J*=15.5 Hz, 1H, NCH_2), 4.44 (d, *J*=11.7 Hz, 1H, OCH_2), 4.26–4.24 (m, 1H, H-5), 4.25 (d, *J*=11.7 Hz, 1H, OCH_2), 3.76 (s, 3H, OCH_3), 3.82 (s, 1H, OH, exchangeable), 3.58 (s, 2H, $BnCH_2O$), 2.56 (ddd, *J*=6.6, 9.5, 17.7 Hz, 1H, H-3), 2.29 (ddd, *J*=5.2, 6.2, 17.7 Hz, 1H, H-3), 2.16–2.10 (m, 1H, H-4), 1.98–1.92 (m, 1H, H-4), 0.96 (s, 9H, *t*-Bu–H), −0.32 (s, 3H, $SiCH_3$), −0.41 (s, 3H, $SiCH_3$). ^{13}C NMR (125 MHz, CD_3CN) *δ*: 171.2 (C═O), 159.2 (Ar), 139.2 (Ar), 133.4 (Ar), 129.4 (Ar), 129.0 (Ar), 128.7 (Ar), 128.6 (Ar), 118.4 (4C, Ar), 114.3 (Ar), 88.0 (C-6), 73.7 (C-5), 71.9 (CH_2O), 70.6 (CH_2O), 55.8 (OCH_3), 44.2 (NCH_2), 28.4 (C-3), 26.2 (C-4), 24.8 (3C, *t*-BuC), 18.7 (SiC), −4.09 ($SiCH_3$), −4.86 ($SiCH_3$). MS (ESI): 486 ($M+H^+$, 100). HRESIMS calcd for ($C_{27}H_{39}NO_5Si+Na$): 508.2495, found: 508.2491.

To a suspension of 10% Pd/C (1.1 g, 30%) in EtOH (5 mL) was added a solution of diastereomeric **14b** (3.811 g, 7.86 mmol) in EtOH (30 mL) under a H_2 atmosphere at rt. After stirring for 8 h, the mixture was filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **19** as an inseparable mixture (2.794 g, yield: 90%).

Major diastereomer of **19**. Colorless solid. Mp 111–112 °C (CH_3OH). $[\alpha]_D^{20}-71.4$ (*c* 1.0, CH_3OH). IR (film) *ν*: 3410, 2956, 2929, 2855, 1625, 1512, 1463, 1246, 1105, 1034 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.30–7.20 (m, 2H, Ar-H), 6.84–6.78 (m, 2H, Ar-H), 4.71 (d, *J*=15.5 Hz, 1H, NCH_2), 4.62 (d, *J*=15.5 Hz, 1H, NCH_2), 4.23–4.22 (m, 1H, H-5), 3.80 (s, 3H, OCH_3), 3.61–3.52 (m, 3H, CH_2O and OH), 2.73–2.66 (m, 1H, H-3), 2.47–2.40 (m, 1H, H-3), 2.12–2.07 (m, 1H, H-4), 1.99–1.94 (m, 1H, H-4), 1.92 (br s, 1H, OH), 0.86 (s, 9H, *t*-Bu–H), −0.35 (s, 3H, $SiCH_3$), −0.47 (s, 3H, $SiCH_3$). ^{13}C NMR (125 MHz, CD_3CN) *δ*: 170.9 (C═O), 158.5 (Ar), 131.8 (Ar), 128.1 (2C, Ar), 114.0 (2C, Ar), 87.5 (C-6), 68.2 (CH_2O), 63.7 (C-5), 55.2 (OCH_3), 43.2 (NCH_2), 27.1 (C-3), 25.6 (C-4), 23.9 (3C, *t*-BuC), 17.9 (SiC), −4.4 ($SiCH_3$), −5.3 ($SiCH_3$). MS (ESI): 396 ($M+H^+$, 100). HRESIMS calcd for ($C_{20}H_{33}NO_5Si+Na$): 418.2026, found: 418.2025.

To a mixture of diastereomeric **19** (3.618 g, 9.16 mmol) in anhydrous CH_2Cl_2 (90 mL, 0.1 M) were added dropwise Et_3SiH (14.5 mL, 91.6 mmol) and $BF_3\cdot OEt_2$ (2.8 mL, 23 mmol) at −78 °C under a N_2 atmosphere. The reaction mixture was allowed to warm to rt and stirred for 5–7 h. The reaction was quenched by addition of a saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL×2). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc/PE) to yield **20** (1.978 g, yield: 57%) as a colorless solid. Mp 108–109 °C ($CHCl_3$). $[\alpha]_D^{20}-66.8$ (*c* 0.7, CH_3OH). IR (film) *ν*: 3409, 2955, 2926, 2855, 1611, 1512, 1463, 1248, 1105, 1034 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.22–7.18 (m, 2H, Ar-H), 6.86–6.82 (m, 2H, Ar-H), 5.27 (d, *J*=14.5 Hz, 1H, NCH_2), 4.04 (ddd, *J*=4.2, 4.9, 10.3 Hz, 1H, H-5), 3.96–3.92 (m, 1H, CH_2O), 3.95 (d, *J*=14.5 Hz, 1H, NCH_2), 3.80 (s, 3H, OCH_3), 3.78–3.73 (m, 1H, CH_2O), 3.37 (ddd, *J*=3.4, 4.2, 7.8 Hz, 1H, H-6), 2.91 (br s, 1H, OH), 2.64 (ddd, *J*=3.9, 7.7, 18.2 Hz, 1H, H-3), 2.48 (ddd, *J*=8.0, 8.8, 18.2 Hz, 1H, H-3), 2.14–2.06 (m, 1H, H-4), 1.91–1.85 (m, 1H, H-4), 0.88 (s, 9H, *t*-Bu–H), −0.23 (s, 3H, $SiCH_3$), −0.36 (s, 3H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 169.5 (C═O), 159.1 (Ar), 129.3 (3C, Ar), 114.1 (2C, Ar), 69.7 (OCH_2), 60.7 (C-5), 59.0 (C-6), 55.3 (OCH_3), 47.8 (NCH_2), 28.8 (C-3), 26.2 (C-4), 25.7 (3C, *t*-BuC), 17.9 (SiC), −4.8 ($SiCH_3$), −5.3 ($SiCH_3$). MS (ESI): 380 ($M+H^+$, 100). HRESIMS calcd for ($C_{20}H_{34}NO_4Si+H$): 380.2257, found: 380.2249.

4.1.2. (5*S*,6*S*)-5-(*tert*-Butyldimethylsilyloxy)-6-[(*tert*-butyldimethylsilyloxy)methyl]-1-(4-methoxybenzyl) piperidin-2-one (12a).

To a mixture of *cis*-**20** (1.960 g, 5.17 mmol), imidazole (703 mg, 10.34 mmol) and a catalytic amount of DMAP in anhydrous DMF (15 mL) was added a solution of *tert*-butyldimethylchlorosilane (930 mg, 6.20 mmol) in anhydrous DMF (5 mL). After being stirred at rt for 12 h, water (20 mL) was added, and the mixture was extracted with Et_2O (30 mL×3). The combined organic layers were washed with brine (10 mL×3), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **12a** (2.341 g, yield:92%) as a colorless oil. $[\alpha]_D^{20}-51.9$ (*c* 1.0, $CHCl_3$). IR (film) *ν*: 2954, 2929, 2857, 1650, 1512, 1462, 1250, 1116 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.18 (d, *J*=8.6 Hz, 2H, Ar-H), 6.85 (d, *J*=8.7 Hz, 2H, Ar-H), 5.35 (d, *J*=14.7 Hz, 1H, NCH_2), 3.98 (d, *J*=14.7 Hz, 1H, NCH_2), 3.90 (dd, *J*=4.6, 10.5 Hz, 1H, CH_2O), 3.87–3.84 (m, 2H, H-6 and CH_2O), 3.80 (s, 3H, OCH_3), 3.19 (m, 1H, H-5), 2.62 (ddd, *J*=2.6, 8.2, 18.3 Hz, 1H, H-3), 2.47 (ddd, *J*=8.5, 9.1, 18.3 Hz, 1H, H-3), 2.09–2.01 (m, 1H, H-4), 1.82–1.74 (m, 1H, H-4), 0.92 (s, 9H, *t*-Bu), 0.84 (s, 9H, *t*-Bu), 0.38 (s, 6H, $SiCH_3$), 0.16 (s, 6H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 169.8 (C═O), 158.9 (Ar), 129.8 (Ar), 129.3 (2C, Ar), 113.9 (2C, Ar), 67.7 (C-5), 60.4 (CH_2O), 55.3 (OCH_3), 47.9 (NCH_2), 29.4 (C-5), 27.0 (C-4), 25.8 (3C, *t*-Bu), 25.6 (3C, *t*-Bu), 18.1 ($SiCMe_3$), 17.9 ($SiCMe_3$), −4.9 ($SiCH_3$), −5.2 ($SiCH_3$), −5.6 (2C,

$SiCH_3$). MS (ESI): 494 ($M+H^+$, 100). HRESIMS calcd for ($C_{26}H_{47}NO_4Si_2+Na$): 516.2941, found: 516.2939.

4.1.3. (5*S*,6*S*)-5-(*tert*-Butyldimethylsilyloxy)-6-[(*tert*-butyldimethylsilyloxy)methyl]piperidin-2-one (21). To a solution of **12a** (1.228 g, 2.49 mmol) in CH_3CN (90 mL, 0.025 M) and H_2O (10 mL) was added CAN (6.822 g, 12.45 mmol) in one portion. The mixture was stirred at 0 °C to rt for 4 h. To the resulting mixture was added H_2O (30 mL) and the mixture was extracted with EtOAc (40 mL×3). The combined organic layers were successively washed with saturated aqueous $NaHCO_3$ (20 mL×3) and brine (20 mL×2). The organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **21** (594 mg, yield: 64%) as a colorless oil. $[\alpha]_D^{20}-23.8$ (*c* 0.9, $CHCl_3$). IR (film) *ν*: 3231, 2947, 1652, 1504, 1458, 1246, 1107 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 5.96 (br s, 1H, N-H), 4.06–4.00 (m, 1H, H-5), 3.66–3.59 (m, 2H, OCH_2), 3.43 (ddd, *J*=3.2, 4.0, 8.4 Hz, 1H, H-6), 2.56 (ddd, *J*=6.4, 12.5, 18.1 Hz, 1H, H-3), 2.29 (ddd, *J*=2.1, 5.8, 18.1 Hz, 1H, H-3), 1.94–1.88 (m, 1H, H-4), 1.83–1.77 (m, 1H, H-4), 0.86 (s, 18H, *t*-Bu), 0.32 (s, 12H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.3 (C=O), 64.3 (C-5), 64.1 (CH_2O), 58.8 (C-6), 28.1 (C-3), 26.4 (C-4), 25.8 (3C, *t*-Bu), 25.6 (3C, *t*-Bu), 18.2 ($SiCMe_3$), 18.0 ($SiCMe_3$), −4.5 ($SiCH_3$), −5.2 ($SiCH_3$), −5.5 (2C, $SiCH_3$). MS (ESI): 374 ($M+H^+$). HRESIMS calcd for ($C_{18}H_{40}NO_3Si_2+H$): 374.2547, found: 374.2538.

4.1.4. *tert*-Butyl [(2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-[(*tert*-butyldimethylsilyloxy)methyl]-6-oxo-piperidin-1-yl] carboxylate (22). To a solution of **21** (242 mg, 0.65 mmol) in anhydrous THF (8 mL) was added *n*-BuLi (1.6 M in hexane, 0.40 mL, 0.63 mmol) at −78 °C. After being stirred at −78 °C for 10 min, a solution of Boc_2O (0.23 mL, 0.97 mmol) in anhydrous THF (2 mL) was added dropwise. After being stirred for 30 min at the same temperature, the mixture was quenched with saturated aqueous NH_4Cl (2 mL), diluted with EtOAc (10 mL) and brine (5 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (10 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **22** (275 mg, yield:90%) as a colorless oil. $[\alpha]_D^{20}+25.8$ (*c* 0.9, $CHCl_3$). IR (film) *ν*: 2954, 2930, 2858, 1775, 1720, 1471, 1367, 1294, 1253, 1160, 1115 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 4.08 (dd, *J*=5.2, 10.4 Hz, 1H, CH_2O), 4.06–4.04 (m, 1H, H-5), 3.90–3.89 (m, 1H, H-6), 3.83 (dd, *J*=4.2, 10.4 Hz, 1H, CH_2O), 2.54–2.42 (m, 2H, H-3), 2.28–2.22 (m, 1H, H-4), 1.77–1.73 (m, 1H, H-4), 1.52 (s, 9H, *t*-Bu–H), 0.96 (s, 9H, *t*-Bu–H), 0.87 (s, 9H, *t*-Bu–H), 0.40 (s, 6H, $SiCH_3$), −0.20 (s, 6H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.2 (C=O), 153.1 (C=O), 82.6 (Boc *t*-C), 67.6 (C-5), 59.7 (CH_2O), 59.0 (C-6), 33.0 (C-4), 28.0 (C-3), 25.9 (3C, *t*-Bu–C), 25.7 (3C, *t*-BuC), 25.7 (3C, *t*-BuC), 18.2 ($SiCMe_3$), 18.0 ($SiCMe_3$), −4.6 ($SiCH_3$), −4.9 ($SiCH_3$), −5.8 ($SiCH_3$), −5.9 ($SiCH_3$). MS (ESI): 496 ($M+Na^+$, 100). HRESIMS calcd for ($C_{23}H_{47}NO_5Si_2+Na$): 496.2890, found: 496.2891.

4.1.5. *tert*-Butyl [(2*S*,3*S*)-1,3-bis(*tert*-butyldimethylsilyloxy)-6-oxo-octadecan]-2-yl carbamate (23). To a solution of **22** (105 mg, 0.22 mmol) in anhydrous THF (10 mL) was added *n*-$C_{12}H_{25}MgBr$ (0.289 mmol) at −78 °C. After being stirred for 3 h at −78 °C, the reaction was allowed to warm to −40 °C and stirred for 40 min. The mixture was quenched with a saturated aqueous NH_4Cl (2 mL), diluted with EtOAc (10 mL) and brine (5 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (10 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **23** (81 mg) in 70% yield based on the recovered starting **22** (20 mg). Compound **23**: colorless oil. $[\alpha]_D^{20}+8.2$ (*c* 0.9, $CHCl_3$). IR (film) *ν*: 3450, 2927, 2855, 1718, 1491, 1471, 1365, 1254, 1171, 1101 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 4.70 (d, *J*=8.5 Hz, 1H, NH), 3.93 (dd, *J*=5.7, 7.8 Hz, 1H, CH_2O), 3.55–3.53 (m, 2H, CHOTBS and CH_2O), 3.45–3.41 (m, 1H, $NCHCH_2O$), 2.46–2.33 (m, 4H, CH_2COCH_2), 1.77–1.69 (m, 2H), 1.56 (m, 4H), 1.42 (s, 9H, *t*-Bu–H), 1.24 (m, 20H), 0.96 (s, 18H, *t*-Bu–H), 0.40 (s, 6H, $SiCH_3$), −0.2 (s, 6H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 204.6 (C=O) 155.9 (C=O), 79.2 (Boc *t*-C), 69.0 (C–TBS), 61.8 (CH_2O), 53.7 (C–HCH), 43.0 ($COCH_2$), 38.4 ($COCH_2$), 31.9 ($COCH_2CH_2$ CHOTBS), 29.6 (2C), 29.5 (2C), 29.4, 29.3, 29.2, 28.4 (3C, *t*-BuC), 28.0, 25.9 (3C, *t*-BuC), 25.8 (3C, *t*-BuC), 23.9, 22.7, 18.1 (2C, $SiCMe_3$), 14.1 (CH_3), −4.3 ($SiCH_3$), −4.9 ($SiCH_3$), −5.3 ($SiCH_3$), −5.4 ($SiCH_3$). MS (ESI): 644 ($M+H^+$). HRESIMS calcd for ($C_{35}H_{74}NO_5Si_2+H$): 644.5106, found: 644.5094, calcd for (M+Na): 666.4925, found: 666.4903.

4.1.6. (+)-2-*epi*-Deoxoprosopinine (11). Trifluoroacetic acid (1 mL) was added dropwise to **23** (120 mg, 0.187 mmol) at 0 °C, and the resulting solution was stirred at rt for 3 h. To the reaction mixture was added, at 0 °C, a 30% aqueous sodium hydroxide until pH was 11–12. The resulting mixture was extracted with ether (20 mL×3), washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product, without further purification, was dissolved in EtOH (4 mL), to which was added 20% $Pd(OH)_2/C$ (60 mg) under H_2 atmosphere (1 atm). After being stirred for 2 h, concd HCl (0.4 mL) was added and the mixture was stirred for 28 h. The reaction mixture was filtered, washed with MeOH, and concentrated in vacuum. The residue was dissolved in water (5 mL) and extracted with ether (6 mL). The aqueous layer was basified by addition of 1 N NaOH solution and extracted thoroughly with $CHCl_3$ (10 mL×5). The combined extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/$NH_3\cdot H_2O$ 100:15:2) to give **11** (28 mg, yield:51%) as a colorless solid. Mp 56–57 °C (Et_2O/MeOH) [lit.[18]mp 59 °C (acetone/pentane)]. $[\alpha]_D^{20}+3.0$ (*c* 0.6, CH_3OH) {lit.[18] $[\alpha]_D^{26}+2.7$ (*c* 1.0, CH_3OH)}. IR (film) *ν*: 3341, 3239 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) *δ*: 3.84–3.82 (m, 1H, H-3), 3.67–3.60 (m, 2H, CH_2OH), 2.76–2.74 (m, 1H, H-2), 2.62–2.58 (m, 1H, H-6), 1.91–1.86 (m, 1H, H-4), 1.64–1.38 (m, 3H, H-4 and H-5), 1.39–1.22 (m, 22H), 0.89 (t, *J*=6.7 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD) *δ*: 66.2 (C-3), 64.7 (CH_2OH), 61.1 (C-2), 56.9 (C-6), 36.8, 31.9, 31.8, 29.7–29.4 (7C), 26.2, 25.8, 22.7,

14.1. MS (ESI): ($M+H^+$, 100). HRESIMS calcd for ($C_{18}H_{37}NO_2+H$): 300.2903, found: 300.2922.

4.1.7. (5*S*,6*S*)-5-(*tert*-Butyldimethylsilyloxy)-6-[(*tert*-butyldimethylsilyloxy)methyl]-1-(4-methoxybenzyl)-5,6-dihydropyridin-2(1*H*)-one (26). To a freshly prepared solution of LDA (0.34 mmol, 1.4 mL THF) was added dropwise a solution of **12a** (80 mg, 0.23 mmol) in THF (0.8 mL) at −78 °C, and the mixture was stirred at the same temperature for 1.5 h. To the resulting mixture was added a THF solution (0.8 mL) of PhSeBr (76 mg, 0.32 mmol), and the mixture was stirred at −78 °C for 5 h. The mixture was poured into a saturated aqueous $NaHCO_3$ (2 mL) and extracted with EtOAc (4 mL×3). The combined organic layers were washed successively with water (3 mL×2) and brine (3 mL×2), dried over Na_2SO_4, filtered and concentrated in vacuum. To a solution of the residue in wet CH_2Cl_2 (4 mL containing 0.01 mL of H_2O) was added a solution of 30% H_2O_2 (0.06 mL, 0.49 mmol). After stirred for 1 h, a second portion of H_2O_2 (0.45 mL, 3.69 mmol) was added and the stirring was continued for another 1 h. The resulting mixture was quenched with water (2 mL). The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (4 mL×3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by chromatography on silica gel (EtOAc/PE) to give **26** (45 mg, yield: 58%) as a colorless oil. $[\alpha]_D^{20}+4.5$ (*c* 1.1, $CHCl_3$). IR (film) *ν*: 2957, 2926, 2854, 1677, 1607, 1506, 1252 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.20–6.80 (m, 5H, Ar-H), 6.15 (d, *J*=10.0 Hz, 1H, H-3), 5.76 (dd, *J*=2.2, 10.0 Hz, 1H, H-4), 5.41 (d, *J*=14.8 Hz, 1H, NCH_2), 4.60–4.56 (m, 1H, H-5), 3.98 (d, *J*=14.8 Hz, 1H, NCH_2), 3.97–3.89 (m, 2H, CH_2O), 3.76 (s, 3H, OCH_3), 3.38–3.33 (m, 1H, H-6), 0.87 (s, 9H, *t*-Bu), 0.78 (s, 9H, *t*-Bu), 0.01 (s, 6H, $SiCH_3$), −0.09 (s, 6H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 163.0 (C=O), 158.9 (Ar), 143.2 (C-4), 130.6 (Ar), 129.4 (2C), 123.7 (C-3), 113.9 (Ar) 67.7 (C-5), 61.5 (CH_2O), 60.8 (C-6), 55.3 (OCH_3), 48.7 (NCH_2), 25.9 (3C, *t*-Bu), 25.6 (3C, *t*-Bu), 18.2 ($SiCMe_3$), 18.0 ($SiCMe_3$), −5.2 ($SiCH_3$), −5.4 ($SiCH_3$), −5.6 (2C, $SiCH_3$). HRESIMS calcd for ($C_{26}H_{46}NO_4Si_2+H$): 492.2965, found: 492.2962.

4.1.8. (3*S*,4*R*,5*R*,6*S*)-5-(*tert*-Butyldimethylsilyloxy)-6-[(*tert*-butyldimethylsilyloxy)methyl]-3,4-dihydroxy-1-(4-methoxybenzyl)piperidin-2-one (27). To a solution of **26** (28 mg, 0.08 mmol) and *N*-methylmorpholine *N*-oxide (NMMO, 10 mg, 0.32 mmol) in *t*-BuOH (1.6 mL) was added a solution of OsO_4 (3 mg, 0.01 mmol) in water (0.4 mL) at rt. After stirring for 3 h, the mixture was quenched with an excess of solid Na_2SO_3. The solvent was removed under reduced pressure until the color of the reaction mixture began to turn gray. The mixture was diluted with MeOH, filtered and washed successively with CH_2Cl_2 and MeOH (10 mL×3). The crude product was purified by chromatography on silica gel (EtOAc/PE) to give **27** (23 mg, yield: 75%) as a colorless oil. $[\alpha]_D^{20}-47.7$ (*c* 1.9, $CHCl_3$). IR (film) *ν*: 3409, 2952, 2926, 2851, 1645, 1513, 1470, 1252, 1108 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.18–6.83 (m, 5H, Ar-H), 5.13 (d, *J*=15.0 Hz, 1H, NCH_2), 4.50 (d, *J*=4.8 Hz, 1H, H-3), 4.21 (d, *J*=15.0 Hz, 1H, NCH_2), 4.15 (dd, *J*=4.8, 5.7 Hz, 1H, H-5), 3.99 (dd, *J*=4.8, 4.8 Hz, 1H, H-4), 3.83 (dd, *J*=6.0, 10.7 Hz, 1H, CH_2O), 3.80–3.75 (m, 1H, H-6), 3.57 (dd, J=5.5, 10.7 Hz, 1H, CH_2O), 0.90 (s, 9H, *t*-Bu–H), 0.86 (s, 9H, *t*-Bu–H), 0.22 (s, 6H, $SiCH_3$), −0.20 (s, 6H, $SiCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 172.0 (C=O), 158.9 (Ar), 128.8 (2C, Ar), 128.4 (Ar), 114.0 (2C, Ar), 70.9, 70.3, 67.1, 60.9, 59.3, 55.3, 47.9, 25.8 (3C, *t*-Bu), 25.7 (3C, *t*-Bu), 18.2 ($SiCMe_3$), 17.9 ($SiCMe_3$), −4.7 ($SiCH_3$), −5.4 ($SiCH_3$), −5.5 ($SiCH_3$), −5.5 ($SiCH_3$). HRESIMS calcd for ($C_{26}H_{48}NO_6Si_2+H$): 526.3020, found: 526.3010.

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Synthesis and determination of absolute configuration of tetracetate 4a-carba-D-xylofuranoside

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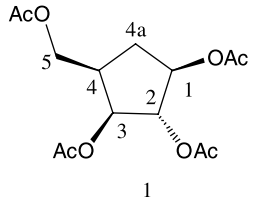
Abstract—The synthesis of carbasugar analogue tetracetate 4a-carba-D-xylofuranoside (**1**) was reported. The new route involved the conversion of D-(−)-tartatic acid into an enyne compound, which was then cyclized via a key ring-closing enyne metathesis to form the key intermediate 1-vinyl cyclopentene **9**, which was then stereoselectively converted to our target. The absolute configuration of the enyne was determined by modified Mosher's method, while that of tetracetate 4a-carba-D-xylofuranoside by ROSEY spectroscopy and γ-*gauche* effect.

1. Introduction

Carbasugars and analogues are attractive because they possess chemical stability and display a wide range of biological activities mainly as glycosidase inhibitors and as antiviral and antitumor agents. For example, acarbose and voglibose[1] are now clinically useful therapeutic agents to control diabetes, and many carbasugar-containing nucleoside analogues, such as abacavir,[2] neplanocin A,[3] can be used for the treatment of HIV and tumor, respectively.

Numerous researchers have reported chemical synthesis of carbocyclic rings for more than two decades. During the last decade ring-closing olefin metathesis has been developed as a very versatile method for converting carbohydrates to carbocycles.[2–4] However, few examples exist where the ring-closing enyne metathesis[5] is applied in the synthesis of carbasugars. Herein, we report a novel approach to the furan carbasugar, tetracetate 4a-carba-D-xylofuranoside (**1**),[6]



1

Figure 1.

by ring-closing enyne metathesis, as well as the determination of its absolute configuration (Fig. 1).

2. Results and discussion

2.1. Synthesis of tetracetate 4a-carba-D-xylofuranoside (1)

The synthesis of **1** began with aldehyde **2**, which was prepared from D-(−)-tartaric acid according to the protocol reported by Ley.[7] We first focused on the synthesis of enyne compound **7**. According to the report by Abad,[8] we used $NaCH_2SOCH_3$, prepared from sodium hydride and DMSO, as the base to react with methyl triphenylphosphonium iodide to form ylide. Compound **2** was treated with the Wittig reagent at room temperature to provide compound **3** as a colorless oil in 86% yield. Treatment of **3** with TBAF and glacial acetic acid, followed by standard Swern oxidation of the residual alcohol **4**, we obtained the aldehyde **5** in good yield. **5** could be used directly to the next reaction without further purification. Diastereoselective nucleophilic addition of trimethylsilylacetylide magnesium bromide[9] to aldehyde **5** at −78 °C gave the diastereoisomeric mixture of **6a** and **6b**, which were easily separated by silica gel chromatography to give **6a** in 70%, **6b** in 4.6% yield, respectively. Thus, the diastereoselectivity of the reaction was 87% de. The enyne compound **7** was obtained by desilylation of **6a** with TBAF (Scheme 1).

We then came to the ring-closing enyne metathesis of the enyne compound. When compound **8**, which was prepared by acetylation of **7**, was used as the substrate for the ring-closing enyne metathesis, only reactant was

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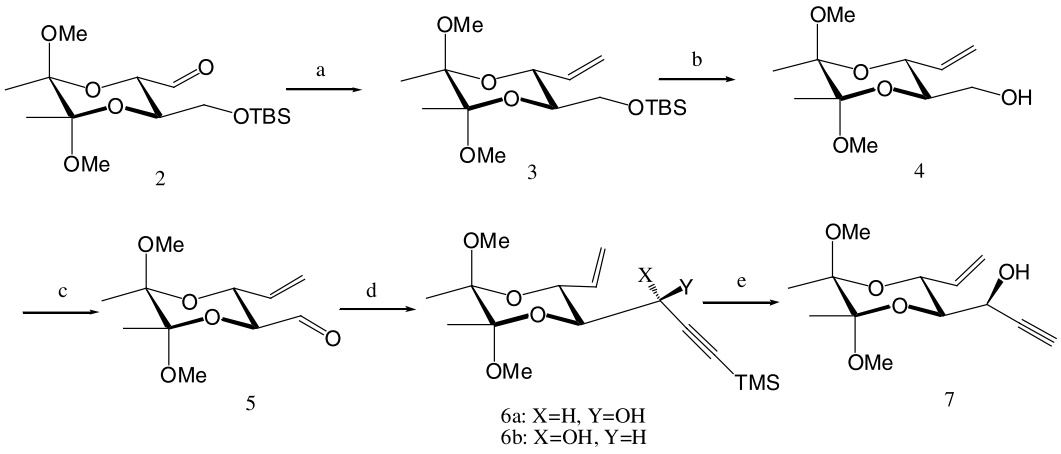
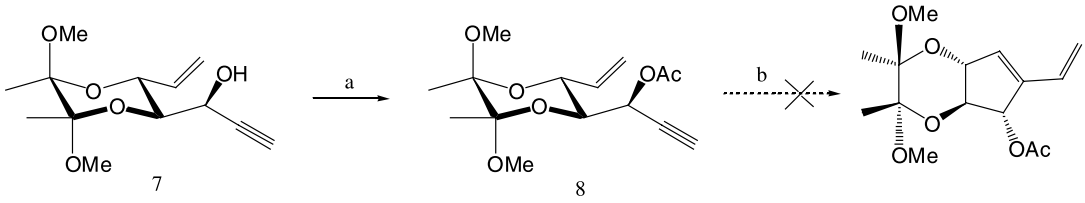
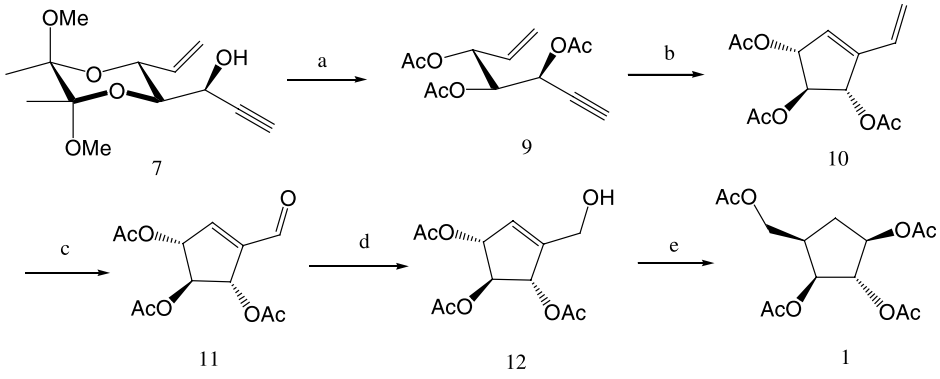


Scheme 1. Reagents and conditions: (a) NaH, DMSO, Ph_3PCH_3I, rt, 2 h, 86% (two steps); (b) TBAF, HOAc, THF, 50 °C, 3 h, 100%; (c) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78 °C. (d) trimethylsilylacetylene, Mg, EtBr, THF, −78 °C, 1 h, 65% (two steps), 87% de; (e) TBAF, THF, rt, 0.5–1 h, 100%.



Scheme 2. Reagents and conditions: (a) Ac_2O, pyridine, DMAP, rt, 10 h, 100%; (b) Grubbs 1st, CH_2Cl_2, rt, 10 h, no product.

recovered (Scheme 2). Kigoshi and co-workers reported[10] that the two groups being attached to one another must be located relatively close to each other. So we synthesized compound **9** from **7** (84%, two steps), which was then cyclized via ring-closing enyne metathesis under ethylene atmosphere[11] to afford the key compound **10** successfully. Oxidation cleavage of the exocyclic double bond under standard conditions of OsO_4–$NaIO_4$ gave the desired aldehyde **11** in only 30% yield. The reaction was improved moderately by addition of 2,6-lutidine[12] and gave **11** in 45% yield. Reduction of the aldehyde **11** with $NaBH_4$–HOAc[13] gave the desired alcohol **12** in 91% yield. Another key step was the stereoselective hydrogenation of **12**. According to the literature,[2] we used Wilkinson's catalyst ($(Ph_3P)_3RhCl$) under an atmosphere of hydrogen to reduce **12**. However, the products were complicated and the yield of target product was trivial. We found that the solvent was critical to the reduction. Using dichloromethane as the solvent, hydrogenation of **12** only resulted in the formation of **9**. We suggested that Wilkinson's catalyst reacted with dichloromethane to afford the rhodium carbene, which reacted with **12** by ring-opening metathesis to give **9**. When toluene was used as the solvent, no compound **9** was detected. So acetylation of compound **12**, then stereoselective hydrogenation with Wilkinson's catalyst in toluene, tetracetate 4a-carba-D-xylofuranoside (**1**) was achieved successfully in 83% yield, and no other stereoisomeric reduction product was isolated (Scheme 3).



Scheme 3. Reagents and conditions: (a) i: 3 M HCl/MeOH rt 24 h; ii: Ac_2O, pyridine, DMAP, rt, 84% (two steps); (b) Grubbs 1st, CH_2Cl_2, rt, 10 h, 87%; (c) OsO_4–$NaIO_4$, 2,6-lutidine, acetone–H_2O (1/1), rt, 2 h, 45%; (d) $NaBH_4$, HOAc, THF, rt, 20 h, 91%; (e) i: Ac_2O, pyridine, DMAP, rt; ii: $(Ph_3P)_3RhCl$ (30 mol%), H_2 (1 atm), toluene, rt, 83%.

2.2. Determination of absolute configuration of 7 and 1

A modified Mosher's method was employed to elucidate the absolute configuration of **7**. Compound **7** was treated with

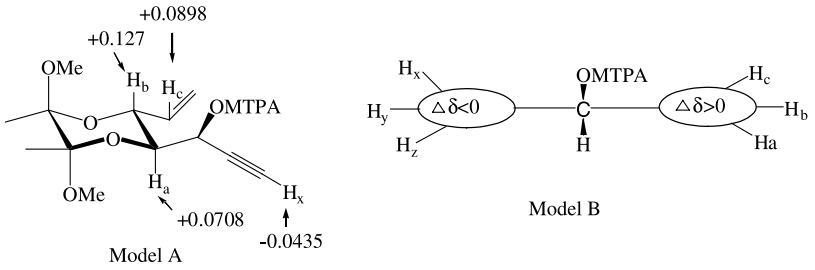


Figure 2. Models to elucidate the absolute configuration of **7**.

(*R*)-(+)- and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) in the presence of DCC and DMAP at room temperature for 4 h to afford (*R*)-(+)- and (*S*)-(−)-MTPA esters,[14] then the ^{1}H NMR spectroscopy was analyzed. The value of the chemical shift differences between the (*R*)- and the (*S*)-MTPA esters [$\Delta\delta=\delta_S-\delta_R$] were calculated (Fig. 2 Model A), and then put the protons with positive $\Delta\delta$ on the right side and those with negative $\Delta\delta$ on the left side of model B. So compound **7** was assigned *S*-configuration at the new chiral center.

The absolute configurations of the stereocenters in tetracetate 4a-carba-D-xylofuranoside (**1**) were assigned using a combination of NMR methods (HSQC, ROSEY, and ^{13}C NMR). Information of HSQC spectra led to assignment of all protons and carbons as listed in Table 1. H-3, H-1, H-4aβ, and H-4 had correlations in ROSEY spectroscopy (Fig. 3, Table 1), which implied cis between C-1, C-3, and C-4. We knew that C-1 was *R*-configuration as in D-(−)-tartaric acid, so we assigned *R*-configuration at C-4 and *S*-configuration at C-3. Furthermore, HSQC and ^{13}C NMR exhibited that the order of chemical shift values was C-2>C-1>C-3, but calculation gave the result of C-2>C-3>C-1.[15] The reason why C-3 shifted to higher field was due to the γ-*gauche* effect between C-5 and C-3, which also favored cis correlation between the two carbons and assigned *R*-configuration at C-4.

Table 1. ^{1}H NMR, ROSEY, and ^{13}C NMR spectral date for **1** in $CDCl_3$[a]

Position	δ_H mult, intgr, (*J*, Hz)	δ_C mult	ROSEY
1	5.04, m, 1H	76.39	H-4aβ, H-3, H-4
2	5.15, m, 1H	81.10	H-4aα, H-5
3	5.12, m, 1H	75.56	H-1, H-4
4	2.62, m, 1H	38.29	H-4aβ, H-3, H-1, H-5
4aα	2.41, m, 1H	31.96	H-4aβ, H-2, H-5
β	1.68, m, 1H		H-4aα, H-1, H-4
5	4.11, m, 2H	62.23	H-2, H-4, H-4aα

[a] Chemical shifts values are in parts per million relative to TMS, spectra were recorded at room temperature.

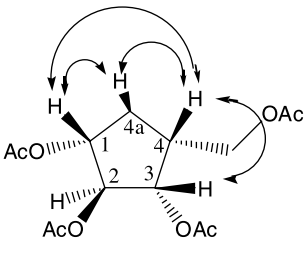


Figure 3. ROSEY of **1**.

3. Conclusion

In conclusion, we have developed a novel route for the preparation of carbasugar analogue tetracetate 4a-carba-D-xylofuranoside (**1**). Starting from D-(−)-tartaric acid, the target compound was obtained via a key ring-closing enyne metathesis and stereoselective hydrogenation in 10 steps and 14% overall yield. This route can also be applied to the preparation of other carbasugar analogues through introducing different double or triple bonds and substitution of D-(−)-tartaric acid with other tartaric acids. The absolute configuration of **7** and **1** were also determined by a combination of NMR methods.

4. Experimental

4.1. General

Solvents were distilled from the appropriate drying agents before use. Melting points were uncorrected. Spots were detected under UV light or by charring with 5% H_2SO_4 in ethanol. NMR spectra were recorded at 400 MHz. The column chromatographies were run on silica gel Gerudan SI 60, 200–300 mesh.

4.1.1. (2*R*,3*R*,5*S*,6*S*)-2-Butyldimethylsilyloxymethanol-3-vinyl-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane (3). A solution of methyl triphenylphosphonium iodide (35 g, 86.6 mmol) in DMSO (100 mL) was added to a solution of $NaCH_2SOCH_3$, prepared from sodium hydride (3.5 g, of 60% dispersion in oil, 87 mmol) and DMSO (75 mL), at room temperature. After 15 min of stirring, a solution of **2** (5 g, 14.4 mmol) in CH_2Cl_2 (20 mL) was added, and stirred at room temperature for 2 h. The reaction mixture was quenched by the addition of saturated aqueous NH_4Cl solution, and the product was isolated by extraction with ether. The combined organic extracts were washed with brine, dried with anhydrous $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel (petroleum ether/ether 15:1) to give compound **3** (3.8 g, 77%) as a colorless oil. $[\alpha]_D^{20}$ +140.7 (*c* 2.0, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ cm^{-1}: 1647, 1464, 1473, 1253. ^{1}H NMR ($CDCl_3$, 400 MHz): 0.054 (s, 6H), 0.89 (s, 9H), 1.29 (s, 3H), 1.34 (s, 3H), 3.24 (s, 3H), 3.27 (s, 3H), 3.58–3.64 (m, 3H), 4.07 (dd, *J*=7.52, 9.34 Hz, 1H), 5.24 (dm, *J*=10.40 Hz, 1H), 5.37 (dm, *J*=17.05 Hz, 1H), 5.85 (ddd, *J*=7.52, 10.40, 17.05 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): −5.1 (2C), 17.8, 17.9, 18.4, 26.0 (3C), 47.8, 47.9, 63.1, 71.2, 72.4, 98.5 (2C), 119.0, 134.1. MS (EI): 315 (M^+-OCH_3). HRMS

(ESI): Calcd $C_{17}H_{34}O_5Si$: 369.2073 (M+Na); found: 369.2062.

4.1.2. (2*R*,3*R*,5*S*,6*S*)-2-Methanol-3-vinyl-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane (4). To a solution of **3** (7.0 g, 20.2 mmol) in THF (200 mL) was added a mixture of TBAF (1 M solution in THF; 100 mL, 100 mmol) and acetic acid (5.5 mL, 95 mmol). After stirring at 50 °C for 3 h, the solvent was removed by evaporation and to the residue was added ether (300 mL), and washed with brine. Organic layers were dried over sodium sulfate, filtered and the solvent evaporated affording a crude product that was purified by column chromatography on silica gel (petroleum ether/ether 3:2) to yield compound **4** (4.6 g, 100%) as a colorless oil. $[\alpha]_D^{20}$ +194.6 (*c* 2.0, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 3481, 1645, 1456, 1375, 1207. ^1H NMR ($CDCl_3$, 400 MHz): 1.31 (s, 3H), 1.32 (s, 3H), 3.27 (s, 6H), 3.56–3.68 (m, 3H), 4.14 (dd, *J*=7.72, 9.74 Hz, 1H), 5.30 (dm, *J*=10.41 Hz, 1H), 5.41 (dm, *J*=17.12 Hz, 1H), 5.80 (ddd, *J*=7.72, 10.41, 17.12 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 17.7, 17.8, 48.0 (2C), 61.9, 70.6, 71.3, 98.5, 98.6, 119.9, 133.5. MS (EI): 201 (M^+-OCH_3). HRMS (ESI): Calcd $C_{11}H_{20}O_5$: 255.1208 (M+Na); found: 255.1210.

4.1.3. (2*S*,3*R*,5*S*,6*S*)-3-Vinyl-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane-2-carbaldehyde (5). A solution of DMSO (6.5 mL, 89.6 mmol) in CH_2Cl_2 (20 mL) was added to a solution of oxalyl chloride (3.9 mL, 44.8 mmol) in CH_2Cl_2 (120 mL) at −78 °C and stirred at this temperature for 15 min before alcohol **4** (5.2 g, 22.4 mmol) was added dropwise as a solution in CH_2Cl_2 (20 mL) over 30 min. The reaction mixture was then left stirring at −78 °C for 1 h before Et_3N (16 mL, 114 mmol) was added over 15 min, and then allowed to warm to room temperature over 1 h. The solvent was concentrated in vacuo and to the residue was added ether (400 mL), and washed with brine. The organic layer was dried with anhydrous $MgSO_4$, and evaporated. The residue was directly used for the next step without further purification. $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 2833, 1739, 1645, 1456, 1375, 1211. ^1H NMR ($CDCl_3$, 400 MHz): 1.33 (s, 3H), 1.39 (s, 3H), 3.28 (s, 3H), 3.29 (s, 3H), 4.07 (dd, *J*=10.07, 1.34 Hz, 1H), 4.25 (dd, *J*=10.07, 6.71 Hz, 1H), 5.34 (d, *J*=10.68 Hz, 1H), 5.43 (d, *J*=17.29 Hz, 1H), 5.80 (ddd, *J*=6.71, 10.68, 17.29 Hz, 1H), 9.60 (d, *J*=1.34 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 17.3, 17.6, 48.1, 48.3, 68.4, 76.1, 98.7, 98.9, 119.9, 132.2, 198.5. MS (EI): 199 (M^+-OCH_3). HRMS (ESI): Calcd $C_{11}H_{18}O_5$: 253.1052 (M+Na); found: 253.1055.

4.1.4. (2*S*,3*R*,5*S*,6*S*)-2-(1′-Hydroxy-3′-trimethylsilyl-prop-2-yne-1′-yl)-3-vinyl-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane (6). Mg (2.64 g, 0.11 mol) was covered with dry THF (50 mL) and freshly distilled ethyl bromide (8.12 mL, 0.11 mol) was added dropwise under argon. Once the reaction was started, the solution was stirred and the addition was maintained in order to obtain a moderate reflux. After the magnesium was completely consumed, the solution was cooled to 0 °C. Additional THF (200 mL) was added, followed by trimethylsilylacetylene (17 mL, 0.605 mol) dropwise. Ice bath was then removed until ethane bubling ceased. The reaction mixture was then cooled to −78 °C and compound **5** (5.0 g, 22 mmol) was added dropwise as a solution in THF (20 mL) over 30 min, then left stirring at −78 °C for 1 h, and allowed to reach room temperature progressively and 10% aqueous NH_4Cl was added. After extraction with ether, the combined organic phases were dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography on silica gel (petroleum ether/ether 3:2) to yield compound **6a** (5.0 g, 70%) and **6b** (330 mg) as a colorless oil. 87%de. Compound **6a**. $[\alpha]_D^{20}$ +130.7 (*c* 0.9, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ cm^{-1}: 2175, 1645, 1375, 1250. ^1H NMR ($CDCl_3$, 400 MHz): 0.13 (s, 9H), 1.30 (s, 3H), 1.34 (s, 3H), 3.24 (s, 3H), 3.31 (s, 3H), 3.64 (dd, *J*=9.88, 1.92 Hz, 1H), 4.28 (dd, *J*=7.94, 9.75 Hz 1H), 4.33 (d, *J*=1.92 Hz, 1H), 5.32 (dd, *J*=10.30, 1.10 Hz, 1H,), 5.46 (d, *J*=17.17 Hz, 1H,), 5.81 (ddd, *J*=10.30, 7.83, 17.17 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): −0.3 (3C), 17.4, 17.5, 47.7, 47.9, 61.5, 70.2, 73.4, 89.1, 98.6, 99.2, 104.5, 120.7, 133.4. MS (ESI): 351.12 (M^++Na). HRMS (ESI): Calcd $C_{16}H_{28}O_5Si$: 351.1604 (M+Na); found: 351.1580. *Compound* **6b**. $[\alpha]_D^{20}$ +168.5 (*c* 1.0, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 2175, 1645, 1375, 1250. ^1H NMR ($CDCl_3$, 400 MHz): 0.18 (s, 9H), 1.30 (s, 3H), 1.32 (s, 3H), 3.24 (s, 3H), 3.26 (s, 3H), 3.76 (dd, *J*=9.89, 3.57 Hz, 1H), 4.22–4.33 (m, 2H), 5.28 (dm, *J*=10.42 Hz, 1H), 5.45 (dm, *J*=17.19 Hz, 1H), 5.84 (ddd, *J*=10.30, 7.83, 17.17 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): −0.3 (3C), 17.4, 17.6, 47.8, 48.0, 62.5, 71.1, 72.2, 91.6, 98.6, 99.0, 102.7, 120.0, 133.3. MS (ESI): 351.12 (M^++Na). HRMS (ESI): Calcd $C_{16}H_{28}O_5Si$: 351.1604 (M+Na); found: 351.1578.

4.1.5. (2*S*,3*R*,5*S*,6*S*)-2-(1′-Hydroxy-prop-2-yne-1′-yl)-3-vinyl-5,6-dimethoxy-5,6-dimethyl-1,4-dioxane (7). To a solution of **6** (5.0 g, 15.24 mmol) in THF (100 mL) was added TBAF (1 M solution in THF; 10 mL, 10 mmol). After stirring at room temperature for 30 min, aqueous NH_4Cl solution was added, extracted with ether, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ether 1:1) to yield compound **7** (3.7 g, 95%) as a white solid. Mp 74–76 °C; $[\alpha]_D^{20}$ +198.7 (*c* 1.7, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 3296, 2121, 1641, 1405, 1378. ^1H NMR ($CDCl_3$, 400 MHz): 1.30 (s, 3H), 1.34 (s, 3H), 2.41 (d, *J*=2.29 Hz, 1H,), 3.25 (s, 3H), 3.31 (s, 3H), 3.67 (dd, *J*=9.93, 1.68 Hz, 1H), 4.30 (dd, *J*=9.93, 7.94 Hz, 1H,), 4.36 (t, *J*=1.68 Hz, 1H), 5.34 (dm, *J*=10.39 Hz, 1H), 5.46 (dm, *J*=17.11 Hz, 1H), 5.80 (*J*=10.39, 7.94, 17.11 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 17.6, 17.8, 48.0 (2C), 61.1, 70.1, 72.6, 73.2, 82.7, 98.5, 99.2, 120.9, 133.2. MS (ESI): 279 (M^++Na). Anal. Calcd for $C_{13}H_{20}O_5$: C, 60.92, H, 7.86; found: C, 60.95, H, 7.80.

4.1.6. (3*R*,4*R*,5*S*)-3,4,5-Triacyloxy-6-yne-1-heptene (9). To a solution of **7** (1.13 g, 4.43 mmol) was added concd HCl (15 mL). After stirring at room temperature for 24 h, solid $NaHCO_3$ was added to neutralize the reaction mixture to PH 7. The solvent was evaporated to dryness. The residue was dissolved in ethyl acetate, filtered and evaporated to dryness. The residue was dissolved in pyridine (50 mL). Ac_2O (5 mL, 35.4 mmol) and DMAP were added to the solution. After stirring at room temperature for 10 h, ether (300 mL) was added to the mixture and washed with water. The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography on silica gel

(petroleum ether/ether 3:2) to yield compound **9** (1.0 g, 84%) as a white solid. Mp: 84–86 °C; $[\alpha]_D^{20}$ +82.4 (*c* 0.6, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 3253, 2125, 1743, 1429, 1375, 1232. ^1H NMR ($CDCl_3$, 400 MHz): 2.1 (s, 9H), 2.51 (d, J= 2.1 Hz, 1H), 5.27–5.38 (m, 3H), 5.52 (dd, J=2.3, 6.8 Hz, 1H), 5.64–5.78 (m, 2H,). ^{13}C NMR ($CDCl_3$, 400 MHz): 20.5, 20.6, 20.8, 62.6, 72.4, 72.9, 76.0, 76.8, 119.4, 131.5, 169.3, 169.5, 169.7. EI-MS: 253 (M^+-CH_3). Anal. Calcd for $C_{13}H_{16}O_6$: C, 58.21, H, 6.01; found: C, 58.25, H, 6.06.

4.1.7. (3*R*,4*R*,5*S*)-3,4,5-Triacyloxy-1-vinylcyclopentene (10). The enyne compound **9** (1.0 g, 3.73 mmol) was dissolved in anhydrous CH_2Cl_2 (300 mL), and ethylene gas was passed through the solution for 20 min. Grubb's first generation catalyst was then added, and the solution was then degassed again with ethylene for 20 min. The mixture was stirred under an atmosphere of ethylene at room temperature until TLC revealed full conversion. The solvent was removed in vacuo, and the crude product was purified by column chromatography on silica gel (petroleum ether/ether 3:2) to yield compound **10** (870 mg, 87%) as an oil. $[\alpha]_D^{20}$ −32.8 (*c* 0.9, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 1740, 1600, 1433, 1371, 1221. ^1H NMR ($CDCl_3$, 400 MHz): 2.06 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 5.27–5.36 (m, 3H), 5.54 (dd, J=2.5, 0.8 Hz, 1H), 5.92–5.94 (m, 2H), 6.35 (dd, J= 11.5, 17.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 20.8, 20.8, 20.9, 78.1, 79.5, 82.9, 119.5, 129.4 (2C), 143.2, 170.0, 170.2, 170.3. EI-MS: 268 (M^+). HRMS (EI): Calcd $C_{13}H_{16}O_6$: 268.0947; found: 268.0944.

4.1.8. Aldehyde 11. To a solution of compound **10** (0.8 g, 2.99 mmol) in acetone–water (3:1, 32 mL) was added 2,6-lutidine (0.8 mL, 6.0 mmol), OsO_4 (cat.), and then aqueous $NaIO_4$ solution (1.28 g, 5.98 mmol in 20 mL H_2O) was added dropwise over 1 h. The reaction mixture was stirred at room temperature and monitored by TLC. After the reaction was complete, ethyl acetate (30 mL) was added, the organic layer was separated, and the water layer was extracted three times with ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the crude product was purified by column chromatography on silica gel (petroleum ether/ether 1:1) to yield compound **11** (360 mg, 45%) as a colorless oil. $[\alpha]_D^{20}$ −117.6 (*c* 0.6, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 1738, 1701, 1600, 1382, 1226. ^1H NMR ($CDCl_3$, 400 MHz): 2.08 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 5.39 (dd, J=3.9, 3.8 Hz, 1H), 5.70–5.73 (m, 1H), 6.05 (d, J=3.8 Hz, 1H), 6.84–6.86 (m, 1H), 9.76 (s, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 20.8 (3C), 75.5, 78.5, 83.0, 143.7, 145.8, 169.8, 170.1, 170.2, 187.4. EI-MS: 269 (M^+-H). HRMS (EI): Calcd $C_{12}H_{14}O_7$: 270.0740; found: 270.0743.

4.1.9. 1,2,3-Triacyloxy-4-dehydro-4-deoxy-4a-carba-β-D-xylofuranoside (12). To a stirred suspension of sodium borohydride powder (88 mg, 2.33 mmol) in dry THF (15 mL) at 25 °C was added over 2 min glacial acetic acid (140 μL, 2.33 mmol). After 0.5 h, **11** (380 mg, 1.42 mmol) was added, and the resulting mixture was stirred for 20 h at 25 °C. The mixture was poured into saturated aqueous sodium acetate (30 mL), stirred for 0 h, and extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4, and concentrated in vacuo, and the crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 2:1), to yield compound **12** (350 mg, 91%) as a colorless oil. $[\alpha]_D^{20}$ −36.7 (*c* 0.8, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 3458, 1740, 1659, 1433, 1373, 1230. ^1H NMR ($CDCl_3$, 400 MHz): 2.07 (s, 3H), 2.10 (s, 3H), 2.14 (s, 3H), 4.14 (AB, J=4.6 Hz, 2H), 5.37 (dd, J= 3.5, 3.2 Hz, 1H), 5.54–5.60 (m, 1H), 5.65 (d, J=3.8 Hz, 1H), 5.83–5.91 (m, 1H). ^{13}C NMR ($CDCl_3$, 400 MHz): 20.8 (3C), 58.6, 79.0, 79.2, 82.7, 127.2, 145.7, 170.2, 170.3, 171.2. EI-MS: 254 (M^+-H_2O). HRMS (ESI): Calcd $C_{12}H_{16}O_7$: 295.0794 (M+Na); found: 295.0799.

4.1.10. Tetracetate 4a-carba-D-xylofuranoside (1). Compound **12** (120 mg, 0.44 mmol) was dissolved in pyridine (3 mL). Ac_2O (60 μL, 1.27 mmol) and DMAP were added to the solution. After stirring at room temperature for 10 h, ether (50 mL) was added and washed with water, and the organic phase was dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography on silica (petroleum ether/ether 3:2). To a stirred solution of $(Ph_3P)_3RhCl$ (90 mg, 30 mol%) in toluene (50 mL) at 25 °C was added the acetylation product (90 mg, 0.287 mmol in 20 mL toluene). The reaction mixture was stirred under an atmosphere of H_2 until TLC revealed full conversion. The solvent was evaporated and the product was purified by column chromatography on silica gel (petroleum ether/ether 3:2) and decolorized with active carbon to yield compound **1** (70 mg, 83%) as a colorless oil. $[\alpha]_D^{20}$ −32.2 (*c* 0.7, $CHCl_3$); $IR_{\nu\text{-max}}^{KBr}$ (cm^{-1}): 1739, 1435, 1367, 1228. ^1H NMR ($CDCl_3$, 300 MHz): 1.61–1.72 (m, 1H), 2.04 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.37–2.46 (m, 1H), 2.55–2.67 (m, 1H), 4.05–4.17 (m, 2H), 5.02–5.08 (m, 1H), 5.14–5.20 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz): 20.7, 20.8 (2C), 20.9, 32.0, 38.3, 62.2, 75.6, 76.4, 81.1, 169.7, 169.8, 170.3, 170.8. HRMS (EI): Calcd $C_{14}H_{20}O_8$: 317.1236 (M^+ + H); found: 317.1219.

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Syntheses of glucose-containing E5564 analogues and their LPS-antagonistic activities

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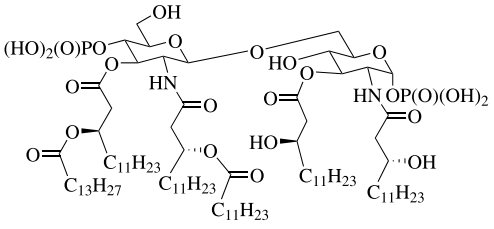
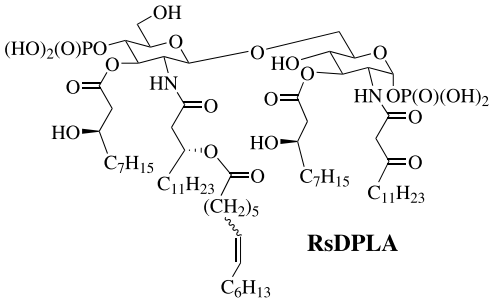
Abstract—Lipid A analogues containing a glucose moiety on their non-reducing end were synthesized, and their LPS-antagonistic activities were measured. The inhibitory activities (IC_{50}) on LPS-induced TNFα production of these six compounds, **26**, **33**, **44β**, **52β**, **59**, and **61**, toward human whole blood cells were 0.49, 0.65, 0.51, 0.98, 0.46, and 1.11 nM, respectively. Inhibitory doses (ID_{50}) of compounds **26**, **33**, **44β**, **59**, and **61** on TNFα production induced by coinjection of galactosamine and LPS in C3H/HeN mice were measured. The ID_{50} values of these compounds were 0.45, 0.96, <0.2, 1.08, and <0.2 mg/kg, respectively. Moreover, C3H/HeN mice preinjected with compounds **26**, **33**, and **44β** were protected from lethality induced by coinjection of galactosamine and LPS. Out of eight mice preinjected with 1 mg/kg of compounds **26**, **33**, and **44β**, five, eight and six mice were protected, respectively.

1. Introduction

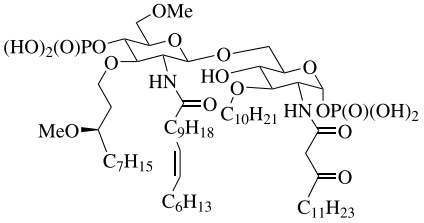
The study of endotoxin, which was named by R. Pfeiffer in 1892,[1] has developed extensively[2] since Shiba and Kusumoto's[3] total synthesis of lipid A, a toxic component of endotoxin (lipopolysaccharide, LPS) existing in the outer surface membrane of Gram-negative bacteria. In an earlier investigation to search for drugs, lipid A-related compounds



***E. coli* Lipid A**



RsDPLA



E5564

Figure 1. Structures of *E. coli* lipid A, RsDPLA, and E5564.

Keywords: Synthesis; Disaccharide; LPS-antagonist; E5564.
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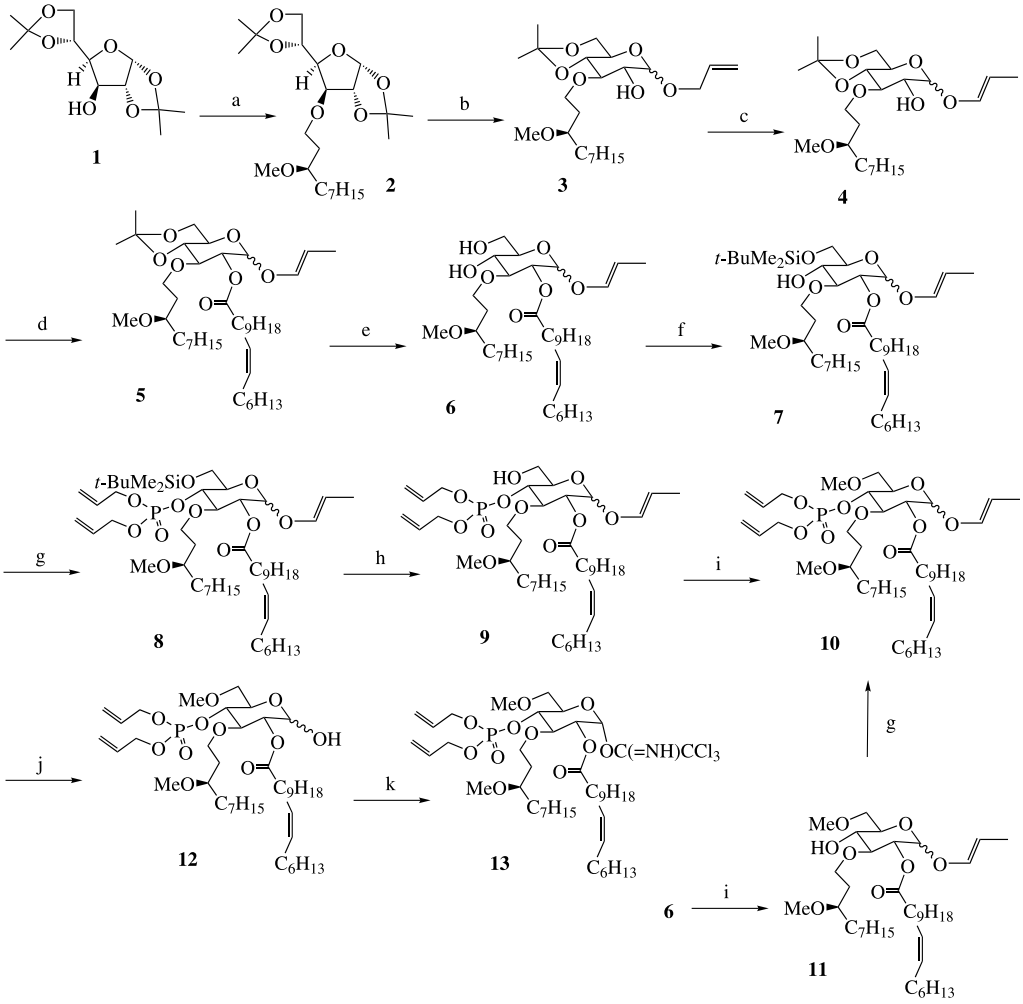
were investigated as anticancer drugs[2] by stimulating the immune system.[4] However, such attempts have not been successful so far, because of difficulty of separation between the medicinal virtues and toxicity as an LPS agonist. Conversely, a nontoxic natural lipid A-related compound (RsDPLA)[5] was isolated from *Rhodobacter sphaeroides* by an Eisai group. This compound did not show LPS-agonistic activity toward mouse macrophages.[6] Furthermore, the Eisai group found that many RsDPLA-related compounds having an olefinic double bond in their molecules behave as LPS antagonists toward both human and murine macrophages,[6] and E5564,[7] a compound related to RsDPLA, has been developed as a potent anti-septicemia drug (Fig. 1).

The active structures of all natural lipid A- and also RsDPLA-related compounds such as E5564 are constructed with a β(1-6)-linked glucosamine–glucosamine disaccharide moiety, and the configuration of the anomeric position of the reducing end is α without exception. Therefore, we were interested in E5564-related compounds, which have a glucose analogue instead of the glucosamine at the reducing end, and we synthesized some β(1-6)-linked glucosamine–glucose disaccharides, and reported their activities toward both human blood cells and murine macrophages.[8] It was proved that these novel synthetic compounds had almost the same or stronger activities towards both human blood cells and murine macrophages than against classic lipid A-type disaccharides having the glucosamine–glucosamine moiety. This result aroused our interest regarding the biological activities for reverse-linked compounds (that is, glucose–glucosamine β(1-6)-linked disaccharides) having a glucose analogue instead of the glucosamine at the non-reducing end, and also for some unnatural α(1-6)-linked glucose–glucosamine disaccharides. Consequently, we attempted to synthesize these compounds. As a result, we successfully synthesized β(1-6)-linked disaccharides **26**, **33**, **44β**, **52β**, **59**, and **61**. However, it is not known exactly why we could not synthesize α(1-6)-linked disaccharides.

This paper describes the syntheses of six compounds, **26**, **33**, **44β**, **52β**, **59**, and **61** and their LPS-antagonistic activities toward both human whole blood cells[9] and



Scheme 1. Reagents and conditions: (a) (*R*)-3-methoxydecyl *p*-toluenesulfonate, NaH, rt, 30 min, 50 °C, 1.5 h, 82%; (b) (1) 2% HCl in allyl alcohol, reflux, 30 min, (2) 2,2-dimethoxypropane, *p*-TsOH, DMF, rt, 16 h, 71% (an anomeric mixture, α:β=2:1); (c) $[IrC_8H_{12}(MePh_2P)_2]PF_6$, THF, N_2, rt, 3.5 h, 100%; (d) (*Z*)-11-octadecenoic acid, WSC·HCl, DMAP, CH_2Cl_2, rt, 3 h 66%; (e) aq 80% AcOH, 60 °C, 1.5 h, 100%; (f) *t*-$BuMe_2SiCl$, DMAP, CH_2Cl_2, rt, 16 h, 89%; (g) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt 15 min, then aq 30% H_2O_2, CH_2Cl_2–THF (1/1), rt, 10 min, 97% (**8** from **7**) and 87% (**10** from **11**); (h) aq 5% H_2SO_4, acetone, rt, 5 h, 91%; (i) 5 equiv Me_3OBF_4, 5 equiv 2,6-di-*t*-butyl-4-methylpyridine, CH_2Cl_2, rt, 3 h, 95% (**10** from **9**), or 1.6 equiv Me_3OBF_4, 2.5 equiv 2,6-di-*t*-butyl-4-methylpyridine, CH_2Cl_2, rt, 1 h, 76% (**11** from **6**); (j) NBS, acetone–H_2O (4/1), 0 °C, 2 h, 90%; (k) CCl_3CN, Cs_2CO_3, CH_2Cl_2, N_2, 95%.

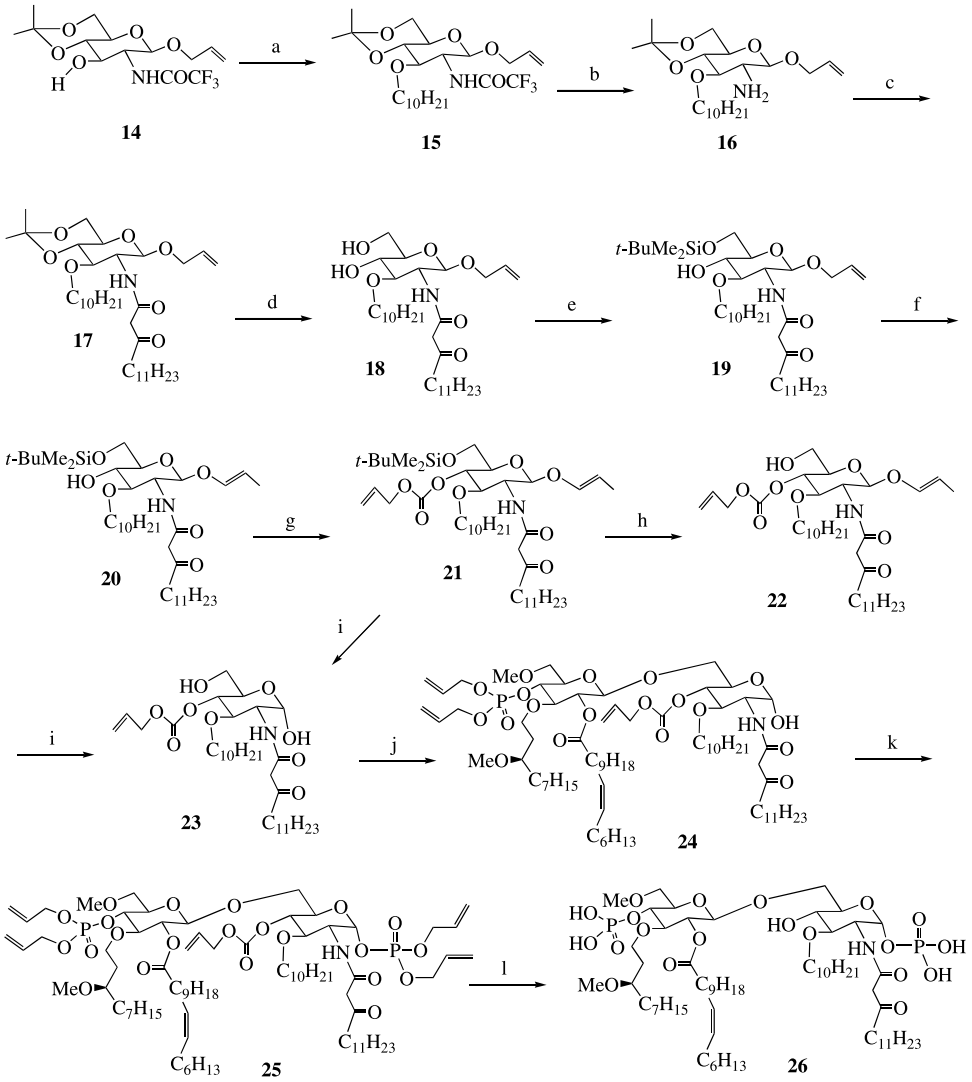
galactosamine-loaded C3H/HeN mice,[10] and improvement of the lethality toward C3H/HeN mice[10] by compounds **26**, **33**, and **44β**.

2. Results and discussion

2.1. Synthesis

Firstly, compound **26** was synthesized from both imidoyl D-glucoside analogue **13** obtained from diacetone D-glucose **1** and glucosamine derivative **23** obtained from known compound **14**.[11]

Synthesis of glycosyl donor **13**: diacetone D-glucose **1** was alkylated with (*R*)-3-methoxydecyl *p*-toluenesulfonate in *N*,*N*-dimethylformamide (DMF) using sodium hydride (NaH) as a base to give ether **2**. Furanoside **2** was heated with allyl alcohol containing hydrochloric acid to yield allyl pyranoside, and then treatment of the resulting 4,6-diol with 2,2-dimethoxypropane in DMF using *p*-toluenesulphonic acid (*p*-TsOH) as an acid catalyst gave a 2:1 anomeric mixture of allyl α- and β-D-pyranoside derivatives **3**. The allyl group of compound **3** was converted to (*E*)-vinyl compound **4** by using an iridium catalyst ($[IrC_8H_{12}(MePh_2P)_2]PF_6$) to differentiate it from the ally groups introduced in the later phase. The alcohol at the C2 position of **4** was esterified with (*Z*)-11-octadecenoic acid using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC·HCl) and 4-dimethylaminopyridine (DMAP) to yield anomeric mixture **5**. The acetonide protecting group of **5** was deprotected with aq 80% acetic acid (AcOH) at 80 °C to afford diol **6**. The C6 primary alcohol of **6** was protected with a *tert*-butyldimethylsilyl group to yield silyl ether **7**. The remaining C4 secondary alcohol of **7** was treated with diallyl diisopropylphosphoramidite and 1*H*-tetrazole to give a phosphite, and successively oxidized with 30% hydrogen peroxide (H_2O_2) to give phosphate **8**.

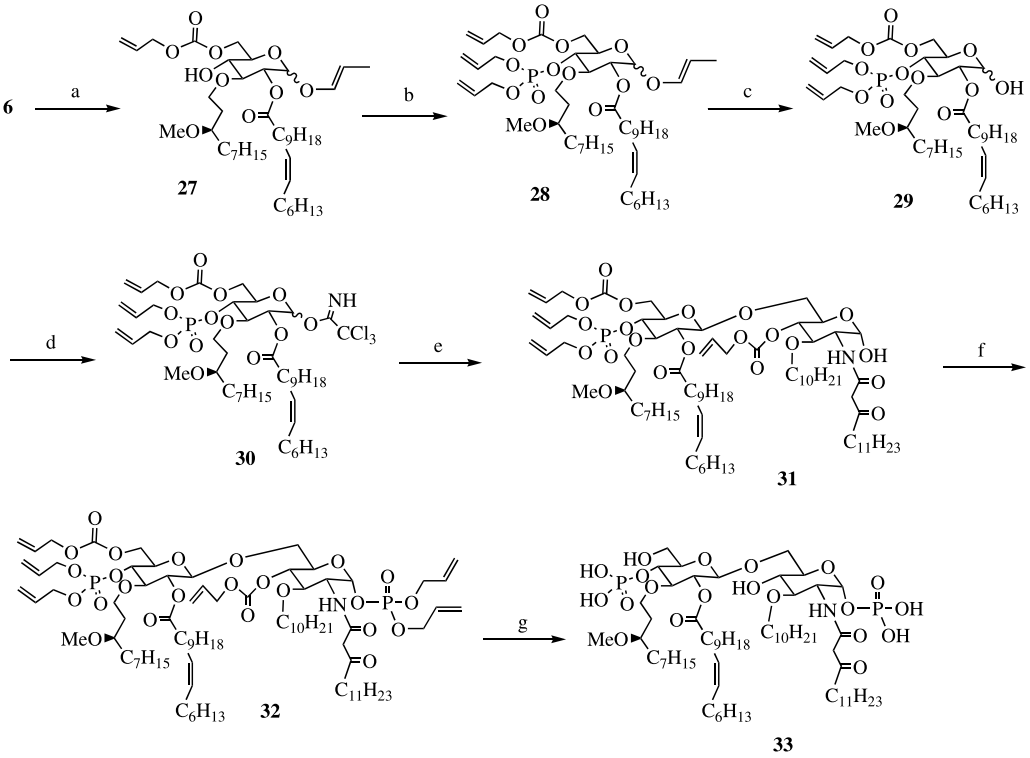


Scheme 2. Reagents and conditions: (a) decyl methanesulfonate, NaH, DMF, rt 16 h, 75%; (b) 1 M KOH, EtOH, reflux, 16 h, 61%; (c) 3-oxotetradecanoic acid, WSC·HCl, rt, 30 min, 87%; (d) aq 80% AcOH, 60 °C, 1 h, 90%; (e) *t*-butyldimethylsilylchloride, DMAP, CH_2Cl_2, rt, 5 h, 80%; (f) $[IrC_8H_{12}(MePh_2P_2)]PF_6$, THF, N_2, rt, 3.5 h, 100%; (g) triphosgene, pyridine, toluene, 0 °C, 10 min, then allyl alcohol, 0 °C, 1 h, 75%; (h) aq 5% H_2SO_4–acetone (1/10), rt, 5 h, 88%; (i) aq 48% HF, CH_2Cl_2–MeCN (1/2), rt, 2 h, 66% (from **21**) and 71% (from **22**); (j) **13**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, 24 °C, 16 h, 61%; (k) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, Na_2SO_4, CH_2Cl_2, rt, 30 min, then aq 30% H_2O_2, THF, 0 °C, 30 min, 58%; (l) $(PPh_3)_4Pd$, PPh_3, Et_3N–COOH, THF, under N_2, 55 °C, 16 h, 84%.

The silyl protecting group of **8** was deprotected with aq 5% sulfuric acid (aq 5% H_2SO_4) in acetone to yield alcohol **9**. Treatment of **9** with 5 equiv of trimethyloxonium tetrafluoroborate (Me_3OBF_4) in dichloromethane (CH_2Cl_2) gave a 2:1 anomeric mixture of α- and β-anomers **10**. This compound was also synthesized from **6** without protection of the C6 alcohol as silyl ether. Treatment of **6** with 2.5 equiv of Me_3OBF_4 gave C6 methyl ether **11**. The remaining C4 secondary alcohol of **11** was treated with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and successively with 30% H_2O_2 to give phosphate **10**. The anomeric propenyl group of **10** was treated with *N*-bromosuccinimide (NBS) to give anomeric mixture **12**. Treatment of the anomeric alcohol of **12** with trichloroacetonitrile (CCl_3CN) using cesium carbonate (Cs_2CO_3) as a base in CH_2Cl_2 gave trichloroimidoyl α-D-glucopyranoside derivative **13** containing the β-anomer in trace proportions (Scheme 1).

Synthesis of glycosyl acceptor **23** proceeded as follows: allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetylamino-β-D-glucoside **14** was alkylated with decyl methanesulfonate in DMF using sodium hydride to afford **15**. Hydrolysis of **15** with 1 M potassium hydroxide (KOH) in ethanol (EtOH) at reflux temperature gave free amine **16**. Acylation of **16** with 3-oxotetradecanoic acid and WSC·HCl in CH_2Cl_2 gave **17**, which was treated with aq 80% AcOH to yield diol **18**. Compound **18** was silylated to give **19** as described according to the same procedure for the synthesis of **7** from **6**. The allyl group of **19** was converted to a vinyl group according to the same procedure for the formation of **4** from **3** to yield anomeric (*E*)-vinyl ether **20**. The C4 alcohol **20** was converted to C4 phosphate **21** according to the same procedure for the formation of **8** from **7**. The silyl protecting group of **21** was deprotected with aq 5% H_2SO_4 in acetone according to the same procedure from **8** to **9** to yield alcohol **22**. Compound **21** or **22** was further treated with aq 48% hydrofluoric acid (HF) in CH_2Cl_2–CH_3CN (1/2) at room temperature for 2 h to yield **23** as mostly α-anomer (from ^1H NMR). Treatment of imidate **13** and alcohol **23** with silver trifluoromethanesulfonate (AgOTf) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[8] in CH_2Cl_2 gave β(1-6) disaccharide **24** without detection of α(1-6) disaccharide or orthoester via oxonium ion by involvement of the C2 acyl group. Treatment of **24** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and successively with aq 30% H_2O_2 gave phosphate **25** as an α-anomer. Treatment of **25** with tetrakis(triphenylphosphine)palladium(0) ($(PPh_3)_4Pd$), triphenylphosphine (Ph_3P), and triethylamine-formic acid (Et_3N–COOH) in THF at 55 °C for 16 h gave **26** (Scheme 2).

Secondly, compound **33** was synthesized from both alcohol **23** and imidate **30**. Compound **30** was synthesized in four steps from diol **6**. Treatment of **6** with allyl chloroformate using pyridine in CH_2Cl_2 gave C6 allyloxycarbonyl compound **27**. The remaining C4 alcohol of **27** was phosphorylated with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and successively with aq 30% H_2O_2 to give phosphate **28** according to the same procedure from **7** to **8**. Deprotection of the anomeric vinyl ether from **28** was accomplished by treatment with NBS in acetone to give **29**, which was further converted to a 7:3 anomeric mixture of α- and β-trichloroimidoyl compound **30** with CCl_3CN using Cs_2CO_3 according to the same procedure from **12** to **13**.



Scheme 3. Reagents and conditions: (a) $ClCOOCH_2CH{=}CH_2$, pyridine, CH_2Cl_2, N_2, 0 °C, 30 min, 90%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 15 min, then H_2O_2, CH_2Cl_2–THF (1/1), rt, 10 min, 95%; (c) NBS, acetone–H_2O (4/1), rt, 0 °C, 1.5 h, 81%; (d) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 1 h, N_2, 98%; (e) **23**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, 24 °C, 16 h, 54%; (f) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, Na_2SO_4, CH_2Cl_2, rt, 30 min, then aq 30% H_2O_2, THF, rt, 15 min, 70%; (g) $(PPh_3)_4Pd$, PPh_3, Et_3N–COOH, THF, under N_2, 50 °C, 16 h, 57%.

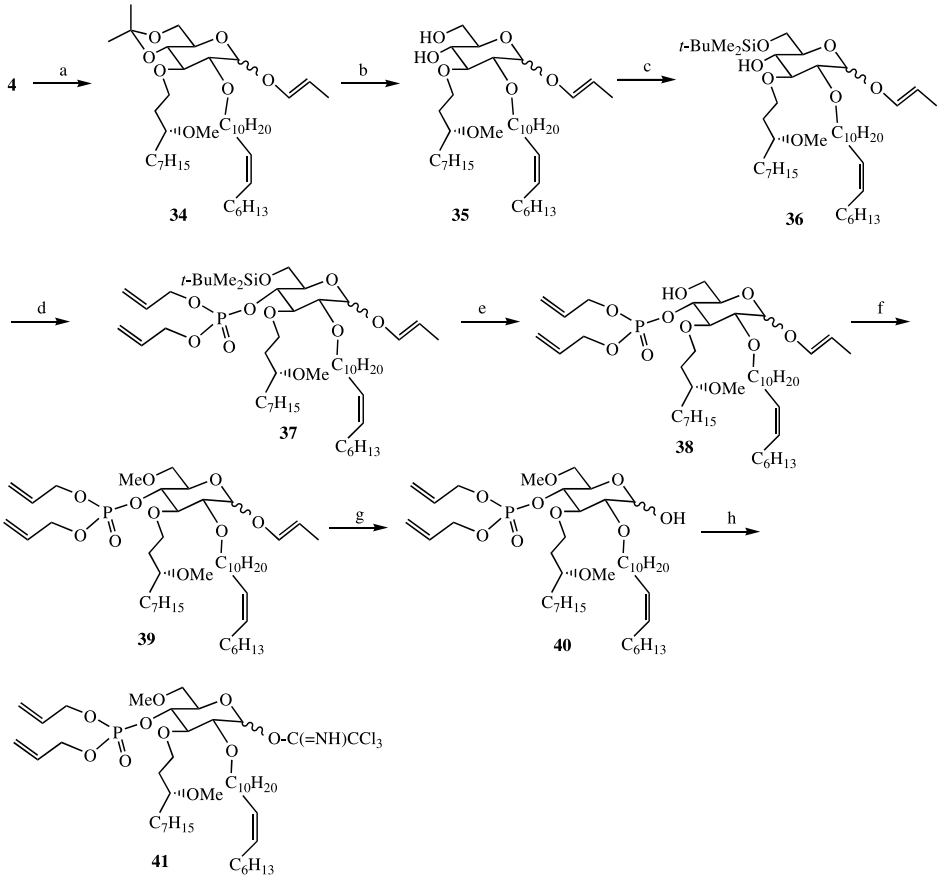
Interestingly, by varying between the C6 methoxy group in **13** and the C6 allyloxycarbonyl group in **30**, most of **13** was an α-anomer, however, compound **30** existed as an anomeric mixture. Treatment of alcohol **23** and imidate **30** with AgOTf and TMSOTf in CH_2Cl_2 gave β(1-6) disaccharide **31** without detection of any α(1-6) disaccharide. Treatment of **31** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and successively with aq 30% H_2O_2, gave phosphate **32** as an α-anomer. Treatment of **32** with $(PPh_3)_4Pd$, Ph_3P, and Et_3N–COOH in THF at 55 °C for 16 h gave **33** (Scheme 3).

Thirdly, syntheses of compounds **44α** and **44β** were attempted from both alcohol **23** and imidate **41** obtained from **4**. However, despite existence of the protected compound **43α**, the deprotected α-anomer **44α** could not be obtained, because of its instability. An anomeric mixture of compound **4** was alkylated with (*Z*)-11-octadecenyl methanesulfonate using NaH to yield **34**. Compound **34** was treated stepwise in the same manner as conversion of compound **5** to imidate **13** via intermediates **6**, **7**, **8**, **9**, **10**, and **12** to yield an anomeric mixture of trichloroacetimidoyl compound **41** (Scheme 4).

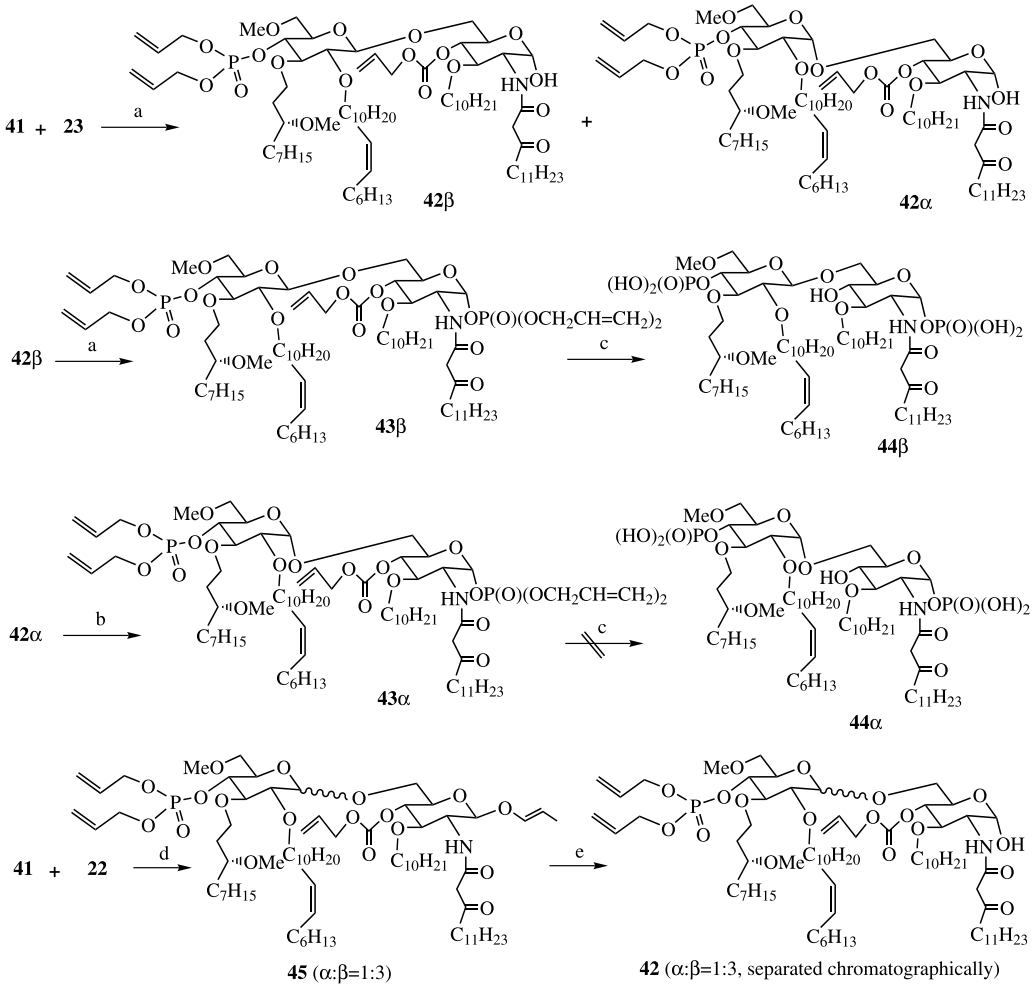
Treatment of alcohol **23** and imidate **41** with AgOTf and TMSOTf in CH_2Cl_2 at room temperature and chromatographic separation gave β(1-6) disaccharide **42β** (32% yield) and α(1-6) disaccharide **42α** (15% yield). Compounds **42β** and **42α** treated with diallyl diisopropylphosphoramidite and 1*H*-tetrazole followed by aq 30% hydrogen peroxide were converted to an anomeric mixture of phosphates **43β** and **43α**, respectively. Treatment of **43β** with $(Ph_3)_4Pd$, PPh_3, and Et_3N–COOH in THF at 55 °C for 4 h gave **44β** in 60% yield. The same treatment of **43α** did not afford **44α** at all. Alternatively, treatment of **22** and imidate **41** with AgOTf and TMSOTf catalysts gave a 1:3 anomeric mixture of α(1-6) and β(1-6) anomers **45**, which was further converted to a 1:3 anomeric mixture of **42α** and **42β** using aq 48% HF in CH_2Cl_2–MeCN (5/11). This two step method for compound **42** via **45** was almost the same yield as that from compounds **23** and **41** (Scheme 5).

Fourthly, compound **52β** was synthesized from imidate **49** and alcohol **23** via **50β** and **51β**. The imidate **49** was obtained from diol **35** via **46**, **47**, and **48** according to the same procedures for the synthesis of **30** from **6**. In this case again, the protected α(1-6) anomer **51α** was not able to yield deprotected α(1-6) anomer **52α** at all (Scheme 6).

Finally, 1-*O*-(phosphonooxy)ethyl compounds **59** and **61** were synthesized from allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetylamino-α-D-glucopyranoside **53**.[11]



Scheme 4. Reagents and conditions: (a) (*Z*)-11-octadecenyl methanesulfonate, NaH, DMF, 60 °C, 1.5 h, 81%; (b) aq 81% AcOH, 60 °C, 2.5 h, 61%; (c) ^{t}BuMe$_2$SiCl, DMAP, CH_2Cl_2, rt, 6 h, 93%; (d) *i*-Pr$_2$NP(OCH$_2$CH=CH$_2$)$_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 15 min, then H_2O_2, THF, rt, 10 min, 83%; (e) aq 5% H_2SO_4, acetone, rt, 5 h, 100%; (f) Me_3OBF_4, 2,6-di-*tert*-butyl-4-methylpyridine, rt, 3 h, 87%; (g) NBS, acetone–H_2O (4/1), 0 °C, 2 h, 75%; (h) CCl_3CN, Cs_2CO_3, CH_2Cl_2, N_2, rt 20 min, 100%.



Scheme 5. Reagents and conditions: (a) AgOTf, TMSOTf, MS-4A, CH_2Cl_2, N_2, rt, 16 h, 15% (**42**α) and 32% (**42**β) (separated chromatographically); (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2 rt, 10 min, then aq 30% H_2O_2, THF, 0 °C, 30 min, 58% (**43**β) and 58% (**43**α); (c) $Pd(Ph_3P)_4$, Ph_3P, Et_3N, HCOOH, THF, 55 °C, 4 h, 60% (**44**β) and unobtainable (**44**α); (d) AgOTf, TMSOTf, MS-4A, CH_2Cl_2, N_2, rt, 16 h, 100% (α-anomer/β-anomer=1:3); (e) aq 48% HF, CH_2Cl_2–MeCN (5/11), rt, 16 h, 12% (**42**α) and 34% (**42**β).

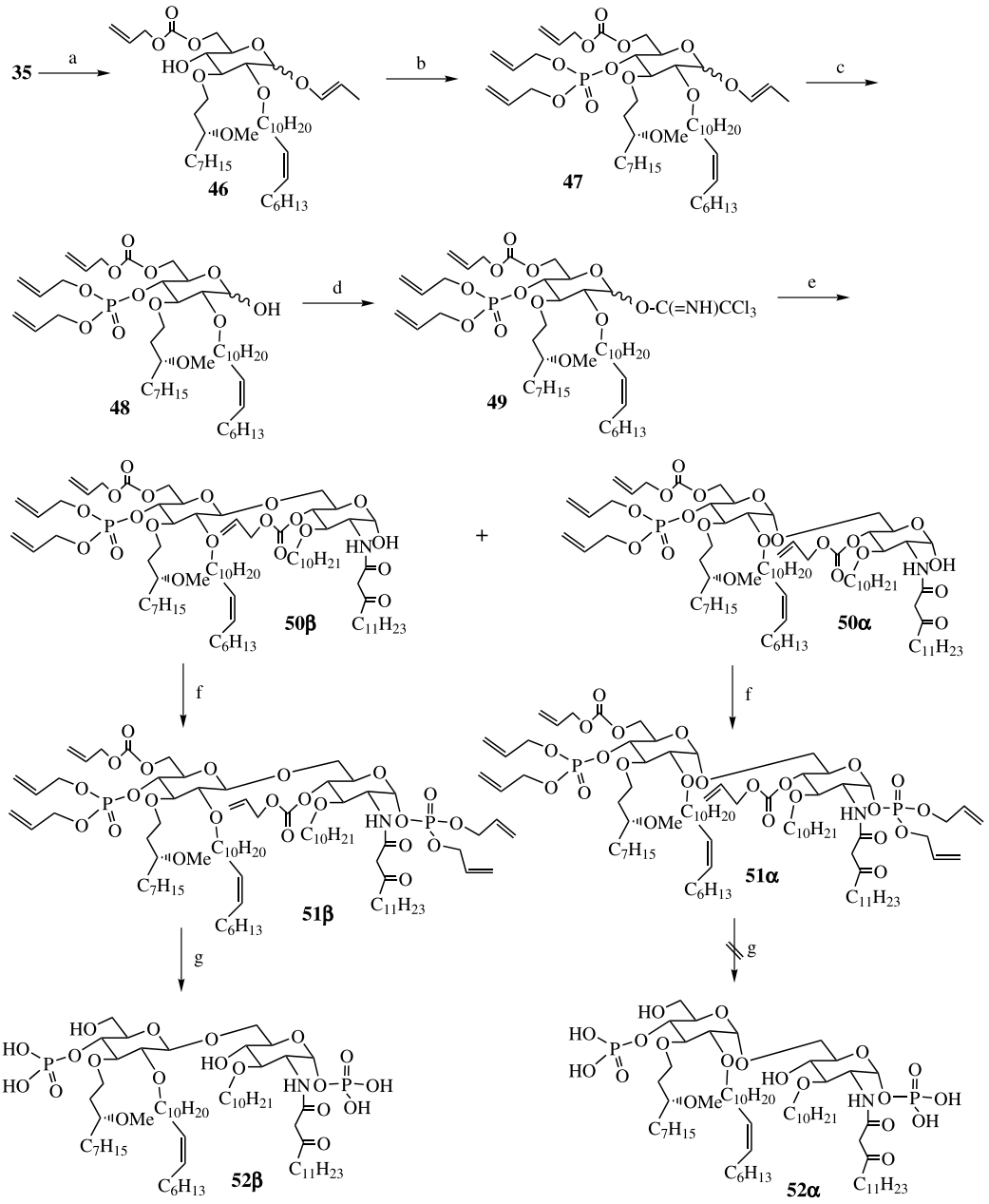
Because it was previously reported by Kusama et al. that 1-*O*-(phosphonooxy)ethyl analogues of lipid A showed essentially the same LPS-agonistic activity.[12] Treatment of compound **53** with decyl methanesulfonate in DMF using NaH gave **54**, which was further converted to amino alcohol **55** in three steps: (1) oxidative fission of the allyl double bond using OsO_4 and $NaIO_4$ in THF–H_2O (3/1); (2) reduction with $NaBH_4$ of the obtained aldehyde; and (3) hydrolysis of trifluoroactamide with aq 1 M NaOH in EtOH at reflux temperature. The primary alcohol of **55** was treated with diallyl *N,N*-diisopropylphosphoramidite and 1*H*-tetrazole, and successive oxidation of the resulting phosphite with aq oxone in THF–CH_2C1_2–H_2O gave C2 free amino phosphate, which was further acylated with 3-oxotetradecanoic acid using WSC·HCl as a dehydrating agent to give **56**. The acetonide of **56** was deprotected with aq 80% AcOH at 85 °C to yield diol **57**. Coupling of **57** with **13** or **30** using AgOTf and TMSOTf as catalysts gave β(1-6)-linked disaccharide **58** or **60**, respectively. Deprotection of **58** and **60** with $(PPh_3)_4Pd$, PPh_3, and Et_3N–COOH in THF at 55 °C for 16 h yielded **59** and **61**, respectively (Scheme 7).

Thus, we could synthesize six disaccharides **26**, **33**, **44β**, **52β**, **59**, and **61**.

2.2. Biological activity

The inhibitory activity on LPS-induced TNFα production, LPS-antagonistic activity, of six synthetic compounds, **26**, **33**, **44β**, **52β**, **59**, and **61**, was investigated in vitro using human whole blood cells by comparison with E5564. The IC_{50} values (nM) of these compounds, **26**, **33**, **44β**, **52β**, **59**, and **61**, and E5564, toward human whole blood cells were 0.49, 0.65, 0.51, 0.98, 0.46, 1.11, and 0.97 nM, respectively. The activities of these compounds were generally as strong as that of E5564.

Inhibitory activities of compounds **26**, **33**, **44β**, **59**, and **61** on TNFα production induced by coinjection of galactosamine and LPS in C3H/HeN mice were measured by comparison with E5564. The ID_{50} values of these five compounds and E5564 were 0.45, 0.96, <0.2, 1.08, <0.2, and 1.82∼2.12 mg/kg, respectively. Compounds **44β** and **61** were much stronger than E5564.



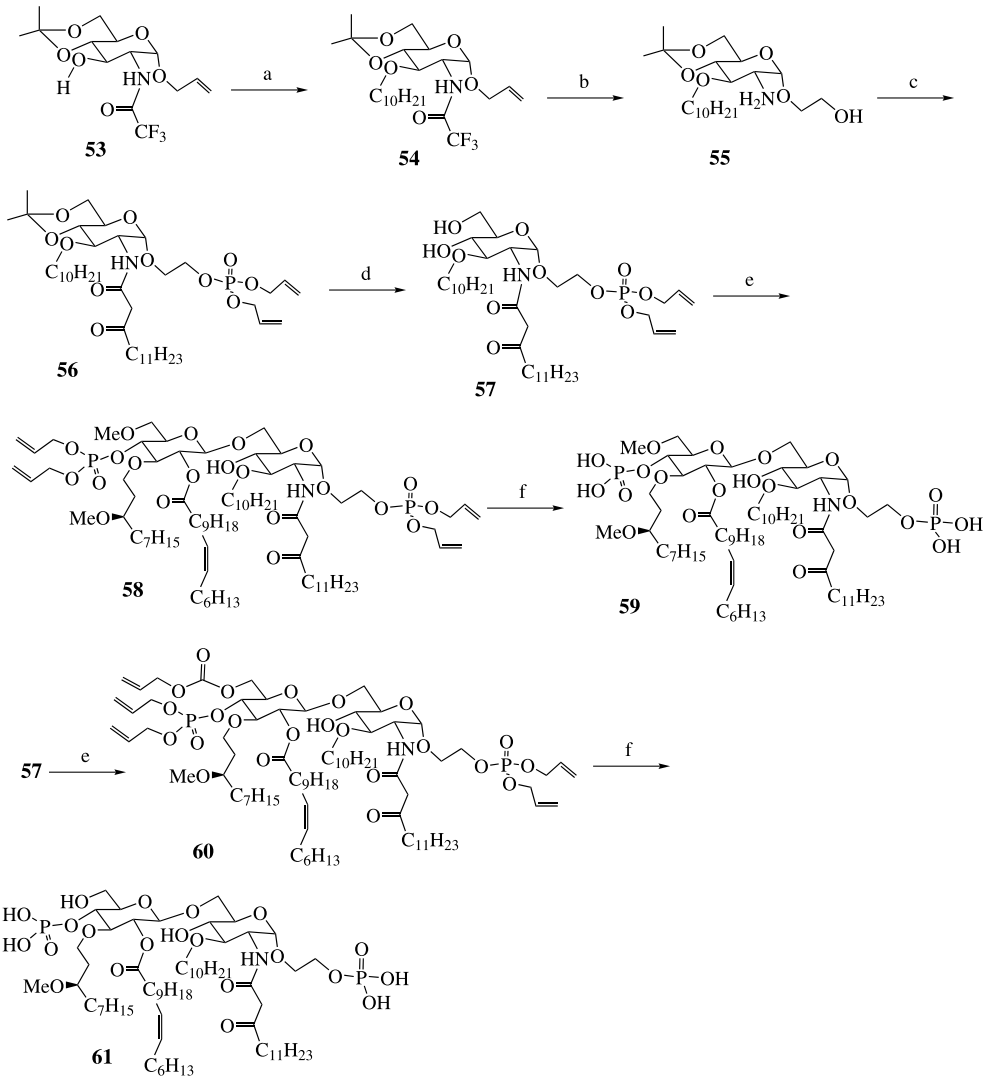
Scheme 6. Reagents and conditions: (a) $ClCOOCH_2CH{=}CH_2$, pyridine, 0 °C, 30 min, 64%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$ 1*H*-tetrazole, CH_2Cl_2 rt, 15 min, then H_2O_2, THF, rt 10 min, 85%; (c) NBS acetone–H_2O (4/1), 0 °C, 1.5 h, 78%; (d) CCl_3CN, Cs_2CO_3, CH_2Cl_2, N_2, rt, 20 min, 100% (e) **23**, AgOTf, TMSOTf, MS-4A, CH_2Cl_2, N_2, rt, 16 h, 54% (**50α**) and 28% (**50β**) (separated chromatographically); (f) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2 rt, 10 min, then aq 30% H_2O_2, THF, 0 °C, 30 min, 61% (**51β**) and 44% (**51α**); (g) $Pd(Ph_3P)_4$, Ph_3P, Et_3N, HCOOH, THF, 35 °C, 4 h, 66% (**52β**) and unobtainable (**52α**).

Out of eight mice preinjected with 1 mg/kg of compounds **26**, **33**, or **44β**, or E5564, five, eight, six and six mice were protected, respectively, from lethality induced by coinjection of galactosamine and LPS. Compound **33** within these compounds was most effective to reduce the lethality. The lethality of the two other compounds was approximately equivalent to that of E5564.

Usually, lipid A analogs having six fatty acid chains show LPS-agonistic (endotoxic) activity toward both human U-937 and mouse peritoneal resident macrophages, and lipid IVa[13] having four fatty acid chains shows LPS-antagonistic activity toward human blood cells and adversely endotoxic activity toward mouse peritoneal resident macrophages. These facts show, interestingly enough, that a difference exists in molecular recognition between human and mouse LPS receptors.[14] However, the synthetic compounds **26**, **33**, **44β**, **52β**, **59**, and **61**, this time, showed LPS-antagonistic activity toward both human and mouse blood cells (no data for **52β**). This tendency was the same as that for a nontoxic natural RsDPLA[5] having a cis-double bond in one of the fatty acid chains isolated from *Rhodobacter sphaeroides*.

3. Conclusion

Thus, we could synthesize six E5564-related disaccharides containing a glucose instead of the glucosamine at the non-reducing end. As a result, it was proved that these novel



Scheme 7. Reagents and conditions: (a) decyl methanesulfonate, NaH, DMF, rt 16 h, 89%; (b) (1) OsO_4, $NaIO_4$ THF–H_2O (3/1), rt, 1 h, (2) $NaBH_4$, EtOH, rt, 10 min; (3) 1 M NaOH–EOH (3/4), reflux, 1.5 h, 75%; (c) (1) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$ 1*H*-trtrazole CH_2Cl_2 rt, 30 min, then oxone, CH_2Cl_2–THF–H_2O (8/10/5), rt, 15 min, (2) 3-oxotetradecanoic acid, WSC·HCl, DMAP, rt, 30 min, two steps 50%; (d) aq 80% AcOH, 85 °C, 75 min, 66%; (e) **13** or **30**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, 24 °C , 16 h, 45% (**58**) and 38% (**60**); (f) $(PPh_3)_4Pd$, PPh_3, Et_3N–COOH, THF, under N_2, 55 °C, 16 h, 82% (**59**) and 55% (**61**).

synthetic compounds had almost the same or stronger activities toward both human blood cells and murine macrophages than against classic lipid A-type disaccharides having the glucosamine–glucosamine moiety.[15] The activity of compound **33** (1 mg/kg) for protection from lethality induced by coinjection of galactosamine and LPS was higher than that of E5564.

4. Experimental

4.1. General procedure

^{1}H NMR spectra were recorded with JEOL-GSX 400 and JNM-ECT 500 spectrometers using tetramethylsilane (TMS) as an internal standard. IR absorption spectra were measured with an IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-700 mass spectrometer. Separation of compounds by column chromatography was done with silica gel 60 (230–400 mesh ASTM) under a slightly elevated pressure (111–182 kPa) for easy elution. Commercially available anhydrous tetrahydrofuran (THF) and CH_2Cl_2 were used for the reactions. DMF and pyridine were dried by storage over 4 Å molecular sieves.

4.1.1. 1,2:5,6-Di-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-α-D-glucofuranose (2). To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**, 6.15 g, 23.638 mmol) and (*R*)-3-methoxydecyl *p*-toluenesulfonate (7.36 g, 21.489 mmol) in DMF (25 ml) was added NaH (55% oil dispersion, 1.41 g, 32.233 mmol) at room temperature. After stirring for 30 min, the mixture was stirred for 1.5 h at 50 °C. The reaction mixture was quenched with MeOH under ice cold temperature, and diluted with EtOAc. The solution was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (15/1, then 3/1) gave **2** (7.60 g, 82%) as an oil. IR ν_{max}(film) 2987, 2932, 2860 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J= 6.6 Hz), 1.28 (10H, br s), 1.32 (3H, s), 1.35 (3H, s), 1.43–1.52 (8H, m, containing two 3H, s, at 1.43 and 1.50 ppm),

1.68–1.74 (2H, m), 3.30 (1H, m), 3.32 (3H, s), 3.57 (1H, m), 3.73 (1H, m), 3.86 (1H, d, J=2.9 Hz), 3.99 (1H, m), 4.08 (1H, m), 4.12 (1H, dd, J=2.9, 7.3 Hz), 4.30 (1H, dd, J=5.9, 13.9 Hz), 4.55 (1H, d, J=3.7 Hz), 5.87 (1H, d, J=3.7 Hz). FABMS (positive-ion) m/z, 431 $[M+H]^+$. HRFABMS, Calcd for $C_{23}H_{43}O_7$: 431.3009. Found: 431.3018.

4.1.2. Allyl 4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-α,β-D-glucopyranoside (3). A solution of **2** (5.79 g, 13.446 mmol) in allyl alcohol (80 ml) containing 2% HCl was refluxed for 30 min, and concentrated in vacuo to give an anomeric mixture of allyl pyranosides, which was dissolved in DMF (12 ml) and 2,2-dimethoxypropane (15 ml). To this solution was added p-TsOH·H_2O (200 mg). After stirring for 16 h at room temperature, the reaction mixture was diluted with EtOAc. The solution was washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3/1) gave an oily 2:1 anomeric mixture of α- and β-anomers **3** (4.12 g, 71%). A small part of this mixture was separated on a preparative silica gel plate. Physical data of α-anomer: IR ν_{max}(film) 3461 (br), 2994, 2929, 2874, 2859 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.28 (10H, br s), 1.41 (3H, s), 1.42–1.48 (2H, m), 1.49 (3H, s), 1.72–1.76 (2H, m), 3.04 (1H, d, J=6.6 Hz, OH), 3.33 (3H, s), 3.38 (1H, m), 3.47–3.93 (8H, m), 4.05 (1H, m), 4.21 (1H, m), 4.93 (1H, d, J=3.7 Hz), 5.22–5.35 (2H, m), 5.95 (1H, m). FABMS (positive-ion) m/z; 431 $[M+H]^+$, 453 $[M+Na]^+$. HRFABMS, Calcd for $C_{23}H_{43}O_7$: 431.3009. Found: 431.2985.

4.1.3. (*E*)-1-Propenyl 4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-α,β-D-glucopyranoside (4). To a solution of **3** (4.10 g, 9.522 mmol) in dry THF (100 ml) was added $Ir[C_8H_{12}(MePh_2P)_2]PF_6$ (50 mg). The reaction flask was replaced with nitrogen, then hydrogen to activate the Ir-complex. When the color changed from red to colorless (ca. 20 s), hydrogen was replaced with nitrogen. After stirring for 6 h under nitrogen atmosphere at room temperature, the reaction mixture was concentrated in vacuo to give an oily 2:1 anomeric mixture of α- and β-anomers **4** (4.10 g, 100%). This mixture was employed for the next reaction without further purification. If needed, this mixture was separated chromatographically on a silica gel column (R_f values of the α- and β-anomers: 0.413 and 0.511, respectively) (cyclohexane–EtOAc (4/1)). Elution with cyclohexane–EtOAc (2/1) gave the α- and β-anomers as oils. Physical data of α-anomer: IR ν_{max}(film) 3441 (br), 2928, 2859, 1679 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.28 (10H, br s), 1.40–1.60 (11H, m, containing two 3H, s, at 1.41 and 1.49 ppm, and 3H, dd, J=1.5, 6.6 Hz, at 1.56 ppm), 1.72–1.77 (2H, m), 1.85 (1H, br, OH), 3.26 (1H, m), 3.33 (3H, s), 3.39 (1H, m), 3.51–3.95 (7H, m), 5.08 (1H, d, J=3.7 Hz), 5.20 (1H, m), 6.15 (1H, J=1.9, 12.1 Hz). FABMS (positive-ion) m/z; 431 $[M+H]^+$, 453 $[M+Na]^+$. HRFABMS, Calcd for $C_{23}H_{43}O_7$: 431.3009. Found: 431.3012. Physical data of β-anomer: IR ν_{max}(film) 3424 (br), 2929, 2859, 1681 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.4 Hz), 1.28 (10H, br s), 1.38–1.60 (11H, m, containing two 3H, s, at 1.40 and 1.48 ppm, and 3H, dd, J=1.6, 7.0 Hz, at 1.55 ppm), 1.68–1.77 (2H, m), 3.25–3.98 (12H, m, containing 3H, s, at 3.33 ppm), 4.58 (1H, d, J=7.4 Hz), 5.17 (1H, m), 6.23 (1H, J=1.6, 10.9 Hz). FABMS (positive-ion) m/z; 431 $[M+H]^+$, 453 $[M+Na]^+$. HRFABMS, Calcd for $C_{23}H_{43}O_7$: 431.3009. Found: 431.3028.

4.1.4. (*E*)-1-Propenyl 4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (5). To a solution of **4** (1.70 g, 3.948 mmol) in CH_2Cl_2 (25 ml) were added (*Z*)-11-octadecenoic acid (1.67 g, 5.922 mmol), DMAP (727 mg, 5.946 mmol), and WSC·HCl (1.14 g, 5.946 mmol). The solution was stirred for 1 h at room temperature, and this solution was applied to a silica gel short column. Elution with cyclohexane–EtOAc (9/1) gave a 2:1 anomeric mixture of α- and β-anomers **5** (1.81 g, 66%) as an oil. Part of this mixture was separated chromatographycally on a silica gel TLC plate to measure the physical data. Development with cyclohexane–EtOAc (9/1) gave α-anomer as a single constituent. Physical data of α-anomer: IR ν_{max}(film) 2927, 2856, 1743, 1680 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (28H, br s), 1.41 (3H, s), 1.42–1.46 (2H, m), 1.49 (3H, s), 1.53 (3H, dd, J=1.5, 6.6 Hz), 1.59–1.69 (6H, m), 1.99–2.03 (4H, m), 2.33–2.37 (2H, m), 3.30 (1H, m), 3.31 (3H, s), 3.60–3.86 (7H, m), 4.77 (1H, m), 5.16–5.18 (2H, m, containing 1H, d, J=3.7 Hz, at δ 5.17 ppm), 5.33–5.36 (2H, m), 6.07 (1H, dd, J=1.5, 12.5 Hz). FABMS (positive-ion) m/z; 637, 695 $[M+H]^+$, 717 $[M+Na]^+$. HRFABMS, Calcd for $C_{41}H_{74}O_8Na$: 717.5282. Found: 717.5275.

4.1.5. (*E*)-1-Propenyl 3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (6). A solution of **5** (250 mg, 0.360 mmol) in aq 80% AcOH (10 ml) was stirred for 1.5 h at 60 °C. The reaction mixture was concentrated in vacuo to give a 2:1 anomeric mixture of α- and β-anomers **6** (235 mg, 100%). Part of this mixture was chromatographed on a silica gel column to measure the physical data. Elution with cyclohexane–EtOAc (2/1, then 1/1) gave α- and β-anomers as oils. Physical data of α-anomer: IR ν_{max} (film) 3407 (br), 2927, 2856, 1741, 1680 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (28H, br s), 1.41–1.49 (2H, m), 1.55 (3H, dd, J=1.5, 6.6 Hz), 1.57–1.77 (4H, m), 1.98–2.05 (4H, m), 2.32–2.41 (2H, m), 3.31 (3H, s), 3.35 (1H, m), 3.60 (1H, m), 3.65–3.74 (3H, m), 3.76–3.85 (2H, m), 3.87 (1H, d, J=1.5 Hz, OH), 4.97 (1H, m), 4.71 (1H, m, C2-H), 5.15 (1H, dd, J=7.3, 12.5 Hz), 5.21 (1H, d, J=3.7 Hz, anomeric H), 5.33–5.37 (2H, m), 6.10 (1H, dd, J=1.5, 12.5 Hz). FABMS (positive-ion) m/z; 655 $[M+H]^+$, 677 $[M+Na]^+$. HRFABMS, Calcd for $C_{38}H_{71}O_8$: 655.5149. Found: 655.5153. Physical data of β-anomer: 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (28H, br s), 1.41–1.49 (2H, m), 1.53 (3H, dd, J=1.5, 6.6 Hz), 1.59–1.72 (4H, m), 1.98–2.02 (4H, m), 2.32–2.35 (2H, m), 3.22 (1H, m), 3.29 (3H, s), 3.31–3.46 (2H, m), 3.57–3.64 (2H, m), 3.78–3.95 (3H, m), 4.60 (1H, d, J=7.3 Hz), 4.97 (1H, m), 5.08 (1H, m), 5.33–5.36 (2H, m), 6.16 (1H, dd, J=1.5, 12.5 Hz). FABMS (positive-ion) m/z; 655 $[M+H]^+$, 677 $[M+Na]^+$.

4.1.6. (*E*)-1-Propenyl 6-*O*-(*tert*-butyldimethylsilyl)-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (7). To a solution of **6** (1.729 g, 2.640 mmol) in CH_2Cl_2 (20 ml) were added t-$BuMe_2SiCl$

(437 mg, 2.904 mmol) and DMAP (362 mg, 2.962 mmol) for 16 h at room temperature. The reaction mixture was applied directly to a silica gel column. Elution with cyclohexane–EtOAc (4/1) gave a 2:1 anomeric mixture of α- and β-anomers **7** (1.784 g, 88%) as an oil. Part of this mixture was chromatographed on a preparative silica gel plate. Development with cyclohexane–EtOAc (4/1) gave α- and β-anomers, respectively as oils. Physical data of α-anomer: IR ν_{max} (film) 3510–3430, 2928, 2857, 1744, 1680 (w), 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.08 (6H, s), 0.88 (6H, t, $J=6.6$ Hz), 0.90 (9H, s), 1.27 (30H, br s), 1.43–1.50 (2H, m), 1.54 (3H, dd, $J=1.5$, 6.6 Hz), 1.59–1.65 (2H, m), 1.72–1.76 (2H, m), 1.99–2.05 (4H, m), 2.33–2.38 (2H, m), 3.30 (3H, s), 3.35 (1H, m), 3.56–3.93 (7H, m), 4.70 (1H, dd, $J=3.7$, 9.5 Hz), 5.14 (1H, m), 5.18 (1H, d, $J=3.7$ Hz, anomeric H), 5.33–5.36 (2H, m), 6.12 (1H, dd, $J=2.2$, 12.5 Hz). FABMS (positive-ion) *m/z*; 769 $[M+H]^+$, 791 $[M+Na]^+$. Physical data of β-anomer: IR ν_{max}(film) 3510–3430, 2928, 2857, 1753, 1682 (w), 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.08 (6H, s), 0.88 (6H, t, $J=6.6$ Hz), 0.90 (9H, s), 1.27 (30H, br s), 1.40–1.44 (2H, m), 1.53 (3H, dd, $J=1.5$, 6.6 Hz), 1.61–1.75 (4H, m), 1.99–2.02 (4H, m), 2.31–2.35 (2H, m), 3.29 (3H, s), 3.30–3.41 (2H, m), 3.60–3.90 (6H, m), 4.55 (1H, d, $J=8.1$ Hz), 4.95 (1H, m), 5.08 (1H, m), 5.33–5.36 (2H, m), 6.16 (1H, dd, $J=1.5$, 12.5 Hz). FABMS (positive-ion) *m/z*; 791 $[M+Na]^+$ (on addition of aq 1 M NaI).

4.1.7. (*E*)-1-Propenyl 6-*O*-(*tert*-butyldimethylsilyl)-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (8). To a solution of silyl ether **7** (1.784 g, 2.319 mmol) and 1*H*-tetrazole (325 mg, 4.639 mmol) in CH_2Cl_2 (30 ml) was added diallyl diisopropylphosphoramidite (853 mg, 3.479 mmol) at room temperature. After stirring for 15 min, the reaction mixture was diluted with THF (30 ml). To this solution, aq 30% H_2O_2 (1.20 ml) was added at room temperature. The mixture was stirred for 10 min at room temperature, diluted with EtOAc, washed with aq 10% $Na_2S_2O_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel short column. Elution with cyclohexane–EtOAc (3/1) gave a 2:1 anomeric mixture of α- and β-anomers **8** (2.100 g, 97%) as an oil. Part of this mixture was chromatographed on a preparative silica gel plate. Development with cyclohexane–EtOAc (3/1) gave α-anomer ($R_f=0.462$) as an oil and β-anomer ($R_f=0.410$, difficult to separate from α-anomer in pure form). Physical data of α-anomer: IR ν_{max}(film) 2928, 2857, 1745, 1680 (w), 1463 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.88 (6H, t, $J=6.6$ Hz), 0.89 (9H, s), 1.27 (28H, br s), 1.40–1.43 (2H, m), 1.53 (3H, dd, $J=1.5$, 7.3 Hz), 1.59–1.75 (6H, m), 1.99–2.02 (4H, m), 2.34–2.39 (2H, m), 3.24 (1H, m), 3.26 (3H, s), 3.73–3.93 (7H, m), 4.26 (1H, m), 4.55–4.58 (4H, m), 4.71 (1H, m), 5.13–5.38 (7H, m), 5.90–5.97 (2H, m), 6.12 (1H, dd, $J=1.5$, 12.5 Hz). FABMS (positive-ion) *m/z*; 929 $[M+H]^+$, 951 $[M+Na]^+$.

4.1.8. (*E*)-1-Propenyl 4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (9). A solution of phosphate **8** (2.100 g, 2.260 mmol) in acetone (20 ml) and aq 5% H_2SO_4 (2 ml) was stirred for 5 h at room temperature. The reaction mixture was concentrated in vacuo to half volume, and diluted with EtOAc. The solution was washed with H_2O and aq satd $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3/2) gave a 2:1 anomeric mixture of α- and β-anomers **9** (1.670 g, 91%) as an oil. Part of this mixture was chromatographed on a preparative silica gel plate. Development with cyclohexane–EtOAc (2/1) gave α-anomer ($R_f=0.308$) and β-anomer ($R_f=0.256$, difficult to separate from α-anomer). Physical data of α-anomer: IR ν_{max} (film) 3439, 2927, 2856, 1744, 1679 (w), 1461 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.27 (28H, br s), 1.39–1.45 (4H, m), 1.54 (3H, dd, $J=1.5$, 7.3 Hz), 1.61–1.75 (4H, m), 1.99–2.03 (4H, m), 2.31–2.41 (2H, m), 3.23 (1H, m), 3.27 (3H, s), 3.65–3.96 (7H, m), 4.40 (1H, m), 4.57–4.66 (4H, m), 4.77 (1H, dd, $J=3.7$, 9.5 Hz), 5.16 (1H, m), 5.41 (1H, dd, $J=1.5$, 3.7 Hz), 5.26–5.38 (5H, m), 5.89–5.99 (2H, m), 6.11 (1H, dd, $J=1.5$, 12.5 Hz). FABMS (positive-ion) *m/z*; 815 $[M+H]^+$, 837 $[M+Na]^+$. HRFABMS, Calcd for $C_{44}H_{80}O_{11}P$: 815.5438. Found: 815.5444.

4.1.9. (*E*)-1-Propenyl 4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (10). (a) To a solution of alcohol **9** (167 mg, 0.205 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (210 mg, 1.024 mmol) in CH_2Cl_2 (5 ml) was added Me_3OBF_4 (152 mg, 1.027 mmol). After stirring for 5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3/1) gave a 2:1 anomeric mixture of α- and β-anomers **10** (161 mg, 95%) as an oil. The R_f values of the two anomers were the same.

(b) The α- and β-anomers of **10** were obtained in 50 and 52% yield in six steps from the α- and β-anomers of **4**, respectively, according to the same procedure via the corresponding α- and β-anomers of compounds **5**, **6**, **7**, **8**, and **9**. Physical data of α-anomer of **10**: IR ν_{max}(film) 2927, 2856, 1745, 1680 (w), 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) 0.88 (6H, t, $J=6.6$ Hz), 1.26 (30H, br s), 1.39–1.43 (2H, m), 1.54 (3H, dd, $J=1.5$, 7.3 Hz), 1.58–1.78 (4H, m), 1.99–2.04 (4H, m), 2.31–2.40 (2H, m), 3.24 (1H, m), 3.26 (3H, s), 3.39 (3H, s), 3.60–3.90 (6H, m), 4.39 (1H, m), 4.44–4.61 (4H, m), 4.78 (1H, m), 5.14–5.40 (8H, m), 5.90–5.98 (2H, m), 6.12 (1H, m). FABMS (positive-ion) *m/z*; 829 $[M+H]^+$, 851 $[M+Na]^+$. HRFABMS, Calcd for $C_{45}H_{82}O_{11}P$: 829.5595. Found: 829.5561. Physical data of β-amomer of **10**: 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.27 (30H, br s), 1.40–1.45 (2H, m), 1.51–1.72 (7H, m), 1.99–2.02 (4H, m), 2.31–2.35 (2H, m), 3.22 (1H, m), 3.26 (3H, s), 3.39 (3H, s), 3.49–3.77 (6H, m), 4.34 (1H, m), 4.55–4.60 (4H, m), 4.96–5.10 (2H, m), 5.24–5.40 (6H, m), 5.89–5.98 (2H, m), 6.17 (1H, dd, $J=1.5$, 12.5 Hz). FABMS (positive-ion) *m/z*; 829 $[M+H]^+$, 851 $[M+Na]^+$.

(c) To a solution of alcohol **11** (216 mg, 0.323 mmol) in CH_2Cl_2 (5 ml) were added 1*H*-tetrazole (68 mg, 0.969 mmol) and diallyl diisopropylphosphoramidite (190 mg, 0.775 mmol). After stirring for 20 min at room temperature, the reaction

mixture was diluted with THF (5 ml). To this solution, aq 30% H_2O_2 (210 mg) was added at room temperature. The mixture was stirred for 10 min at room temperature, diluted with EtOAc, washed with aq 10% $Na_2S_2O_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (2/1) gave a 2:1 anomeric mixture of α- and β-anomers **10** (233 mg, 87%) as an oil.

4.1.10. (*E*)-1-Propenyl 3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (11). To a solution of alcohol **6** (177 mg, 0.270 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (139 mg, 0.675 mmol) in CH_2Cl_2 (3 ml) was added Me_3OBF_4 (64 mg, 0.432 mmol). After stirring for 2 h at room temperature, another amount of Me_3OBF_4 (17 mg, 0.115 mmol) was added. After stirring for 1 h, the reaction mixture was diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (2/1) gave a 2:1 anomeric mixture of α- and β-anomers **11** (136 mg, 76%) as an oil. Part of this mixture was chromatographed on a preparative silica gel plate. Development with cyclohexane–EtOAc (2/1) gave α-anomer (R_f=0.576) and β-anomer (R_f=0.455) as oils. Physical data of α-anomer: IR ν_{max} (film) 3439, 2926, 2856, 1743, 1680 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.28 (30H, br s), 1.41–1.46 (2H, m), 1.54 (3H, dd, J=1.5, 6.6 Hz), 1.61–1.65 (2H, m), 1.71–1.76 (2H, m), 1.98–2.10 (4H, m), 2.33–2.39 (2H, m), 3.31 (3H, s), 3.34 (1H, m), 3.40 (3H, s), 3.59–3.79 (6H, m), 3.93 (1H, m), 4.74 (1H, dd, J=3.7, 10.3 Hz), 5.16 (1H, m), 5.22 (1H, d, J=3.7 Hz, anomeric H), 5.33–5.36 (2H, m), 6.13 (1H, dd, J=1.5, 12.5 Hz). FABMS (positive-ion) *m/z*; 669 $[M+H]^+$, 691 $[M+Na]^+$. HRFABMS, Calcd for $C_{39}H_{73}O_8$: 669.5306. Found: 669.5316. Physical data of β-anomer: IR ν_{max}(film) 3430 (br), 2927, 2856, 1752, 1682 (w), 1663 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (30H, br s), 1.41–1.44 (2H, m), 1.52 (3H, dd, J=1.5, 6.6 Hz), 1.59–1.73 (4H, m), 1.98–2.02 (4H, m), 2.30–2.35 (2H, m), 3.28–3.82 (14H, m, containing two 3H, s, at 3.29 and 3.41 ppm), 4.56 (1H, d, J=8.1 Hz), 4.98 (1H, dd, J=8.1, 9.5 Hz), 5.07 (1H, m), 5.33–5.36 (2H, m), 6.18 (1H, dd, J=1.5, 12.5 Hz). FABMS (positive-ion) *m/z*; 667 $[M-H]^+$, 691 $[M+Na]^+$, 707 $(M+K)^+$ (on addition of aq 1 M KI). HRFABMS; Calcd for $C_{39}H_{72}O_8K$: 707.4864. Found: 707.4883.

4.1.11. 4-*O*-(Diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranose (12). To a solution of **10** (433 mg, 0.522 mmol) in acetone–H_2O (4/1, 5 ml) was added NBS (140 mg, 0.787 mmol) at 0 °C with stirring. After stirring for 1.5 h at 0 °C, the reaction mixture was diluted with EtOAc. The solution was washed with aq 10% $Na_2S_2O_3$, aq satd $NaHCO_3$, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1/1) gave **12** (370 mg, 90%) as an oil. IR ν_{max}(film) 3327 (br), 2927, 2856, 1743, 1461 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (30H, br s), 1.41–1.46 (2H, m), 1.60–1.76 (4H, m), 1.99–2.03 (4H, m), 2.35–2.40 (2H, m), 3.23 (1H, m), 3.26 (3H, s), 3.39 (3H, s), 3.58–3.78 (5H, m), 3.86 (1H, t, J=9.5 Hz), 4.12 (1H, m), 4.27 (1H, m), 4.57–4.61 (4H, m), 4.76 (1H, m), 5.23–5.40 (6H, m), 5.90–6.00 (2H, m). FABMS (positive-ion) *m/z*; 789 $[M+H]^+$, 811 $[M+Na]^+$. HRFABMS, Calcd for $C_{42}H_{78}O_{11}P$: 789.5282. Found: 789.5286.

4.1.12. Trichloroacetimidoyl 4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-α-D-glucopyranoside (13). To a solution of **12** (368 mg, 0.466 mmol) and trichloroacetonitrile (673 mg, 4.664 mmol) in CH_2Cl_2 (7 ml) was added Cs_2CO_3 (76 mg, 0.233 mmol). After stirring for 1.5 h at room temperature, the reaction mixture was directly chromatographed on a silica gel short column. Elution with cyclohexane–EtOAc (2/1) gave α-anomer **13** (415 mg, 95%) as an oil containing β-anomer in trace proportions. IR ν_{max}(film) 3349 (w), 2927, 2856, 1750, 1675, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (30H, br s), 1.40–1.44 (2H, m), 1.65–1.80 (4H, m), 1.99–2.02 (4H, m), 2.23 (2H, t, J=7.3 Hz), 3.24–3.28 (4H, m, containing 3H, s, at 3.27 ppm), 3.38 (3H, s), 3.64–3.80 (4H, m), 3.91 (1H, t, J=9.5 Hz), 4.03 (1H, m), 4.50 (1H, q, J=9.5 Hz), 4.57–4.61 (4H, m), 5.00 (1H, dd, J=3.7, 10.3 Hz), 5.25–5.40 (6H, m), 5.90–6.00 (2H, m), 6.50 (1H, d, J=3.7 Hz, anomeric H), 8.61 (1H, s). FABMS (positive-ion) *m/z*; 771, 954 [^{35}Cl, $M+Na]^+$, 956 $[M+Na]^+$.

4.1.13. Allyl 3-*O*-decyl-2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-β-D-glucopyranoside (15). To a solution of allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetylamino-β-D-glucopyranoside **14** (3.553 g, 10.00 mmol) and decyl methanesulfonate (2.836 g, 12.00 mmol) in DMF (10 ml) was added NaH (55% oil dispersion, 800 mg, 18.33 mmol) at ice cold temperature. After stirring for 30 min, the mixture was stirred at room temperature for 16 h and 50 °C for 1.5 h, diluted with EtOAc, washed with ice water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3/1) gave **15** (3.718 g, 75%) as an amorphous solid. IR ν_{max}(KBr) 3318 (w), 2925, 2854, 1705, 1561 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.25 (14H, br s), 1.41 (3H, s), 1.42–1.53 (5H, m, containing 3H, s, at 1.49 ppm), 3.29–3.41 (2H, m), 3.47 (1H, m), 3.61 (1H, m), 3.74–3.80 (2H, m), 3.81–3.95 (2H, m), 4.05 (1H, m), 4.31 (1H, m), 4.92 (1H, d, J=8.1 Hz), 5.19–5.28 (2H, m), 5.78–5.86 (1H, m), 6.43 (1H, d, J=8.1 Hz, NH). FABMS (positive-ion) *m/z*; 496 $[M+H]^+$.

4.1.14. Allyl 2-amino-3-*O*-decyl-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (16). A solution of **15** (1.760 g, 2.018 mmol) in EtOH (20 ml) and aq 1 M KOH (20 ml) was refluxed for 16 h, and concentrated in vacuo to give a residue, which was extracted with Et_2O. The ether solution was washed with H_2O and brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1/4) gave **16** (870 mg, 61%) as an oil. IR ν_{max}(film) 3395 (w), 2926, 2857 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.26 (14H, br s), 1.41 (3H, s), 1.49 (3H, s), 1.50–1.59 (4H, m,

containing NH_2), 2.83 (1H, dd, J=7.3, 9.5 Hz), 3.19–3.28 (2H, m), 3.56 (1H, m), 3.67 (1H, dd, J=8.8, 9.5 Hz), 3.77–3.92 (3H, m), 4.11 (1H, m), 4.32 (1H, d, J=8.1 Hz), 4.35 (1H, m), 5.20–5.33 (2H, m), 5.92 (1H, m). FABMS (positive-ion) *m/z*; 400 $[M+H]^+$. HRFABMS, Calcd for $C_{22}H_{42}NO_5$: 400.3063. Found: 400.3056.

4.1.15. Allyl 3-*O*-decyl-2-deoxy-4,6-*O*-isopropylidene-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (17). To a solution of **16** (974 mg, 2.437 mmol) and 3-oxotetradecanoic acid (886 mg, 3.655 mmol) in CH_2Cl_2 (5 ml) was added WSC·HCl (800 mg, 4.173 mmol). After stirring for 30 min at room temperature, the reaction mixture was diluted with EtOAc, which was washed with H_2O and aq satd $NaHCO_3$, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3/1) gave **17** (1.320 g, 87%) as an amorphous solid. IR ν_{max}(KBr) 3265, 3097, 2921, 2851, 1724, 1716, 1648, 1565, 1447 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (30H, br s), 1.40 (3H, s), 1.44–1.48 (5H, m, containing 3H, s, at 1.48 ppm), 2.52 (2H, t, J=7.3 Hz), 3.29 (1H, m), 3.39 (2H, s), 3.43–3.59 (2H, m), 3.61 (1H, m), 3.68–3.80 (2H, m), 3.91 (1H, dd, J=5.1, 11.0 Hz), 4.05 (1H, m), 4.30 (1H, m), 4.79 (1H, d, J=8.8 Hz), 5.14–5.26 (2H, m), 5.82 (1H, m), 7.11 (1H, d, J=8.1 Hz, NH). FABMS (positive-ion) *m/z*; 624 $[M+H]^+$, 646 $[M+Na]^+$. HRFABMS, Calcd for $C_{36}H_{66}NO_7$: 624.4839. Found: 624.4844.

4.1.16. Allyl 3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (18). A solution of **17** (1.310 g, 2.100 mmol) in aq 80% AcOH (100 ml) was stirred for 1 h at 60 °C, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with EtOAc, then 5% MeOH in EtOAc, gave **18** (1.100 g, 90%) as an amorphous solid. IR ν_{max}(KBr) 3270, 3094 (w), 2955, 2921, 2851, 1726, 1716, 1648, 1559 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (30H, br s), 1.50–1.60 (4H, m), 2.16 (1H, br s, OH), 2.50–2.54 (3H, m, containing OH), 3.40–3.41 (3H, m, containing 2H, s, at 3.40 ppm), 3.58–3.64 (5H, m), 3.81 (1H, dd, J=4.4, 11.7 Hz), 3.91 (1H, dd, J=3.3, 11.7 Hz), 4.06 (1H, dd, J=5.9, 13.2 Hz), 4.06 (1H, d, J=5.5 Hz), 4.73 (1H, d, J=7.3 Hz), 5.15–5.27 (2H, m), 5.84 (1H, m), 7.22 (1H, d, J=7.3 Hz, NH). FABMS (positive-ion) *m/z*; 584 $[M+H]^+$, 606 $[M+Na]^+$. HRFABMS, Calcd for $C_{33}H_{62}NO_7$: 584.4526. Found: 584.4537.

4.1.17. Allyl 6-*O*-(*tert*-butyldimethylsilyl)-3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (19). To a solution of **18** (1.030 g, 1.764 mmol) in CH_2Cl_2 (25 ml) were added *tert*-$BuMe_2SiCl$ (293 mg, 1.940 mmol) and DMAP (237 mg, 1.940 mmol). After stirring for 16 h at room temperature, the reaction mixture was directly chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (4/1) gave **19** (1.072 g, 87%) as an amorphous solid. IR ν_{max}(KBr) 3516 (w), 3270, 3087 (w), 2956, 2922, 2852, 1715, 1647, 1556, 1467 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.09 (6H, s), 0.88 (6H, m), 0.90 (9H, s), 1.26 (30H, br s), 1.50–1.58 (4H, m), 2.52 (2H, t, J=7.3 Hz), 3.11 (1H, br s, OH), 3.36 (1H, m), 3.39 (2H, s), 2.52–2.62 (4H, m), 3.72 (1H, m), 3.84 (1H, m), 3.91 (1H, m), 4.04 (1H, m), 4.28 (1H, dd, J=5.1, 13.2 Hz), 4.71 (1H, d, J=8.1 Hz), 5.13–5.25 (2H, m), 5.83 (1H, m), 7.09 (1H, d, J=8.1 Hz, NH). FABMS (positive-ion) *m/z*; 698 $[M+H]^+$, 720 $[M+Na]^+$. HRFABMS, Calcd for $C_{39}H_{76}NO_7Si$: 698.5391. Found: 698.5383.

4.1.18. (*E*)-1-Propenyl 6-*O*-(*tert*-butyldimethylsilyl)-3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (20). To a solution of **19** (315 mg, 0.451 mmol) in dry THF (10 ml) was added $Ir[C_8H_{12}(MePh_2P)_2]PF_6$ (5 mg). The reaction flask was replaced with nitrogen, then hydrogen to activate the Ir complex. When the color changed from red to colorless (ca. 15 s), hydrogen was replaced with nitrogen. After stirring for 3 h under nitrogen atmosphere at room temperature, the reaction mixture was concentrated in vacuo to give **20** (315 mg, 100%) as an amorphous solid. IR ν_{max}(KBr) 3509 (w), 3276 (w), 2956, 2922, 2852, 1714, 1642, 1556, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.08 (3H, s), 0.10 (3H, s), 0.86–0.92 (15H, m, containing 9H, s, at 0.90 ppm), 1.26 (30H, br s), 1.50–1.58 (7H, m), 2.51 (2H, t, J=7.7 Hz), 3.19 (1H, br s, OH), 3.39 (2H, s), 3.43 (1H, m), 3.51–3.75 (5H, m), 3.84 (1H, m), 3.91 (1H, m), 4.94 (1H, d, J=8.1 Hz, anomeric H), 5.06 (1H, qd, J=6.6, 12.5 Hz), 6.15 (1H, dd, J=1.5, 12.5 Hz), 7.19 (1H, d, J=8.1 Hz, NH). FABMS (positive-ion) *m/z*; 698 $[M+H]^+$, 720 $[M+Na]^+$. HRFABMS, Calcd for $C_{39}H_{76}NO_7Si$: 698.5391. Found: 698.5407.

4.1.19. (*E*)-1-Propenyl 4-*O*-(allyloxycarbonyl)-6-*O*-(*tert*-butyldimethylsilyl)-3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (21). To a solution of **20** (840 mg, 1.203 mmol) in toluene (10 ml) containing pyridine (381 mg, 4.813 mmol) was added triphosgene (179 mg, 0.602 mmol, 0.5 equiv) at 0 °C under nitrogen. After stirring for 10 min at 0 °C, to the reaction mixture was added allyl alcohol (700 mg, 12.050 mmol), and the mixture was stirred for 1 h at 0 °C, and diluted with EtOAc. The solution was washed with H_2O, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (7/1) gave **21** (704 mg, 75%) as a wax. IR ν_{max}(KBr) 3269, 3088 (w), 2926, 2855, 1752, 1717, 1681 (w), 1647, 1558, 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.03 (3H, s), 0.04 (3H, s), 0.87 (15H, m), 1.22–1.26 (30H, br s), 1.40–1.44 (2H, m), 1.51 (3H, dd, J=1.5, 6.6 Hz), 1.52–1.58 (2H, m), 2.50 (2H, t, J=7.3 Hz), 3.38 (2H, s), 3.49–3.57 (4H, m), 3.68–3.78 (2H, m), 3.93 (1H, t, J=9.2 Hz), 4.62–4.73 (3H, m), 4.99 (1H, d, J=8.1 Hz, anomeric H), 5.08 (1H, m), 5.26–5.39 (2H, m), 5.92 (1H, m), 6.16 (1H, dd, J=1.5, 12.5 Hz), 7.22 (1H, d, J=7.3 Hz, NH). FABMS (positive-ion) *m/z*; 804 $[M+Na]^+$. HRFABMS, Calcd for $C_{43}H_{79}NO_9SiNa$: 804.5422. Found: 804.5413.

4.1.20. (*E*)-1-Propenyl 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (22). A solution of **21** (700 mg, 0.895 mmol) in acetone (20 ml) and aq 5% H_2SO_4 (2 ml) was stirred for 5 h at room temperature. The reaction mixture was concentrated to half volume in vacuo, and diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column.

Elution with cyclohexane–EtOAc (3/2) gave **22** (528 mg, 88%) as a wax. IR ν_{max}(film) 3291, 3085 (w), 2924, 2855, 1759, 1722, 1680, 1648, 1551 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.22–1.26 (30H, br s), 1.40–1.49 (2H, m), 1.52 (3H, dd, J=1.5, 6.6 Hz), 1.53–1.59 (2H, m), 2.24 (1H, m, OH), 2.50 (2H, t, J=7.3 Hz), 3.39 (2H, s), 3.47–3.60 (4H, m), 3.67 (1H, m), 3.75 (1H, m), 4.01 (1H, t, J=9.5 Hz), 4.64–4.66 (2H, m), 4.74 (1H, m), 5.04–5.11 (2H, m), 5.27–5.39 (2H, m), 5.93 (1H, m), 6.16 (1H, dd, J=1.5, 11.7 Hz), 7.29 (1H, d, J=8.1 Hz, NH). FABMS (positive-ion) m/z; 668 $[M+H]^+$, 690 $[M+Na]^+$. HRFABMS, Calcd for $C_{37}H_{66}NO_9$: 668.4737. Found: 668.4758.

4.1.21. 4-*O*-(Allyloxycarbonyl)-3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (23). (a) To a solution of **21** (630 mg, 0.805 mmol) in CH_2Cl_2–MeCN (1/2, 63 ml) was added aq 48% HF (14 ml). After stirring for 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed with H_2O and aq satd $NaHCO_3$, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel short column. Elution with cyclohexane–EtOAc (1/2) and then EtOAc–MeOH (19/1) gave **23** (336 mg, 66%) as a wax. IR ν_{max}(KBr) 3546, 3406, 3292, 3085, 2925, 2854, 1758, 1721, 1641, 1555 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (30H, br s), 1.40–1.49 (2H, m), 1.51–1.60 (2H, m), 2.45 (1H, br, OH), 2.52 (2H, t, J=7.3 Hz), 3.42 (2H, s), 3.46 (1H, br, OH), 3.52–3.80 (5H, m), 4.00 (1H, m), 4.16 (1H, m), 4.66 (2H, d, J=5.9 Hz), 4.80 (1H, t, J=9.5 Hz, anomeric H), 5.28–5.40 (3H, m), 5.94 (1H, m), 7.33 (1H, d, J=8.8 Hz, NH). FABMS (positive-ion) m/z; 610, 628 $[M+H]^+$, 650 $[M+Na]^+$. HRFABMS, Calcd for $C_{34}H_{61}NO_9Na$: 650.4244. Found: 650.4254.

(b) Compound **22** was treated as described above in (a) to give **23** (71%).

4.1.22. 4-*O*-(Allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (24). To a solution of imidate **13** (164 mg, 0.175 mmol) and amide alcohol **23** (100 mg, 0.159 mmol) in CH_2Cl_2 (5 ml) was added MS 4 Å (250 mg) under nitrogen. After the mixture was stirred for 20 min at room temperature, AgOTf (100 mg, 0.389 mmol) and TMSOTf (10 mg, 0.045 mmol) were added to this mixture, which was stirred for 16 h at room temperature under nitrogen, and diluted with CH_2Cl_2. The solution was washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1/2) gave **24** (135 mg, 61%) as a gum. IR ν_{max}(film) 3650–3200, 2927, 2855, 1752, 1650, 1546, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.26 (60H, br s), 1.41–1.44 (4H, m), 1.55–1.74 (6H, m), 1.99–2.04 (4H, m), 2.31–2.35 (2H, m), 2.52 (2H, t, J=7.3 Hz), 3.22 (1H, m), 3.26 (3H, s), 3.40 (5H, m), 3.49–3.79 (11H, m), 4.18 (1H, m), 4.25 (1H, m), 4.41 (1H, m), 4.52–4.67 (9H, m, containing OH), 4.93 (1H, m), 5.24–5.40 (8H, m), 5.89–5.98 (3H, m), 7.13 (1H, d, J=9.5 Hz, NH). FABMS (positive-ion) m/z; 1420 $[M+Na]^+$. HRFABMS, Calcd for $C_{76}H_{136}NO_{19}PNa$: 1420.9342. Found: 1420.9341.

4.1.23. Diallylphosphono 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (25). To a solution of **24** (167 mg, 0.119 mmol) in CH_2Cl_2 (6 ml) were added Na_2SO_4 (350 mg), 1*H*-tetrazole (121 mg, 1.727 mmol), and diallyl diisopropylphosphoramidite (179 mg, 0.730 mmol) under nitrogen at room temperature. After stirring for 30 min, the suspension was charged on a silica gel short column, and eluted with cyclohexane–EtOAc (2/3) to give crude phosphite (199 mg). The obtained phosphite was dissolved in THF (7 ml), and aq 30% H_2O_2 (0.4 ml) was added to this solution. After stirring for 15 min at room temperature, the reaction mixture was diluted with EtOAc, which was washed with aq 10% $Na_2S_2O_3$, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (2/3) gave **25** (108 mg, 58%) as an oil. IR ν_{max}(film) 3290, 3085 (w), 2926, 2855, 1755, 1723 (w), 1678, 1650, 1552 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.25 (60H, br s), 1.42–1.44 (4H, m), 1.55–1.74 (6H, m), 2.00–2.10 (4H, m), 2.33 (1H, m), 2.39 (1H, m), 2.49 (2H, t, J=7.3 Hz), 3.20 (1H, m), 3.25 (3H, s), 3.38 (3H, s), 3.39 (2H, s), 3.51–3.75 (10H, m), 3.95 (1H, m), 4.08 (1H, m), 4.26–4.34 (2H, m), 4.40 (1H, d, J=7.3 Hz, anomeric H), 4.58–4.64 (10H, m), 4.93 (1H, m), 4.76 (1H, dd, J=9.5, 10.3 Hz), 4.90 (1H, dd, J=8.1, 9.5 Hz), 5.24–5.42 (12H, m), 5.69 (1H, m, anomeric H), 5.89–6.03 (5H, m), 7.40 (1H, d, J=8.8 Hz, NH). FABMS (positive-ion) m/z, 1379, 1580 $[M+Na]^+$. HRFABMS, Calcd for $C_{82}H_{145}NO_{22}PNa$: 1580.9631. Found: 1580.9618.

4.1.24. Phosphono 3-*O*-decyl-2-deoxy-6-*O*-{3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-4-*O*-phosphono-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (26). To a solution of **25** (95 mg, 0.061 mmol) in dry THF (5 ml) were added PPh_3 (11 mg, 0.042 mmol), Et_3N (43 mg, 0.425 mmol), HCOOH (36 mg, 0.782 mmol) and $Pd(PPh_3)_4$ (11 mg, 0.010 mmol) in this sequence. The solution was stirred for 20 h at 55 °C under nitrogen, and concentrated in vacuo to give a mixture, which was chromatographed on a DEAE-cellulose (Whatman Ion-Exchange Cellulose, wet 5 g) column. The column was prepared by preliminary consecutive washing with 50 ml each of 0.5 M HCl, H_2O, 0.5 M NaOH, H_2O, and 20 ml of aq 1 M AcOH, and 50 ml each of H_2O and aq 0.05 M $AcO\cdot NH_4$, followed by $CHCl_3$–MeOH–H_2O (2/3/1), and finally $CHCl_3$–MeOH (2/1). The column was eluted with 5 ml each of $CHCl_3$–MeOH (2/1), then 0.05 M $AcO\cdot NH_4$ in $CHCl_3$–MeOH–H_2O (2/3/1). Six fractions containing **26** [R_f=0.233; $CHCl_3$–EtOH–AcOH–H_2O (8/5/1/1), on a silica gel TLC plate] were collected. To this solution were added another volume of $CHCl_3$ (5 ml) and aq 0.15 M HCl (10 ml), and the mixture was shaken well to adjust to pH 2–3. The lower $CHCl_3$ layer was separated, and concentrated in vacuo to give **26** (67 mg, 84%) as a wax. IR ν_{max}(KBr) 3537, 3301, 2955, 2923, 2852, 1722, 1655, 1544, 1467 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$+CD_3OD,

4:1) δ 0.87–0.90 (12H, m), 1.26–1.28 (60H, m), 1.42–1.65 (8H, m), 1.71–1.76 (2H, m), 2.00–2.04 (4H, m), 2.30–2.43 (2H, m), 2.56 (2H, t, J=7.3 Hz), 3.26–3.46 (9H, m, containing two 3H, s, at 3.30 (C6–6CH_3) and 3.41 (side chain OCH_3), 3.48–4.22 (15H, m), 4.52 (1H, d, J=8.1 Hz, anomeric H), 4.88 (1H, t, J=8.8 Hz, O–C2′–H), 5.34–5.36 (2H, m), 5.50 (1H, m, anomeric H). FABMS (negative-ion) m/z; 1312 $[M-H]^-$. HRFABMS, Calcd for $C_{66}H_{124}NO_{20}P_2$: 1312.8192. Found: 1312.8104. Anal. Calcd for $C_{66}H_{125}NO_{20}P_2\cdot 3H_2O$ (1368.7): C, 57.92; H, 9.65; N, 1.02; P, 4.53. Found: C, 57.86; H, 9.55; N, 1.22; P, 4.30.

4.1.25. (*E*)-1-Propenyl 6-*O*-(allyloxycarbonyl)-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (27). To a solution of an anomeric 1:1 mixture **6** (1.490 g, 2.275 mmol) in CH_2Cl_2 (90 ml) containing pyridine (5.00 g, 63.211 mmol) was added allyl chloroformate (5.00 g, 41.480 mmol) at 0 °C with stirring under nitrogen. After stirring for 30 min, the reaction mixture was diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (4/1) gave an anomeric mixture of α- and β-anomers **27** (1.52 g, 90% yield). Part of the mixture was separated on a TLC plate. Physical data of α-anomer (R_f=0.353, cyclohexane:EtOAc=4:1): IR ν_{max}(film) 3500–3400, 2927, 2856, 1749, 1680 (w), 1660 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.28 (30H, br s), 1.40–1.48 (4H, m), 1.54 (3H, dd, J=1.6, 7.0 Hz), 1.57–1.65 (2H, m), 1.67–1.76 (2H, m), 1.98–2.03 (4H, m), 2.31–2.40 (2H, m), 3.30 (3H, s), 3.34 (1H, m), 3.55 (1H, m), 3.65–3.74 (3H, m), 3.83 (1H, m), 3.95 (1H, m), 4.40 (1H, d, J=3.5 Hz, anomeric H), 4.63 (1H, td, J=1.2, 5.0 Hz), 4.72 (1H, dd, J=3.5, 10.2 Hz), 5.15 (1H, m), 5.22 (1H, d, J=3.9 Hz), 5.25–5.39 (4H, m), 5.93 (1H, m), 6.11 (1H, dd, J=1.6, 12.1 Hz). FABMS (positive-ion) m/z, 681, 739 $[M+H]^+$, 761 $[M+Na]^+$. Physical data of β-anomer (R_f=0.265, cyclohexane/EtOAc=4:1): IR ν_{max}(film) 3500–3400, 2927, 2856, 1752, 1682 (w), 1662 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (30H, br s), 1.40–1.48 (4H, m), 1.53 (3H, d, J=7.0 Hz), 1.60–1.64 (2H, m), 1.68–1.72 (2H, m), 1.99–2.10 (4H, m), 2.31–2.34 (2H, m), 3.29 (3H, s), 3.31–3.39 (2H, m), 3.56–3.64 (3H, m), 3.80–3.85 (2H, m), 4.36 (1H, m), 4.50 (1H, d, J=11.7 Hz), 4.56 (1H, d, J=7.8 Hz), 4.64 (1H, dd, J=1.0, 5.7 Hz), 4.98 (1H, t, J=9.0 Hz), 5.08 (1H, m), 5.27–5.39 (4H, m), 5.93 (1H, m), 6.18 (1H, d, J=12.0 Hz). FABMS (positive-ion) m/z; 681, 761 $[M+Na]^+$.

4.1.26. (*E*)-1-Propenyl 6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (28). Compound **27** (1.45 g, 1.962 mmol) was treated as described in the formation of **8** from **7** to give **28** (1.68 g, 95%) as an oil. Part of the mixture was separated on a TLC plate. Physical data of α-anomer (R_f=0.395, cyclohexane:EtOAc=3:1): IR ν_{max} (film) 2927, 2856, 1750, 1680 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.27 (30H, br s), 1.40–1.48 (2H, m), 1.54 (3H, d, J=5.8 Hz), 1.61–1.78 (4H, m), 1.99–2.04 (4H, m), 2.32–2.40 (2H, m), 3.25 (1H, m), 3.26 (3H, s), 3.77–3.94 (4H, m), 4.31–4.38 (2H, m), 4.46 (1H, dd, J=2.0, 11.7 Hz), 4.56–4.63 (6H, m), 4.76 (1H, dd, J=3.9, 9.8 Hz), 5.17 (1H, m), 5.20 (1H, d, J=3.9 Hz), 5.24–5.39 (8H, m), 5.89–5.98 (3H, m), 6.10 (1H, dd, J=2.0, 12.7 Hz). FABMS (positive-ion) m/z, 899 $[M+H]^+$, 921 $[M+Na]^+$. HRFABMS, Calcd for $C_{48}H_{83}O_8PNa$: 921.5469. Found: 921.5450. Physical data of β-anomer (R_f=0.303, cyclohexane:EtOAc=3:1): 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.88 (6H, m), 1.26 (30H, br s), 1.40–1.48 (2H, m), 1.53 (3H, d, J=8.8 Hz), 1.60–1.76 (4H, m), 1.99–2.08 (4H, m), 2.32–2.39 (2H, m), 3.23 (1H, m), 3.26 (3H, m), 3.58 (1H, t, J=8.8 Hz), 3.66–3.80 (3H, m), 4.32–4.38 (2H, m), 4.53–4.59 (7H, m), 4.62 (1H, d, J=5.9 Hz), 5.01 (1H, t, J=8.3 Hz), 5.08 (1H, m), 5.25–5.39 (8H, m), 5.90–5.97 (3H, m), 6.16 (1H, dd, J=2.0, 12.7 Hz).

4.1.27. 6-*O*-(Allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranose (29). Compound **28** (1.55 g, 1.724 mmol) was treated as described in the formation of **12** from **10** to give anomeric mixture **29** (1.20 g, 81%) as an oil. IR ν_{max}(film) 3324, 2927, 2856, 1750, 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (30H, br s), 1.42–1.50 (2H, m), 1.58–1.77 (4H, m), 1.99–2.02 (4H, m), 2.36–2.40 (2H, m), 2.86 (1H, br s, OH), 3.25 (1H, m), 3.26 (3H, s), 3.76–3.79 (2H, m), 3.87 (1H, t, J=9.2 Hz), 4.20 (1H, m), 4.28–4.37 (3H, m), 4.51–4.64 (7H, m), 4.76 (1H, dd, J=3.5, 9.8 Hz), 5.24–5.39 (8H, m), 5.88–5.99 (3H, m). FABMS (positive-ion) m/z; 859 $[M+H]^+$, 881 $[M+Na]^+$. HRFABMS, Calcd for $C_{45}H_{79}O_{13}PNa$: 881.5156. Found: 881.5153.

4.1.28. Trichloroacetimidoyl 6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-α,β-D-glucopyranoside (30). Compound **29** (1.10 g, 1.280 mmol) was treated as described in the formation of **13** from **12** to give a 7:3 anomeric mixture of α- and β-anomers **30** (1.26 g, 98%) as an oil. IR ν_{max}(film) 3348 (w), 2928, 2856, 1752, 1677, 1651 (w), 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.90 (6H, m), 1.24–1.28 (30H, m), 1.41–1.45 (2H, m), 1.58–1.77 (4H, m), 1.99–2.04 (4H, m), 2.28–2.32 (2H, m), 3.24 (1H, m), 3.26 (3H, s), 3.67–4.15 (5H, m), 4.36–4.62 (8H, m), 4.99 (1H, dd, J=3.5, 8.8 Hz), 5.24–5.40 (8H, m), 5.83 (0.3H, d, J=7.4 Hz, anomeric H), 5.88–5.99 (3H, m), 6.49 (0.7H, d, J=3.9 Hz, anomeric H), 8.63 (0.7H, s), 8.65 (0.3H, s). FABMS (positive-ion) m/z, 1026, 1024 $[M+Na]^+$.

4.1.29. 4-*O*-(Allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (31). Compounds **30** (576 mg, 0.576 mmol) and **23** (314 mg, 0.500 mmol) were treated as described in the formation of **24** from **13** and **23** to give **31** (393 mg, 54%) as an oil. IR ν_{max}(film) 3293, 3085 (w), 2924, 2854, 1752, 1729 (shoulder), 1707 (shoulder), 1635, 1557, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.26 (60H, br s), 1.40–1.50 (4H, m), 1.52–1.75 (6H, m), 2.01–2.04 (4H, m), 2.33–2.35 (2H, m), 2.50–2.54 (2H, m), 3.25 (1H, m), 3.26 (3H, s), 3.39 (2H, s), 3.44–3.76 (11H, m), 4.11–4.65 (14H, m), 4.92 (1H, t, J=7.0 Hz), 5.18 (1H, s), 5.24–5.39 (10H, m), 5.90–6.00 (4H, m), 7.13 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) m/z; 1490 $[M+Na]^+$.

HRFABMS, Calcd for $C_{79}H_{138}NO_{21}PNa$: 1490.9397. Found: 1490.9419.

4.1.30. Diallylphosphono 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-(allyloxycarbonyl)-6-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (32). Compound **31** (390 mg, 0.266 mmol) was treated as described in the formation of **25** from **24** to give **32** (305 mg, 70%) as an oil. IR ν_{max}(film) 3289 (w), 3086 (w), 2926, 2856, 1754, 1679, 1650, 1552, 1462 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.25 (60H, br s), 1.40–1.46 (4H, m), 1.50–1.70 (6H, m), 1.99–2.03 (4H, m), 2.34 (1H, m), 2.40 (1H, m), 2.42–2.51 (2H, m), 3.22 (1H, m), 3.25 (3H, s), 3.39 (2H, s), 3.51–3.72 (10H, m), 3.92 (1H, m), 4.08 (1H, m), 4.25–4.35 (2H, m), 4.42 (1H, d, J=8.2 Hz, anomeric H), 4.57–4.65 (12H, m), 4.76 (1H, m), 4.90 (1H, m), 5.24–5.42 (14H, m), 5.70 (1H, m, anomeric H), 5.90–5.99 (6H, m), 7.38 (1H, d, J=8.2 Hz, NH). FABMS (positive-ion) *m/z*; 1450, 1650 $[M+Na]^+$. HRFABMS, Calcd for $C_{85}H_{147}NO_{24}P_2Na$: 1650.9686. Found: 1650.9692.

4.1.31. Phosphono 3-*O*-decyl-2-deoxy-6-*O*-{3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-4-*O*-phosphono-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (33). Compound **32** (190 mg, 0.117 mmol) was treated as described in the formation of **26** from **25** to give **33** (86 mg, 57%) as a wax [R_f=0.212; $CHCl_3$–EtOH–AcOH–H_2O (8/5/1/1), on a silica gel TLC plate]. IR ν_{max}(KBr) 3299 (br), 2955, 2924, 2853, 1722, 1650, 1545, 1467 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$+CD_3OD, 5:1) δ 0.87–0.90 (12H, m), 1.26 (60H, br s), 1.42–1.70 (8H, m), 1.70–1.76 (2H, m), 1.98–2.02 (4H, m), 2.35–2.40 (2H, m), 2.54–2.57 (2H, m), 3.26–4.11 (21H, m, containing 3H, s, and 2H, s, at 3.31 and 3.38 ppm, respectively), 4.24 (1H, m), 4.50 (1H, d, J=7.4 Hz, anomeric H), 4.86 (1H, m), 5.35–5.39 (2H, m), 5.49 (1H, m, anomeric H). FABMS (negative-ion) *m/z*; 1298 $[M-H]^-$. Anal. Calcd for $C_{65}H_{123}NO_{20}P_2\cdot 3H_2O$: C, 57.63; H, 9.60; N, 1.03; P, 4.57. Found: C, 57.59; H, 9.38; N, 1.29; P, 4.49.

4.1.32. (*E*)-1-Propenyl 4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (34). To a solution of anomeric mixture **4** (3.140 g, 7.293 mmol) and (*Z*)-11-octadecenyl methanesulfonate (3.040 g, 8.771 mmol) in DMF (20 ml) was added NaH (55% oil dispersion, 400 mg, 9.167 mmol). The mixture was stirred for 1 h at room temperature, and then for 1.5 h at 60 °C under nitrogen. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (19/1, then 9/1) gave a 7:3 anomeric mixture of α- and β-anomers **34** (4.000 g, 81%) as an oil. IR ν_{max}(film) 2926, 2856, 1680 (w), 1661 (w), 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (6H, m), 1.29 (30H, br s), 1.40–1.80 (17H, m, containing two 3H, s, at 1.42 and 1.49 ppm), 2.01–2.04 (4H, m), 3.19–3.93 (14H, m, containing 3H, s, at 3.34 ppm), 4.53 (0.3H, d, J=7.4 Hz, anomeric H), 5.07 (0.7H, d, J=3.5 Hz, anomeric H), 5.11–5.22 (1H, m), 5.34–5.38 (2H, m), 6.14–6.23 (1H, m). FABMS (positive-ion) *m/z*; 681 $[M+H]^+$, 703 $[M+Na]^+$. HRFABMS, Calcd for $C_{41}H_{76}O_7Na$: 703.5490. Found: 703.5527.

4.1.33. (*E*)-1-Propenyl 3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (35). A solution of anomeric mixture **34** (4.000 g, 5.873 mmol) in aq 80% AcOH (100 ml) was stirred for 2.5 h at 60 °C, and concentrated in vacuo to give a diol, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (2/1, then 2/3) gave a 7:3 anomeric mixture of α- and β-anomers **35** (2.310 g, 61%) as an oil. IR ν_{max}(film) 3423 (br), 2926, 2856, 2525 (br), 1680 (w), 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27 (30H, br s), 1.45–1.61 (9H, m, containing two 3H, dd, J=1.6, 6.6 Hz at 1.56 ppm), 1.71–1.80 (2H, m), 1.99–2.08 (4H, m), 3.18 (1H, m), 3.23–3.92 (12H, mcontaining 3H, s, at 3.32 ppm), 4.10 (1H, m), 4.52 (0.3H, d, J=7.0 Hz, anomeric H), 5.11 (0.7H, d, J=3.1 Hz, anomeric H), 5.20 (1H, m), 5.34–5.36 (2H, m), 6.16–6.24 (1H, m). FABMS (positive-ion) *m/z*; 641 $[M+H]^+$, 663 $[M+Na]^+$. HRFABMS, Calcd for $C_{38}H_{72}O_7Na$: 663.5176. Found: 663.5182.

4.1.34. (*E*)-1-Propenyl 6-*O*-*tert*-butyldimethylsilyl-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (36). Compound **35** (1.200 g, 1.872 mmol) was treated as described in the formation of **7** from **6** to give a 3:2 anomeric mixture of α- and β-anomers **36** (1.31 g, 93%) as an oil. IR ν_{max}(film) 3432 (br), 2927, 2856, 1681 (w), 1661 (w), 1463 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.07 (6H, s), 0.86–0.92 (15H, m), 1.24–1.40 (30H, m), 1.41–1.60 (9H, m), 1.75–1.79 (2H, m), 1.99–2.05 (4H, m), 3.13–4.03 (14H, m, containing 3H, s, at 3.31 ppm), 4.46 (0.4H, d, J=7.4 Hz, anomeric H), 5.08–5.21 (1.6H, m, containing 0.6H, anomeric H), 5.33–5.38 (2H, m), 6.18–6.23 (1H, m). FABMS (positive-ion) *m/z*, 755 $[M+H]^+$. HRFABMS (on addition of NaI), Calcd for $C_{44}H_{86}O_7SiNa$: 777.6040. Found: 777.6022.

4.1.35. (*E*)-1-Propenyl 6-*O*-*tert*-butyldimethylsilyl-4-*O*-diallylphosphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (37). Compound **36** (1.200 g, 1.589 mmol) was treated as described in the formation of **8** from **7** to give anomeric mixture **37** (1.338 g, 92%) as an oil. IR ν_{max}(film) 2928, 2856, 1681 (w), 1463 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.86–0.92 (15H, m), 1.24–1.28 (30H, br s), 1.43–1.50 (2H, m), 1.53–1.62 (7H, m), 1.78–1.81 (2H, m), 1.99–2.04 (4H, m), 3.18–4.20 (14H, m), 4.46 (0.4H, d, J=7.8 Hz, anomeric H), 4.54–4.58 (4H, m), 5.09–5.39 (8H, m), 5.90–5.98 (2H, m), 6.18–6.23 (1H, m). FABMS (positive-ion) *m/z*, 915 $[M+H]^+$, 937 $[M+Na]^+$. HRFABMS, Calcd for $C_{50}H_{96}O_{10}Si$: 915.6510. Found: 915.6527.

4.1.36. (*E*)-1-Propenyl 4-*O*-diallylphosphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (38). Compound **37** (1.338 g, 1.462 mmol) was treated as described in the formation of **9** from **8** to give a 3:2 anomeric mixture of α- and β-anomers **38** (1.171 g, 100%) as an oil. IR ν_{max}(film) 3431 (br), 2927, 2856, 1680 (w), 1661 (w), 1462 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.26 (30H, br s), 1.40–1.44 (2H, m), 1.54–1.61 (7H, m), 1.73–1.78 (2H, m), 1.99–2.03 (4H, m),

3.20–3.39 (5H, m, containing 3H, s, at 3.29 ppm), 3.56–3.93 (8H, m), 4.31 (1H, m), 4.47 (0.4H, d, J=7.8 Hz, anomeric H), 4.55–4.58 (2H, m), 4.61–4.65 (2H, m), 5.08–5.21 (1.6H, m, containing 0.6H, d, J=3.9 Hz, at 5.13 ppm, anomeric H), 5.25–5.41 (6H, m), 5.90–5.98 (2H, m), 6.17–6.22 (1H, m). FABMS (positive-ion) *m/z*; 801 $[M+H]^+$, 823 $[M+Na]^+$. HRFABMS, Calcd for $C_{44}H_{81}O_{10}PNa$: 823.5465. Found: 823.5477.

4.1.37. (*E*)-1-Propenyl 4-*O*-diallylphosphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (39). Compound **38** (1.180 g, 1.473 mmol) was treated as described in the formation of **10** from **9** to give a 3:2 anomeric mixture of α- and β-anomers **39** (1.040 g, 87%) as an oil. IR ν_{max}(film) 2926, 2856, 1681 (w), 1661 (w), 1459 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (30H, br s), 1.40–1.46 (2H, m), 1.51–1.60 (7H, m), 1.74–1.80 (2H, m), 1.98–2.01 (4H, m), 3.19–3.91 (16H, m, containing two 3H, s, at 3.28 and 3.37 ppm), 4.42 (0.6H, q, J=9.4 Hz), 4.34 (0.4H, q, J=9.4 Hz), 4.45 (0.4H, d, J=7.8 Hz), 4.53–4.58 (4H, m), 5.06–5.38 (7.6H, m), 5.88–5.97 (2H, m), 6.16–6.22 (1H, m). FABMS (positive-ion) *m/z*; 815 $[M+H]^+$, 837 $[M+Na]^+$. HRFABMS, Calcd for $C_{45}H_{83}O_{10}PNa$: 837.5621. Found: 837.5635.

4.1.38. 4-*O*-Diallylphosphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranose (40). Compound **39** (1.030 g, 1.264 mmol) was treated as described in the formation of **11** from **10** to give an anomeric mixture **40** (739 mg, 75%) as an oil. IR ν_{max}(film) 3354 (br), 2927, 2856, 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (32H, br s), 1.40–1.45 (2H, m), 1.57–1.61 (2H, m), 1.75–1.81 (2H, m), 1.99–2.04 (4H, m), 3.09–3.40 (16H, m, containing two 3H, s, at 3.29 and 3.39 ppm), 3.54–3.88 (7H, m), 4.04–4.29 (3H, m), 4.54–4.65 (4H, m), 5.24–5.40 (6H, m), 5.91–5.99 (2H, m). FABMS (positive-ion) *m/z*; 775 $[M+H]^+$, 797 $[M+Na]^+$. HRFABMS, Calcd for $C_{42}H_{79}O_{10}PNa$: 797.5309. Found: 797.5279.

4.1.39. Trichloroacetimidoyl 4-*O*-diallylphosphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (41). Compound **40** (733 mg, 0.946 mmol) was treated as described in the formation of **12** from **11** to give a 1:1 anomeric mixture of α- and β-anomers **41** (870 mg, 100%) as an oil. IR ν_{max}(film) 3350–3000, 2927, 2856, 1733, 1674, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.24–1.55 (34H, m), 1.77–1.80 (2H, m), 1.99–2.05 (4H, m), 3.25–4.00 (16H, m, containing two 3H, s, at 3.30 and 3.38 ppm), 4.37–4.48 (1H, m), 4.56–4.62 (4H, m), 5.24–5.41 (6H, m), 5.67 (0.5H, d, J=7.4 Hz, anomeric H), 5.90–6.00 (2H, m), 6.50 (0.5H, d, J=3.2 Hz, anomeric H), 8.59 (0.5H, s), 8.66 (0.5H, s). FABMS (positive-ion) *m/z*; 940 $[M+Na]^+$.

4.1.40. 4-*O*-(Allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (42α) and 4-*O*-(Allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (42β). (a) Imidate **41** (307 mg, 0.334 mmol) and alcohol **23** (201 mg, 0.320 mmol) were treated as described in the formation of **24** from imidate **13** and alcohol **23** to give **42α** (66 mg, 15%) and **42β** (143 mg, 32%) as oils. Physical data of **42α**: R_f=0.714 (cyclohexane–EtOAc (2/3). IR ν_{max}(film) 3450–3300, 2926, 2854, 1751, 1723 (w), 1646, 1554, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.26 (62H, br s), 1.40–1.45 (4H, m), 1.55–1.59 (4H, m), 1.70–1.80 (2H, m), 1.99–2.03 (4H, m), 2.52 (2H, t, J=7.2 Hz), 3.20–3.91 (25H, m, containing two 3H, s, and 2H, s, at 3.28, 3.38 and 3.39 ppm, respectively), 4.13–4.24 (2H, m), 4.37 (1H, m, OH), 4.52–4.63 (6H, m), 4.87 (1H, d, J=3.5 Hz, anomeric C1′–H), 5.13 (1H, t, J=3.1 Hz, changed to a doublet on addition of D_2O, anomeric C1–H), 5.23–5.40 (8H, m), 5.88–5.98 (3H, m), 7.08 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) *m/z*; 1406 $[M+Na]^+$. HRFABMS, Calcd for $C_{76}H_{138}NO_{18}PNa$: 1406.9550. Found: 1406.9556. Physical data of **42β**: R_f=0.514 (cyclohexane–EtOAc (2/3). IR ν_{max}(film) 3307 (w), 2926, 2855, 1754, 1722, 1645, 1555 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (62H, br s), 1.40–1.50 (4H, m), 1.50–1.60 (4H, m), 1.75–1.79 (2H, m), 1.99–2.02 (4H, m), 2.52 (2H, t, J=7.4 Hz), 3.16–3.88 (25H, m, containing two 3H, s, and 2H, s, at 3.28, 3.38 and 3.40 ppm, respectively), 4.20 (1H, m), 4.31 (1H, m), 4.37 (1H, d, J=7.0 Hz, anomeric C1′–H), 4.54–4.64 (6H, m), 5.20 (1H, m, changed to a doublet, J=3.1 Hz, on addition of D_2O, anomeric C1–H), 5.25–5.39 (8H, m), 5.89–5.98 (3H, m), 7.12 (1H, d, J=9.3 Hz, NH). FABMS (positive-ion) *m/z*; 1406 $[M+Na]^+$. HRFABMS, Calcd for $C_{76}H_{138}NO_{18}PNa$: 1406.9550. Found: 1406.9575.

(b) To a solution of anomeric mixture **45** (247 mg, 0.173 mmol) in CH_2Cl_2 (5 ml) and MeCN (11 ml) was added aq 48% HF (3.5 ml). The mixture was stirred for 16 h at room temperature, and diluted with EtOAc, washed with water, aq satd $NaHCO_3$, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1/1, then 1/3) gave **42α** (29 mg, 12%) and **42β** (82 mg, 34%).

4.1.41. Diallylphosphono 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (43β). Compound **42β** (204 mg, 0.146 mmol) was treated as described in the formation of **25** from **24** to give **43β** (132 mg, 58%) as an oil. IR ν_{max}(film) 3293, 3086 (w), 2926, 2856, 1757, 1721, 1678, 1650, 1552, 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.25 (62H, br s), 1.40–1.49 (4H, m), 1.52–1.60 (4H, m), 1.74–1.79 (2H, m), 2.00–2.13 (4H, m), 2.48–2.54 (2H, m), 3.11 (1H, dd, J=7.8, 8.6 Hz), 3.23 (1H, m), 3.28 (3H, s), 3.31–3.38 [22H, m, containing 3H, s, 2H, s, and 1H, d, J=7.4 Hz (anomeric H), at 3.37, 3.40, and 4.27 ppm, respectively], 4.54–4.65 (10H, m), 4.84 (1H, m), 5.23–5.42 (12H, m), 5.72 (1H, dd, J=3.1, 6.3 Hz, anomeric H), 5.87–6.01 (5H, m), 7.41 (1H, d, J=8.6 Hz, NH). FABMS (positive-ion) *m/z*; 1366, 1406, 1566 $[M+Na]^+$. HRFABMS, Calcd for $C_{82}H_{147}NO_{21}P_2Na$: 1566.9838. Found: 1566.9841.

4.1.42. Phosphono 3-*O*-decyl-2-deoxy-6-*O*-{3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-4-*O*-phosphono}-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (44β). Compound **43β** (124 mg, 0.080 mmol) was treated as described in the formation of **26** from **25** to give **44β** (63 mg, 60%) as a wax [R_f=0.128; $CHCl_3$–EtOH–AcOH–H_2O (8/5/1/1), on a silica gel TLC plate]. IR ν_{max}(KBr) 3296 (w, br), 2924, 2853, 1717, 1649, 1546, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$+CD_3OD, 4:1) δ 0.86–0.90 (12H, m), 1.26–1.28 (62H, br s), 1.51–1.60 (8H, m), 1.81–1.85 (2H, m), 1.99–2.04 (4H, m), 2.54–2.58 (2H, m), 3.14 (1H, m), 3.28–4.11 (26H, m, containing two 3H, s, at 3.34 (C_6–OMe) and 3.40 (side chain OMe) ppm), 4.39 (1H, d, J=7.4 Hz, anomeric H), 5.34–5.39 (2H, m, olefinic H), 5.53 (1H, dd, J=3.1, 6.9 Hz, anomeric H). FABMS (negative-ion) *m/z*; 1298 $[M-H]^-$. Anal. Calcd for $C_{66}H_{127}NO_{19}P_2$ (1300.7): C, 60.95; H, 9.84; N, 1.08; P, 4.76. Found: C, 60.78; H, 9.61; N, 1.32; P, 4.53.

4.1.43. (*E*)-1-Propenyl 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-β-D-glucopyranoside (45). Imidate **13** (510 mg, 0.437 mmol) and alcohol **22** (253 mg, 0.379 mmol) were treated as described in the formation of **24** from imidate **13** and an alcohol **23** to give a 1:3 anomeric mixture of α- and β-anomers **45** (546 mg, quantitatively) as an oil. Part of the β-anomer was separated from the mixture on a silica gel TLC plate. R_f values of α- and β-anomers were 0.476 and 0.547, respectively [cyclohexane–EtOAc (3/2)]. Developmemt with cyclohexane–EtOAc (3/2) gave the β-anomer as a single constituent. Physical data of β-anomer of **45**: IR ν_{max}(film) 3370–3180, 2926, 2856, 1755, 1726, 1681, 1663, 1610, 1556 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.89 (12H, m), 1.24–1.40 (62H, br s), 1.40–1.44 (4H, m), 1.51–1.56 (7H, m), 1.75–1.80 (2H, m), 2.01–2.05 (4H, m), 2.51 (2H, t, J=7.2 Hz), 3.10–3.94 (26H, m, containing two 3H, s, and 2H, s, at 3.28, 3.37 and 3.39 ppm, respectively), 4.23 (1H, m), 4.38 (1H, d, J=7.8 Hz, C1′–H), 4.54–4.64 (6H, m), 4.97 (1H, d, J=7.8 Hz, C1–H), 5.08 (1H, m), 5.23–5.39 (8H, m), 5.88–5.98 (3H, m), 6.21 (1H, dd, J=1.7, 12.3 Hz), 7.28 (1H, d, J=8.2 Hz, NH). FABMS (positive-ion) *m/z*; 1446 $[M+Na]^+$. HRFABMS, Calcd for $C_{79}H_{142}NO_{18}PNa$: 1446.9862. Found: 1446.9869.

4.1.44. (*E*)-1-Propenyl 6-*O*-(allyloxycarbonyl)-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (46). The 1:1 anomeric mixture **35** (1.09 g, 1.700 mmol) was treated as described in the formation of **27** from **6** to give 1:1 anomeric mixture **46** (784 g, 64%) as an oil. IR ν_{max}(film) 3500–3400, 2927, 2856, 1752, 1681 (w), 1661 (w), 1458 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.24–1.38 (30H, m), 1.42–1.49 (4H, m), 1.54–1.62 (7H, m), 1.71–1.79 (2H, m), 1.99–2.05 (4H, m), 3.18 (1H, m), 3.27–3.82 (11H, m, containing 3H, s, at 3.31 ppm), 4.08 (1H, m), 4.30–4.50 (1.5H, m, containing 0.5H anomeric H), 4.62–4.64 (2H, m), 5.08–5.23 (1.5H, m, containing 0.5H, d, J=3.5 Hz, at 5.12 ppm, anomeric H), 5.25–5.39 (4H, m), 5.88–5.98 (1H, m), 6.13–6.24 (1H,). FABMS (positive-ion) *m/z*; 725 $[M+H]^+$, 747 $[M+Na]^+$.

4.1.45. (*E*)-1-Propenyl 6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (47). The 1:1 anomeric mixture **46** (780 mg, 1.076 mmol) was treated as described in the formation of **8** from **7** to give a 3:2 anomeric mixture of α- and β-anomers **47** (809 mg, 85%) as an oil. IR ν_{max}(film) 2927, 2856, 1753, 1680 (w), 1660–1651 (w), 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (32H, br s), 1.40–1.45 (2H, m), 1.54–1.60 (5H, m, containing 3H, dd, J=1.6, 6.6 Hz, at 1.55 ppm), 1.75–1.80 (2H, m), 1.99–2.05 (4H, m), 3.24 (1H, m), 3.29 (3H, s), 3.31–3.38 (1H, m), 3.56–3.92 (7H, m), 4.21–4.52 (2.4H, m, containing 0.4H, d, J=7.8 Hz, at 4.47 ppm, anomeric H), 4.56–4.63 (6H, m), 5.09–5.20 (1.6H, m, containing 0.6H, d, J=3.9 Hz, at 5.10 ppm, anomeric H), 5.23–5.39 (8H, m), 5.87–5.97 (3H, m), 6.16–6.20 (1H, m). FABMS (positive-ion) *m/z*; 461, 885 $[M+H]^+$, 907 $[M+Na]^+$. HRFABMS, Calcd for $C_{48}H_{85}O_{12}PNa$: 907.5677. Found: 907.5654.

4.1.46. 6-*O*-(Allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranose (48). The anomeric mixture **47** (800 mg, 0.904 mmol) was treated as described in the formation of **12** from **10** to give anomeric mixture **48** (595 mg, 78%) as an oil. IR ν_{max}(film) 3338 (br), 2927, 2856, 1753, 1650 (w), 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.7 Hz), 1.27 (32H, br s), 1.42–1.46 (2H, m), 1.59–1.63 (2H, m), 1.75–1.79 (2H, m), 1.99–2.02 (4H, m), 3.12–3.42 (6H, m, containing 3H, s, at 3.29 ppm), 3.60–3.69 (2H, m), 3.78–3.87 (2H, m), 4.16–4.53 (4H, m), 4.56–4.63 (6H, m), 5.23–5.39 (9H, m), 5.87–5.97 (3H, m). FABMS (positive-ion) *m/z*; 845 $[M+H]^+$, 867 $[M+Na]^+$. HRFABMS, Calcd for $C_{45}H_{81}O_{12}PNa$: 867.5363. Found: 867.5362.

4.1.47. Trichloroacetimidoyl 6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α,β-D-glucopyranoside (49). Compound **48** (590 mg, 0.698 mmol) was treated as described in the formation of **13** from **12** to give a 1:1 anomeric mixture of α- and β-anomers **49** (691 mg, 100%) as an oil. IR ν_{max}(film) 3350–3080, 2928, 2856, 1752, 1675, 1651 (w), 1611 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.7 Hz), 1.22–1.29 (32H, m), 1.43–1.53 (4H, m), 1.74–1.82 (2H, m), 1.99–2.04 (4H, m), 3.25 (1H, m), 3.30 (3H, s), 3.44–4.11 (7H, m), 4.33–4.51 (3H, m), 4.56–4.62 (6H, m), 5.23–5.40 (8H, m), 5.71 (0.5H, d, J=7.4 Hz, anomeric H), 5.86–5.99 (3H, m), 6.48 (0.5H, d, J=3.5 Hz, anomeric H), 8.61 (0.5H, s), 8.66 (0.5H, s). FABMS (positive-ion) *m/z*; 827, 1010 [^{35}Cl, $M+H]^+$, 1012 $[M+H]^+$.

4.1.48. 4-*O*-(Allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (50α) and 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranose (50β). Imidate **49** (765 mg, 0.576 mmol) and alcohol **23** (438 mg, 0.698 mmol) were treated as described in the formation of **24** from imidate **13** and alcohol **23** to give α-anomer **50α** (543 mg, 53%) [R_f=0.467, cyclohexane–

EtOAc (3/2)] as an oil containing a small amount of **48** [R_f=0.511, cyclohexane–EtOAc (3/2)] derived from the starting imidate **49**, and β-anomer **50β** (280 mg, 28%) [R_f=0.189, cyclohexane–EtOAc (3/2)] as an oil. It was difficult to separate **48** from **50α**. Physical data of α-anomer: IR ν_{max}(film) 3380–3080, 2927, 2856, 1752, 1725, 1690–1672 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.90 (12H, m), 1.26 (62H, br s), 1.40–1.46 (4H, m), 1.53–1.80 (4H, m), 1.70–1.80 (2H, m), 1.98–2.05 (4H, m), 2.53 (2H, t, J=7.4 Hz), 3.21–3.93 (19H, m, containing 3H, s, at 3.28 ppm), 4.15–4.41 (4H, m, containing OH), 4.49–4.64 (10H, m), 4.87 (1H, d, J=3.1 Hz, anomeric C1′–H), 5.16 (1H, d, J=3.9 Hz, anomeric C1–1), 5.21–5.40 (10H, m), 5.87–5.99 (4H, m), 7.15 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) *m/z*; 867 (contamination of **48**), 1476 $[M+Na]^+$. Physical data of β-anomer: IR ν_{max}(film) 3312 (w, br), 2926, 2855, 1754, 1650, 1547 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (62H, br s), 1.40–1.50 (4H, m), 1.52–1.60 (4H, m), 2.52 (2H, t, J=7.4 Hz), 3.19 (1H, t, J=8.0 Hz), 3.26 (1H, m), 3.29 (3H, s), 3.35 (1H, m), 3.37, 3.42 (2H, AB-q, J=14.3 Hz), 3.50–3.89 (11H, m), 4.20 (1H, dt, J=3.4, 10.0 Hz), 4.27–4.39 (2H, m), 4.36 (1H, d, J=7.4 Hz, anomeric C1′–H), 4.43 (1H, br s, OH), 4.56–4.66 (10H, m), 5.22–5.40 (11H, m), 5.88–5.98 (4H, m), 7.18 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) *m/z*; 1476 $[M+Na]^+$. HRFABMS, Calcd for $C_{79}H_{140}NO_{20}PNa$: 1476.9604. Found: 1476.9606.

4.1.49. Diallylphosphono 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-(allyloxycarbonyl)-6-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (51β). Compound **50β** (276 mg, 0.190 mmol) was treated as described in the formation of **25** from **24** to give **51β** (188 mg, 61%) as an oil. IR ν_{max}(film) 3294 (w), 3089 (w), 2926, 2856, 1754, 1678 (w), 1650 (w), 1550 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.86–0.90 (12H, m), 1.26 (62H, br s), 1.42–1.46 (4H, m), 1.51–1.57 (4H, m), 1.74–1.80 (2H, m), 2.00–2.02 (4H, m), 2.48–2.51 (2H, m), 3.13 (1H, m), 3.23 (1H, m), 3.29 (3H, s), 3.35 (1H, t, J=9.0 Hz), 3.40 (2H, s), 3.50–4.29 (14H, m), 4.50–4.65 (14H, m), 4.84 (1H, t, J=9.5 Hz), 5.22–5.42 (14H, m), 5.72 (1H, dd, J=3.1, 6.3 Hz), 5.89–5.98 (6H, m), 7.42 (1H, d, J=9.0 Hz, NH). FABMS (positive-ion) *m/z*; 1436, 1636 $[M+Na]^+$. HRFABMS, Calcd for $C_{85}H_{149}NO_{23}P_2Na$: 1636.9894. Found: 1636.9932.

4.1.50. Diallylphosphono 4-*O*-(allyloxycarbonyl)-3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-(allyloxycarbonyl)-6-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (51α). Compound **50α** (531 mg, 0.365 mmol) was treated as described in the formation of **25** from **24** to give **51α** (260 mg, 44%) as an oil contaminated by a small amount of diallylphosphono 6-*O*-(allyloxycarbonyl)-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-α- and β-D-glucopyranosides derived from **48**. Physical data of **51α**: IR ν_{max}(film) 3370–3100, 2927, 2856, 1753, 1730, 1668, 1651, 1611, 1549, 1461 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.22–1.30 (62H, m), 1.42–1.44 (4H, m), 1.52–1.62 (4H, m), 1.75–1.80 (2H, m), 1.98–2.06 (4H, m), 2.53 (2H, t, J=7.4 Hz), 3.24 (1H, m), 3.28 (3H, s), 3.29 (2H, s), 3.40–4.30 (18H, m), 4.35–4.64 (18H, m), 4.87 (1H, d, J=3.1 Hz, anomeric C1′–H), 5.15–5.39 (14H, m), 5.60 (1H, m), 5.88–5.99 (6H, m), 7.11 (1H, d, J=9.2 Hz, NH). FABMS (positive-ion) *m/z*; 1476, 1636 $[M+Na]^+$. FABMS (negative-ion) *m/z*; 1612 $[M-H]^-$.

4.1.51. Phosphono 3-*O*-decyl-2-deoxy-6-*O*-{3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenyl]-4-*O*-phosphono-β-D-glucopyranosyl}-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (52β). Compound **51β** (82 mg, 0.051 mmol) was treated as described in the formation of **26** from **25** except for a temperature of 35 °C to give **52β** (43 mg, 66%) as a wax [R_f=0.275; $CHCl_3$–EtOH–AcOH–H_2O (8/5/1/1), on a silica gel TLC plate]. IR ν_{max}(KBr) 3700–3000, 2925, 2854, 1717, 1652, 1543, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$+CD_3OD, 5:1) δ 0.88 (6H, t, J=6.6 Hz), 0.89 (6H, t, J=6.6 Hz), 1.22–1.34 (62H, m), 1.50–1.58 (8H, m), 1.78–1.84 (2H, m), 1.99–2.04 (4H, m), 2.55 (2H, t, J=7.2 Hz), 3.14 (1H, t, J=8.4 Hz), 3.31–3.38 (6H, m, containing 3H, s, at 3.34 ppm), 3.50 (1H, d, J=9.8 Hz), 3.60–4.15 (16H, m), 4.39 (1H, d, J=7.4 Hz, anomeric C1′–H), 5.31–5.39 (2H, m), 5.52 (1H, q, J=3.1 Hz, anomeric C1–H). FABMS (negative-ion) *m/z*; 1284 $[M-H]^-$. Anal. Calcd for $C_{65}H_{125}NO_{19}P_2\cdot 2H_2O$: C, 59.03; H, 9.83; N, 1.06; P, 4.68. Found: C, 59.10; H, 9.77; N, 1.07; P, 4.29.

4.1.52. Allyl 3-*O*-decyl-2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetylamino-α-D-glucopyranoside (54). To a solution of allyl 2-deoxy-4,6-*O*-isopropylidene-2-(trifluoroacetyl)amino-α-D-glucofuranoside **53** (10.60 g, 29.83 mmol) and decyl methanesulfonate (9.04 g, 38.25 mmol) in DMF (40 ml) was added NaH (55% oil dispersion, 3.00 g, 39.60 mmol) under ice cold temperature. After stirring for 30 min at 0 °C, the mixture was stirred for 16 h at room temperature. The reaction mixture was quenched with MeOH under ice cold temperature, and diluted with EtOAc. The solution was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (9/1) gave **54** (13.20 g, 89%) as an oil. IR ν_{max}(film) 3320, 2927, 2857, 1715, 1556–1543, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.24 (14H, br s), 1.41 (3H, s), 1.42–1.50 (2H, m), 1.51 (3H, s), 3.40–3.51 (2H, m), 3.64–3.88 (5H, m), 3.99 (1H, m), 4.15–4.21 (2H, m), 4.88 (1H, d, J=3.5 Hz, anomeric H), 5.24–5.32 (2H, m), 5.87 (1H, m), 6.40 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) *m/z*; 496 $[M+H]^+$.

4.1.53. 2-Hydroxyethyl 2-amino-3-*O*-decyl-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (55). To a solution of **54** (13.20 g, 26.63 mmol) in THF–H_2O (3/1, 260 ml) were added $NaIO_4$ (43 g, 201 mmol) and OsO_4 (4.5 ml, 2.5% solution in *t*-BuOH). The mixture was stirred for 1.5 h at room temperature. The reaction mixture was diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give crude aldehyde (16.47 g), which was dissolved in 99.5% EtOH (300 ml). To this solution was added $NaBH_4$ (2.00 g, 52.87 mmol), and the mixture was stirred for 20 min at room temperature. The reaction mixture was cooled to 0 °C, quenched with AcOH, concentrated

in vacuo till one sixth volume and diluted with EtOAc. The solution was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a crude alcoholic mixture of a 2-trifluoroacetylamino compound and corresponding 2-amino compound (ca. 9.00 g), which was employed for the next reaction. The mixture was refluxed in EtOH (100 ml) and aq 1 M NaOH (50 ml) for 1.5 h, concentrated to half volume, and extracted with EtOAc, which was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give amine, which was chromatographed on an NH silica gel short column. Elution with EtOAc–MeOH (19/1) gave amine **55** (8.10 g, 76%). IR ν_{max}(film) 3700–3000, 2926, 2857, 1682, 1593 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.88 (3H, t, J=6.6 Hz), 1.27 (14H, br s), 1.40 (3H, s), 1.49 (3H, s), 1.52–1.56 (2H, m), 2.86 (1H, dd, J=3.4, 9.8 Hz, C2–2), 3.40 (1H, t, J=9.3 Hz), 3.53–3.87 (10H, m), 4.94 (1H, d, J=3.4 Hz, anomeric H). FABMS (positive-ion) *m/z*; 404 $[M+H]^+$, 426 $[M+Na]^+$. HRFABMS, Calcd for $C_{21}H_{42}NO_6$: 404.3012. Found: 404.3011.

4.1.54. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-2-deoxy-4,6-*O*-isopropylidene-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (56). To a solution of **55** (2.02 g, 5.000 mmol) in CH_2Cl_2 (20 ml) were added 1*H*-tetrazole (1.56 g, 22.3 mmol) and diallyl diisopropylphosphoramidite (1.69 g, 6.89 mmol), and the mixture was stirred for 30 min at room temperature, and then a solution of oxone (4.55 g, 7.40 mmol) in H_2O (12.5 ml) and THF (25 ml) was added to this reaction mixture. After stirring for 15 min at room temperature, the reaction mixture was diluted with CH_2Cl_2, which was washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a phosphate, which was dissolved in CH_2Cl_2 (150 ml). To this solution were added 3-oxotetradecanoic acid (1.46 g, 6.00 mmol), WSC·HCl (1.44 g, 7.51 mmol) and DMAP (0.92 g, 7.53 mmol). After stirring for 30 min at room temperature, the reaction mixture was diluted with CH_2Cl_2, which was washed with H_2O and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc–cyclohexane (3/1) gave **56** (1.96 g, two steps 50%). IR ν_{max}(film) 3290, 3085 (w), 2926, 2856, 1820, 1673, 1658 (shoulder), 1550, 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.25 (30H, br s), 1.40 (3H, s), 1.42–1.48 (2H, m), 1.49 (3H, s), 1.53–1.58 (2H, m), 2.52–2.56 (2H, m), 3.43–3.50 (4H, m, containing 2H, s, at 3.43 ppm), 3.60–3.88 (7H, m), 4.19–4.26 (3H, m), 4.52–4.61 (4H, m), 4.78 (1H, d, J=3.5 Hz, anomeric H), 5.26–5.44 (4H, m), 5.90–6.02 (2H, m). 7.62 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) *m/z*; 788 $[M+H]^+$, 810 $[M+Na]^+$. HRFABMS, Calcd for $C_{41}H_{74}NO_{11}PNa$: 810.4897. Found: 810.4916.

4.1.55. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-2-deoxy-2-(3-oxotetradecanoylamino)-α-D-glucopyranoside (57). A solution of **56** (79 mg, 0.10 mmol) in aq 80% AcOH (8 ml) was stirred at 85 °C for 75 min, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with EtOAc–MeOH (19/1) gave **57** (49 mg, 66%) as a solid. IR ν_{max}(KBr) 3427, 3298, 3081 (w), 2921, 2851, 1708, 1639, 1549 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.25 (28H, br s), 1.50–1.70 (8H, m, containing 2×OH), 2.54 (2H, t, J=7.4 Hz), 3.43–3.92 (11H, m, containing 2H, s, at 3.45 ppm), 4.19–4.27 (3H, m), 4.52–4.59 (4H, m), 4.78 (1H, d, J=3.5 Hz, anomeric H), 5.26–5.42 (4H, m), 5.89–5.99 (2H, m). 7.71 (1H, d, J=9.4 Hz, NH). FABMS (positive-ion) *m/z*; 748 $[M+H]^+$, 770 $[M+Na]^+$. HRFABMS, Calcd for $C_{38}H_{71}NO_{11}P$: 748.4765. Found: 748.4776.

4.1.56. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-2-deoxy-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-2-[(3-oxotetradecanoyl)amino]-α-D-glucopyranoside (58). Compounds **13** and **57** were treated as described in the formation of **24** from **13** and **23** to give **58** (45%) as a wax. IR ν_{max}(film) 3302, 2925, 2854, 1747, 1720, 1636, 1549, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.86–0.89 (12H, m), 1.25 (60H, br s), 1.40–1.72 (10H, m), 1.99–2.02 (4H, m), 2.31–2.35 (2H, m), 2.54 (2H, t, J=7.0–8.2 Hz), 3.21 (1H, m), 3.25 (3H, s), 3.39 (3H, s), 3.43 (2H, s), 3.45–3.78 (12H, m), 3.85 (1H, m), 3.98 (1H, dd, J=2.3, 11.0 Hz), 4.15–4.23 (3H, m), 4.35 (1H, q, J=9.4 Hz), 4.49–4.60 (10H, m), 4.72 (1H, d, J=3.9 Hz), 4.97 (1H, dd, J=8.2, 9.4 Hz), 5.24–5.43 (10H, m), 5.89–6.00 (4H, m), 7.67 (1H, d, J=9.4 Hz). FABMS (positive-ion) *m/z*; 1518 $[M+H]^+$, 1540 $[M+Na]^+$.

4.1.57. 2-(Phosphonooxy)ethyl 3-*O*-decyl-2-deoxy-6-*O*-{3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-4-*O*-phosphono-β-D-glucopyranosyl}-2-[(3-oxotetradecanoyl)amino]-α-D-glucopyranoside (59). Compound **58** was treated as described in the formation of **26** from **25** to give **59** (82%) as a wax [R_f=0.156; $CHCl_3$–EtOH–AcOH–H_2O (8/5/1/1), on a silica gel TLC plate]. IR ν_{max}(KBr) 3600–3200, 2925, 2854, 1723, 1649, 1547, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+CD_3OD$, 5:1) δ 0.86–0.89 (12H, m), 1.20–1.35 (60H, m), 1.38–1.62 (8H, m), 1.71–1.73 (2H, m), 2.00–2.04 (4H, m), 2.31–2.34 (2H, m), 2.55–2.58 (2H, m), 3.29 (3H, s), 3.32–4.18 (25H, m, containing 3H, s, at 3.40 ppm), 4.49 (1H, d, J=7.7 Hz, anomeric C1′–H), 4.72 (1H, d, J=3.5 Hz, anomeric C1–H), 4.88 (1H, dd, J=8.2, 9.0 Hz), 5.32–5.35 (2H, m). FABMS (negative-ion) *m/z*, 1356 $[M-H]^-$. Anal. Calcd for $C_{68}H_{128}NO_{21}P_2\cdot 1.4\ H_2O$: C, 59.02; H, 9.60; N, 1.01; P, 4.48. Found: C, 59.10; H, 9.51; N, 1.09; P, 4.37.

4.1.58. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-2-deoxy-6-*O*-{6-*O*-allyloxycarbonyl-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-2-[(3-oxotetradecanoyl)amino]-α-D-glucopyranoside (60). Compounds **30** and **57** were treated as described in the formation of **24** from **13** and **23** to give **60** (38%) as a wax. IR ν_{max}(film) 3400–3200, 2926, 2855, 1751, 1669, 1651, 1547, 1461 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.86–0.89 (12H, m), 1.25 (60H, br s), 1.40–1.78 (10H, m), 2.00–2.02 (4H, m), 2.30–2.34 (2H, m), 2.51–2.55 (2H, m), 3.21 (1H, m), 3.25 (3H, s), 3.43 (2H, s), 3.53–3.74 (12H, m), 3.85 (1H, m), 4.07 (1H, d, J=10.2 Hz), 4.15–4.40 (4H, m), 4.45 (1H, d, J=7.8 Hz), 4.50–4.65 (11H, m), 4.70 (1H, d, J=3.5 Hz), 4.95 (1H, m), 5.25–5.42 (12H, m), 5.90–5.97 (5H, m), 7.72 (1H, d, J=9.8 Hz). FABMS (positive-ion) *m/z*; 1588 $[M+H]^+$, 1610 $[M+Na]^+$.

4.1.59. 2-(Phosphonooxy)ethyl 3-*O*-decyl-2-deoxy-6-*O*-{3-*O*-[(*R*)-3-methoxydecyl]-2-*O*-[(*Z*)-11-octadecenoyl-4-*O*-phosphono]-β-D-glucopyranosyl}-2-[(3-oxotetradecanoyl)amino]-α-D-glucopyranoside (61). Compound **60** (203 mg, 0.128 mmol) was treated as described in the formation of **26** from **25** to give **61** (95 mg, 55%) as a wax. IR ν_{max}(KBr) 3600–3200, 2924, 2853, 1723, 1650, 1547, 1467 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+CD_3OD$, 4:1) δ 0.86–0.90 (12H, m), 1.26 (60H, br s), 1.40–1.62 (8H, m), 1.71–1.73 (2H, m), 2.00–2.04 (4H, m), 2.32–2.36 (2H, m), 2.56–2.58 (2H, m), 3.30 (3H, s), 3.31–4.24 (22H, m), 4.48 (1H, d, $J=8.2$ Hz, anomeric H), 4.73 (1H, d, $J=3.4$ Hz, anomeric H), 4.87 (1H, t, $J=8.2$ Hz), 5.33–5.37 (2H, m). FABMS (negative-ion) *m/z*; 1342 $[M-H]^-$. Anal. Calcd for $C_{67}H_{127}NO_{21}P_2\cdot 1.4\ H_2O$: C, 59.85; H, 9.52; N, 1.04; P, 4.61. Found: C, 59.96; H, 9.32; N, 1.19; P, 4.47.

4.2. Methods for measurement of biological activity

4.2.1. Production of TNFα by human whole blood.[9] *Materials.* Lipopolysaccharide (LPS, *E. coli* 026:B6), human tumor necrosis factor alpha (TNFα) immunoassay kit and 96-well assay plates were purchased from Sigma, BioSource International Inc., and Corning Inc. (Cat. No. 3956), respectively.

Whole blood TNFα production. Fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. The subjects did not have any apparent inflammatory conditions and had taken no drugs for at least 7 days prior to blood collection. Written informed consent was obtained from all volunteers before the experiment. In each well of the 96-well assay plates, a 360 μL aliquot of blood was mixed with 20 μL of LPS solution (200 ng/ml) dissolved in PBS in the presence (for the test sample) or absence (for the positive control samples) of a test compound solution (dissolved in 10% DMSO/PBS solution). For the negative control samples, the same amount of blood was cultured with PBS and a test compound solution. After 6 h of incubation at 37 °C, the plates were centrifuged at 490×g for 15 min, and the plasma was collected and stored at −20 °C. The concentrations of TNFα in the plasma were measured with commercially available immunoassay kits.

Statistical analysis. The percentage inhibition of TNFα production was calculated by the following formula: [1−(concentration of TNFα in the test sample−concentration of TNFα in the negative control sample)/(concentration of TNFα in the positive control sample−concentration of TNFα in the negative control sample)]×100. The suppressive activity of each test compounds is expressed as the fifty percent inhibitory concentration (IC_{50}) of the test compound, the concentration at which the test compound suppresses TNFα production by 50%. The IC_{50} was calculated from the percentage inhibition using the SAS System for Windows. The results are expressed as the mean IC_{50} of triplicate experiments.

4.2.2. Production of TNFα and lethality in C3H/HeN mice coinjected galactosamine and LPS.[10] *Materials: animals*. Male C3H/HeN mice were purchased from Charles River Japan (Tokyo, Japan). All mice were used at the age of 7 weeks, and housed at Sankyo Laboratories (Tokyo, Japan) with free access to standard rodent chow diet.

Reagents. Lipopolysaccharide (LPS, from *E. coli* O26:B6) and D-galactosamine (GalN) were purchased from Sigma (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits of murine TNFα were from R&D Systems (Minneapolis, MN).

TNFα production. Naïve C3H/HeN mice (five per group) were intravenously injected with the test compound solution (10 ml/kg; dissolved in 0.1% triethylamine /saline solution), and immediately after, mice were intravenously injected with a mixture of LPS (0.05 mg/10 ml saline/kg) and GalN (1 g/10 ml saline/kg). Mice were injected with vehicle (0.1% triethylamine/saline solution) and saline for negative control samples, and with vehicle and LPS/GalN for positive control samples. One hour after injection, venous blood was collected under ether anesthesia with heparinized syringes fitted with 23-gauge needles from the abdominal vena, and was centrifuged at 4 °C for 3 min at 13,230×g to obtain the plasma. Plasma was stored at −30 °C before measuring the TNFα level by ELISA. The concentration of TNFα in mouse plasma was measured using ELISA analysis according to the manufacturer's instructions.

Statistical analysis. The percentage inhibition of TNFα production was calculated by the following formula: [1−(concentration of TNFα in the test sample−concentration of TNFα in the negative control sample)/(concentration of TNFα in the positive control sample−concentration of TNFα in the negative control sample)]×100. The suppressive activity of each test compounds is expressed as the fifty percent inhibitory dose (ID_{50}) of the test compound, the dose at which the test compound suppresses TNFα production by 50%. The ID_{50} was calculated from the percentage inhibition using the SAS System for Windows (version 5).

Lethality. Naïve C3H/HeN mice (eight per group) were intravenously injected with a test compound solution (1 mg/10 ml/kg; dissolved in 0.1% triethylamine/saline solution), and immediately after, were intravenously injected with a mixture of LPS (0.05 mg/10 ml saline/kg) and GalN (1 g/10 ml saline/kg). Mice were injected with vehicle (0.1% triethylamine/saline solution) and LPS/GalN for control samples. Deaths occurring up to 3 days following administration were recorded.

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Trapping of carbamic acid species with (trimethylsilyl)diazomethane

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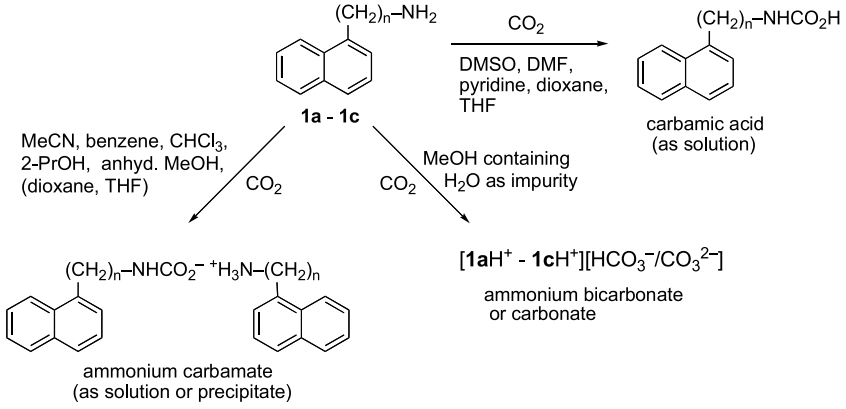
Abstract—Methoxycarbonylation of a variety of amines into the corresponding methyl carbamates was accomplished by allowing them to react with (trimethylsilyl)diazomethane $TMSCHN_2$ under bubbling of CO_2. The reaction was performed at room temperature for a period of ca. 2 h in benzene–MeOH (4/1 v/v), which was the solvent of choice. In this mixed solvent, undesirable bicarbonate is formed in equilibrium along with carbamate anion. Owing to the irreversibility in the esterification step by $TMSCHN_2$, however, the yield of methyl carbamate can reach very high.

1. Introduction

We and others have recently reported studies on the solvent dependence of the carbamic acid formation from ω-(1-naphthyl)alkylamines **1a–c** and carbon dioxide.[1,2] On the basis of NMR and IR analyses in situ after bubbling of CO_2 through solutions of these amines in DMSO, DMF or pyridine, it is concluded that they are completely converted to the corresponding carbamic acids in the protophilic, highly dipolar, aprotic solvents (see Scheme 1).[1] In dioxane (protophilic, dipolar, aprotic solvent), the carbamic acid and a small amount of the ammonium carbamate are formed. By contrast, in MeCN (protophobic, dipolar, aprotic solvent), in benzene or $CHCl_3$ (apolar, aprotic solvent), or in 2-PrOH or MeOH (dipolar, amphiprotic solvent), ammonium carbamates rather than carbamic acids are formed, although the ammonium bicarbonates/carbonates are competitively formed in hydrous MeOH. Not only solvent polarity and protic character but also its protophilicity was found to be a crucial factor. The selective generation of the undissociated carbamic acids in preference to the ammonium carbamates in protophilic, dipolar, aprotic solvent (DMSO, DMF, pyridine, or dioxane) was ascribed to the larger pK_a values[3] for the carbamic acids than for the amines **1a–c** in this class of solvent, that is, the equilibrium reaction 1 is shifted far to the left.



Scheme 1.

Keywords: Amine; Carbon dioxide; Carbamic acid; (Trimethylsilyl)diazomethane; Methyl carbamate.
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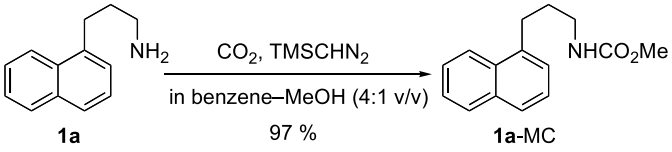
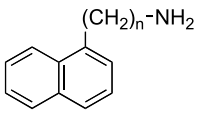
$$RNHCO_2H + RNH_2 \rightleftharpoons RNHCO_2^- + RNH_3^+ \quad \text{in protophilic, dipolar, aprotic solvent} \qquad (1)$$

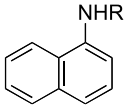
The ammonium carbamates often precipitated out from the solution and hence they could be separated. In order to trap unstable carbamic acids as the stable esters, we reacted 3-(1-naphthyl)propylamine (**1a**) in 4:1 v/v benzene/MeOH with (trimethylsilyl)diazomethane $TMSCHN_2$ under bubbling of CO_2.[1a] The corresponding methyl carbamate (**1a**-MC) was successfully obtained in essentially quantitative yield (Eq. 2). $TMSCHN_2$ is a well-known reagent for methylation of carboxylic acids.[4] In this paper, application of this *N*-methoxycarbonylation reaction to other amines such as **1**–**5** is investigated. Lately, amine has been utilized to fix carbon dioxide through various types of chemical reaction.[5] An example is the formation of carbamate ester (urethane) by using alkyl halide,[6] alcohol,[7] carbonate ester,[8] alkene,[9] alkyne,[9] or epoxide[9] as the alkylating agent, but $TMSCHN_2$ had not been employed to this aim before our previous report.[1a]



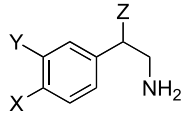
(2)



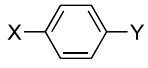
1a: n = 3
1b: n = 2
1c: n = 1



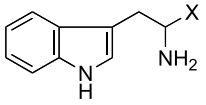
1d: R = H
1e: R = Me



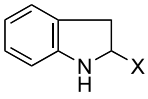
2a: X = Y = Z = H
2b: X = OH, Y = Z = H
2c: X = Y = OMe, Z = H
2d: X = Y = H, Z = OH (rac-)
2e: X = Y = OH, Z = H
2f: X = Y = Z = OH (rac-)



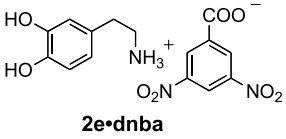
2g: X = H, Y = NH_2
2h: X = H, Y = NHMe
2i: X = H, Y = NMe_2
2j: X = OMe, Y = NH_2
2k: X = NMe_2, Y = NH_2



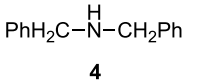
3a: X = H
3b: X = CO_2Me (L-)



5a: X = H
5b: X = Me



2e•dnba



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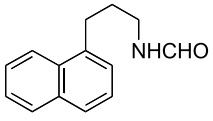

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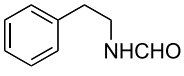

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2. Results and discussion

2.1. Methoxycarbonylation with $TMSCHN_2$

First, we looked for a suitable solvent for the *N*-methoxycarbonylation reaction. Since, according to Shioiri and co-workers,[4] methanol is indispensable for methylation of acids with $TMSCHN_2$, we carried out the reaction in benzene, dioxane, pyridine or DMF, each containing 20 (or 10) volume % of MeOH, or in pure MeOH.[†] Thus, to solutions of amines **1a**, **2a**, **2c**, **3a**, and **5a** in one of these solvents were added at room temperature 2–3equiv of $TMSCHN_2$ (an Aldrich 2.0 M solution in hexanes was used) with stirring under bubbling of CO_2. In less than half an hour, a yellow color of $TMSCHN_2$ disappeared and the corresponding methyl carbamates (MC's) resulted with excellent yields (83–100%) when benzene–MeOH (4/1) or dioxane–MeOH (4/1) was used (Table 1).[‡] However, the yields were much lower in the solvent like pyridine–MeOH (4/1 or 9/1), DMF–MeOH (4/1) or MeOH (22–61%). In the case of pyridine–MeOH, formation of a large amount of brown tar greatly impaired the reaction. When pure MeOH was used, $TMSCHN_2$ decomposed more rapidly as compared with the benzene–MeOH system. This is probably due to some side reactions. In DMF–MeOH, formamides **6** and **7** were obtained as additional products from **1a** and **2a**, respectively. From these results, benzene–MeOH (4/1) was employed as the solvent of choice for further experiments.

Solutions of amines **1**–**5** in 4:1 benzene–MeOH were treated with a $TMSCHN_2$ solution under bubbling of CO_2, as described above. As summarized in Table 2, primary alkyl amines **1a–c**, **2a–d**, **3a**, **3b** and secondary alkylamine **4** afforded their methyl carbamates (MC's) in excellent to good yields (76–100%). MC was obtained even from dopamine (**2e**), an important neurotransmitter in brain,[10] in a modest yield (17%) by using dopamine·3,5-dinitrobenzoic acid salt (**2e**·dnba)[11] as the starting amine. This methoxycarbonylation reaction is interesting, because **2e** itself is easily susceptible to oxidative polymerization to generate melanin, a structurally unelucidated black pigment.[11,12] Similar treatment of commercially available dopamine hydrochloride **2e**·HCl with $TMSCHN_2$ gave a very low yield of the crude MC (6%). MC was also obtained from DL-noradrenaline (**2f**) in 12% yield. One reason for the low yields of MC from catecholamines **2e** and **2f** is that their reactions had to be carried out in 4:1 pyridine/MeOH, since

[†] The possibility that the Me group comes from methanol in the methylation reaction was excluded by Shioiri by using methanol–^{13}C.[4]

[‡] The use of 1 equiv of $TMSCHN_2$ instead of 2 equiv decreased the yield of **2a**-MC from 97% (Table 1) to 78% in benzene–MeOH (4/1).

Table 1. Solvent effect on methoxycarbonylation of several amines with (trimethylsilyl)diazomethane under atmospheric pressure of CO_2 at room temperature

Amine	Solvent (v/v)	Product yield (%)[a]		Recovered amine (%)[b]
		Methyl carbamate	Others	
1a	Benzene–MeOH (4/1)	99[#]		Trace
1a	Dioxane–MeOH (4/1)	98[#]		0
1a	Pyridine–MeOH (9/1)	42[#]	[c]	51
1a	Pyridine–MeOH (4/1)	40	[c]	51
1a	DMF–MeOH (4/1)	22[#]	**6**, 52	0
1a	MeOH	38[#]		55
2a	Benzene–MeOH (4/1)	97[#]		0
2a	DMF–MeOH (4/1)	25	**7**, 7	0
2c	Benzene–MeOH (4/1)	100[#]		0
2c	MeOH	29		45
3a	Benzene–MeOH (4/1)	83[#]		10
3a	Pyridine–MeOH (4/1)	61	[c]	Trace
5a	Benzene–MeOH (4/1)	98 (97[#])		2
5a	Pyridine–MeOH (4/1)	32	[c]	50

[a] Isolation yield, unless marked with #, where it is the NMR yield. Values are based on the initial amount of amine.
[b] Estimated by NMR.
[c] A large amount of brown tar was formed.

2e·dnba, **2e**·HCl and **2f** are insoluble in 4:1 benzene/MeOH.

Other significant points found from inspection of Table 2 are that methoxycarbonylation reactions of arylamines **1d**, **2g**, **2h**, **2j**, **2k**, and **5b** are difficult (0–27% yields), although indoline (**5a**), which is an *N*-alkyl-substituted arylamine whose nitrogen atom is expected to be sterically less hindered than that of **5b** or **2h**, gave its methyl carbamate (**5a**-MC) in an excellent yield (98%). The MC yields from electron-rich *p*-anisidine (**2j**) and *N*,*N*-dimethyl-*p*-phenylenediamine (**2k**) (27 and 19%, respectively) were better than aniline (**2g**) (8%).

Alcohols react with CO_2 to form alkylcarbonic acids in the presence of tertiary amine bases under high-pressure or in the presence of 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU).[13] Under our reaction conditions, however,

Table 2. Methoxycarbonylation of various amines with (trimethylsilyl)diazomethane under atmospheric pressure of CO_2 at room temperature

Amine	Product yield (%)[a]		Recovered amine (%)[b]
	Methyl carbamate	Others	
Primary alkylamine: $RNH_2 \rightarrow RNH–CO_2Me$			
1a	99[#]		Trace
1b	95		0
1c	95		0
2a	97[#]		0
2b	88		7
2c	100[#]		0
2d	82		0
2e[c]	17	[d]	[e,f]
2f[g]	12	[d]	15
3a	83[#]		10
3b	76		14[h]
Secondary alkylamine: $RR'NH \rightarrow RR'N–CO_2Me$			
4	78		3
Arylamine: $ArRNH \rightarrow ArRN–CO_2Me$			
1d	0	**1e**, 13	82
2g	8		[e,i]
2h	0	**2i**, 7	>75[i]
2j	27		52
2k	19		66
5a	98 (97[#])		2
5b	24 (40[#])		57

The solvent employed is benzene–MeOH (4/1 v/v) except for **2e** and **2f**.
[a] Isolation yield, unless marked with #, where it is the NMR yield. Values are based on the initial amount of amine.
[b] Estimated by NMR.
[c] Dopamine·3,5-dinitrobenzoic acid salt (**2e**·dnba)[11] dissolved in 4:1 pyridine/MeOH was reacted (**2e**·dnba is insoluble in 4:1 benzene/MeOH). The reaction of dopamine hydrochloride (**2e**·HCl) under the same conditions gave a lower yield of MC (6%).
[d] A large amount of brown tar was formed.
[e] Not estimated.
[f] Polymerized to melanin during the workup.
[g] The reaction was carried out in 4:1 pyridine/MeOH, since DL-noradrenaline (**2f**) is insoluble in 4:1 benzene/MeOH.
[h] Isolated.
[i] The recovered amine was partially lost on evaporation of the solvent.

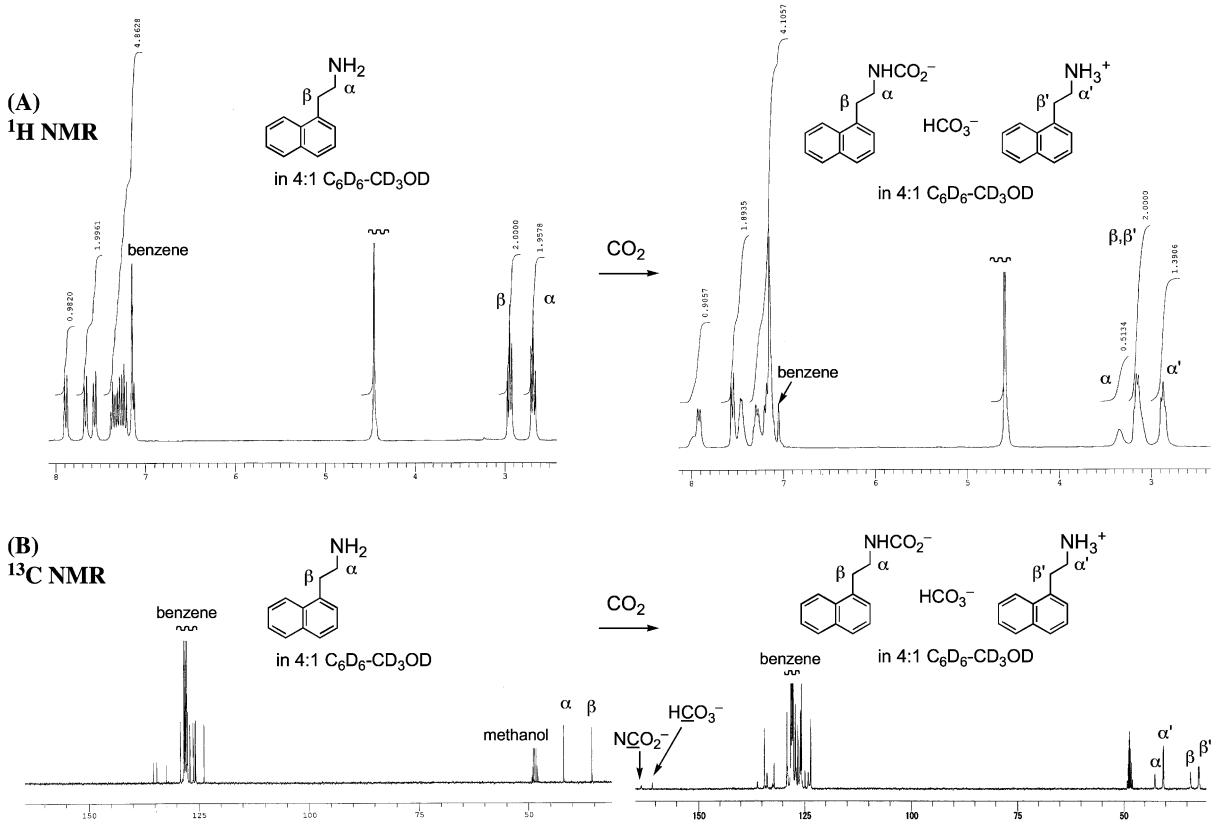

Figure 1. The ^{1}H and ^{13}C NMR spectra of **1b** in 4:1 C_6D_6/CD_3OD before and after CO_2 bubbling.

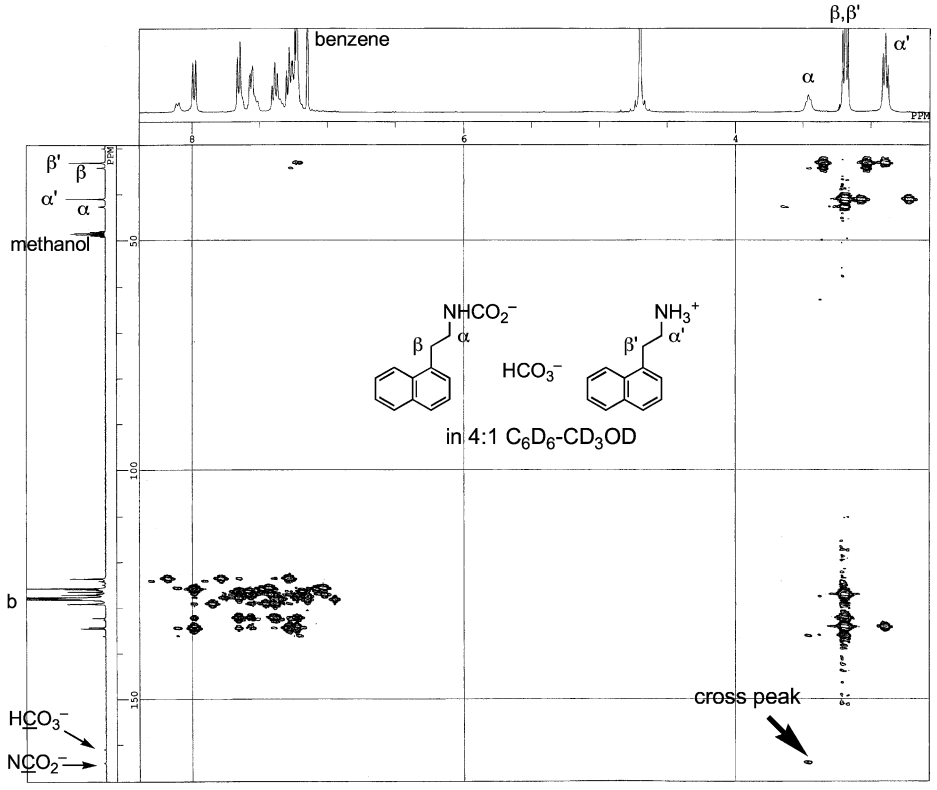

Figure 2. The HMBC spectrum of **1b** in 4:1 C_6D_6/CD_3OD after CO_2 bubbling.

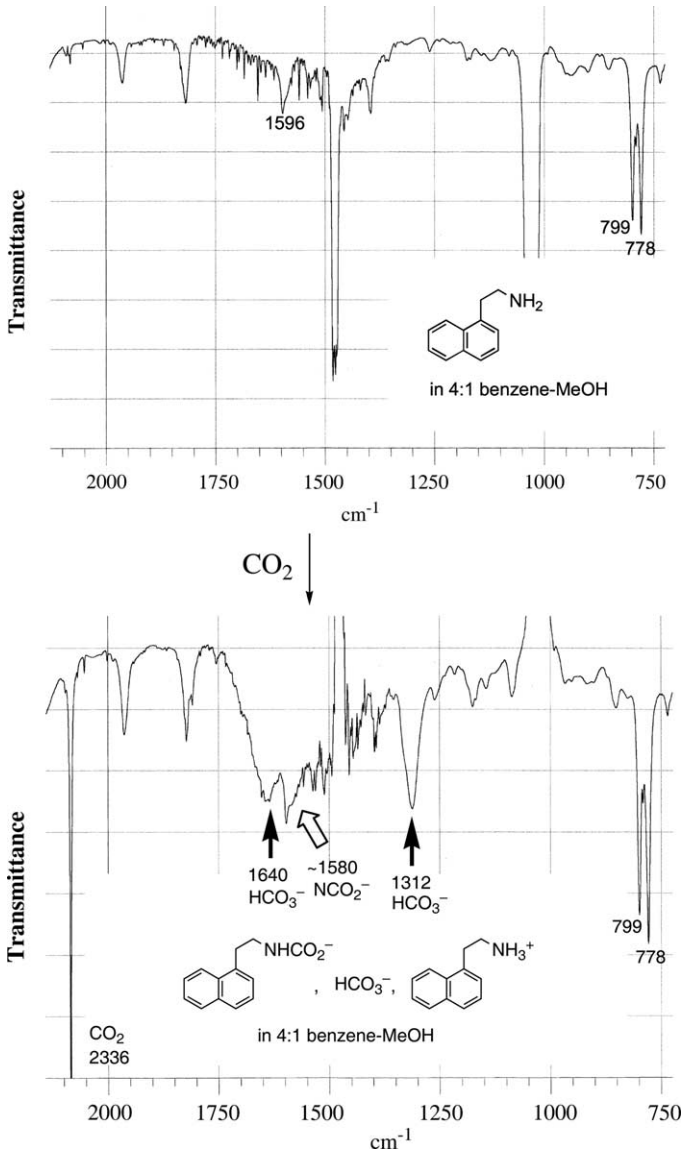

Figure 3. The IR spectra of **1b** in 4:1 benzene/MeOH before and after CO_2 bubbling.

methylcarbonic acid $CH_3OCO_2^-$ was probably not formed from the following reasons. Dimethyl carbonate, which is a possible product from the reaction of methylcarbonic acid with $TMSCHN_2$, was undetectable (NMR) in the reaction mixtures. Furthermore, the signals corresponding to $CD_3OCO_2^-$ were none by the in situ ^{13}C NMR experiments in C_6D_6–CD_3OD or in CD_3OD (Section 2.2 and Ref. 1a).

2.2. Carbamate anion formation in 4:1 benzene/MeOH

When we previously studied the solvent dependence of carbamic acid formation,[1] we did not investigate the benzene–MeOH mixed solvent. Therefore, by selecting 2-(1-naphthyl)ethylamine (**1b**) as a typical example, the ^1H and ^{13}C NMR, HMBC, and IR spectra in 4:1 benzene–MeOH before and after bubbling CO_2 were measured in situ (Figs. 1–3). It can be seen from these figures that a mixture of ammonium carbamate and bicarbonate was predominantly formed in this mixed solvent. From the ^1H NMR spectrum after CO_2 bubbling (Fig. 1A, right), the molar ratio between ammonium carbamate and ammonium bicarbonate is estimated as 54:46. The carbamyl carbon appeared at δ 163.5 and the bicarbonate carbon at δ 160.9 (Fig. 1B, right). In the HMBC spectrum (Fig. 2), there is an expected cross peak between the α-methylene proton and the carbamyl carbon, demonstrating the N– Cbond formation between the amine nitrogen and the CO_2 carbon. The IR spectrum (Fig. 3, bottom) showed a broad band at about 1580 cm^{-1}, which is assignable to the $NHCOO^-$ group of the carbamate anion.[14] The bands at 1640 and 1312 cm^{-1} should be attributed to bicarbonate.[1a] For comparison, the pertinent spectra in DMSO and DMF are displayed in Figures 4–6. As reported previously,[1] **1b** was quantitatively converted to the carbamic acid (1-Naph)$CH_2CH_2NHCO_2H$ in DMSO, DMF or pyridine (e.g., as can be seen from the NMR spectra in Figures 4 and 5).

2.3. Mechanistic consideration

Interconversion reactions among amine, carbamic acid, ammonium carbamate, and ammonium bicarbonate (formed

if H_2O is present as a contaminant in MeOH; see Scheme 1) are reversible processes,[5,15] whereas the formation of methyl carbamate (MC) is irreversible (Eqs. 3–6). It is assumed that $TMSCHN_2$ can esterify either undissociated or dissociated carbamic acid (Eq. 6). As aforementioned, upon CO_2 bubbling through the solution of **1b** in 4:1 benzene/MeOH, formation of both ammonium carbamate and bicarbonate (molar ratio 54:46) but no carbamic acid was observed (Figs. 1–3). Of course, there may exist a low concentration of the carbamic acid below the detection limit of the NMR and IR analysis. Apart from the ambiguity about the true species involved in the esterification, however, production of the methyl carbamate **1b**-MC in high yield (95%, Table 2) from the ammonium carbamate–bicarbonate mixture (carbamate content 54%) in 4:1 benzene/MeOH is feasible owing to the irreversibility of the reaction 6.

$$RR'NH + CO_2 \rightleftharpoons RR'NCO_2H \tag{3}$$

$$RR'NCO_2H + RR'NH \rightleftharpoons RR'NH_2^+ + RR'NCO_2^- \tag{4}$$

$$RR'NH + CO_2 + H_2O \rightleftharpoons RR'NH_2^+ + HCO_3^- \tag{5}$$

$$\left\{ \begin{array}{c} RR'NCO_2H \\ \rightleftharpoons \\ RR'NCO_2^- + H^+ \end{array} \right\} \xrightarrow[MeOH]{TMSCHN_2} RR'NCO_2Me \tag{6}$$

In the preceding paper[1] we have mentioned that the in situ yield of carbamic acid in CO_2-saturated DMSO-d_6 is generally much lower for arylamines than for alkylamines. More complete data supporting this statement are shown in Table 3. Many alkylamines underwent nearly complete conversion (~100%) into their respective carbamic acids. Among arylamines, those having an electron-donating group (**2j** and **2k**) and cyclic ones (**5a** and **5b**) exhibit relatively high carbamic acid yields (7–24%). It is interesting to note that the carbamic acid yields in DMSO-d_6 for alkyl- and arylamines (Table 3) are roughly correlated with their methoxycarbonylation yields (Table 2). On the assumption that the structural effect by different amines on the formation of either carbamic acid or carbamate anion is similar, it seems that $TMSCHN_2$ is a good reagent for trapping unstable carbamic acid species (carbamic acid or carbamate anion).

Table 3. Carbamic acid formation from amines in DMSO-d_6 through bubbling of CO_2 at room temperature

Alkylamine	Carbamic acid yield (%)	Arylamine	Carbamic acid yield (%)
1a–c	~100	**1d**, **1e**, **2h**	0
2a–d, **2f**	~100	**2g**	1
2e·dnba	12 (31[a])	**2j**	11
3a	~100	**2k**	24
4	82	**5a**	22
		5b	7

The conversion or the carbamic acid yield was estimated in situ by ^1H NMR analysis. Some data are from Ref. 1a.
[a] In pyridine-d_5.

3. Conclusion

Methoxycarbonylation of a variety of amines into the corresponding methyl carbamates was accomplished by allowing them to react with (trimethylsilyl)diazomethane $TMSCHN_2$ under bubbling of CO_2 at room temperature. Benzene–MeOH (4/1 v/v) was the solvent of choice. Thus, primary alkyl amines **1a–c**, **2a–d**, **3a**, **3b** and secondary alkylamine **4** afforded their methyl carbamates in excellent to good yields (76–100%). On the other hand, methoxycarbonylation of arylamines (**1d**, **2g**, **2h**, **2j**, **2k** and **5b**) was difficult (0–27% yield), although indoline (**5a**), whose nitrogen atom is sterically less hindered, gave its methyl carbamate in an excellent yield (98%). Even oxidatively labile dopamine (**2e**) and DL-noradrenaline (**2f**) were successfully methoxycarbonylated, albeit a low yield (17 and 12%, respectively). By selecting 2-(1-naphthyl)ethylamine (**1b**) as a typical example, the ^1H and ^{13}C NMR, HMBC, and IR spectra in 4:1 benzene–MeOH were measured before and after CO_2 bubbling. These spectra have revealed that about an equimolar amount of the ammonium carbamate and the ammonium bicarbonate is formed in equilibrium in this mixed solvent. However, since the methyl esterification step by $TMSCHN_2$ is irreversible, the conversion of amine to methyl carbamate can be nearly quantitative.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were measured on a JEOL EX-270J, AL-300, or JUM-A400 spectrometer. Measurements of 2D NMR were carried out with JEOL JUM-A400. Mass and IR spectra were recorded on JEOL JMS-HX 110A and SHIMADZU FTIR-8400 spectrometers, respectively.

The in situ measurements of ^1H and ^{13}C NMR, HMBC, and IR spectra in benzene–MeOH (4/1 v/v) before and after bubbling CO_2 were carried out as described previously.[1a]

4.2. Methoxycarbonylation

4.2.1. A typical procedure: isolation of methyl *N*-[3-(1-naphthyl)propyl]carbamate (1a-MC). Through 5 mL of a 4:1 benzene/MeOH solution containing 73 mg (0.393 mmol) of amine **1a** was bubbled CO_2 gas for 15 min. Into this solution was added 0.5 mL (1.0 mmol) of (trimethylsilyl)diazomethane $TMSCHN_2$ (Aldrich 2.0 M solution in hexanes) in one portion at room temperature with both stirring and CO_2 bubbling. The yellow color of $TMSCHN_2$ disappeared in 20 min. The mixture was stirred for an additional 2 h under CO_2 bubbling. The resultant colorless solution was rotary-evaporated to afford 93 mg of a colorless viscous oil, which was almost pure **1a**-MC containing only a trace of **1a** on the basis of the NMR analysis. Further purification was carried out by preparative TLC on silica gel ($CHCl_3$/MeOH 20:1 v/v) to give 93 mg (97% yield) of **1a**-MC as a colorless viscous oil.

4.2.2. Isolation of methyl *N*-[2-(3,4-dihydroxyphenyl)-ethyl]carbamate (2e-MC). Through 12.5 mL of a 4:1

pyridine/MeOH solution containing 150 mg (0.411 mmol) of the salt **2e**·dnba[11] was bubbled CO_2 gas for 15 min. Into this solution was added 1.1 mL (2.2 mmol) of $TMSCHN_2$ in one portion at room temperature with both stirring and CO_2 bubbling. The mixture was stirred at room temperature for 2.5 h under CO_2 bubbling. Then the solvent was rotary-evaporated to leave 452 mg of a dark brown residue, which was dissolved in 10 mL of MeOH and was left overnight. An insoluble black solid (probably melanin) appeared and this was removed by filtration. The filtrate was subjected to repeated preparative TLC on silica gel ($CHCl_3$/MeOH 10:1 v/v) to afford 14 mg (17% yield) of **2e**-MC as a pale brown semisolid. Methyl 3,5-dinitrobenzoate was undetectable in the reaction mixture (NMR and TLC).

4.2.3. Spectral data for isolated products. Other amines were reacted in a manner similar to that described at Section 4.2.1. The products were separated by preparative TLC on silica gel ($CHCl_3$–MeOH) and were characterized by ^1H and ^{13}C NMR, IR, MS, and HRMS measurements. Further purification of the isolated products by recrystallization or distillation was usually not done. Spectral data for known compounds were well in agreement with the reported data.

4.2.3.1. Methyl *N*-[3-(1-naphthyl)propyl]carbamate (1a-MC). Obtained as a colorless thick oil; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (1H, d, J=8.2 Hz), 7.85 (1H, dd, J=8, 1.7 Hz), 7.71 (1H, d, J=8.1 Hz), 7.53–7.44 (2H, m), 7.38 (1H, t, J=7.6 Hz), 7.31 (1H, d, J=7 Hz), 4.74 (1H, br s), 3.67 (3H, s), 3.28 (2H, quar, J=6.5 Hz), 3.10 (2H, t, J=7.6 Hz), 1.96 (2H, quin, J=7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.13, 137.44, 133.91, 131.72, 128.84, 126.83, 125.97, 125.88, 125.53, 125.52, 123.58, 52.06, 40.95, 30.88, 30.11; IR (neat) 3336 (m), 1705 (br s), 1538 (br s), 1258 (s), 778 (s) cm^{-1}; MS (FAB$^+$) *m/z* 244 (MH^+, 100), 243 (M^+, 58), 212 (13), 168 (21); HRMS (FAB$^+$) calcd for $C_{15}H_{17}NO_2$ 243.1259, found 243.1259.

4.2.3.2. *N*-[3-(1-Naphthyl)propyl]formamide (6). Obtained as a colorless thick oil; ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (0.84H, s), 8.05 (0.16H, d, J=12.3 Hz), 8.01–7.95 (1H, m), 7.87–7.83 (1H, m), 7.76–7.70 (1H, m), 7.54–7.28 (4H, m), 5.8 and 5.59 (ca. 0.15 and 0.85H, two br s), 3.41 (1.66H, quar, J=6.8 Hz), 3.28 (0.34H, quar, J=6.7 Hz), 3.12 (2H, t, J=7.8 Hz), 1.99 (2H, quin, J=7.4 Hz); ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 164.55,[§] 161.09, 137.06, 136.49,[§] 133.84,[§] 133.78, 131.54, 131.47,[§] 129.42,[§] 128.84,[§] 128.77, 127.03,[§] 126.83, 126.00,[§] 125.94, 125.85, 125.57,[§] 125.47, 125.44, 125.40,[§] 123.41, 123.28,[§] 41.41,[§] 38.07, 31.92,[§] 30.40, 30.31, 29.73[§]; IR (neat) 3287 (m), 1666 (s), 1537 (m), 1386 (m), 779 (s) cm^{-1}; MS (EI$^+$) *m/z* 213 (M^+, 89), 168 (100), 153 (71), 141 (95), 115 (48); HRMS (EI$^+$) calcd for $C_{14}H_{15}NO$ 213.1154, found 213.1155.

4.2.3.3. Methyl *N*-[2-(1-naphthyl)ethyl]carbamate (1b-MC). Obtained as a pale yellow thick oil; ^1H NMR (300 MHz, $CDCl_3$) δ 8.08 (1H, d, J=7.8 Hz), 7.85 (1H, d, J=7.5 Hz), 7.79 (1H, d, J=7.8 Hz), 7.55–7.45 (2H, m), 7.39 (1H, t, J=7.7 Hz), 7.31 (1H, d, J=6.6 Hz), 4.78 (1H, br s), 3.67 (3H, s), 3.54 (2H, quar, J=6.6 Hz), 3.28 (2H, t, J=7.1 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 157.05, 134.77, 133.90, 131.87, 128.79, 127.31, 126.77, 126.14, 125.66, 125.45, 123.55, 52.04, 41.65, 33.37; IR (neat) 3337 (m), 1705 (s), 1530 (s), 1254 (s), 777 (s) cm^{-1}; MS (EI$^+$) *m/z* 229 (M^+, 31), 154 (83), 141 (100), 115 (58), 88 (52); HRMS (EI$^+$) calcd for $C_{14}H_{15}NO_2$ 229.1103, found 229.1105.

4.2.3.4. Methyl *N*-[(1-naphthyl)methyl]carbamate (1c-MC). Obtained as a pale yellow solid, mp 84–88 °C (lit.[16] mp 66–69 °C); ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (1H, d, J=7.8 Hz), 7.86 (1H, d,[¶] J=8.1 Hz), 7.79 (1H, dd, J=6.5, 2.8 Hz), 7.56–7.47 (2H, m), 7.42–7.37 (2H, m), 5.00 (1H, br s), 4.81 (2H, d, J=5.4 Hz), 3.69 (3H, s);[8b] ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.82, 133.82, 133.62, 131.23, 128.77, 128.49, 126.52, 126.13, 125.91, 125.36, 123.29, 52.24, 43.19; IR (neat) 3298 (m), 1695 (s), 1550 (m), 1296 (m), 1259 (m), 799 (m) cm^{-1}; MS (EI$^+$) *m/z* 215 (M^+, 100), 200 (72), 156 (61), 141 (77), 129 (85); [8b] HRMS (EI$^+$) calcd for $C_{13}H_{13}NO_2$ 215.0946, found 215.0944.

4.2.3.5. Methyl *N*-(2-phenylethyl)carbamate (2a-MC). Obtained as a colorless oil; ^1H NMR (270 MHz, $CDCl_3$) δ 7.38–7.21 (5H, m), 4.75 (1H, br s), 3.70 (3H, s), 3.48 (2H, quar, J=6.5 Hz), 2.85 (2H, t, J=6.9 Hz);[6c] ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 156.84, 138.64, 128.68, 128.53, 126.41, 52.07, 42.22, 36.19;[6c] IR (neat) 3335 (m), 1705 (s), 1532 (s), 1258 (s), 700 (m) cm^{-1}; MS (EI$^+$) *m/z* 179 (M^+, 32), 104 (60), 91 (59), 88 (100); HRMS (EI$^+$) calcd for $C_{10}H_{13}NO_2$ 179.0946, found 179.0944.

4.2.3.6. *N*-(2-Phenylethyl)formamide (7). Obtained as a colorless oil; ^1H NMR (270 MHz, $CDCl_3$) δ 8.07 (0.85H, s), 7.85 (0.15H, d, J=11.9 Hz), 7.28–7.09 (5H, m), 5.7 and 5.56 (1H, two br s), 3.52 (1.7H, quar, J=6.7 Hz), 3.42 (0.3H, quar, J=6.7 Hz), 2.78 (2H, t, J=6.9 Hz);[17] ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 164.33,[§] 161.07, 138.31, 137.41,[§] 128.78,[§] 128.75,[§] 128.65 (two peaks), 126.86,[§] 126.60, 43.20,[§] 39.25, 37.73,[§] 35.50;[17] IR (neat) 3283 (m), 1667 (s), 1534 (m), 1384 (m), 1239 (m), 699 (m) cm^{-1};[17] MS (EI$^+$) *m/z* 149 (M^+, 13), 104 (100), 91 (44); HRMS (EI$^+$) calcd for $C_9H_{11}NO$ 149.0841, found 149.0842.

4.2.3.7. Methyl *N*-[2-(4-hydroxyphenyl)ethyl]carbamate or *N*-(methoxycarbonyl)tyramine (2b-MC). Obtained as a colorless thick oil; ^1H NMR (270 MHz, $CDCl_3$) δ 6.99 (2H, d,[¶] J=8.5 Hz), 6.76 (2H, d,[¶] J=8.5 Hz), 4.80 and 4.69 (1H, two br s), 3.64 (3H, s), 3.37 (2H, quar, J=6.7 Hz), 2.69 (2H, t, J=7.0 Hz); ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 157.23, 154.64, 130.03, 129.70, 115.45, 52.27, 42.47, 35.21; IR (neat) 3335 (s), 1700 (s), 1515 (s), 1260 (s), 827 (m) cm^{-1}; MS (EI$^+$) *m/z* 195 (M^+, 14), 120 (100), 107 (95); HRMS (EI$^+$) calcd for $C_{10}H_{13}NO_3$ 195.0895, found 195.0896.

4.2.3.8. Methyl *N*-[2-(3,4-dimethoxyphenyl)ethyl]carbamate (2c-MC). Obtained as a colorless thick oil; ^1H NMR (270 MHz, $CDCl_3$) δ 6.81 (1H, d, J=7.9 Hz), 6.74–6.70 (2H, m), 4.69 (1H, br s), 3.87 (3H, s), 3.86 (3H, s), 3.66 (3H, s), 3.42 (2H, quar, J=6.6 Hz), 2.76 (2H, t, J=6.9 Hz);[18] ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.95, 148.99,

[§] The peaks probably belonging to the minor isomer.

[¶] Each peak is finely split (J=1–2 Hz).

147.66, 131.21, 120.64, 111.87, 111.32, 55.89, 55.84, 52.03, 42.28, 35.69;[18] IR (neat) 3365 (m), 1710 (s), 1516 (s), 1260 (s), 1236 (s), 1142 (s), 1028 (s) cm^{-1};[18] MS (FAB^+) *m/z* 240 (MH^+, 87), 239 (M^+, 100), 165 (59); HRMS (FAB^+) calcd for $C_{12}H_{17}NO_4$ 239.1158, found 239.1164.

4.2.3.9. Methyl *N*-(2-hydroxy-2-phenylethyl)carbamate (2d-MC). Obtained as a colorless solid, mp 85–89 °C (lit.[8a] mp 88–90 °C); ^1H NMR (300 MHz, $CDCl_3$) δ 7.29–7.19 (5H, m), 5.11 (1H, br s), 4.74 (1H, dd, $J=8$, 3 Hz), 3.60 (3H, s), 3.44 (1H, br d, $J=14$ Hz), 3.21 (1H, br dd, $J=14$, 8 Hz), 2.56 (1H, br s);[8a] ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 157.81, 141.52, 128.54, 127.93, 125.84, 73.58, 52.33, 48.53;[8a] IR (nujol) 3374 (m), 3272 (m), 1689 (s), 1551 (m), 1285 (m), 1277 (m), 704 (m) cm^{-1};[8a] MS (EI^+) *m/z* 195 (M^+, 11), 177 (20), 120 (29), 107 (58), 89 (100); HRMS (EI^+) calcd for $C_{10}H_{13}NO_3$ 195.0895, found 195.0895.

4.2.3.10. Methyl *N*-[2-(3,4-dihydroxyphenyl)ethyl]-carbamate or *N*-(methoxycarbonyl)dopamine (2e-MC). Obtained as a pale brown semisolid; ^1H NMR (400 MHz, CD_3OD) δ 6.66 (1H, d, $J=8.1$ Hz), 6.62 (1H, d, $J=2.0$ Hz), 6.50 (1H, dd, $J=8.0$, 2.0 Hz), 3.60 (3H, s), 3.23 (2H, t, $J=$ 7.5 Hz), 2.60 (2H, t, $J=7.5$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 159.54, 146.23, 144.74, 132.04, 121.02, 116.85, 116.33, 52.36, 43.76, 36.57; IR (neat) 3335 (s), 1700 (s), 1525 (s), 1283 (s) cm^{-1}; MS (EI^+) *m/z* 211 (M^+, 21), 136 (100), 123 (92); HRMS (EI^+) calcd for $C_{10}H_{13}NO_4$ 211.0845, found 211.0842.

4.2.3.11. Methyl *N*-[2-hydroxy-2-(3,4-dihydroxy-phenyl)ethyl]carbamate or *N*-(methoxycarbonyl)nor-adrenaline (2f-MC). Obtained as a pale brown semisolid; ^1H NMR (400 MHz, CD_3OD) δ 6.80 (1H, d, $J=1.8$ Hz), 6.72 (1H, d, $J=8.1$ Hz), 6.66 (1H, dd, $J=8.1$, 1.8 Hz), 4.55 (1H, dd, $J=7.9$, 4.9 Hz), 3.61 (3H, s), 3.29–3.17 (2H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 159.70, 146.25, 145.89, 135.39, 118.73, 116.12, 114.39, 73.71, 52.49, 49.47; IR (KBr) 3335 (s), 1700 (s), 1535 (m), 1290 (s) cm^{-1}; MS (EI^+) *m/z* 227 (M^+, 9), 209 (82), 177 (23), 152 (21), 139 (100), 93 (45); HRMS (EI^+) calcd for $C_{10}H_{13}NO_5$ 227.0794, found 227.0793.

4.2.3.12. Methyl *N*-phenylcarbamate (2g-MC). Obtained as a colorless thick oil; ^1H NMR (270 MHz, $CDCl_3$) δ 7.33–7.19 (4H, m), 7.00 (1H, t,¶ $J=7.2$ Hz), 6.54 (1H, br s), 3.71 (3H, s);[19] ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 153.91, 137.70, 128.98, 123.41, 118.62, 52.37;[19] IR (neat) 3318 (m), 1713 (s), 1602 (m), 1544 (s), 1448 (s), 1235 (s), 1070 (m), 755 (m), 691 (m) cm^{-1};[19] MS (EI^+) *m/z* 151 (M^+, 100), 119 (47), 106 (60); HRMS (EI^+) calcd for $C_8H_9NO_2$ 151.0633, found 151.0636.

4.2.3.13. Methyl *N*-(4-methoxyphenyl)carbamate (2j-MC). Obtained as a pale brown semisolid; ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (2H, d, $J=8.2$ Hz), 6.82 (2H, d, $J=8.2$ Hz), 6.58 (1H, br s), 3.76 (3H, s), 3.74 (3H, s);[20] ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.04, 154.49, 130.87, 120.83, 114.23, 55.47, 52.25; [20] IR (neat) 3319 (m), 1710 (s), 1602 (m), 1514 (s), 1298 (m), 1231 (s), 1180 (m), 1073 (m), 1034 (m), 829 (m) cm^{-1};[20] MS (EI^+) *m/z* 181 (M^+, 100), 149 (96), 122 (85); HRMS (EI^+) calcd for $C_9H_{11}NO_3$ 181.0739, found 181.0740.

4.2.3.14. Methyl *N*-[4-(dimethylamino)phenyl]carbamate (2k-MC). Obtained as a dark plates (from benzene–hexane), mp 87–99 °C (dec); ^1H NMR (300 MHz, $CDCl_3$) δ 7.20 (2H, br d, $J=7.8$ Hz), 6.68 (2H, d,¶ $J=8$ Hz), 6.52 (1H, br s), 3.73 (3H, s), 2.88 (6H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 154.76, 147.71, 127.67, 121.00, 113.28, 52.11, 40.97; IR (KBr) 3331 (m), 1700 (s), 1536 (s), 1323 (m), 1244 (s), 1074 (m), 811 (m) cm^{-1}; MS (EI^+) *m/z* 194 (M^+, 100), 162 (89), 135 (66); HRMS (EI^+) calcd for $C_{10}H_{14}N_2O_2$ 194.1055, found 194.1055.

4.2.3.15. Methyl *N*-[2-(3-indolyl)ethyl]carbamate or *N*-(methoxycarbonyl)tryptamine (3a-MC). Obtained as a colorless semisolid; ^1H NMR (270 MHz, $CDCl_3$) δ 8.11 (1H, br s), 7.62 (1H, d, $J=7.6$ Hz), 7.38 (1H, d,¶ $J=7.9$ Hz), 7.22 (1H, t,¶ $J=7.5$ Hz), 7.14 (1H, t,¶ $J=7.5$ Hz), 7.04 (1H, s¶), 4.78 (1H, br s), 3.67 (3H, s), 3.53 (2H, quar, $J=6.8$ Hz), 2.98 (2H, t, $J=6.8$ Hz);[21] ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 156.89, 136.21, 127.08, 122.03, 121.91, 119.32, 118.57, 112.74, 111.08, 51.97, 41.20, 25.75; IR (neat) 3412 (s), 3334 (s), 1700 (s), 1530 (m), 1454 (m), 1260 (m), 744 (m) cm^{-1}; MS (FAB^+) *m/z* 219 (MH^+, 73), 218 (M^+, 100), 144 (69), 130 (72);[21] HRMS (FAB^+) calcd for $C_{12}H_{14}N_2O_2$ 218.1055, found 218.1055.

4.2.3.16. *N*α-Methoxycarbonyl-L-tryptophan methyl ester (3b-MC). Obtained as a colorless semisolid; ^1H NMR (270 MHz, CD_3OD) δ 7.56 (1H, d, $J=7.9$ Hz), 7.38 (1H, d, $J=7.9$ Hz), 7.14 (1H, t, $J=7.1$ Hz), 7.11 (1H, s), 7.05 (1H, t, $J=7.0$ Hz), 4.54 (1H, t, $J=6.7$ Hz), 3.69 (3H, s), 3.64 (3H, s), 3.32 (1H, dd, $J=14.6$, 6 Hz), 3.18 (1H, dd, $J=14.6$, 7.7 Hz);[22] ^{13}C NMR (67.7 MHz, CD_3OD) δ 174.28, 158.87, 137.90, 128.53, 124.33, 122.33, 119.71, 118.96, 112.22, 110.60, 56.44, 52.63, 28.71;[22] IR (neat) 3400 (m), 1733 (s), 1700 (s), 1454 (m), 1356 (m), 1221 (m), 745 (m) cm^{-1}; MS (EI^+) *m/z* 276 (M^+, 26), 130 (100); HRMS (EI^+) calcd for $C_{14}H_{16}N_2O_4$ 276.1110, found 276.1116.

4.2.3.17. Methyl *N*,*N*-dibenzylcarbamate (4-MC). Obtained as a colorless oil; ^1H NMR (270 MHz, $CDCl_3$) δ 7.33–7.15 (10H, m), 4.43 (2H, s), 4.38 (2H, s), 3.79 (3H, s);[23] ^{13}C NMR (67.7 MHz, $CDCl_3$) δ 157.29, 137.26, 128.50, 128.03, 127.31, 52.98, 49.43, 48.90; IR (neat) 1700 (s), 1471 (m), 1454 (m), 1407 (m), 1238 (s), 1119 (m), 699 (m) cm^{-1};[23] MS (EI^+) *m/z* 255 (M^+, 21), 164 (93), 121 (25), 91 (100); HRMS (EI^+) calcd for $C_{16}H_{17}NO_2$ 255.1259, found 255.1257.

4.2.3.18. 1-(Methoxycarbonyl)indoline (5a-MC). Obtained as a pale yellow oil; ^1H NMR (300 MHz, $CDCl_3$) δ 7.9–7.15 (1H, br), 7.07–7.00 (2H, m), 6.81 (1H, t, $J=7.5$ Hz), 3.86 (2H, t, $J=8.3$ Hz), 3.69 (3H, s), 2.97 (2H, t, $J=8.7$ Hz);[24] ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 153.68, 142.47, 130.82, 127.41, 124.65, 122.48, 114.66, 52.45, 47.36, 27.46; IR (neat) 1710 (s), 1488 (s), 1444 (s), 1395 (s), 1337 (m), 1317 (m), 1137 (m), 1058 (m), 752 (m) cm^{-1};[24] MS (EI^+) *m/z* 177 (M^+, 100), 162 (19), 132 (30), 118 (48), 91 (38); HRMS (EI^+) calcd for $C_{10}H_{11}NO_2$ 177.0790, found 177.0787.

4.2.3.19. 1-Methoxycarbonyl-2-methylindoline (5b-MC). Obtained as a pale brown thick oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.6 (1H, very br), 7.07–7.00 (2H, m), 6.82 (1H, t, J=7.3 Hz), 4.41 (1H, br quin, J=7 Hz), 3.70 (3H, s), 3.22 (1H, dd, J=15.9, 9.6 Hz), 2.49 (1H, dd, J=15.9, 2.4 Hz), 1.15 (3H, d, J=6.3 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 153.62, 130.03, 127.42, 125.01, 122.67, 115.32, 55.33, 52.40, 35.83, 21.06; IR (neat) 1708 (s), 1485 (s), 1441 (s), 1390 (s), 1285 (m), 1060 (m), 751 (m) cm^{-1}; MS (EI$^+$) *m/z* 191 (M$^+$, 83), 176 (100), 132 (40), 117 (76); HRMS (EI$^+$) calcd for $C_{11}H_{13}NO_2$ 191.0946, found 191.0942.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.09.116. The ^{1}H and ^{13}C NMR spectra of **1b** in DMF-d_7 before and after CO_2 bubbling (Fig. 4). The HMBC spectrum of **1b** in DMSO-d_6 after CO_2 bubbling (Fig. 5). The IR spectra of **1b** in DMSO before and after CO_2 bubbling (Fig. 6).

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Factors affecting the efficiency and stereoselectivity of α-amino acid synthesis by the Petasis reaction

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Abstract—The use of chiral secondary amines containing only one branched substituent has been shown to give optimal yields and stereoselectivities in the preparation of α-amino acids using the Petasis reaction. While the use of chiral primary amines generally gives products in low to moderate diastereoselectivity, chiral secondary amines generally give products in >95:5 diastereoselectivity. Additionally, the use of amines with two chiral (and by definition, branched) *N*-alkyl substituents results in significantly reduced yields with respect to to secondary amines with one or no branched *N*-alkyl substituents.

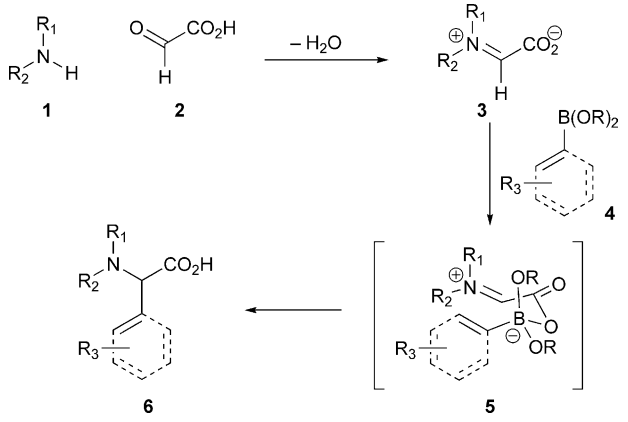
1. Introduction

The Petasis reaction, or boronic acid Mannich reaction, involves the three-component coupling of an amine, aldehyde and organoboronic acid, and has developed over the last few years into a powerful synthetic tool.[1–5] The methodology has been extended to the solid phase,[6–9] and tandem processes involving a Petasis reaction and subsequent Ugi[10–12] or palladium-catalysed process[13] have expanded the scope of the reaction. Recent advances in microwave conditions[14,15] and the use of fluorinated solvents[16] have allowed for high conversions within reasonable reaction times, which had remained one of the major limitations.

One of the most important uses of the Petasis reaction is the synthesis of α-amino acids using glyoxylic acid as the aldehyde component (Scheme 1). A wide range of aryl- and vinyl-boronic acids have been employed in such reactions, allowing the production of a wide variety of arylglycine and vinylglycine derivatives. The use of chiral amines allows the stereoselective production of α-amino acid derivatives, but the range of chiral amines investigated remains limited.

The Petasis reaction proceeds through condensation of the amine **1** and aldehyde to give the corresponding iminium ion **3**. It is believed that when glyoxylic acid (**2**) is employed as the aldehyde, the organoboron species **4** coordinates to the carboxylic acid group, with subsequent intramolecular transfer of the aryl/vinyl group from the activated 'ate'-complex (**5**) of the organoboronic acid yielding the amino acid product **6** (Scheme 1).[6]

Two general trends are apparent from the reported examples of Petasis reactions; vinylboronic acids are more reactive than arylboronic acids, and secondary amines generally give better yields than primary amines (though some branched primary amines appear to be suitable substrates for the reaction). However, despite the wide appeal of the Petasis reaction as a mild method for the preparation of α-amino



Scheme 1. α-Amino acid synthesis from the Petasis reaction.

Keywords: Amino acids; Petasis reaction; Multi-component coupling; Boronic acid Mannich reaction.
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acids, few systematic studies have been conducted to explore the scope and limitations of the process, particularly with regard to stereoselective reactions.

Scobie and co-workers[17] recently investigated the use of chiral boronate esters in the Petasis reaction, but the ee of the products obtained from chiral boronates were very low. However, it was not ascertained whether the low selectivity was due to low stereoinduction by the chiral boronate, or the result of the chiral boronates hydrolysing to the achiral boronic acids, which then dominate the reaction in a stereorandom manner. Scobie also investigated the use of pinacolyl boronate esters in Petasis reactions.[18] Whereas reactions of pinacolyl vinylboronates with secondary amines proceeded in good yield, the corresponding reactions of arylboronates proceeded in only low yield, and the reactions of all pinacolyl boronates with primary amines failed completely.

Given the enormous potential of the Petasis reaction as a general tool for the synthesis of α-amino acids, we have conducted a systematic study of the factors affecting the yield and stereoselectivity of the Petasis reaction, focussing on the effects of the nature and chirality of the amine and organoboron species used.

2. Results and discussion

2.1. Effect of boronate esters and amine

We initially sought to expand the scope of Scobie's study of the reactivity of organoboronate esters in Petasis reactions.[18] We speculated that two factors could result in the lower reactivity of pinacolyl boronate esters compared to the corresponding boronic acids. Pinacolyl boronates form tetrahedral 'ate'-complexes much less readily than boronic acids due to steric interactions between the boron ligand and the methyl groups of the pinacolyl group, which would impede formation of the active species **5**. Alternatively, the bulk of the pinacolyl group could simply hinder the approach of the organoboron group to the iminium ion and/or disrupt the conformation of the active species **5**. Ethyleneglycolyl boronates were therefore employed alongside pinacolyl boronates and boronic acids in order to provide organoboron reagents of intermediate steric bulk. It was expected that ethylene glycol boronates would form tetrahedral 'ate'-complexes relatively easily but experience a degree of steric clash during nucleophilic attack on the iminium ion intermediate between that of a boronic acid and a pinacolyl boronate ester.

An examination of the reactivity of glyoxylic acid **2** and benzylamine **1a** with phenylboronic acid **4a** and the corresponding ethyleneglycolyl and pinacolyl boronates, **4b** and **4c**, was undertaken. Additionally, the reactivity of styrenylboronic acid **7a** and the corresponding ethylene glycolyl and pinacolyl boronates, **7b** and **7c**, were investigated. Phenylboronic acid **4a** was found to react very slowly under standard conditions (room temperature, 48 h, CH_2Cl_2), giving the product *N*-benzylphenylglycine **6a** in low yield (10%) (Scheme 2 and Table 1, row 1). No product was obtained from the corresponding ethylene glycol boronate ester **4b**. The pinacolyl ester **4c** was not investigated given that this was expected to be less reactive than the ethyleneglycolyl ester, and that Scobie and co-workers demonstrated that such reagents are unreactive with primary amines. A similar trend was observed in reaction of the styrenylboron reagents **7a–c** with amine **1b**. As previously demonstrated by Petasis,[1] use of styrenylboronic acid **7a** gave vinylglycine derivative **8a** in good yield (Table 1, row 1). When the corresponding ethyleneglycolyl ester **7b** was employed, the product **8a** was obtained in considerably lower yield (20% cf. 79%). No product was obtained when the pinacolyl ester **7c** was employed, in accordance with the findings of Scobie and co-workers.[18]

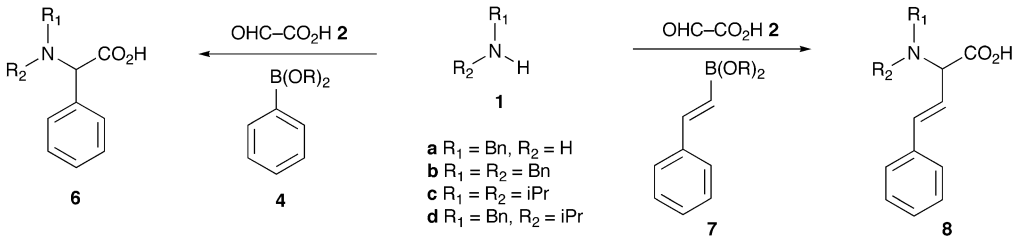
Importantly, the fact that **7b** affords the corresponding α-amino acid, albeit in low yield, indicates that it is possible for boronate esters to participate in the Petasis reaction with primary amines. This reaction proceeded in an identical manner in the presence of dehydrating agents (molecular sieves, magnesium sulfate), indicating that the boronate ester **7b** is participating in the reaction, with hydrolysis to the boronic acid **7a** not being a significant factor.

An analogous series of reactions was then performed with the secondary amine, dibenzylamine **1b**. Phenylboronic acid **4a** reacted in the presence of dibenzylamine **1b** to form the corresponding α-amino acid **6b** in good yield (Table 1, row 2). Both the ethyleneglycol boronate **4b** and pinacolyl boronate **4c** reacted with dibenzylamine **1b** to give the α-amino acid product **6b** in reasonable yield. This is in stark contrast to the result obtained from the reaction of boronate **4b** with benzylamine **1a**, where no product was obtained.

A similar contrast in reactivity was found when the styrenylboronate series **7a–c** was treated with dibenzylamine **1b** and glyoxylic acid **2**. Reactions of the boronic acid **7a**, ethyleneglycolyl boronate **7b** and pinacolyl boronate **7c** all proceeded to give the product **8b** in good yield. These results indicate that sterically demanding boronates can participate in the Petasis reaction providing a secondary amine is used, in accordance with the results of Scobie and co-workers.[18] Again, the addition of dehydrating agents (molecular sieves, magnesium sulfate), did not affect outcome of the reaction, suggesting that the boronate esters **7b**,**c** are participating directly in the reaction, rather than via hydrolysis to the corresponding boronic acid **7a**.

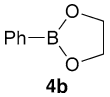
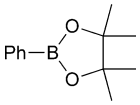
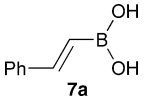
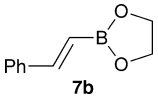
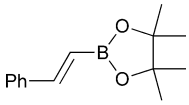
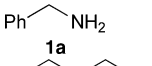
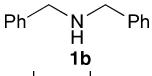
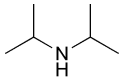
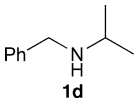
The reaction with diisopropylamine **1c** was next investigated. Phenylboronic acid **4a** reacted with diisopropylamine **1c** and glyoxylic acid **2** to afford the *N*,*N*-diisopropyl α-amino acid **6c** in high yield (84%). The ethyleneglycolyl boronate **4b** gave the product **6c** in low yield (19%). Furthermore, the pinacolyl boronate **4c** failed to react at all. Reaction of diisopropylamine **1c** with the styrenylboron reagents **7a–c** proceeded in an identical manner to the corresponding phenylboron reagents (Table 1, row 3).

A comparison of the yields obtained from diisopropylamine **1c** with those obtained from dibenzylamine **1b** shows that much lower yields of the amino acid product are isolated from reactions of diisopropylamine and boronate esters, particularly in the case of styrenyl pinacolyl boronate **7c** (0% cf. 90%, Table 1, column 6).[19] It is apparent, therefore,



Scheme 2. Preparation of phenylglycine derivatives **6** and styrenylglycine derivatives **8** using the Petasis reaction.

Table 1. Yields of phenylglycine derivatives **6a–d** and styrenylglycine derivatives **8a–d** from Petasis reaction (see Scheme 3)

	 4a	 4b	 4c	 7a	 7b	 7c
 1a	10	0	—	79	20	0
 1b	64	35	35	64	77	90
 1c	84	19	0	84	19	0
 1d	89	43	0	90	39	5

that an increased degree of branching in the iminium ion, in combination with increased steric bulk about the boron, results in a decrease in yield.

In order to gain a broader picture of the effect of branching upon the outcome of the Petasis reaction with boronates, the amine substrate *N*-isopropylbenzylamine **1d** was employed, since the degree of branching of **1d** can be viewed as intermediate between dibenzylamine **1b** and diisopropylamine **1c**. As shown in Table 1, row 4, the boronic acids **4a** and **7a** both afforded the corresponding α-amino acids **6d** and **8d** in high yield. When the ethylene glycol boronates **4b** and **7b** were used in place of the boronic acid, the products were formed in yields intermediate between those obtained from the corresponding reactions with diisopropylamine **1c** and dibenzylamine **1b**. The use of pinacolyl boronates **4c** and **7c** gave no product or only very low yield of the products. It is therefore apparent that monobranched amine substrates are tolerated by ethylene glycol boronates but not by pinacolyl boronates.

These results presented in Table 1 can be interpreted through three factors that influence the outcome of the Petasis reaction. Two of these effects have already been discussed: first, the reactivity of the organoboron species, with vinylboron reagents being more reactive than arylboron reagents, and second, the reactivity of the amine, with secondary amines being generally more reactive than primary amines. The third effect is a steric effect, being the combined effect of steric bulk of both the boronate and amine. It should be noted that the steric bulk of the boronate and degree of branching of the amine by themselves are not predictive of the reaction outcome, but must be considered in combination. That is, combination of a bulky boronate ester with a branched amine greatly reduces the yield of the Petasis reaction product. The ability of the organoboron reagent to form a tetrahedral 'ate'-complex appears not to be a critical factor in the progress of the Petasis reaction, given that pinacolyl boronates can give high yields under certain conditions.

2.2. Stereoselective reactions with chiral amines

We next investigated the effects of the amine and organoboron reagents on the stereoselectivity of Petasis reactions. The reaction of (*S*)-1-phenylethylamine **1e** with styrenylboronic acid **7a** yielded the Petasis reaction product **8e** in high yield (Scheme 3 and Table 2, row 1), as previously demonstrated by Petasis and co-workers.[1] The use of the corresponding ethylene glycol boronate **7b** afforded the product **8e** in moderate yield and with similar stereoselectivity to that obtained from the boronic acid **7a**. Use of the pinacolyl boronate **7c**, however, afforded the product **8e** in low yield and with poor stereoselectivity (Table 2, row 1). Most surprisingly, the direction of the diastereoselectivity was reversed. The major change in stereoselectivity when employing the pinacolylboronate presumably indicates a change in the reaction mechanism or conformation of the transition state. However, whether these results relate to the steric bulk around this system or its lack of propensity to form tetracoordinate species cannot be delineated at this time.

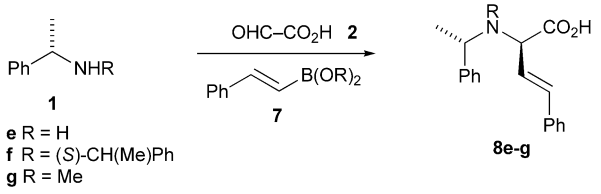
The use of the chiral secondary amine, *N*,*N*-bis-((*S*)-1-phenylethyl)amine **1f** as a substrate in the Petasis reaction was also investigated. Three-component coupling of **1f** with

glyoxylic acid **2** and styrenylboronic acid **7a** gave the product **8f** in moderate yield but with very high stereoselectivity. Only one diastereomer was observed by ^{1}H NMR spectroscopy, indicating the product **8f** was formed in >95:5 diastereomeric ratio. The corresponding ethylene glycol boronate **7b** afforded **8f** in low yield but again with very high stereoselectivity.

Although the stereoselectivity obtained in the reaction of the secondary amine **1f** is much greater that that from the primary amine **1e**, the yields are lower, consistent with the fact that secondary amine **1f** has a high degree of branching, similar to diisopropyl amine. Indeed, the isolated yield for the product formed from the boronic acid **7a** with amine **1f** is much lower than any other obtained from **7a** in this study.

Despite the poor yields obtained from amine **1f**, the high degree of stereoselectivity obtained prompted the investigation of a chiral secondary amine with a reduced degree of branching. Accordingly, a series of reactions with (*S*)-*N*-methyl-1-phenylethylamine **1g** was conducted (Scheme 3 and Table 2, row 3). The results from this series of reactions were very promising. Petasis reaction of amine **1g** with styrenylboronic acid **7a** and glyoxylic acid **2** gave the corresponding amino acid product **8g** in good yield (89%) and high stereoselectivity (>95:5) (Table 2). The yields from the corresponding ethylene glycolyl and pinacolyl boronates, **7b** and **7c**, reduced to moderate and poor, respectively, but the diastereoselectivity remained very high.

Similar results with chiral secondary amines have been observed by Nanda et al.,[16] in which the three-component coupling of phenylboronic acid, glyoxylic acid and various chiral 2-substituted pyrrolidines were studied. In all cases



Scheme 3. Chiral amines in diastereoselective α-amino acid synthesis.

the products were obtained in good yield and >95:5 diastereomeric ratio. Use of 2,5-dimethylpyrrolidine, however—containing two chiral (branched) substituents—resulted in no reaction.

In general, it is apparent that chiral secondary amines give Petasis reaction products in much greater stereoselectivity than reactions of related chiral primary amines. However, secondary amines containing two chiral alkyl substituents by definition have a high degree of branching, which leads to low yields. Nevertheless, we have shown that only one chiral alkyl substituent is required for high degree of stereoselectivity. Chiral secondary amines such as **1g**, containing one chiral alkyl substituent and one achiral (i.e., unbranched) substituent are the optimal reagents for Petasis reactions as they give rise to both high yields and high diastereoselectivities.

2.3. Combination of chiral amines and chiral boronates

While much attention has focused on the participation of chiral amines and aldehydes in the Petasis reaction very little has been placed upon the potential role of chiral boronates. Scobie and co-workers[17] have reported the use of chiral boronate esters such as tartrate- and pinanediol-derived boronates, **7d–f**, in enantioselective Petasis reactions with glyoxylic acid and morpholine. However, in all cases the ee of the amino acid products were very low (ee 6–15%). Despite the poor asymmetric induction provided by chiral boronate esters, we investigated the combination of chiral boronates and chiral amines in order to assess whether the establishment of matched/mismatched systems may lead to improvements in diastereoselectivity. Treatment of (*S*)-1-phenyl-ethylamine **1e** with the enantiomeric tartrate-derived boronates **7d** and **7e** under standard conditions gave the Petasis reaction product **8e** in moderate yield, with the major stereoisomer in each case being the same as that observed from the corresponding reaction with the boronic acid **7a** (Table 3). Use of the D-tartrate derivative **7d** gave the product in a 3.5:1 diastereomeric ratio, only slightly higher than that observed from the boronic acid **7a**. Use of the L-tartrate derivative **7e** gave the product with slightly lower diastereoselectivity (2.5:1). No reaction was observed when the pinane-diol derived

Table 2. Comparison of Petasis reaction yields and stereoselectivities with chiral amines **1e–g** (see Scheme 3)

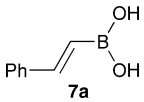
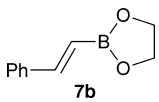
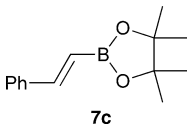
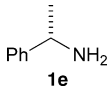
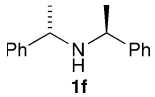
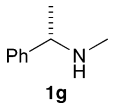
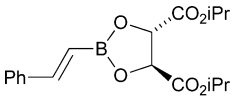
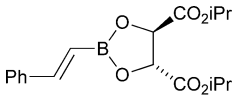
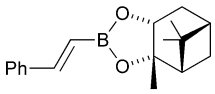
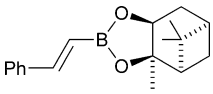
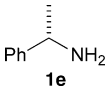
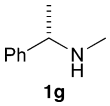
	 **7a**		 **7b**		 **7c**	
	Yield	dr	Yield	dr	Yield	dr
 **1e**	81	3.3:1	47	3.2:1	7	1:1.4
 **1f**	38	>95:5	13	>95:5	—	—
 **1g**	89	>95:5	49	>95:5	21	>95:5

Table 3. Comparison of yields and stereoselectivities of Petasis reaction of **2** and **1e**,**g** with chiral boronates **7d–g**

	 **7d**		 **7e**		 **7f**		 **7g**	
	Yield	dr	Yield	dr	Yield	dr	Yield	dr
 **1e**	75	3.5:1	55	2.5:1	—	—	—	—
 **1g**	60	>95:5	50	>95:5	7	>95:5	1	>95:5

boronates **7f**,**g** were used. Analogous reactions were conducted with the chiral secondary amine **1g**. In all cases the product was obtained in >95:5 diastereomeric ratio, consistent with other reactions of amine **1g**. Products were obtained from the tartrate-derived boronates in reasonable yield, while from the pinane-diol derived bornates the yields were very low. These results indicate that while the use of chiral boronates in combination with chiral amines does result in the generation of matched/mismatched systems, the effects of the chiral boronates are only small and the configuration of the amine dominates the stereochemical outcome of the reaction. However, the use of chiral boronates in the improvement of stereoselectivity of Petasis reactions may find use in limited cases.

3. Conclusion

In conclusion, the yield and stereoselectivity of a Petasis reaction is determined by an interplay of several factors, including the type of amine employed, either primary or secondary, the degree of branching in the amine reagent and the degree of steric hindrance which is imposed by the groups around boron.

The use of chiral secondary amines containing only one branched substituent has been shown to give optimal yields and stereoselectivities in the preparation of α-amino acids using the Petasis reaction. While the use of chiral primary amines generally gives products in low to moderate diastereoselectivity, chiral secondary amines generally give products in >95:5 diastereoselectivity. Additionally, the use of amines with two chiral (and by definition, branched) *N*-alkyl substitutents results in significantly reduced yields with respect to secondary amines with one or no branched *N*-alkyl substituents.

4. Experimental procedures

4.1. General

Infrared absorption spectra were acquired using a Perkin–Elmer 1600 FTIR spectrometer. Compounds were prepared as KBr discs or as thin films between sodium chloride plates. Absorption maxima are expressed in wavenumbers (cm^{-1}). ^1H nuclear magnetic resonance spectra were recorded using a Bruker AC 200B recorded at a frequency of 200.13 MHz or a Bruker Avance 300 recorded at a frequency of 300.13 MHz and are expressed as parts per million (ppm) downfield shift, with deuterochloroform (δ 7.26) as an internal reference, unless otherwise stated. The spectral data is recorded as chemical shift (δ_H), relative integral, multiplicity (s=singlet, br=broad, d=doublet, t=triplet, dd=doublet of doublets, dt=doublet of triplets, m=multiplet, app=apparent) and coupling constant (*J* Hz). ^{13}C nuclear magnetic resonance spectra were recorded using a Bruker AC 200B recorded at a frequency of 50.32 MHz or a Bruker Avance 300 recorded at a frequency of 75.47 MHz. The ^{13}C NMR data are recorded as parts per million (ppm) downfield shift with deuterochloroform (δ 77.2) as an internal reference, unless otherwise stated. Low resolution mass spectra were recorded on a Finnigan PolarisQ ion trap mass spectrometer using electron impact (EI) ionisation mode at 70 eV and a Finnigan LCQ ion trap mass spectrometer (ESI). The molecular ion, designated as $[M^{+\cdot}]$, major fragment peaks are quoted as percentages relative to the base peak intensity. High resolution mass spectra were recorded on a VG Autospec mass spectrometer using EI ionisation mode at 70 eV and a Bruker BioApex FTICR with an Analytica ESI source and magnet strength of 4.7 T. Melting points were determined on a Reichert hot stage microscope and are uncorrected.

Amines **1a–g** were obtained from Sigma–Aldrich. Compounds **4a**, **4c**, **7a** were obtained from BoronMolecular Pty. Ltd.

4.2. Preparation of boronate esters

Boronate esters **7d–g** were prepared according to the method of Scobie et al.[17,18] Other boronate esters were prepared by the following method: to a solution of the appropriate diol in diethyl ether (1 mL/mmol) was added the boronic acid (1 equiv) and dried magnesium sulfate (1 equiv) and the resulting mixture was stirred for 20 h. The mixture was filtered, the solid was washed with ether, and the filtrate concentrated in vacuo to yield the boronate ester.

4.2.1. 2-Phenyl-1,3,2-dioxaborolane (4b). Colourless oil, 90%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.85–7.80 (2H, m), 7.49–7.35 (3H, m), 4.38 (4H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.0, 131.6, 128.0, 66.2 (carbon bearing boron substituent not observed); data in accordance with literature values.[20]

4.2.2. 2-((*E*)-2-Phenylethenyl)-1,3,2-dioxaborolane (7b). Colourless liquid which solidified on standing, 93%; mp 47–48 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.53–7.48 (3H, m), 7.39–7.32 (3H, m), 6.20 (1H, d, J=18.4 Hz), 4.29 (4H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 150.2, 129.1, 128.7, 127.2 65.7 (carbon bearing boron substituent not observed); MS *m/z* (EI) 173.9 [$M^{+\cdot}$] (100%); HRMS *m/z* (EI) found 174.0857, $C_{10}H_{11}BO_2$ requires 174.0852.

4.2.3. 4,4,5,5-Tetramethyl-2-((*E*)-2-phenylethenyl)-1,3,2-dioxaborolane (7c). Colourless liquid, 93%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.50–7.26 (6H, m), 6.17 (1H, d, J= 18.4 Hz), 1.30 (12H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ 149.6, 137.5, 128.9, 128.6, 127.1, 83.4, 24.8 (carbon bearing boron substituent not observed); MS *m/z* (EI) 230 [$M^{+\cdot}$] (20%), 129 (100), 144 (66); spectral data in accordance with literature values.[21]

4.3. Petasis reactions

Method A. To a suspension of glyoxylic acid monohydrate **2** (1 mmol) in dichloromethane (5 mL) was added the amine **1** (1 mmol) and organoboron reagent (1 mmol) and the reaction was stirred under N_2 for 48 h. The resulting precipitate was isolated by filtration and washed with dichloromethane to yield the product.

Method B. To a suspension of glyoxylic acid monohydrate **2** (1 mmol) in dichloromethane (5 mL) was added the amine (1 mmol) and organoboron reagent (1 mmol) and the reaction was stirred under N_2 for 48 h. The solvent was removed in vacuo and the crude product purified by chromatography on silica to yield the product.

4.3.1. *N*-Benzylphenylglycine (6a). *Method A*. White solid, 10%; mp 219–220 °C (lit.[22] 220–221 °C); ^{1}H NMR (200 MHz, $D_2O+K_2CO_3$) δ 7.45–7.34 (10H, m), 4.16 (1H, s), 3.70 (2H, m); data in accordance with literature values.[22]

4.3.2. *N*,*N*-Dibenzyl-α-phenylglycine (6b). *Method B*. Clear colourless oil, 64%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.61 (1H, br s), 7.53–7.25 (15H, m), 4.83 (1H, s), 4.02 (2H, d, J=13.7 Hz), 3.78 (2H, d, J=13.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.2, 137.0, 134.0, 130.2, 129.8, 129.2, 128.7, 128.6, 127.8, 67.1, 54.7; data in accordance with literature values.[23]

4.3.3. *N*,*N*-Diisopropyl-α-phenylglycine (6c). *Method A*. White solid, 94%; mp 135–136 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.82–7.32 (5H, m), 5.08 (1H, s), 3.51 (2H, septet, J=6.5 Hz), 1.31 (12H, d, J=6.5 Hz); ^{13}C NMR (75 MHz, D_2O) δ 176.9, 134.3, 131.9, 131.8, 128.8, 63.2, 47.9, 18.9; IR ν_{max} (cm^{-1}) 3015, 2873, 1696, 1622; MS *m/z* (ESI) 236.1 [$M+H]^+$(100%); HRMS *m/z* (EI) found 235.1566, $C_{14}H_{22}NO_2$ requires 235.1572.

4.3.4. *N*-Benzyl-*N*-isopropyl-α-phenylglycine (6d). *Method A*. White solid, 99%; mp 101–102 °C; ^{1}H NMR (200 MHz, $D_2O+K_2CO_3$) δ 7.60–7.20 (10H, m), 5.09 (1H, br s), 3.82 (2H, br s), 2.94 (1H, septet, J=6.5 Hz), 1.05 (6H, d, J=6.5 Hz); ^{13}C NMR (75 MHz, d_6-DMSO) δ 177.5, 134.9, 133.5, 132.7, 130.6, 129.6, 129.5, 128.2, 126.9, 92.1, 50.2, 48.4, 19.6; IR ν_{max} (cm^{-1}) 2922, 1699; MS *m/z* (ESI) 284 [$M+H]^+$(100%); HRMS *m/z* (ESI) found 284.1650, $C_{18}H_{22}NO_2$ requires 284.1651.

4.3.5. *N*-Benzyl-α-(*E*)-phenylethenylglycine (8a). *Method A*. Off white solid, 79%; ^{1}H NMR (200 MHz, $D_2O+K_2CO_3$) δ 7.35–7.11 (10H, m), 6.47 (1H, d, J=15.9 Hz), 6.12 (1H, dd, J=8.2, 15.9 Hz), 3.70 (1H, d, J=8.1 Hz), 3.56 (2H, m); ^{13}C NMR (50 MHz, $D_2O+K_2CO_3$) δ 171.2, 136.1, 133.3, 129.0, 128.6, 128.5, 128.4, 128.2, 128.0, 127.7, 126.9, 63.5, 49.2; data in accordance with literature values.[1]

4.3.6. *N*,*N*-Dibenzyl-α-(*E*)-phenylethenylglycine (8b). *Method B*. Colourless oil, 64%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.26 (1H, br s), 7.45–7.24 (15H, m), 6.70 (1H, d, J=16.0 Hz), 6.38 (1H, dd, J=8.4, 16.0 Hz), 4.23 (1H, d, J=8.4 Hz), 4.06 (2H, d, J=13.5 Hz), 3.78 (2H, d, J=13.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 138.1, 136.1, 136.0, 129.3, 128.9, 128.7, 128.5, 128.2, 126.9, 120.7, 65.5, 55.1; IR ν_{max} (cm^{-1}) 3021, 2849, 1711, 1628; HRMS *m/z* (ESI) found 358.1813, $C_{24}H_{24}NO_2$ requires 358.1807.

4.3.7. *N*,*N*-Diisopropyl-α-(*E*)-phenylethenylglycine (8c). *Method A*. White solid, 84%; mp 111–112 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.61–7.41 (5H, m), 7.03 (1H, d, J=15.7 Hz), 6.36 (1H, dd, J=9.8, 15.7 Hz), 4.69 (1H, d, J=9.8 Hz), 3.90 (2H, br m), 1.48–1.37 (12H, m); ^{13}C NMR (75 MHz, D_2O) δ 173.3, 141.3, 136.0, 129.8, 129.6, 119.7, 65.7, 54.7, 20.1; IR ν_{max} (cm^{-1}) 3011, 2973, 1628, 1616; MS *m/z* (EI^+) 284.1 [$M+Na]^+$(100%); HRMS *m/z* (ESI) found 262.1810, $C_{16}H_{24}NO_2$ requires 262.1807.

4.3.8. *N*-Benzyl-*N*-isopropyl-α-(*E*)-phenylethenylglycine (8d). *Method A*. White solid, 90%; mp 116–117 °C; ^{1}H NMR (300 MHz, D_2O/DMSO + K_2CO_3) δ 7.17–6.85 (10H, m), 6.41 (1H, d, J=16.1 Hz), 6.04 (1H, dd, J=8.7, 16.1 Hz), 3.93 (1H, d, J=8.7 Hz), 3.57 (1H, d, J=15.0 Hz), 3.46 (1H, d, J=15.0 Hz), 3.04 (1H, septet, J=6.2 Hz), 0.92 (6H, m); ^{13}C NMR (75 MHz, D_2O/DMSO + K_2CO_3) δ 179.4, 141.5, 137.3, 132.7, 129.5, 129.3, 129.0, 128.6, 128.0, 127.0, 126.9, 71.4, 51.5, 51.1, 20.9; IR ν_{max} (cm^{-1}) 3029, 2983, 1621, 1616; MS *m/z* (ESI) 332.1 [$M+Na]^+$(100%); HRMS *m/z* (ESI) found 310.1810, $C_{20}H_{24}NO_2$ requires 310.1807.

4.3.9. *N*-((*S*)-1-Phenylethyl)-(*R*)-α-(*E*)-phenylethenylglycine (8e). *Method A*. White solid, 97%; ^{1}H NMR (200 MHz, $D_2O+K_2CO_3$) major isomer δ 7.55–7.35 (10H, m), 6.48 (1H, d, J=15.9 Hz), 6.22 (1H, dd, J=8.4, 15.9 Hz), 3.91 (1H, q, J=6.6 Hz), 3.70 (1H, d, J=8.4 Hz), 1.42 (3H, d, J=6.6 Hz); minor isomer δ 7.55–7.35 (10H, m), 6.53 (1H, d, J=16.0 Hz), 6.17 (1H, dd, J=8.0, 16.0 Hz), 3.89 (1H, q, J=6.6 Hz), 3.61 (1H, d, J=8.0 Hz), 1.44 (3H, d, J=6.6 Hz); ^{13}C NMR (75 MHz, D_2O/DMSO + K_2CO_3) major isomer δ 178.9, 144.3, 137.1, 134.2, 129.2, 129.1, 128.5, 128.2, 127.7, 127.3, 126.9, 64.2, 54.7, 24.0; minor isomer δ 179.5, 145.2, 137.1, 132.8, 129.2, 129.1, 128.5, 128.1, 127.5, 127.3, 126.9, 65.1, 55.9, 22.7; data in accordance with literature values.[1]

4.3.10. *N*,*N*-Bis-((*S*)-1-phenylethyl)-(*R*)-α-(*E*)-phenylethenylglycine (8f). *Method B*. Colourless oil, 38%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.22 (15H, m), 6.83 (1H, d, J=15.9 Hz), 6.46 (1H, dd, J=8.9, 15.9 Hz), 4.54 (2H, q, J=6.9 Hz), 4.39 (1H, d, J=8.9 Hz), 1.69 (6H, d, J=6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 141.5, 137.3, 136.2, 129.0, 128.8, 128.4, 128.2, 127.8, 126.7, 123.7, 62.5, 56.9, 17.6; IR ν_{max} (cm^{-1}) 3029, 2976, 1721, 1626; MS *m*/*z* (ESI) 386 (100%) $[M+H]^+$; HRMS *m*/*z* (ESI) found 386.2116, $C_{26}H_{28}NO_2$ requires 386.2120.

4.3.11. *N*-Methyl-*N*-((*S*)-1-phenylethyl)-(*R*)-α-(*E*)-phenylethenylglycine (8g). *Method A*. White solid, 81%; ^{1}H NMR (300 MHz, $D_2O+K_2CO_3$) δ 7.36–7.17 (10H, m), 6.41 (1H, d, J=15.9 Hz), 6.18 (1H, dd, J=8.7, 15.9 Hz), 3.93 (1H, q, J=6.8 Hz), 3.60 (1H, d, J=8.7 Hz), 2.12 (3H, s), 1.28 (3H, d, J=6.8 Hz).

Acknowledgements

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